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October 5, 2004

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OFFICE OF
FOOD ADDITIVE SAFETY

Laura Tarantino, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Tarantino:

In accordance with proposed 21 CFR § 170.36 (notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18937-18964), I am submitting in triplicate, as the representative of the notifier, Griffith Laboratories, 1 Griffith Center, Alsip, IL 60803, a GRAS notification of *Carnobacterium maltaromaticum* strains CB1, CB2, CB3, LV17, UAL26, ATCC 35586 and ATCC43225 for use in ready-to-eat (RTE) and fresh comminuted[1], processed meat products at a maximum inoculation[2] concentration of $1x10^4$ colony forming units (cfu)/g, for use as an inhibitor of *Listeria monocytogenes*. The common name of "*Carnobacterium maltaromaticum*", will be used in ready-to-eat and fresh, comminuted, processed meat products. A GRAS panel report, setting forth the basis for the GRAS determination, as well as CVs of the members of the GRAS panel for review by the Agency, are also enclosed.

Best regards,

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

[1] Comminuted = Reduced to small pieces or particles by pounding or abrading.
[2] Inoculation = Use of a small amount of biological material to initiate a microbial culture.

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1. GRAS Exemption Claim

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36 (c) (1)

Carnobacterium maltaromaticum, at a concentration of up to $1x10^4$ cfu/g of food, has been determined to be generally recognized as safe (GRAS) and; therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

_____ Date_____

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
Burdock Group
888 17th Street, NW, Suite 810
Washington, DC 20006

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(i) Name and Address of the Notifier

George A. Burdock, Ph.D.
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Fellow, American College of Nutrition
Burdock Group
888 17th Street, NW, Suite 810
Washington, DC 20006

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(ii) Common Name of the Notified Substance

Carnobacterium maltaromaticum bacteria

(iii) Conditions of Use

Specific strains of *Carnobacterium maltaromaticum* are intended to be used as an ingredient to inhibit the growth of *Listeria monocytogenes* in ready-to-eat and fresh, comminuted meat products. *C. maltaromaticum* will be used at levels up to $1x10^4$ cfu/g of meat in ready-to-eat (RTE) and fresh comminuted, processed meat products. The estimated mean *per capita* consumption, taking into account the theoretical assumption that the bacteria will grow over an extended period of time in storage and that bacteria are already present on the meat products, by the total population of the United States from all proposed uses at the maximum use levels was determined to be $8.6x10^9$ cfu *per* person *per* day, with a 90th percentile consumption calculated to be $1.6x10^{10}$ cfu *per* person *per* day. However, *C. maltaromaticum* has been shown to be self-limiting in its growth and will plateau between approximately 10^8 and 10^9 cfu/g of meat.

(iv) Basis of GRAS Determination

Pursuant to 21 CFR § 170.3, *C. maltaromaticum* has been determined GRAS by scientific procedures for its intended conditions of use. The safety of *C. maltaromaticum* is supported by isolation of the bacteria on food products already consumed by the population, prior use of the bacteria in sausage fermentation, and animal studies. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of substances used as ingredients in food.

(v) Availability of Information

The data and information that serve as a basis for this GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at a reasonable time at the office of:

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
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Alternatively, copies of data and information can be provided to FDA upon request, by contacting Dr. Burdock.

2. Detailed Information About the Identity of the Notified Substance

A. Identity

The *Carnobacterium maltaromaticum* strains CB1, CB2, CB3, LV17, UAL26, ATCC 35586 and ATCC 43225 are naturally selected, non-genetically modified bacteria.

Common or Usual Name: *Carnobacterium maltaromaticum* bacteria

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Table 1. General description and analytical information of *C. maltaromaticum.*

Bacterial name	*Carnobacterium maltaromaticum* strains
Growth Characteristics	Gram-positive, non-motile, non-sporeforming rods occurring singly or in short chains. Colonies as pinpoint, convex, white, circular and nonpigmented when grown at 25°C for 24 hours on Trypticase Soy Agar. Optimal temperature for growth is 30°C, with a range between 6 to 40°C, with an optimal pH range from 6.0 to 7.0. It is facultatively anaerobic, with folic acid, riboflavin, panthothenate and niacin required for growth. Catalase and oxidase are not produced during growth, while arginine and esculin are hydrolyzed, with variable gas production.
Packaging	The standardized, viable cell blend will be packaged into plastic foil film packages, flushed with nitrogen and the package weight will be customized to the finished product application such that between 10^3 and 10^4 viable *C. maltaromaticum* cells *per* gram of finished product will be achieved.
Storage	Store at ambient temperature (22°C).
Labeling	*Carnobacterium maltaromaticum.*
Functional Use in Food	Inhibition of *Listeria monocytogenes* growth.

B. Composition

The *Carnobacterium maltaromaticum* blend contains the viable *C. maltaromaticum* cells and maltodextrin, as an excipient for freeze drying.

C. Method of Manufacture of *Carnobacterium maltaromaticum*

C. maltaromaticum strains are maintained in lyophilized form under vacuum at 4°C, or as frozen cultures in 20% (v/v) glycerol at –80°C in sealed vials. API® strip analysis (a kit for identification of bacteria to the species level) is conducted to ensure viability and strain purity will be confirmed by the absence of bacteriological contamination and/or by random amplified polymorphic DNA (RAPD) and microbiological analysis. Randomly selected vials undergo microbiological testing to confirm strain purity and the absence of bacteriological contamination.
2 21 CFR 20.61

[3] Maltodextrin = A manufacturing aid (*i.e.*, excipient) for freeze drying.

2. 21 CFR 20.11

Figure 1. ***Carnobacterium maltaromaticum*** **production scheme.**

21 CFR 20.61(c)

21 CFR 20.61(c)

20.61():)

D. Specifications for Food Grade Material

Table 2. Specifications of *C. maltaromaticum* as a lyophilized powder.

Active Ingredients	*Carnobacterium maltaromaticum*	
Excipients	Maltodextrin	
Shelf life	> one year	
Storage conditions	Room Temperature (22°C)	
Physical aspects	**Specifications**	**Method**
Appearance	Pass	Visual inspection on APT plate and comparison to standard plate photograph and description
Concentration	Between $3.2x10^6$/g and $3.2x10^7$ viable cells/g	A.P.H.A./USP
Residual moisture	<5%	O'Haus
	Microbiological Specifications	
Lactic acid bacteria	Between $3.2x10^6$/g and $3.2x10^7$ cfu/g or between $1.3x10^9$ and $1.4x10^{10}$ cfu/package	A.P.H.A./USP
Non-Lactic acid bacteria	<100/g	A.P.H.A./USP
Yeasts	<100/g	A.P.H.A./USP
Molds	<100/g	A.P.H.A./USP
Anaerobic Spore-Forming Bacteria	<10/g	A.P.H.A./USP
Clostridium botulinum	Absent *per* 50 g	A.P.H.A./USP
Total coli forms	<10/g	A.P.H.A./USP
Staphylococcus aureus	<100/g	A.P.H.A./USP
Escherichia coli	Absent *per* 25 g	A.P.H.A./USP
***Salmonella* spp.**	Absent *per* g	A.P.H.A./USP

APT = All Purpose Tween agar; cfu = Colony Forming Units; A.P.H.A. = American Public Health Association; USP = U.S. Pharmacopoeia

III. Self Limiting Levels of Use

The growth of *Carnobacterium maltaromaticum* is self-limiting, inhibiting its own growth to approximately 10^9 cfu/g of meat.

IV. Basis of GRAS Determination

The determination that specific strains of *Carnobacterium maltaromaticum* are GRAS is on the basis of scientific procedures. See attached- DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SPECIFIC STRAINS OF *Carnobacterium maltaromaticum* AS A FOOD INGREDIENT.

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DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SPECIFIC STRAINS OF *Carnobacterium maltaromaticum* AS A FOOD INGREDIENT

September 16, 2004

Panel Members:

Douglas Archer, Ph.D.

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OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SPECIFIC STRAINS OF *Carnobacterium maltaromaticum* AS A FOOD INGREDIENT

TABLE OF CONTENTS

1. History and Phylogenetic Analysis of Carnobacterium maltaromaticum *2*
2. Identity and Characteristics of Carnobacterium maltaromaticum *4*
3. Natural Occurrence of Bacteria in Food *9*
3.1. Occurrence and use of Lactic Acid Bacteria in Food *9*
3.2. Natural Occurrence of Carnobacterium maltaromaticum *on Meat, Fish and Cheese Products* *10*
4. Production of Carnobacterium maltaromaticum *Culture* *12*
4.1. Culture Collection *12*
4.2. Seed and Mother Culture *13*
4.3. Fermentation and Concentration *13*
4.4. Lyophilization *13*
4.5. Microbiological Analysis *13*
4.6. Reconstitution of Starter Culture *15*
5. Growth Characteristics of Carnobacterium maltaromaticum *in Meat Products* *15*
5.1. Growth Characteristics of Carnobacterium maltaromaticum *on Vacuum-Packaged Wieners* *15*
5.2. Growth Characteristics of Carnobacterium maltaromaticum *When Inoculated into Sausages* *16*
7. Exposure/Consumption Assessment *18*
7.1. Carnobacterium maltaromaticum *in Commercial Use* *18*
7.2. Background Exposure to Carnobacterium maltaromaticum *18*
7.3. Per Capita *Consumption of* Carnobacterium maltaromaticum *From Additional Exposure Due to Intended Uses* *20*
8. Biological Toxicity and Pathogenicity *21*
8.1. Biogenic Amine Formation *21*
8.2. Pathogenicity Studies *24*
8.3. Safety of bacteriocins produced by Carnobacterium maltaromaticum *25*
8.4. Observations of Carnobacterium maltaromaticum *in humans* *27*
9. Discussion *28*
10. Conclusion *31*
11. References *32*
12. Appendix A *41*
13. Appendix B *42*
14. Appendix C *43*
15. Appendix D *47*
16. Appendix E *50*

OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SPECIFIC STRAINS OF *Carnobacterium maltaromaticum* AS A FOOD INGREDIENT

Summary

The undersigned, an independent panel of recognized experts (hereafter referred to as the Expert Panel)[1], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to determine the Generally Recognized As Safe (GRAS) status, in accordance with 21 CFR § 170.30, 21 CFR § 170.35 and proposed 21 CFR § 170.36, of *Carnobacterium maltaromaticum* strains CB1, CB2, CB3, LV17, UAL26, ATCC 35586 AND ATCC43225 for use as a preservative in ready-to-eat (RTE) and fresh comminuted[2], processed meat products at a maximum inoculation[3] concentration of 1 x 10^4 colony forming units (cfu)/g. A comprehensive search of the scientific literature for safety and toxicity information on the bacterium commonly known as *Carnobacterium piscicola* (recently renamed from *Lactobacillus piscicola* and reclassified with *Lactobacillus maltaromicus* to the new name of *Carnobacterium maltaromaticum*) was conducted through April 2004 and made available to the Expert Panel. The Expert Panel independently evaluated materials submitted by Griffith Laboratories and other materials deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

1. History and Phylogenetic Analysis of *Carnobacterium maltaromaticum*

Carnobacterium maltaromaticum is one species of a diverse group of bacteria that are generally classified as atypical Lactic Acid Bacteria (LAB). LAB have been utilized for centuries in the food and dairy industries in the production of fermented foods. Important in this capacity is their ability to produce aromatic and flavor-enhancing compounds (Stiles and Holzapfel, 1997; Carr *et al.*, 2002). LAB have been characterized by their ability to produce a variety of isomers of lactic acid from the fermentation of carbohydrates. Atypical LAB are distinct due their inability to grow on acetate agar at pH 5.6, while being able to produce virtually pure L(+)-lactic acid from glucose and, their ability to ferment both glycerol and mannitol, properties that are unusual in lactobacilli (Holzapfel and Gerber, 1983; Shaw and Harding, 1984).

The name, *Lactobacillus piscicola* spp. nov., was first proposed for a group of 17 bacterial strains that were isolated from stressed rainbow trout (*Salmo gairdneri*), cutthroat trout (*Salmo clarki*) and Chinook salmon (*Oncorhynchus tshawytscha*) (Hiu *et al.*, 1984). The characteristics of the isolates from these fish best matched the description of the genus

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[1] Modeled after that described in section 201(s) of the Federal Food, Drug and Cosmetic Act, As Amended. See also attachments (*curriculum vitae*) documenting the expertise of the Panel members.
[2] Comminute = Reduce to small pieces or particles by pounding or abrading.
[3] Inoculation = Use of a small amount of biological material to initiate a microbial culture.

Lactobacillus. They were, however, genetically distinct from other members of the genus, and differed in their fermentation patterns and natural habitat (associated with fish). Therefore, the name *Lactobacillus piscicola* [(pis.ci' co.la. L.n. *piscis* fish; L. suff *–cola* dweller; M.L. n. *piscicola* fish (dweller)] was proposed.

L. piscicola, as well as *L. divergens,* and *L. carnis* and others, were found to possess a number of properties that were different from normal lactobacilli. These properties included the ability to grow at pH 9.5, resistance to thallous acetate, antibiotic resistance, specific vitamin requirements and, the aforementioned inability of *L. piscicola, L. divergens* and *L. carnis* to grow on acetate agar (Collins *et al.*, 1987; Rogosa *et al.*, 1951; Stiles and Holzapfel, 1997). *Lactobacillus* species were also shown to possess predominantly straight-chain, saturated and mono-unsaturated fatty acids, with the predominant $C_{18:1}$ isomer synthesized as *cis*-vaccenic acid in their cell membranes (Turujmaqn *et al.*, 1974; Uchida and Mogi, 1973; Veerkamp, 1971).

However, Collins *et al.* (1987) reported that *L. piscicola, L. divergens* and *L. carnis* synthesize the major $C_{18:1}$ isomer as oleic acid ($\Delta_{9,10}$), indicative of a different unsaturated fatty acid synthase pathway. Genetic homology classifications and chemical as well as physical characteristics also placed *L. piscicola, L. carnis* and *L. divergens* in the same DNA homology group. In addition, biochemical and chemical data indicated that *L. piscicola* and *L. carnis* should be (and were) reduced to the same species, *L. piscicola*. *L. piscicola*, along with *L. divergens,* were then re-classified into a new genus, *Carnobacterium* (L. gen. N. *carnis*, of flesh; Gr. dim. n. *bakterion*, a small rod; M.L. neut. N. *Carnobacterium*, flesh rodlet) by Collins *et al.* (1987). This was further substantiated when a 16S rRNA sequence analysis demonstrated that the *Carnobacterium* genus forms a distinct phylogenetic clade[4] within the lactic acid bacteria and included *C. funditum*, *C. alterfunditum*, *C. gallinarum* and *C. mobile* (Table 1), with *Lactobacillus maltaromaticus* further defined as an objective synonym of *Carnobacterium piscicola* (Miller *et al.*, 1974; Collins *et al.*, 1991; Lai and Manchester, 2000; Lai *et al.*, 2004). In addition, although the *Carnobacterium* spp. were originally classified with the lactobacilli, phylogenetically the genus is more closely related to the genera *Enterococcus* and *Vagococcus* (Hiu *et al.*, 1984).

Recently, phenotypic and genetic characterizations of *Lactobacillus maltaromicus* strains DSM 20342^T, DSM 20344 and JCM1154 determined that these strains also belonged in the genus *Carnobacterium*. Further comparison with *C. piscicola* resulted in the decision that these two species should be considered synonymous. As a result, *C. piscicola* was reclassified as *Carnobacterium maltaromaticum* comb. nov. (Collins *et al.*, 1991; Mora *et al.*, 2003). Therefore, the common name of *Carnobacterium maltaromaticum* will be used for this GRAS determination.

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[4] Clade = A group of biological taxa or species that share features inherited from a common ancestor.

Table 1. *Carnobacterium* species, their relationship to previously described bacteria and their habitat (Collins *et al.*, 1987; Collins *et al.*, 1991; Mora *et al.*, 2003).

Current nomenclature	Previous nomenclature	Habitat
C. divergens	*L. divergens*	Meat, poultry, surface of ripened mold cheeses
C. gallinarum		Poultry
C. mobile		Poultry
*C. maltaromaticum**	*L. piscicola* *L. carnis* *L. maltaromicus*	Meat, poultry or salmonid fish
C. funditum		Antarctic lake
C. alterfunditum		Antarctic lake

*Proposed as *C. maltaromicus* (Collins *et al.*, 1991) and *C. maltaromaticum* (Mora *et al.*, 2003); *C.* = *Carnobacterium*; *L.* = *Lactobacillus.*

2. Identity and Characteristics of *Carnobacterium maltaromaticum*

Naturally-occurring *C. maltaromaticum* historically belongs to a group of LAB that metabolize glucose heterofermentatively to produce equimolar amounts of lactic acid, carbon dioxide, ethanol and acetic acid from sugars and was previously included in the genus *Lactobacillus* (Stanier *et al.*, 1957; Hiu *et al.*, 1984). Although some research has indicated that *Carnobacterium* spp. are homofermentative for L-lactate [with acetate, formate and CO_2 being produced as end-products of some secondary decarboxylation/dissimilation reactions of pyruvate (Hiu *et al.*, 1984; De Bruyn *et al.*, 1988)], the most recent description and characterization of *C. maltaromaticum* states that L(+)-lactic acid, ethanol and acetate are produced heterofermentatively (Mora *et al.*, 2003). Therefore, for this GRAS determination *C. maltaromaticum* has been characterized as having heterofermentative properties. *C. maltaromaticum* was found frequently in fish that had suffered some form of stress, such as that which occurs at spawning or with handling (Hiu *et al.*, 1984; Baya *et al.*, 1991). *C. maltaromaticum* has also been found by Ringo *et al.* (2000) to be associated with the digestive tract of the Atlantic salmon (*Salmo salar* L.). Carnobacteria have been isolated from refrigerated, vacuum-packaged fish and unprocessed beef and lamb, where it was among the predominant bacteria on the meats (Ahn and Stiles, 1990a; Baya *et al.*, 1991; Barakat *et al.*, 2000; Carr *et al.*, 2002; Paludan-Muller *et al.*, 1998; Sakala *et al.*, 2002; Yamazaki *et al.*, 2003). The methods used in these studies did not enrich or select for any specific bacterial class or species.

A biochemical and physiological comparison between *C. divergens* and *C. maltaromaticum* is given in (Table 2). *C. maltaromaticum* strain B270^T was described as having the following characteristics (Hiu *et al.*, 1984; Collins *et al.*, 1987):

- Gram-positive, non-motile, non-sporeforming rods that occur singly and in short chains;
- Grows well on many standard laboratory media, including TSA and Brain Heart Infusion agar and in deMan, Rogosa and Sharpe (MRS) broth and thioglycolate broth[5];
- Colonies are pinpoint, convex, white, circular and nonpigmented when grown at 25°C for 24h on TSA;
- Temperature range for growth is 6°C to 40°C; optimum temperature is approximately 30°C;
- Optimum pH range is from 6.0 to 7.0;
- Facultatively anaerobic. D, L-lactate is produced homofermentatively, but the species may exhibit heterofermentative properties under certain conditions; lactic acid production is enhanced under anaerobic growth conditions;
- Folic acid, riboflavin, panthothenate and niacin are required for growth; vitamin B_{12}, biotin, thiamine and pyridoxal are not required;
- Catalase and oxidase are not produced;
- Nitrate is not reduced to nitrite;
- Gas production is variable (depending on substrate) and frequently negative; gas production from glucose in arginine-MRS broth;
- Acid is produced from glycerol, ribose, galactose, gluconate, glucose, fructose, mannose, mannitol, *N*-acetyl glucosamine, amygdalin, arbutine, salicin, cellobiose, sucrose and trehalose; acid is not produced from arabinose, xylose, sorbose, rhamnose, dulcitol, inositol, methyl-D-mannoside, inulin or melezitose;
- Arginine and esculin are hydrolyzed;
- H_2S is not detected in TSI[6] slants;
- Resistant to 0.4 and 0.6% Teepol;
- Cell wall peptidoglycan contains diaminopimelic acid;
- DNA G+C content is 33.7-36.4 mol%;
- Major cellular fatty acids are of the straight-chain saturated and mono-unsaturated types with myristic, palmitic, palmitoleic and $\Delta_{9,10}$-oleic acids predominating;
- The type strain is B270^T (ATCC 35586), isolated in 1970 from a stressed adult cutthroat trout reared at Bandon Trout Hatchery in Coos County, Oregon.

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[5] MRS = Lactobacilli de Man-Rogosa-Sharpe broth; TSA = Trypticase Soy Agar.

[6] TSI = Triple Sugar Iron Agar.

Table 2. Biochemical and physiological comparison of the *Carnobacterium* species[1].

Characteristic	*C. divergens*	*C. maltaromaticum*[2]
Acid produced from[3]:		
Amidon	-	-
Amygdalin	+	+
Galactose	-	+
β-Gentiobiose	+	+
Gluconate	+(-)[4]	+
Inulin	-	+
Mannitol	-	+
Melibiose	-	+
Melezitose	+(-)	+(-)
α-Methyl-D-glucoside	-	+
α -Methyl-D-mannoside	-	+
D-Tagatose	-	+(-)
D-Turanose	-	-
D-Xylose	-	-
Voges-Proskauer[5]	+	+
Motility	-	-
Δ9,10-Methyleneoctadecanoic acid[6]	+	-

[1]Adapted from (Collins *et al.*, 1987). [2]Previously designated as *Lactobacillus piscicola* and *Carnobacterium piscicola*; [3]Reading performed at seven days. [4]+(-) = Occasional strain negative; [5]Glucose metabolism test performed on API 10E system; both strains produced arginine dihydrolase and β-galactosidase; both strains were negative for lysine decarboxylase, tryptophan desaminase, urease, ornithine decarboxylase, indole and H_2S; [6]Greater than 15% of total cellular fatty acids.

Alkaline pH (up to pH 9.5) promotes the growth of *Carnobacterium* colonies, while inhibiting other *Lactobacillus* species. Differentiation of *C. maltaromaticum* from other bacteria may be accomplished by modification of growth substrates. Differentiation of *C. maltaromaticum* from the enterococci includes microscopic distinction of rods *vs.* cocci and growing on Cresol Red Thallous Acetate Sucrose (CTAS) medium containing 2% inulin instead of sucrose. Enterococci are not able to ferment inulin, while *C. maltaromaticum* ferments inulin, forming yellowish to pinkish colonies with a metallic bronze sheen, a yellow color change of the medium and a clearance of precipitate. *C. maltaromaticum* forms an umbolate or *beta*-type colony when inosine is substituted for sucrose in CTAS Agar. The enterococci also produce a yellowing of the medium and a clearing of the precipitate, but do not have a metallic sheen (Carr *et al.*, 2002). Different strains of *C. maltaromaticum* have been shown to produce bacteriocins[7] that inhibit the growth of *Lactobacillus*, *Listeria* and other *Carnobacterium* species (McMullen and Stiles, 1996; Duffes *et al.*, 1999c; Schillinger *et al.*, 1993).

[7] Bacteriocins = Ribosomally synthesized, low molecular weight, antibacterial, proteinaceous materials that are able to inhibit the growth or kill closely related bacteria.

Three strains of *C. maltaromaticum* (V1, SF668, NCDO2762) have been tested for antibiotic resistance to 37 antibiotics and found to be resistant to cephalosporin (first, second and third generation), clindamycin, nalidixic acid, fosfomycin and aminosides (Duffes *et al.*, 1999b). In general, most antibiotics are directly (or indirectly) derived from chemicals produced from common bacteria that have a "natural" defense towards other bacteria. Consequently, bacteria develop resistance to these antibiotics. The profile of resistance or sensitivity to various chemotherapeutic drugs can be useful in the characterization of specific bacterial species (Antibiogram Committee of the French Society for Microbiology, 1998; Baya *et al.*, 1991; Duffes *et al.*, 1999b; Euzeby, 2004, Vescovo *et al.*, 1982). As an example, *Lactobacillus* species have been accepted as nonpathogenic, commensal[8] bacteria that have a reputation as health promoters in the human gastrointestinal and female urogenital tracts (Charteris *et al.*, 1998). Forty-six *Lactobacillus* strains evenly divided between isolates derived from human and dairy sources (including commercial sources), were tested for antibiotic resistance to 44 antibiotics (Charteris *et al.*, 1998). The *Lactobacillus* strains were found to exhibit resistance to 14 antibiotics, including inhibitors of cell wall synthesis (vancomycin, cefoxitin and aztreonam), nucleic acid synthesis (sulphamethoxazole, trimethoprim, co-trimoxazole and metronidazole), protein synthesis (fusidic acid, amikacin, gentamycin, kanamycin and streptomycin) and cytoplasmic membrane function (polymyxin B and colistin sulphate). Danielsen and Wind (2003) determined the level of susceptibility of *Lactobacillus* spp. to various antimicrobial agents utilizing the Etest (ABBiodisk, Stockholm, Sweden) kit. Most of the 62 *Lactobacillus* strains screened had minimum inhibitory concentrations (MICs) above the maximum value of the Etest strip, for 13 of the 25 antimicrobial agents utilized, which the authors indicated as a high natural resistance. The antibmicrobial agents were bacitracin, cefoxitin, ciprofloxacin, fusidic acid, kanamycin, metronidazole, nitrofuratoin, norfloxacin, streptomycin, sulphadiazine, teicoplanin, trimethoprim/sulphamethoxazole and vancomycin.

The strains specified in this GRAS document (*e.g.*, *Carnobacterium maltaromaticum* strains CB1, CB2, CB3, LV17, UAL26, ATCC 35586 AND ATCC43225) have been tested for their resistance to 27 antibiotics (Table 3; Griffiths Labs, 2004). Overall, the *C. maltaromaticum* strains tested were sensitive to amoxicillin + clavulanic acid, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, imipenem, netilmicin, rifampin, tetracycline and tobramycin. In viewing the antibiotic resistance profiles (Table 3), the *Carnobacterium* strains are sensitive to those major antibiotics that are commonly associated with transferable genetic elements in gram-positive commensal bacteria; specifically, erythromycin, chloramphenicol and tetracycline. Borriello *et al.* (2003) suggested that when used as probiotics, selected strains should be susceptible to greater than two major antibiotics. A comparison with the antibiotics used by Baya *et al.* (1991), Duffes *et al.*, (1999b) or Euzeby (2004) indicate that the sensitivity of the *C. maltaromaticum* strains (CB1, CB2, CB3, LV17, UAL26, ATCC 35586 and ATCC43225) to various antibiotics correlate well with antibiotic resistance found in *C. maltaromaticum* strains isolated from natural fish sources, as noted in Table 3. The antibiotic resistance profile for the *C. maltaromaticum* strains specified in this GRAS dossier correlate well with the antibiotic resistance profiles of *Lactobacillus* species already being added to food or found in food naturally. This indicates that the addition of these strains of *C. maltaromaticum* to foods would

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[8] Of, relating to, or characterized by a symbiotic relationship in which one species is benefited while the other is unaffected.

not be adding any new or significant antibiotic resistance determinants that are not normally found in commensals or probiotic lactobacilli.

Table 3. Antibiotic resistance profile of specific strains of *C. maltaromaticum* (Griffith Labs, 2004).

Antibiotic	CB1	CB2	CB3	LV17	UAL26	ATCC 35586	ATCC 43225	Baya *et al.*, 1991	Euzby, 2004	Duffes *et al.*, 1999b
Amikacin	R	R	R	I	R	R	R			R
Amoxicillin + clavulanic acid	S	S	S	S	R	R	S			S
Aztreonam	R	R	R	R	R	R	R			R
Cefepime	R	R	R	R	R	R	R			R
Cefotaxim	R	R	R	R	R	R	R			R
Cefoxitin	P	P	P	P	P	P	P			R
Ceftazidime	R	R	R	R	R	R	R			R
Cefuroxime	R	R	R	R	R	R	R			R
Chloramphenicol	I	I	S	S	I	S	S	R	S	S
Ciprofloxacin	I	S	S	S	I	S	S			S
Clindamycin	R	R	R	R	R	R	R			R
Colistin	R	R	R	R	R	R	R			R
Erythromycin	I	I	S	I	I	I	I	S	R	S
Gentamicin	I	R	S	I	R	S	I	R		R
Imipenem	S	S	S	S	S	S	S			S
Kanamycin	R	R	I	R	R	I	R	R		R
Minocycline	P	P	P	P	P	P	P			
Moxolactam	P	P	P	P	P	P	P			R
Nalidixic acid	R	R	R	R	R	R	R	R		R
Netilmicin	I	S	S	S	I	S	S			
Piperacillin	R	R	R	R	R	R	R			S
Rifampin	I	I	S	S	I	S	S			S
Streptomycin	R	R	R	R	R	R	R		R	R
Tetracycline	S	I	S	I	I	S	S	S	S	S
Ticarcillin	R	R	R	R	R	R	R			S
Tobramycin	S	S	S	S	S	S	S			R
Vancomycin	R	S	R	S	R	R	R			S

I = Intermediate; P = Pending; R = Resistant; S = Sensitive

3. Natural Occurrence of Bacteria in Food

3.1. Occurrence and use of Lactic Acid Bacteria in Food

Using direct-plating methods to identify bacteriocin-producing LAB isolates from meat and meat products, milk and dairy products, vegetables, fruit and seafoods, a total of 663,533 colonies from 72 food samples (32 milk and dairy, 40 meat) were examined for bacteriocin-production (Coventry *et al.*, 1997). Many of these food samples were judged to have exceeded an acceptable shelf life. A total of 15% of the meat and meat products yielded bacteriocin-producing *Carnobacterium* spp. Of the 72 food samples investigated, 44% yielded bacteriocin-producing bacteria. From the total 663,533 colonies tested, 80,992 colonies (12.2%) were found to be *Carnobacterium* spp., with 0.15% of those producing bacteriocins. The antibacterial activities of filter-sterilized culture supernatant fluids from select strains of the bacteriocin-producers were not affected by catalase, lipase or lysozyme, but were either completely or partially inactivated by at least one of the proteolytic enzymes, indicating that antibacterial activity was associated with proteinaceous substances. This study also shows that humans are already being exposed to *Carnobacterium* spp. and other food-borne bacteria that produce bacteriocins.

Amezquita and Brashears (2002) report the isolation of 49 strains of LAB from commercially available ready-to-eat (RTE) meat products. These were screened for their ability to inhibit the growth of *Listeria monocytogenes* at 5°C on agar spot tests. *Pediococcus acidilactici*, *Lactobacillus casei* and *L. paracasei* were identified as the three species with the greatest inhibitory activity. There was significant inhibition ($P<0.05$) of the growth of *L. monocytogenes* in all of the RTE meat products evaluated (five commercial samples of cooked ham and five commercial samples of frankfurters), when three selected strains of *Pediococcus acidilactici*, *Lactobacillus casei* and *L. paracasei* were added to the RTE meat products. This study showed that select strains of LAB can be isolated from RTE meat products and these strains effectively inhibit the growth of *L. monocytogenes* in frankfurters and cooked ham at 5°C over 28 days of storage. During the time of storage, the numbers of LAB increased by only approximately 1 log cycle and no visible signs of spoilage were evident (*e.g.*, detrimental effect on some organoleptic properties related to external appearance such as color changes, undesirable aromas and stickiness or texture changes) on the surface of the products.

A study was recently conducted by Sakala *et al.* (2002) to investigate the psychrotrophic[9] spoilage microflora on refrigerator-stored, vacuum-packaged beef. This study utilized a less selective glucose-blood-liver agar and Trypticase Soy Agar plating method (allowing for the widest range of bacterial growth) at an incubation temperature of 7°C. Various psychrotrophic species on vacuum packaged beef stored at refrigeration temperatures were identified and quantified over a six-week period to determine alterations in the bacterial species or quantities of the bacteria. Five fresh beef cut samples (acquired and vacuum-packaged approximately 48 hours after slaughter) were utilized to determine the types and quantities of the various bacteria

[9] Psychrotrophic = Bacteria which are able to grow at refrigeration temperatures, but grow optimally at temperatures above 20°C.

found in vacuum-packaged beef. A total of 1493 bacterial strains were identified as: *Brochothrix thermosphacta* (64), *Carnobacterium maltaromaticum* (27), *C. divergens* (79), *Lactobacillus algidus* (637), *Lactobacillus* spp. (4), *Lactococcus piscium* (270), *Leuconostoc gelidum* (375), *Acinetobacter* (3), *Aeromonas* (1), *Bacillus* (10), *Corynebacterium* (3), *Enterobacteriaceae* (1), *Pseudomonas* (13) and *Psychrobacter* (6). *L. gelidum*, *L. piscium* and *L. algidus* increased during the first three weeks of storage from approximately $5x10^3$ cfu/g to approximately $1x10^8$ cfu/g, and remained stable for the rest of the six-week study. *C. maltaromaticum* was inconsistently detected, but when present increased to approximately $5x10^7$ cfu/g during the first three weeks of storage and remained at that level for the last three weeks of the study. Vacuum or modified atmosphere (CO_2) packaging (CO_2-MAP) influences the bacterial species isolated from meat (Labadie, 1999). At low temperatures and with a limited amount of oxygen, LAB comprise the predominant bacterial population of CO_2-MAP packaged meat, at approximately $1x10^7$ cfu/cm^2 (Gill and Newton, 1978). There have been no studies directly comparing the specific quantities of different species of *Lactobacillus*, *Leuconostoc* and *Carnobacterium* on freshly packaged meat under CO_2-MAP conditions. Nilsson *et al.* (1999) isolated $2x10^4$ and $5x10^7$ cfu/g LAB from cold-smoked salmon at the time of purchase and after thirty-two days of incubation, respectively.

3.2. Natural Occurrence of *Carnobacterium maltaromaticum* on Meat, Fish and Cheese Products

Carnobacterium species have been isolated from vacuum-packaged meat, fish and French soft cheese (Ahn and Stiles, 1990a; Buchanan and Klawitter, 1992b; Stoffels *et al.*, 1992; Pilet *et al.*, 1995; Milliere and Lefebvre, 1994a; Milliere *et al.*, 1994b), as summarized in Table 4. A study by Lewus *et al.* (1991) identified two bacteriocin-producing strains of *C. maltaromaticum* from different parts of meat from retail meat products. Other *C. maltaromaticum* strains have been isolated from fish, meat and cheese (Milliere *et al.*, 1994b; Nissen *et al.*, 1994; Pilet *et al.*, 1995; Schillinger *et al.*, 1993; Shaw and Harding, 1984). Leisner *et al.* (1994) found that eighteen of the 80 strains of bacteria originally isolated from vacuum-packed halibut, salmon or mackerel were lactic acid bacteria. Of these, 28% were identified as *C. maltaromaticum*.

Sakala *et al.* (2002) conducted a study to investigate the psychrotrophic spoilage microflora on chill-stored vacuum-packaged beef and determined that out of a total of 1493 strains isolated from five fresh beef cut samples (each from a different meat shop), twenty-seven were identified as *C. maltaromaticum*. This bacterium was detected at 0, 1, 3, 5 and 6 weeks of storage at mean numbers of $2x10^3$, $2x10^4$, $2.5x10^6$, $1x10^7$ and $2.5x10^7$ cfu/g, respectively, for two samples positive for *C. maltaromaticum* and, persisted at the level of approximately $5x10^7$ cfu/g during the last three weeks of the six-week storage period.

The growth of *C. maltaromaticum* in fermented meat products has been noted by Montel (1999), who noted "At the end of the fermentation period, lactic acid bacteria are generally the dominant bacterial flora. The species *Lactobacillus curvatus*, *L. sakei*, *L. plantarum*, *L. viridescens*, *Carnobacterium divergens*, *C. maltaromaticum* and *Leuconostoc* are present naturally, but *Pediococcus* is only found when inoculated as a starter culture. Their count

generally exceeds 10^6 cfu/g and remains at this level during the whole ripening period. *Carnobacterium* is present during the fermentation period, but disappears afterwards."

Table 4. Isolation of *C. maltaromaticum* from food products.

Food Category	Food Product	*C. maltaromaticum* strain*	Reference
Fish	Cultured striped bass, channel catfish and bullhead catfish		(Baya *et al.*, 1991)
	Cold-smoked freshwater fish		(Gonzalez-Rodriguez *et al.*, 2002)
	Salmonid fish		(Hiu *et al.*, 1984)
	Cold-smoked salmon		(Leroi *et al.*, 1998)
	Vacuum-packaged halibut, salmon or mackerel		(Leisner *et al.*, 1994)
	Cold-smoked salmon	A9a, A9b, A9c, A9J, A10a, A10b, A10f, A10J, S1, S2, S3, S4	(Paludan-Muller *et al.*, 1998)
	Fish	V1	(Pilet *et al.*, 1995)
Beef	Vacuum-packaged beef		(Ahn and Stiles, 1990a)
	Raw ground beef		(Buchanan and Klawitter, 1992A)
	Meat	GN, DX	(Lewus *et al.*, 1991)
	Vacuum-packaged beef		(Sakala *et al.*, 2002)
	Meat	LV 61	(Shaw and Harding, 1984)
Lamb	Modified atmosphere-packaged lamb		(Nissen *et al.*, 1994)
Chicken	Modified atmosphere-packaged chicken legs		(Barakat *et al.*, 2000)
	Poultry		(Collins *et al.*, 1987)
Dairy	Soft cheeses	CP5	(Milliere *et al.*, 1994B)

* = Strain given, if known.

Cold-smoked salmon (CSS) is an extremely perishable food product and is highly susceptible to contamination with *Listeria monocytogenes*. CSS spoilage is primarily due to microbial activity during refrigerated storage (Duffes, 1999a). For CSS, it has been estimated that immediately after packing, bacterial counts range from $1x10^3$ to $1x10^4$ cfu/g with a predominance of Gram-negative bacteria (64%) such as *Shewanella putrefaciens* and *Aeromonas* spp. LAB were found to be present (32%), with the majority being *Carnobacterium* spp. (Donald and Gibson, 1992; Huss *et al.*, 1995). At 8°C, the level of bacterial flora increased to $1x10^7 - 1x10^8$ cfu/g over three weeks, with a shift in relative bacterial populations such that LAB predominate (60%), mainly as *Carnobacterium* spp. (47%) and *Lactobacillus* spp. (13%).

Paludan-Muller *et al.* (1998) reported a series of studies that evaluated the role of *C. maltaromaticum* in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5°C. A mixture of LAB and Gram-negative bacteria are usually found on spoiled CSS. Initial numbers of bacteria were low with total psychrotrophic counts less than $5x10^3$ cfu/g and, specifically, LAB counts of $10 - 1x10^2$ cfu/g. In addition, it was determined (by sensory

evaluation) that the shelf life of vacuum-packed cold-smoked salmon peaked at four weeks at 5°C. The microflora at four weeks was composed of LAB ($1x10^6$ – $1x10^7$ cfu/g) with Gram-negative flora at varying levels ($1x10^5$ – $1x10^7$ cfu/g).

Modified-atmosphere packaging reduced the growth of Gram-negative bacteria and selected specifically for LAB, although growth of LAB was below $3x10^5$ cfu/g during five weeks of storage (Paludan-Muller *et al.*, 1998). The LAB microflora was dominated by *C. maltaromaticum*, accounting for 87% of the 255 LAB isolates characterized. The spoilage potential of *C. maltaromaticum* was further studied by inoculation of approximately $1x10^6$ cfu *C. maltaromaticum per* gram in CSS stored at 5°C (Paludan-Muller *et al.*, 1998). In vacuum-packed salmon inoculated with *C. maltaromaticum* strains, LAB counts reached $1x10^7$ cfu/g after only one week of storage and the level was above $1x10^8$ cfu/g for the rest of the storage period. However, after four weeks of storage, the salmon was not rejected by a sensory taste panel, while the vacuum-packed control was rejected after four to five weeks. In inoculated modified-atmosphere-packed salmon, the LAB counts reached final levels of $1x10^6$ – $1x10^7$ cfu/g after two weeks, but the salmon was not sensory-rejected until four to five weeks of storage. It was concluded that the growth of *C. maltaromaticum* even at high numbers ($1x10^7$ – $1x10^8$ cfu/g) for several weeks did not accelerate the spoilage process of packed cold-smoked salmon.

A bacterial study on the composition of the psychrotrophic and mesophilic flora of French surface-mold-ripened soft cheeses made from raw cow's milk found that *C. maltaromaticum* was the dominant bacteria at the end of ripening on five samples of Brie cheese (Milliere and Lefebvre, 1994a). *C. maltaromaticum* bacteria was also isolated from Coulommiers, Camember, Pon-l'Eveue and Munster cheeses. The number of *Carnobacterium* colonies isolated from these cheeses ranged from 5×10^5 to 8×10^8 cfu/g in the various cheese samples. Milliere *et al.* (1994b) went on to characterize *C. maltaromaticum* strains isolated from five samples of Brie cheese. The pH values of the cheeses were between 6.8 and 7.6 and no off-odors or organoleptic defects were noted. The *Carnobacterium* species were dominant in the cheese samples, at between 1×10^8 and 1×10^9 cfu/g. The results of DNA-DNA hybridizations indicated that 33 of the 36 isolates were of the *C. maltaromaticum* species, while the remaining three (all picked from the same sample) were *C. divergens*.

To summarize, *Carnobacterium* spp. are common components of the microflora on vacuum-packaged meat, poultry, fish and cheese products and in some cases, they can represent a predominant constituent population, reaching levels of $1x10^8$ cfu/g or higher, on products such as smoked fish, chicken, beef and cheese, without being considered spoilage organisms.

4. Production of Carnobacterium maltaromaticum Culture

4.1. Culture Collection

C. maltaromaticum strains are maintained in lyophilized form under vacuum at 4°C, or as frozen cultures in 20% (v/v) glycerol at –80°C. API® strip analysis (a kit for identification of bacteria to the species level) is conducted to ensure viability and strain purity will be confirmed by the absence of bacteriological contamination and/or by random amplified polymorphic DNA (RAPD) and microbiological analysis. Seven lyophilized vials are prepared for each strain

(master seed). From a single vial of the master seed, 15 lyophilized vials are prepared under vacuum and stored at 4°C (secondary seed). From each vial of the secondary seed, enough frozen vials are prepared for one year's production requirements and stored at –80°C. One out of every 10 vials undergoes microbiological testing to confirm strain purity and the absence of bacteriological contamination. A process control flow chart for the production of *C. maltaromaticum* is given in Appendix A.

4.2. Seed and Mother Culture

The seed culture is prepared by transferring a loop full of frozen master seed or a vial of lyophilized culture to 10 ml All Purpose Tween (APT). The seed is then grown overnight. The mother culture is prepared from the seed culture by transferring the seed culture (grown overnight) into 6L of APT medium and incubated again overnight.

4.3. Fermentation and Concentration

The mother culture is aseptically transferred to the production fermentor, which contains Griffith CB medium and was maintained at 25°C. The fermentation is monitored spectrophotometrically (650 nm) and by plating onto APT agar until a cell density of approximately 10^9 cfu is reached. An aliquot (aliquot A) of the fermented CB medium (containing *C. maltaromaticum*) is pasteurized. Maltodextrin[10] is added to the pasteurized culture and the resulting slurry was mixed for five minutes to ensure homogeneity. Maltodextrin is added to the remaining aliquot (aliquot B) of the fermented CB medium (which contained viable *C. maltaromaticum*). Aliquot B is then mixed for five minutes to ensure homogeneity. Aliquot A is utilized to dilute the viable *C. maltaromaticum* culture (Aliquot B) to the required bacterial concentration for delivery to meat products.

4.4. Lyophilization

Aliquots A and B are separately aliquoted onto stainless steel trays. Once frozen, the trays are placed under vacuum. The resulting lyophilized material is scraped from the trays, ground and milled, and placed into polyethylene bags and double-bagged prior to refrigeration (4-8°C).

4.5. Microbiological Analysis

The lyophilized pasteurized cell fraction (aliquot A) and the lyophilized live cell fraction (aliquot B) is analyzed microbiologically for total lactic acid bacteria, non lactic acid bacteria, yeast, molds, total coliforms, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. (Table 5). If the results meet specifications, the lot is approved for release; otherwise, the lot is placed in a holding section for review by the Non-Conforming Material committee. If the material is deemed outside specification limits, Quality Assurance personnel will place a tag on the container documenting all appropriate product information, date and the reason for the non-conformance. If the viable *Carnobacterium* count is outside specifications, the product will be re-blended to meet the required specifications. A deviation for high moisture content will result in further drying in order to meet specifications. If the product does not meet the microbiological specifications for Salmonella, *E. coli*, *S. aureus*, total coliforms, yeasts or molds, the product will be denatured by the addition of a 200 ppm solution of sodium hypochlorite.

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[10] Maltodextrin = A manufacturing aid (*i.e.*, excipient) for freeze drying.

Autoclaving may be used as an alternative to hypochlorite denaturing. The product will then be disposed of, as applicable to the jurisdiction of the manufacturing facility.

To determine shelf life stability, triplicate vials of the cell culture, previously stored at 30°C, 45°C or 60°C, will be withdrawn from each storage temperature and a viable cell count determined on All Purpose Tween (APT) agar by serial dilution. The Log cell count will be plotted for each temperature and the linear regression of each line will be determined. The linear regression equation for each temperature will be used to determine the time to take to reach the minimum viable bacterial count for each vial, from which one can determine the shelf life of the bacterial culture at any given temperature.

Table 5. Specifications of *Carnobacterium maltaromaticum* lyophilized bacterial powder.

Active Ingredients	*Carnobacterium maltaromaticum*	
Excipients	Maltodextrin	
Shelf Life	> one year	
Storage Conditions	Room Temperature (22°C)	
Physical Aspects	**Specifications**	**Method**
Appearance	Pass	Visual inspection APT plate and comparison to standard plate photograph and description
Concentration	Between $3.2x10^6$/g and $3.2x10^7$ viable cells/g	A.P.H.A./USP
Residual moisture	<5%	O'Haus
Microbiological Specifications		
Lactic Acid Bacteria	Between $3.2x10^6$/g and $3.2x10^7$ cfu/g or between $1.3x10^9$ and $1.4x10^{10}$ cfu/package	A.P.H.A./USP
Non Lactic acid bacteria	<100/g	A.P.H.A./USP
Yeasts	<100/g	A.P.H.A./USP
Molds	<100/g	A.P.H.A./USP
Anaerobic Spore Forming Bacteria	<10/g	A.P.H.A./USP
Clostridium botulinum	Absent *per* 50 g	A.P.H.A./USP
Total coli forms	<10/g	A.P.H.A./USP
Staphylococcus aureus	<100/g	A.P.H.A./USP
E. coli	Absent *per* 25 g	A.P.H.A./USP
***Salmonella* spp.**	Absent *per* g	A.P.H.A./USP

A.P.H.A. = American Public Health Association; USP = U.S. Pharmacopoeia

4.6. Reconstitution of Starter Culture

Standardization of viable cell numbers will be achieved by blending a portion of the lyophilized pasteurized cell fraction (aliquot A) with a portion of the lyophilized live cell fraction (aliquot B) of viable *C. maltaromaticum* cells, to obtain between 3.2×10^6 and 3.2×10^7 cfu/g. The standardized viable cell blend will be packaged into plastic foil film packages, flushed with nitrogen and package weight will be customized to the finished product application such that between 10^3 and 10^4 viable *C. maltaromaticum* cells *per* gram of finished product will be achieved. The packages will then be stored at ambient temperature. During packaging of RTE meat products, such as wieners, a dose of *C. maltaromaticum* will be applied to each 454 g package *via* a liquid spray applicator, to achieve between 1×10^3 and 1×10^4 cfu/gram of product.

The packaged standardized viable cell blend will be analyzed microbiologically for identity, total lactic acid bacteria, non lactic acid bacteria, yeast, molds, total coliforms, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. (Table 5). If the results of the microbiological testing meet specifications, the lot is approved for release; otherwise, the lot will be placed in a holding section for review by the Non-Conforming Material committee. If the viable *Carnobacterium* count is outside of specifications, the product will be re-blended to meet the required specifications. A deviation for high moisture content will result in further drying in order to meet specifications. If the product does not meet the microbiological specifications for *Salmonella*, *E. coli*, *S. aureus*, total coliforms, yeasts or molds, the product will be denatured by the addition of 200 ppm sodium hypochlorite or autoclaved and then disposed of, as applicable to the jurisdiction of the manufacturing facility.

A dose of reconstituted *C. maltaromaticum*, containing one or more of strains CB1, CB2, CB3, LV17, UAL26, ATCC 35586 or ATCC 43225 (approximately 1×10^3 to 1×10^4 cfu/g of finished product) may also be added directly to ground meat prior to ingredient mixing and further grinding and stuffing into casings. The sausages would then be rapid frozen at –50°C until frozen in the center. These sausages would then be wrapped airtight in plastic wrap and kept frozen until thawed for retail sale.

5. Growth Characteristics of *Carnobacterium maltaromaticum* in Meat Products

5.1. Growth Characteristics of *Carnobacterium maltaromaticum* on Vacuum-Packaged Wieners

A laboratory-scale study was designed to investigate the growth characteristics of *C. maltaromaticum* on vacuum-packaged wieners inoculated with *C. maltaromaticum* under conditions comparable to those proposed for commercial production (Griffith Laboratories, 2002). In addition, the effects on sensory properties, such as aroma and flavor profile, were investigated and are detailed in Appendix B. The methods and results of this study were as follows:

Two strains of *C. maltaromaticum* were chosen for study: LV17 (a synonym for UAL 8) was originally isolated from vacuum-packaged, refrigerated, fresh pork and described by Shaw and Harding (1984) and, strain UAL 26, which was isolated from vacuum-packaged beef (Stiles and Holzapfel, 1997). The inoculum was prepared by adding washed bacterial cells to sterile 0.85% saline to provide an inoculum level of 2.5×10^6 cfu/ml. Individual wieners were dipped into the inoculum suspension for one minute, drain dried and vacuum-packaged in groups of five

wieners *per* bag (high barrier, low O_2 transmission, VP bags). As a control, wieners were dipped in 0.85% sterile saline without bacterial inoculum. Treated and control samples were then placed into refrigerated (4°C) storage for up to 12 weeks. Sampling of the wieners for microbiological analyses and sensory evaluation was performed on day zero and after 2, 4, 6, 7, 8, 10 and 12 weeks of storage.

Samples were prepared for microbial analysis by cutting 1.8 cm length piece of wiener (equivalent to a surface area of 10 cm^2), placing it in a sterile tissue homogenizer bag and homogenizing. Bacterial counts were conducted by standard dilution and plating techniques and included: 1) Total aerobic plate count on Plate Count Agar incubated aerobically at 25°C, 48 hours; 2) Lactic acid bacteria on APT agar incubated anaerobically at 25°C, 48 hours; 3) *Enterobacteriaceae* on Violet Red Bile Agar with one *percent* added glucose incubated at 35°C, 18 hours. Concentrations of bacteria were reported as cfu *per* cm^2 of product (cfu/cm^2).

This study reported that sample wieners that had been inoculated with *C. maltaromaticum* strains LV17 or UAL 26 reached maximum anaerobic lactic acid bacteria (LAB) counts of 2.75×10^6 and 1.2×10^5 cfu/cm^2 after seven or eight weeks of cold storage, respectively. *C. maltaromaticum* grew at a slow rate on vacuum-packaged wieners and growth was accompanied by a relatively small decrease in surface pH during storage. LV17 varied from pH 6.2 at Week 0 to pH 6.1 at Week 10, while UAL 26 varied from an initial pH 6.2 to approximately 5.9 during Weeks 6-8 and Week 12).

It was concluded that in comparison with other lactic acid bacteria, such as *L. gelidum*, *C. maltaromaticum* is a slow-growing species when inoculated onto refrigerated (4°C), vacuum packaged wieners. The levels of *C. maltaromaticum* reached a maximum of 5×10^7 cfu/cm^2 after 12 weeks of cold storage. Based on sensory evaluations using a trained nine-member panel over the 12-week storage period, there were no significant adverse effects on aroma, off-flavors, sour intensity, or overall acceptability resulting from inoculation with *C. maltaromaticum*.

5.2. Growth Characteristics of *Carnobacterium maltaromaticum* When Inoculated into Sausages

C. maltaromaticum CB1 was added to pork in three trials as an inoculant during the production of sausages (Griffith Laboratories, 2003a, Internal Report). Odor intensity and freshness attributes for the sausages (evaluated as both raw and cooked) were treated on a 21-point line scale. Inoculum levels ranged from 1×10^3 to 1×10^5 cfu/g of meat. Bacteriological analyses were conducted on Days 0, 5, 10, 15 and 20 to assess the growth of *C. maltaromaticum* and bacteriocin production.

Chilled pork shoulder and pork fat were weighed, coarse ground and divided into four batches to which 2.76% water and 1.8% of seasoning were added. Test product was inoculated with *C. maltaromaticum* up to 10^5 cfu/g. The ground meat and ingredients were mixed, ground fine and stuffed into collagen casing (UniPac, Edmonton). The stuffed casing was cut into links 3.5-3.75 inches, to give sausages that were approximately 20.4 g/sausage. The individual sausage links were rapid frozen at –50°C for approximately 35 minutes. The frozen sausages were packaged on Styrofoam trays (approximately 10 oz *per* pack) and airtight wrapped and

sealed in plastic wrap. The samples were thawed and stored at 4°C prior to bacterial sampling. Usual manufacturing procedures for meats include the procedure of "flash freezing" meat for shipment, with subsequent thawing for sale or further processing.

Bacteriological sampling was done on samples that had been thawed and stored at 4°C for 0, 5, 10, 15 and 20 days. Duplicate 10 g samples were placed into sterile stomacher bags (VWR International) and blended with 90 ml of sterile 0.1% peptone water. Appropriate serial dilutions in 0.1% peptone water were streaked onto prepoured APT agar and MRS agar plates and incubated at 30°C for 48 h. Duplicate counts (cfu/g of meat) were recorded for each sample after the incubation period.

Over a 20 day period, total anaerobic bacterial counts on MRS agar increased from 10^3 – 10^5 cfu/g, up to 10^9 cfu/g of product. The growth of background microflora in the test product did not differ from that associated with un-inoculated samples, as evidenced by the growth on APT agar. This indicates that the inoculation of the sausage meat with C. maltaromaticum did not increase the overall incidence of bacterial growth in the sausage. The microbiology assays indicated that the total number of bacteria growing on APT and MRS agar was similar on the uninoculated control and the test products. Therefore, added *C. maltaromaticum* cultures did not increase the number of bacteria found on the test products, nor cause the meat to spoil faster than the control.

Bacteriocin production in the sausage samples was tested at Days 0, 5, 10, 15 and 20 by direct and indirect assays and was detected, indicative of bacteriocin production by the added *C. maltaromaticum*. Suppression of the indicator organism, *L. monocytogenes*, was noted by Day 10 *via* the indirect assay [portions of the sausage were heat-treated (to kill the producer organism) and directly embedded into APT agar inoculated with *L. monocytogenes* CDC 7762 (serotype 4b)], with this suppression maintained through Day 20 of the assay. The direct assay for bacteriocin production (heat-treated supernatant of a homogenized sausage was directly added to APT agar plates overlaid with the *L. monocytogenes* indicator organism) in the sausages inoculated with *C. maltaromaticum* indicated that bacteriocin production occurred by Day 15 of sausage storage at 4°C and continued through Day 20 of storage (Griffith Laboratories, 2003b, Internal Report).

6. Intended Use of the Addition of *Carnobacterium maltaromaticum* in Ready-To-Eat and Fresh Comminuted, Processed Meat Products

RTE meat and fresh comminuted, processed meat products require preservation techniques that inhibit the growth of potentially pathogenic bacteria. A deadly *Listeria monocytogenes* outbreak recently spread across the northeast US, resulting in the U.S. Food and Drug Administration and the USDA's Food Safety Inspection Service (FSIS) issuing a health advisory in September, 2003 (Morbidity and Mortality Weekly Report, 2003). Symptoms of listeriosis in healthy individuals are usually short-term and include a severe headache, high fever, abdominal pain, stiffness, nausea and diarrhea. However, in those with weakened immune systems, newborns and the elderly, listeriosis may cause mortality, while pregnant women may have an increased risk to miscarriages and stillbirths (Water Quality Health Council, 2003; Doganay,

2003). Research has indicated that the growth of *L. monocytogenes* is inhibited when co-cultured in the presence of *C. maltaromaticum*, as summarized in Appendix C. *C. maltaromaticum* is to be added to RTE meat and fresh comminuted, processed meat products to inhibit the growth of human pathogenic bacteria, such as *L. monocytogenes*, on these food products.

It is proposed that *C. maltaromaticum* will be added to vacuum- or modified atmosphere-packaged ready-to-eat (RTE) meat products and fresh comminuted, processed meat products as a means of mitigating the effects of contamination caused by human pathogenic bacteria, such as *L. monocytogenes*. During packaging of RTE meat products, such as wieners, it is proposed that a dose (approximately 1.5 ml, or $5x10^6$ cfu) of reconstituted *C. maltaromaticum* will be applied to each 454 g (1 pound) package.

An aliquot of reconstituted *C. maltaromaticum* (to deliver approximately $1x10^3$ to $1x10^4$ cfu/g) would also be added to fresh comminuted, processed meat products prior to ingredient mixing and further grinding and stuffing into casings to produce fresh comminuted, processed meat products. The fresh comminuted, processed meat products would be rapid frozen at –50°C until frozen in the center, then wrapped airtight in plastic wrap and stored frozen.

The inoculation ranges for RTE meat products and fresh comminuted, processed meat products would be approximately $1x10^3$ to $1x10^4$ viable *C. maltaromaticum* cells (cfu) *per* gram of product.

7. Exposure/Consumption Assessment

7.1. *Carnobacterium maltaromaticum* in Commercial Use

Lallemand S.A. of Toulouse, France, produces and markets a range of LAB for use in the French food industry, including sausage fermentation. *C. maltaromaticum* is among the bacterial species listed by this company for this food application. A memo from Institut Rosell (Lallemand) of Montreal, Canada, attesting to this fact, along with a page of a Lallemand S.A. product catalogue, attesting to the fact of use, have been included in Appendix D. The European Commission has made reference to the fact that Carnobacterium species have been and are used to produce fermented foods both in Europe and around the world (European Commission, 2002).

7.2. Background Exposure to *Carnobacterium maltaromaticum*

Studies have shown that lactic acid bacteria in general and, specifically *C. maltaromaticum*, are found in retail food products (modified-atmosphere packaging and refrigeration preferentially selects for anaerobic *Carnobacterium* spp.) within the expiration dates (Milliere and Lefebvre, 1994A; Kelly *et al.*, 1996; Schobitz *et al.*, 1999; Amezquita and Brashears, 2002; Sakala *et al.*, 2002). Therefore, for an accurate assessment of the numbers of *C. maltaromaticum* that could maximally be consumed, one must take into account any theoretical amount of *C. maltaromaticum* that may already be present on the proposed foods.

An extensive search of the literature resulted in two references specifically analyzing the quantity of *C. maltaromaticum* found on commercially available foods. Sakala *et al.* (2002) determined that two beef samples contained *C. maltaromaticum*. It was detected at 0, 1, 3, 5 and 6 weeks of storage (vacuum-packaged and stored at 2°C) at mean numbers of $2x10^3$, $2x10^4$, $2.5x10^6$, $1x10^7$ and $2.5x10^7$ cfu/g of meat, respectively. Montel (2000) found that at the end of the fermentation period of sausages, lactic acid bacteria were generally the dominant bacterial flora, with *C. maltaromaticum* naturally present during the fermentation period at levels of approximately $5x10^7$ cfu/g of sausage, but disappeared afterwards. Sterile cold smoked salmon inoculated with *C. maltaromaticum* at 10^4-10^5 cfu/g of salmon was found to have final counts ranging between $5x10^7$ to 10^9 cfu/g, after two to three weeks of storage at 6°C (Stohr *et al.*, 2001). Nadon *et al* (2001) showed that LAB (which included carnobacteria) increased from an initial 100 cfu/cm^2 to an average level of $1x10^6$ cfu/cm^2 for the first six weeks of storage in vacuum-packaged or carbon dioxide-controlled atmosphere-packaged (CO_2-CAP) treated pork and maintained that level of LAB for the remainder of the thirteen-week study. In the CO_2-CAP pork samples, there was no significant increase in LAB until the 11th week of storage, with a maximum level of LAB at $3.2x10^5$ cfu/cm^2. Unpublished work by Nadon *et al.* (2001) demonstrated that carnobacteria dominate the LAB microflora during storage at –1.5°C in the absence of oxygen.

Therefore, to consider the number of *C. maltaromaticum* that could "theoretically" be found on commercial food products, one could take the highest number of *C. maltaromaticum* found in foods ($5x10^7$ cfu/g of meat) and utilize this number as the amount of potentially naturally-occurring *C. maltaromaticum* found on foods (theoretical). Therefore, a consumption analysis was performed to determine the theoretical amount of *C. maltaromaticum* naturally consumed from the foods stated in Appendix E. Consumption of each food was determined from an initial two-day intake survey acquired by the USDA's Continuing Survey of Food Intakes by Individuals and a weighted average of the amount (in grams) of each food eaten was extrapolated for the US population at the time of the survey. This gram amount of each food was then multiplied by the amount of *C. maltaromaticum* added *per* gram weight and divided by the total US population to obtain a percentage of the total amount of *C. maltaromaticum* ingested, when added to the foods specified.

Theoretical maximal background consumption of *C. maltaromaticum* from the RTE and fresh comminuted, processed food products outlined above has been determined (*via* consumption analysis of the proposed foods that *C. maltaromaticum* would be added) to be $4.3x10^9$ cfu/day (Table 6). This number is based on the highest number of LAB found on commercial food products, as described above ($5x10^7$ cfu/g of meat). Therefore, the theoretical consumption of the foods to which *C. maltaromaticum* would be added would result in a background consumption of $4.3x10^9$ cfu/day.

It must be noted, however, that several studies have determined that the growth of *C. maltaromaticum* plateaus at a maximum of $1x10^9$ cfu/g of meat (a reported range of $1x10^7$ to $1x10^9$ cfu/g when grown under optimal conditions on media or sterile cuts of meat), as the growth of *C. maltaromaticum* is self-limiting, as well as limiting to the growth of other types of bacteria (Paludan-Muller *et al.*, 1998; Stohr *et al.*, 2001; Duffes *et al.*, 2000; Yamazaki *et al.*, 2003). Therefore, it can be assumed that the addition of *C. maltaromaticum* to RTE meat products is not likely to significantly add to the ingestion of these bacteria.

7.3. *Per* Capita Consumption of *Carnobacterium maltaromaticum* From Additional Exposure Due to Intended Uses

Foods included in the analysis were chosen by searching the CSFII database using descriptor words to generate a list of foods to consider for the analysis. The list of foods was reviewed by Griffith Laboratories and Burdock Group and, only those foods that represented a targeted food were selected. Foods from the CSFII database selected for the analysis and treatment with *C. maltaromaticum* are noted in Appendix E.

Per capita estimate of intake, also called the Maximum Survey-derived Daily Intake (MSDI), is based on "disappearance data" from periodic surveys of ingredient manufacturers reporting the volume of ingredients produced during the survey year. The method is easy to use because it divides the total annual production by the population in the survey year and the number of days *per* year[11]. The assumption is that there is a finite amount of substance available and the general population ingests it as an added food ingredient regardless of source at the retail level. It is also assumed in this analysis that only 10% of the population consumes the products.

The intake profile (amount and frequency) by individuals in USDA's Continuing Survey of Food Intakes by Individuals 1994-96, 98 (CSFII 1994-96, 2000) was used to calculate the estimated daily intake (EDI) of *C. maltaromaticum* for individuals consuming the food groups selected for the addition of *C. maltaromaticum per* this GRAS evaluation. Calculation of the EDI is unique in this situation; *C. maltaromaticum* is a dynamic ingredient, multiplying during the storage period. Therefore, the highest quantity of *C. maltaromaticum* that has been found to grow on commercial meats (*i.e.*, $5x10^7$ cfu/g) was used to determine the maximal possible amount of exposure to *C. maltaromaticum*, when added to RTE foods and fresh comminuted, processed meat products. The calculated EDI would be $4.3x10^9$ cfu/day, or $7.2x10^7$ cfu/kg/day for a 60 kg person (Table 6).

Therefore, summation of the potential (theoretical) natural consumption of *C. maltaromaticum* in RTE and fresh comminuted, processed meat products ($4.3x10^9$ cfu/day), with the proposed maximal addition of *C. maltaromaticum* to RTE meats ($4.3x10^9$ cfu/day for the proposed RTE and fresh comminuted, processed meat products) gives a total theoretical maximal consumption of *C. maltaromaticum* equaling $8.6x10^9$ cfu/day (Table 6). The total number of LAB found on meat and cold-smoked salmon was not found to increase after the addition of *C. maltaromaticum* (Griffiths Lab, 2003a; Leroi *et al.*, 1996; Nilsson *et al.*, 1999; Paludan-Muller *et al.*, 1998). LAB has been typically found on cold-smoked salmon at the end of the shelf-life period at levels of $10^7 - 10^9$ cfu/g (Paludan-Muller *et al.*, 1998). Therefore, the addition *C. maltaromaticum* would not significantly increase the consumption of bacteria, as *C. maltaromaticum* growth plateaus at a maximal level of $1x10^9$ cfu/g of meat.

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[11] *Per Capita* Estimate = (Number of pounds of RTE meats (as specified in Appendix E) produced / Annual U.S. population) / 365 days *per* year.

Table 6. Proposed consumption of *C. maltaromaticum* as added to RTE and fresh comminuted, processed meats.

	Theoretical maximal amount of *C. maltaromaticum* ingested	90th Percentile
Naturally occurring bacteria on RTE and fresh comminuted, processed meat products.	4.3×10^9 cfu/day	8.1×10^9 cfu/day
Addition of *C. maltaromaticum* to RTE and fresh comminuted, processed meat products.	4.3×10^9 cfu/day	8.1×10^9 cfu/day
Total number of ingested bacteria from specified RTE and fresh comminuted, processed meat products.	8.6×10^9 cfu/day	1.6×10^{10} cfu/day

cfu = colony forming units; RTE = Ready To Eat.

Addition of *C. maltaromaticum* to the fresh comminuted, processed sausage meat products (as indicated in Appendix E) would not add to the consumption of viable *C. maltaromaticum*, because adequate cooking of the products, as indicated for these products, would render the bacteria nonviable. This was confirmed in a study by Samelis *et al.* (1998) who indicated that prior to cooking, Carnobacteria (*C. divergens* and *C. maltaromaticum*) dominated the distribution of sixty strains of lactic acid bacteria isolated along the processing line and prior to the cooking of ham. *C. divergens* and *C. maltaromaticum* dominated by gradually increasing their percentage distribution from the beginning to the end of processing. The lactic acid flora contained on the ham just before cooking comprised 87% *Carnobacterium* spp. However, carnobacteria were not found on sliced or whole ham after cooking, indicating the lethality of heat treatment.

This consumption analysis has been accomplished with the assumption that the selected foods reasonably represent the proposed sources of *C. maltaromaticum* intake as an added ingredient. This analysis is considered conservative because the concentration of *C. maltaromaticum* added to each food product is based on the proposed maximum amount of *C. maltaromaticum* added *per* product.

8. Biological Toxicity and Pathogenicity

8.1. Biogenic Amine Formation

Biogenic amines are formed in foods as a result of amino acid decarboxylation catalyzed by bacterial enzymes. Histamine and tyramine, two well-characterized biogenic amines, catalyzed by several microorganisms isolated from food products, are believed to cause adverse effects in humans (Jansen *et al.*, 2003). Histamine has been noted in several fish products (pickled fish samples, crab sauce, fish paste and mackeral fish) at levels above 100 mg/kg for fish and fish products (Den Brinker *et al.*, 1995). Histamine production by bacteria in fish

products can potentially cause food poisoning symptoms similar to scombroid poisoning[12] (Leisner *et al.*, 1994; Stratton and Taylor, 1991). Leisner *et al.* (1994) noted that the *C. maltaromaticum* strains tested were not able to produce histamine, but produced tyramine.

Pharmacological and toxicological evidence indicates that tyramine injected intravenously acts mainly by an indirect mechanism of action, releasing noradrenaline from the sympathetic nervous system. Due to this systemic response, tyramine ingestion may increase blood pressure by peripheral vasoconstriction and by increasing the cardiac output. Tyramine also dilates the pupils, dilates the palpebral tissue, causes lacrimation, salivation and increased respiration. At high doses, the blood sugar level is increased (Joosten, 1988). Tyramine metabolism by oxidative deamination to *p*-hydroxyphenylacetic acid is the main degradative pathway. Tyramine is also catalyzed by monoamine oxidase (MAO), present in almost every organ. There are two types of MAO, A and B, with either type having the ability to degrade tyramine. Elimination of tyramine from the body through monoamine oxidases is normally efficient. However, people on nonselective monoamine oxidase inhibitors may be at risk for increased blood pressure after ingesting increased amounts of tyramine. Only one incidence of potential food-induced sickness due to tyramine has been reported from fish products (Nuessle *et al.*, 1965) from a patient taking tranylcypromine, a nonselective monoamine oxidase inhibitor. New monoamine oxidase inhibitor medications inhibit only one of the types of MAO, thereby decreasing the possibility of adverse effects associated with tyramine ingestion.

During storage at –1.5°C, some LAB (including carnobacteria) grow steadily on fresh pork packaged either by vacuum-packaged or carbon dioxide-controlled atmosphere-packaged (CO_2-CAP) treatments (Nadon *et al.*, 2001). Unpublished work by Nadon *et al.* (2001) demonstrated that carnobacteria dominate the LAB microflora during storage at –1.5°C in the absence of oxygen. Nadon *et al* (2001) showed that LAB increased from an initial 100 cfu/cm^2 to an average level of 1×10^6 cfu/cm^2 for the first six weeks of storage in vacuum-packaged pork and maintained that level of LAB for the remainder of the thirteen-week study. In the CO_2-CAP pork samples, there was no significant increase in LAB until the 11th week of storage, with a maximum level of LAB at 3.2×10^5 cfu/cm^2. Histamine concentrations in both packaging treatments did not exceed 16 µg/g. Tyramine concentrations in the vacuum-packaged pork did not change until Week 4, when it increased to nearly 40 µg/g, but on average, the concentrations of tyramine fluctuated between 26 and 38 µg/g. Tyramine concentrations in the CO_2-CAP pork were significantly lower than in the vacuum-packaged samples, with levels averaging 2 µg of tyramine /g for the first 7 weeks of storage, then an increase to an average of 18 µg of tyramine /g for the remainder of the thirteen week study (Nadon *et al.*, 2001). Tyramine production has been shown to increase in salmon that has first been frozen to –20°C or –30°C, then thawed and refrigerated for greater than 21 days. Tyramine concentrations in salmon previously frozen, then thawed and maintained at 2°C attained 56.3±12.5 µg/g after 21 days and 41.8±3.6 µg/g after 34 days (Emborg *et al.*, 2002). Duffes *et al.* (1999b) reported that at 8°C, *C. maltaromaticum* SF668 produced 262 µg of tyramine /g after four weeks of storage, when grown on vacuum-packed, cold-smoked salmon. At 4°C, 82 µg of tyramine/g was produced by *C. maltaromaticum* V1 after one week and 165 µg/g was produced by *C. maltaromaticum* SF668 after four weeks.

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[12] Scombroid poisoning = poisoning from ingestion of heat-stable toxins produced by bacterial action on inadequately preserved dark-meat fish of the order Scombroidea (tuna, bonito, mackerel, albacore, skipjack); characterized by epigastric pain, nausea and vomiting, headache, thirst, difficulty in swallowing, and urticaria.

Natural tyramine concentration in commercial vacuum-packed, cold-smoked salmon after three weeks of refrigerated storage contained between 80 and 230 μg/g; some French cheeses contain tyramine in a range between 130 to 290 μg/g, while in fermented meat, tyramine concentrations could exceed 150 μg/g (Duffes *et al.*, 1999b).

Tyramine formation during the malting and brewing process in the production of beer has been noted, with tyramine concentrations ranging from 4.0 to 45.6 mg/L. These levels were determined to be well below levels that may elicit direct adverse reactions (Izquierdo-Pulido *et al.*, 1994).

The toxic threshold dose of tyramine is not precisely known (Nadon *et al.*, 2001). *Carnobacterium* spp. produced tyramine in amounts higher than 426 μg/ml/day, when grown in Maijala's liquid medium supplemented with 2% histamine and 2% tyrosine (Masson *et al.*, 1996). The bacteria were incubated for five days at 30°C prior to biogenic amine measurement *via* HPLC. The production of tyramine by *C. maltaromaticum* bacteria has been shown to be approximately 1.02 mmol/liter/day (*i.e.*, 140 μg/g/day) in laboratory broth supplemented with two *percent* tyrosine (Leisner *et al.*, 1994).

Ingestion of tyramine HCl (200 mg, approximately 2.86 mg/kg bodyweight) in 12 healthy men after an overnight fast was well tolerated (Grind *et al.*, 1986). However, ingestion of 400 mg tyramine resulted in three of the twelve subjects having an increase in systolic blood pressure greater than 30 mm Hg, within 20 minutes after tyramine ingestion and lasting approximately 20 minutes. Simultaneously, there was a decrease in heart rate. Therefore, the threshold dose for the blood pressure increase after oral tyramine was found to be between 200 and 400 mg in normal healthy subjects. The mean concentrations of tyramine found in the blood after ingestion of 200 or 400 mg tyramine was 31±19 and 79±74 ng/ml, respectively.

Tyramine has been shown to have a reduced bioavailability when ingested in conjunction with food (VanDenBerg *et al.*, 2003). Tyramine (200 mg, given orally as a 253 mg dose of tyramine HCl) was given to eight healthy males, either in a fasted state or during a meal. Results showed that the maximum serum concentration ranged from 13.6 to 85.7 ng/ml when 200 mg tyramine was administered to fasting subjects and from 2.96 to 27.9 ng/ml when the same dose was administered to subjects during a meal. The difference averaged 72% (P=0.0056). Administration of 200 mg tyramine during a meal resulted in a median delay in time of maximum concentration of 0.5 hours (P=0.0156). No reported adverse events were serious, and all resolved completely without problem. The most frequently reported adverse event was hypertension, as defined as a semi-recumbent systolic blood pressure greater than 160 mm Hg and was observed in three subjects. In one subject, hypertension was observed after tyramine was administered during a fast and was possibly treatment related. In two subjects, hypertension was observed after tyramine was administered during a meal but was not considered treatment related. This indicates that ingestion of tyramine during a meal results in decreased concentrations of tyramine reaching the bloodstream.

In addition, conflicting research has been published questioning the hypothesis that food intolerance reactions are related to biogenic amine ingestion. A review of thirteen oral challenge studies with biogenic amines (five with positive results and eight with negative results) found that only ten were randomized, double-blind, placebo-controlled studies (Jansen *et al.*, 2003). Of

these, six were found to be inconclusive, with the remaining four reporting no relation between biogenic amine ingestion and food intolerance. Jansen *et al.* (2003) therefore concluded that the currently published data do not substantiate the hypothesis that oral ingestion of biogenic amines and food intolerance reactions are related.

8.2. Pathogenicity Studies

C. maltaromaticum has been implicated in producing pathogenicity in stressed fish, such as occurs during spawning or handling. Measurements of the number of *C. maltaromaticum* in control *vs.* stressed (*e.g.,* starved) fish showed that fasting does not change total numbers of viable autochthonous[13] aerobic bacteria or composition of carnobacteria. Daily, repeated stress and starvation of the fish over eleven days had no influence on the total culturable bacterial numbers or population level of *C. maltaromaticum* associated with the digestive tract. A comparative analysis was conducted of the phenotypic and serological properties of *Carnobacterium* strains associated with mortalities of cultured striped bass and channel catfish and the properties of isolates obtained from wild brown bullhead catfish (Baya *et al.*, 1991). Experimental virulence tests conducted to assess the pathogenicity of selected *Carnobacterium* strains indicated that only the isolates HB-425 and HB-426 (isolated from striped bass) and the *C. maltaromaticum* reference strain (ATCC 35586) proved to be pathogenic for rainbow trout, with 50% lethal doses of 1.4×10^6, 2.3×10^6 and 2.0×10^6 cfu/g, respectively (Baya *et al.*, 1991). The trout died within 5-9 days after challenge.

Conversely, no mortalities were recorded in striped bass during a two-week period (50% lethal dose, $>8 \times 10^7$ cfu/g). However, the *C. maltaromaticum* strains were recovered from the kidneys of all survivor fish (trout and bass), even those injected with the lowest doses. Histological examination of dead and carrier fish revealed abundant melanomacrophage centers in the kidneys, necrotic areas in the livers and acute inflammation of the brains. Interestingly, none of the *Carnobacterium* strains showed lipolytic, phospholipolytic, amylolytic or hemolytic activity. No strain produced gelatinase, but most exhibited caseinolytic activity. This study indicates that *Carnobacterium* strains are associated with fish mortalities and that a carrier state can be established in surviving fish populations. However, it should be noted that these studies are not be realistic in determining the virulence of *C. maltaromaticum* through an oral route, since the method of inoculation was by injection into the intraperitoneal cavity. Natural exposure to *C. maltaromaticum* is *via* a skin wound or oral ingestion in a water environment. Interestingly, although the exposure was injection directly into the fish, thereby removing any natural barrier systems, the authors state that stress conditions are important in predisposing fish to infection by *Carnobacterium* spp. The authors also noted that *Carnobacterium* infection frequently occurs in association with secondary pathogenic bacteria, such as *Aeromonas hydrophila* and *Pseudomonas fluorescens*, with the fish mortalities often following spawning or repeated stressful handling practices (Baya *et al.*, 1991). Taken as a whole, *C. maltaromaticum* is not believed to be a pathogenic species *via* the oral route.

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[13] autochthonous = native to the place inhabited; aboriginal.

8.3. Safety of bacteriocins produced by *Carnobacterium maltaromaticum*

One of the methods that *C. maltaromaticum* may inhibit potentially pathogenic bacteria is through the production of bacteriocins. Bacteriocins are ribosomally synthesized, low molecular weight antibacterial proteinaceous materials that are able to kill closely related bacteria (Klaenhammer, 1993). Bacteriocins have been isolated from beef, spoiled ham, as well as from French mold-ripened soft cheese (Jack *et al.*, 1996; Herbin *et al.*, 1997). Because bacteriocins are isolated from foods such as meat and dairy products, which normally contain LAB, both LAB and bacteriocins have been consumed for centuries. Bacteriocins produced from *C. maltaromaticum* have been shown to be susceptible to proteolytic enzymes. The bacteriocin from *C. maltaromaticum* LV17 is stable during heat treatment at 62°C, boiling for 30 min and, after autoclaving at 121°C for 15 minutes. Trypsin, protease types I, IV, VIII, XIV, α-chymotrypsin, β-chymotrypsin and papain inactivated the bacteriocin, while non-proteolytic enzymes did not (Ahn and Stiles, 1990b). Piscicolin 126, a bacteriocin produced by *C. maltaromaticum* JG126 was inactivated by α- and β-chymotrypsin, proteases (types I, XIV, XXIII and trypsin), but catalase, lipase or lysozyme had no effect (Jack *et al.*, 1996). Similarly, the bacteriocin produced by *C. maltaromaticum* LV61 is resistant to heat (100°C for 20 minutes), while being inactivated by α-chymotrypsin, trypsin, pepsin, papain and proteinase K. Treatment with catalase, α-amylase, lipase, phosphlipase C, DNase I and lysozyme did not affect the antibacterial activity (Schillinger *et al.*, 1993). This evidence has indicated that ingestion of bacteriocins would not have an effect on the beneficial gut microorganisms. Trypsin has been shown to inactivate the bacteriocin, nisin[14] (Hara *et al.*, 1962).

Strains of *C. maltaromaticum* produce several different carnobacteriocins (Quadri *et al.*, 1994), which have been identified as heat-resistant peptides, stable over a wide pH range and capable of acting as bactericides (Jack *et al.*, 1996). A bacteriocin from *C. maltaromaticum* L103 was recently tested in a study to determine the ability of this bacteriocin to control the growth of *Listeria monocytogenes* in vacuum-packaged meat (Schobitz *et al.*, 1999). Steaks from beef semitendinosus muscle were inoculated with the partially purified bacteriocin at a concentration of 100 AU[15]/ml. *L. monocytogenes* was added to the meat as an indicator strain at a final concentration of $1x10^3$ cfu/cm^2. After assuring good contact with the meat, the steaks were vacuum-packaged and stored at 4°C for 21 days. Non-inoculated controls and meat containing only the indicator strain were included for each sampling date. Duplicate steaks were sampled at time 0 and every seven days for growth of *L. monocytogenes* and LAB growth. After seven days of storage at 4°C, a significant decrease in the *L. monocytogenes* counts were observed, from an initial count of $2x10^3$ cfu/cm^2 to 4 cfu/cm^2, with complete inhibition of the pathogen (<1 cfu/cm^2) on day 14 of storage. The LAB multiplied on the vacuum-packaged meats, reaching counts of $1x10^7$ cfu/cm^2 after 14 days, with a starting level of $1.6x10^2$ cfu/cm^2. Color and odor of the meat remained acceptable during the 14 days of storage. The results of this study indicate that the bacteriocin from *C. maltaromaticum* was able to inhibit *L. monocytogenes* on vacuum-packaged meat, while maintaining edible characteristics of the meat for up to 14 days (Schobitz *et al.*, 1999). *C. maltaromaticum* V1 produces a bacteriocin that was active against *C. maltaromaticum* 2762 and *L. monocytogenes* (strains R2, Lud 1033, Br124 6,

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[14] nisin = A bacteriocin produced by Lactococcus lactis (a LAB) that is selectively active against members of the same or other species.
[15] AU/ml = Arbitrary units of activity.

Lud 905 and T), but was inactivated by pronase E, proteinase K and trypsin (Pilet *et al.*, 1995). Other research has indicated that the purified bacteriocin from *C. maltaromaticum* LV61 inhibits several strains of *Carnobacterium* and *Enterococcus*, but does not inhibit several strains of *Listeria* (Holck *et al.*, 1994). It was therefore concluded that *C. maltaromaticum* LV61 produces, in addition to piscicolin 61, another factor involved in the anti-listerial activity.

The following table (Table 7) indicates the ability of simulated gastric fluid (SGF[16]) to inhibit the activity of *C. maltaromaticum* bacteriocins (Griffith Laboratories, 2003b). The bacteriocin was small and estimated to be 4.5 kDa. The stability of protein or protein fragments to digestion in simulated gastric fluid may be used to assess the potential allergenicity of a protein. Stability is dependent on the ratio of pepsin to the test protein. Proteins that are rapidly degraded in SGF (within 15 to 30 minutes) are generally assumed to be incapable of inducing a toxic and/or allergenic response.

The SGF digestion stability assay was initiated by first growing the producer organism, *C. maltaromaticum* CB1 overnight at 25°C in APT[17] broth. The resulting culture was then centrifuged at 15,000 x g for 5 minutes, with the supernatant removed and heat-treated at 60°C for 30 min. The supernatant was concentrated to 10x and the protein content and activity (AU) of the supernatant and 10x concentrated supernatant against *L. monocytogenes* CDC 7762 was determined. Supernatant and concentrated supernatant were added to pepsin at a starting ratio of 13:1 (w/w). The samples were incubated for 0, 0.5, 5, 10, 15 and 30 min to test for bacteriocin activity. The plate was incubated overnight and visually inspect for zones of inhibition.

As shown by the absence of bacteriocin activity noted in Table 7, the results of this study indicate that the bacteriocin produced from CB1 is readily digested in simulated gastric fluid and, therefore, has a low potential for the production of allergenicity or toxicity.

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[16] SGF was produced (in accordance with US Pharmacopoeia (2000) methods) by first dissolving 0.2 g of sodium chloride and 0.32 g of purified pepsin (derived from porcine stomach mucosa), with an activity of 800 to 2500 units *per* mg of protein (with an activity of 3,460 units/mg protein), in 0.70 ml of hydrochloric acid and sufficient water to make 100 ml, with a pH of approximately 1.2.

[17] APT = All purpose Tween medium (Difco Laboratories, Detroit, Michigan).

Table 7. Effect of treatments on the presence (+) or absence (-) of *C. maltaromaticum* bacteriocin activity.

	CB1 Time (minutes)					10x CB1 Time (minutes)				
Treatments	0	0.5	2	10	30	0	0.5	2	10	30
Pepsin										
0.32% pH 1.2	+	-	-	-	-	+	+	-	-	-
0.16% pH 1.2	+	-	-	-	-	+	+	-	-	-
0.08% pH 1.2	+	-	-	-	-	+	+	-	-	-
0.04% pH 1.2	+	-	-	-	-	+	+	-	-	-
0.02% pH 1.2	+	-	-	-	-	+	+	-	-	-
pH										
0.32% pH 1.2	+	-	-	-	-	+	-	-	-	-
0.32% pH 2	+	-	-	-	-	+	+	-	-	-
0.32% pH 3	+	Slight +	-	-	-	+	+	+	+	Slight +
0.32% pH 4	+	-	-	-	-	+	+	+	+	Slight +
Bacteriocin Added										
5 µl to 95 µl SGF	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
15 µl to 95 µl SGF	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
35 µl to 95 µl SGF	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
55 µl to 95 µl SGF	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
75 µl to 95 µl SGF	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
95 µl to 95 µl SGF	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
						N/A	N/A	N/A	N/A	N/A
SGF Added						N/A	N/A	N/A	N/A	N/A
95 µl to 5 µl of 307	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
75 µl to 5 µl of 307	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
55 µl to 5 µl of 307	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A
35 µl to 5 µl of 307	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A

SGF = Simulated gastric fluid; N/A = Not applicable

8.4. Observations of *Carnobacterium maltaromaticum* in humans

There have been no reports of human pathogenicity associated with the oral consumption of *C. maltaromaticum*. Extensive searches of various electronic medical and toxicological databases with the terms "*Carnobacterium maltaromaticum*", "*Carnobacterium piscicola*", "*Lactobacillus piscicola*" or "*Lactobacillus carnis*" and, "pathogenicity", "toxicity" or "human" failed to recover any information on human pathogenicity connected with the oral consumption of this specific bacterial species. For the purposes of the Human Pathogens Importation Regulations (SOR/94-558), the genus *Carnobacterium* has been characterized in Canada and France as having non-pathogenic organism status (Health Canada, 2003; Republic of France, 1994). It is believed that ingestion of *C. maltaromaticum*, by the addition to RTE meats and fresh comminuted, processed meat products, will not have significant adverse effects on people with suboptimal health.

In addition, the American Type Culture Collection Repository (ATCC) has designated *C. maltaromaticum* strains ATCC 35586 and ATCC 43225 as requiring a biosafety level (BSL) of

1. As stated by the ATCC, those items in BSL-1 are not known to cause disease in healthy adult humans. This classification system is based on assessment of the potential risk using U.S. Public Health Service guidelines, with assistance provided by ATCC scientific advisory committees (American Type Culture Collection (ATCC): Collections & Repository, 2004a, b).

Reviews of the available literature on the safety of lactic acid bacteria, in general, concluded that except for a few cases of endocarditis occurring in patients without past cardiac problems (or possible cardiac disease that was overlooked), there has always been a severe underlying disease prior to LAB pathogenicity. Often, the infection was not through normal ingestion of the bacteria, but through an obvious aberrant portal of entry, *i.e.*, a breach of the barrier between a septic site known to contain LAB and a sterile site in the body (Aguirre and Collins, 1993; Gasser, 1994; Borriello *et al.*, 2003).

There has been one published report of the isolation of *C. maltaromaticum* from humans (Chmelar *et al.*, 2002). This report details a man being treated for the loss of his arm after accidental amputation in a water sawmill. The arm stump had been polluted with water and debris before the patient entered the hospital. The amputation was completed at the carpometacarpal joint. Suturing of the arm was completed after leveling and a post-surgical antibiotic was given (cefuroxim). Six days after the surgery the stump was revised (cleaned and any necrotic tissue removed). At this time an abscess was evacuated and a pus sample collected for bacterial cultivation. *Serratia marcescens*, a known harmful human pathogen that has been known to cause urinary tract infections, wound infections and pneumonia, was isolated from the pus. This bacterium was shown to be resistant to cefuroxim and colistin. In addition, a white, relatively large nonhemolytic colony of Gram-positive rods was isolated from the wound, on sheep blood agar plates. The general description was as follows: Gram-positive rods, facultatively anaerobic; fermentation products from glucose were lactic acid and some acetic acid; motility-negative; negative spore formation; negative catalase formation; negative gas formation from glucose. The configuration of lactic acid was L-(+)-, meso-2,6-diamino-pimelic acid in positive cell hydrolyzates, with an undetermined peptidoglycan-type. The initial conventional biochemical tests performed on the bacteria yielded a profile of 99.5% identity to *C. maltaromaticum*. As indicated, the source of this infection may have been pollution of the wound itself, as well as exposure to the open water. Subsequently, the *S. marcescens* and *C. maltaromaticum* bacteria may have entered the wound and caused the infection. This infection by *C. maltaromaticum* was under extreme conditions and, was likely not to exemplify potential pathogenicity of *C. maltaromaticum* through ingestion.

9. Discussion

C. maltaromaticum is a Gram-positive, non-motile, non-sporeforming, rod-shaped bacterium, recently redefined from the genus *Lactobacillus* to *Carnobacterium*. *C. maltaromaticum* has been indicated as one of a large, diverse group of lactic acid-producing bacteria, which metabolize glucose to produce lactic acid and other acids that inhibit the growth of several pathogenic bacteria. *C. maltaromaticum* was initially found in salmonid fish, but has since been found on various food products, from meats and fish to fruits and vegetables, produced and stored by current agricultural practices, at levels exceeding $1x10^7$ cfu/g. Lactic acid bacteria have been used for centuries in the fermentation and preservation of food products

(*e.g.*, yogurts, sausages, vegetables, breads, wine, cheeses and milk). *C. maltaromaticum* has already been used as part of a starter bacterial culture in sausage fermentation in France.

Carnobacterium spp. are psychrotrophic, grow at elevated pH values of 8 to 9 and ferment inulin. In culture conditions in the presence of inulin, *C. maltaromaticum* forms yellowish to pinkish colonies with a metallic bronze sheen, a yellow color change of the medium and a clearance of precipitate. Various *C. maltaromaticum* strains have been shown to produce bacteriocins, proteinaceous compounds with the ability to inhibit the growth of other *Carnobacterium*, *Lactobacillus* and *Listeria* species.

It is proposed that *C. maltaromaticum* will be inoculated into various ready-to-eat and fresh comminuted, processed meat products at a range of $1x10^3$ to $1x10^4$ cfu/g for enhanced preservation and decreased pathogenic bacterial growth. Based on these inoculation ranges, as well as the theoretical assumption that the bacteria will grow over an extended period of time in storage, the mean *per capita* consumption estimate of *C. maltaromaticum* as an addition to the selected RTE foods would be $4.3x10^9$ cfu/day or $7.2x10^7$ cfu/kg/day for a 60 kg person.

The suppression of the pathogen, *L. monocytogenes,* by *C. maltaromaticum* occurs when assessed in salmon, chicken, pork, beef and other commercial meat products. Co-culturing of *C. maltaromaticum* with *L. monocytogenes* results in log reductions in *L. monocytogenes* growth. Compared to *L. monocytogenes* growth at low temperatures, the suppressive effect of *C. maltaromaticum* on *L. monocytogenes* growth is enhanced at low temperatures. *L. monocytogenes* suppression may be mediated through production of lactic acid, competition for nutrients, as well as the production of bacteriocins. Bacteriocin production is correlated with increased suppression of *L. monocytogenes* growth. The activity of bacteriocins produced by *C. maltaromaticum* is rapidly degraded when subjected to simulated gastric acid or proteolytic enzymes, an indication of a non-toxic and non-allergenic protein.

C. maltaromaticum has been implicated in the pathogenicity of stressed salmonid fish. *C. maltaromaticum* has been found to be associated with the digestive tract of Atlantic salmon, while carrier states of *C. maltaromaticum* infections in fish have been shown to occur. The induction of stress on a fish is important in predisposing fish to pathogenic infection by the *Carnobacterium* spp., while further evidence indicates that the *Carnobacterium* infection frequently occurs in association with secondary bacteria.

C. maltaromaticum has been shown to produce the biogenic amine, tyramine, formed as a result of amino acid decarboxylation. Tyramine releases noradrenaline from the sympathetic nervous system, which may increase blood pressure and increase cardiac output. Tyramine elimination is normally very efficient *via* monoamine oxidase enzymes (MAO), but can be inhibited by the use of a class of pharmaceuticals, monoamine oxidase inhibitors. Ingestion of tyramine, in conjunction with food, has been shown to reduce the bioavailability of tyramine. Tyramine has been hypothesized to play a part in the formation of food intolerance; however, recent research has found that levels of tyramine production in food (*e.g.*, µg/g concentrations) are significantly below the doses known to cause adverse effects (mg/g concentrations ingested). Other research has indicated that a correlation between tyramine consumption and adverse food intolerance reactions could not be verified.

C. maltaromaticum increases the storage time of RTE and vacuum-packaged meat products, while decreasing the growth of pathogenic bacteria. *C. maltaromaticum* growth has been found to be self-limiting, with levels of *C. maltaromaticum* on RTE meat products and vacuum-packaged, cold-smoked salmon stabilizing at approximately $1x10^9$ cfu/g. Addition of *C. maltaromaticum* to the proposed RTE and fresh comminuted, processed meat products at a level between $1x10^3$ and $1x10^4$ cfu/g, would not significantly increase the overall human consumption of LAB from these food products (theoretical natural consumption determined at $4.3x10^9$ cfu/day). *C. maltaromaticum* has been shown to be self-limiting in its growth; the growth of *C. maltaromaticum* will plateau between approximately $1x10^8$ and$1x10^9$ cfu/g of meat. This has been speculated to be due to the release of specific bacteriocins that limit a higher bacterial density.

C. maltaromaticum has been determined to be nonpathogenic by both Canada and France. The ATCC has also defined the biosafety requirement of *C. maltaromaticum* to the category of organisms not causing disease to humans. *C. maltaromaticum* strains have been isolated from fish, meat and cheese at levels exceeding $1x10^7$ cfu/g. Although one case of human pathogenicity by *C. maltaromaticum* has been reported, it occurred through the infection of an accidental amputation of a limb, not through oral ingestion. There have been no human illnesses or mortalities associated with ingestion of *C. maltaromaticum* bacteria.

In summary, *C. maltaromaticum* has been found to be safe when added to RTE and fresh comminuted, processed meats at inoculation ranges between $1x10^3$ to $1x10^4$ cfu/g of product, resulting in the added consumption of bacteria from RTE and fresh comminuted, processed meat products to be approximately $4.3x10^9$ cfu/day of food. The inoculation of RTE and fresh comminuted, processed meat products with this level of *C. maltaromaticum* does not significantly increase the levels of *Carnobacterium* already being consumed in the American diet.

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10. Conclusion

Based on a critical evaluation of the information available, the Expert Panel has determined that *Carnobacterium maltaromaticum*, meeting Food Chemical Codex specifications, is Generally Recognized As Safe (GRAS), by scientific procedures, when used as a food ingredient in meat products. Concentrations for use range from 10^3 to 10^4 cfu/g of food for fresh comminuted, processed meat products and 10^3 to 10^4 cfu/g of food for ready-to-eat meat products, produced in accordance with current good manufacturing practice.

Signatures

Douglas Archer, Ph.D.	10/3/04 Date
Todd Klaenhammer, Ph.D.	9/21/04 Date
Mary Ellen Sanders, Ph.D.	9/28/04 Date

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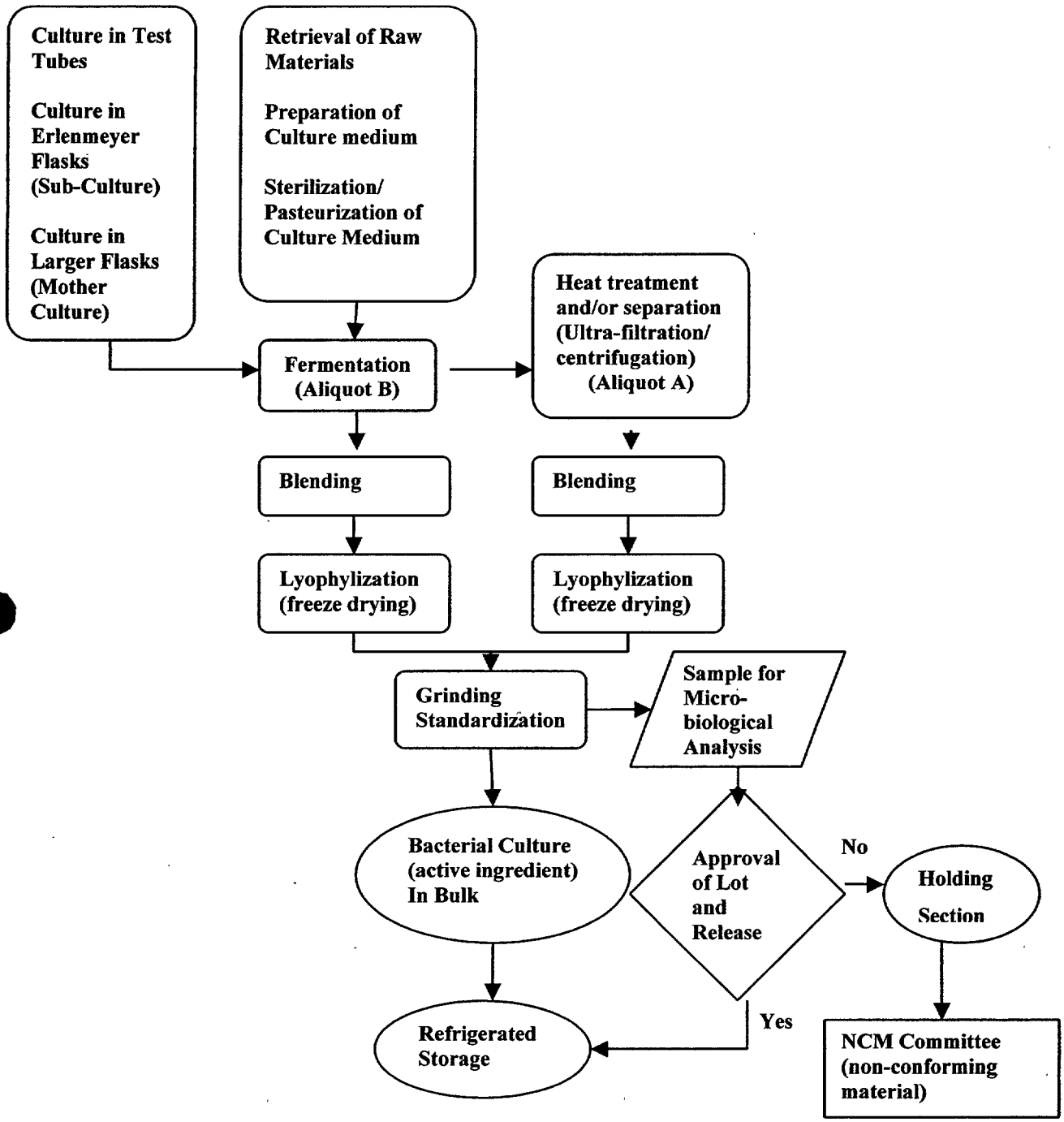
12. Appendix A



Process control flow chart for the production of *Carnobacterium maltaromaticum.*

000051

13. Appendix B

Sensory Characteristics of *Carnobacterium maltaromaticum* on Vacuum-Packaged Wieners

A laboratory-scale study was designed to investigate the sensory characteristics, such as aroma and flavor profile, of *C. maltaromaticum* on vacuum-packaged wieners inoculated with *C. maltaromaticum* under conditions comparable to those proposed for commercial production (Griffith Laboratories, 2002). The methods and results of this study were as follows:

Two strains of bacteriocin-producing *C. maltaromaticum* were chosen for study: UAL 8 (synonym LV17) was originally isolated from vacuum-packaged, refrigerated, fresh pork and described by Shaw and Harding (1984) and, strain UAL 26, which was isolated from vacuum-packaged beef (Stiles and Holzapfel, 1997). The inoculum was prepared by adding 10 ml of washed bacterial cells to 4 L of sterile 0.85% saline to provide an inoculum level of 2.5×10^6 cfu/ml. Individual wieners were dipped into the inoculum suspension for one minute, drain dried and vacuum-packaged in groups of five wieners *per* bag (high barrier, low O_2 transmission, VP bags). As a control, wieners were dipped in 0.85% sterile saline without bacterial inoculum. Treated and control samples were then placed into refrigerated (4°C) storage for up to 12 weeks. Sampling of the wieners for microbiological analyses and sensory evaluation was performed on day zero and after 2, 4, 6, 7, 8, 10 and 12 weeks of storage.

The wieners to be evaluated for sensory characteristics were cooked in "just boiled" water and allowed to stand for five minutes (internal wiener temperature approximately 83°C). Wieners were cut into pieces, placed in coded foil-covered jars and heated for 15 min in a 94°C oven just prior to evaluation. Sensory evaluation was conducted by a group of nine panelists trained over a three-month period. Samples were evaluated for overall aroma intensity, meat flavor intensity, seasoned flavor, smoke intensity, sourness/acidity, off-flavor and overall acceptability using a 15 cm unstructured line scale with 0 = very bland and 15 = very strong. Between samples, palates were cleansed with crackers and a 1:1 dilution of 7-Up®.

This study reported that sample wieners that had been inoculated with *C. maltaromaticum* strains UAL 8 or UAL 26 reached maximum anaerobic lactic acid bacteria (LAB) counts of 2.75×10^6 and 1.2×10^5 cfu/cm^2 after seven or eight weeks of cold storage, respectively. *C. maltaromati`cum* grew at a slow rate on vacuum-packaged wieners and growth was accompanied by a relatively small decrease in surface pH during storage. UAL 8 varied from pH 6.20 at Week 0 to 6.10 at Week 10, while UAL 26 varied from an initial pH 6.20 to approximately 5.9 during Weeks 6-8 and Week 12. Based on sensory evaluations using a trained nine-member panel over the 12-week storage period, there were no significant adverse effects on aroma, off-flavors, sour intensity, or overall acceptability resulting from inoculation with *C. maltaromaticum*.

It was concluded that in comparison with other lactic acid bacteria, such as *L. gelidum*, *C. maltaromaticum* is a slow-growing species when inoculated onto refrigerated (4°C), vacuum packaged wieners. The levels of *C. maltaromaticum* reached a maximum of 5×10^7 cfu/cm^2 after 12 weeks of cold storage, while having no adverse effects on the overall acceptability as an edible food product.

14. Appendix C

Inhibition of Pathogenic Bacteria by *Carnobacterium maltaromaticum* in Meat Products

The U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition has stated in the document "Processing parameters needed to control pathogens in cold-smoked fish" that *C. maltaromaticum* and *C. divergens* inhibit the growth of *L. monocytogenes* in food products. Specifically, the growth of *L. monocytogenes* in cold-smoked salmon can be completely inhibited by addition of *C. maltaromaticum* (U. S. Food and Drug Administration, 2003).

Examination of the potential of a bacteriocin-producing strain of *C. maltaromaticum* (strain SF668) isolated from commercial, vacuum-packaged cold-smoked salmon (CSS) to inhibit *L. monocytogenes* growth on CSS by Duffes *et al.* (2000), found that *C. maltaromaticum* SF668 was able to grow from $1x10^5$ to $3x10^7$ cfu/ml in 21 days on vacuum-packaged, cold-smoked salmon stored at 4°C (Table 8). *L. monocytogenes* co-cultured with *C. maltaromaticum* grew from $1x10^3$ cfu/ml to $3.5x10^3$ cfu/ml after three weeks at 4°C. This co-culturing of *C. maltaromaticum* with *L. monocytogenes* resulted in a significant bacteriostatic effect on *L. monocytogenes* growth on cold-smoked salmon (*L. monocytogenes* growth in the absence of *C. maltaromaticum* reached approximately $5x10^4$ cfu/ml).

When screened against 21 strains of *Listeria* spp., distinct zones of inhibition formed by *C. maltaromaticum* LK5 were evident with seventeen of the strains (Buchanan and Klawitter, 1992a). *C. maltaromaticum* LK5 was found to lack hydrogen peroxide formation, while producing a bacteriocin. The ability of *C. maltaromaticum* LK5 to inhibit *Listeria* spp. was temperature-dependent (determined at both 5°C and 19°C), with a substantially greater suppression of *L. monocytogenes* co-cultured with *C. maltaromaticum* LK5 occurring at 5°C than at 19°C. *C. maltaromaticum* LK5 was shown to be capable of substantially faster growth than *L. monocytogenes* at refrigeration temperatures, whereas the growth rates were approximately the same at 19°C. At 19°C, suppression of *L. monocytogenes* (the inoculation concentration was kept constant at $1x10^3$ cfu/ml) was dependent on inoculum ratio, with only LK5:*L. monocytogenes* ratios of $\geq$1:1 producing a substantial degree of inhibition. At 5°C, an increased level of anti-listerial activity was observed with the higher inoculum ratios during the early stages of the incubation, but by approximately 300 h of incubation, LK5 inoculum size had no effect on inhibitory activity; the degree of suppression was equivalent for ratios ranging from 0.01:1 to 1000:1 ($10:1x10^3$ and $1x10^6:1x10^3$ cfu/ml, respectively). The *Carnobacterium* isolate was extremely competitive at refrigeration conditions, indicating that even small inocula could be used to control *L. monocytogenes* in refrigerated foods. This study confirms the report by Schillinger and Holzapfel (1990), who reported that out of thirteen strains of *C. maltaromaticum*, ten significantly inhibited the growth of *L. monocytogenes* DSM 20600, as determined by the agar spot test.

Table 8. Inhibition of *L. monocytogenes* growth by *C. maltaromaticum* at refrigeration temperatures.

Food Product	Initial *C. maltaromaticum* (cfu/g of meat)*#	Initial *L. monocytogenes* (cfu/g of meat)*	Incubation period (days)	Final *L. monocytogenes* (cfu/g of meat)*	Reference
Crabmeat	$1x10^4$	$1x10^3$	21	$1x10^4$†	(Buchanan and Klawitter, 1992b)
Vacuum-packaged cold-smoked salmon	$3.2x10^4$ (strain V1)	$5.1x10^2$	28	<10 (DL)	(Duffes *et al.*, 1999b)
Vacuum-packaged cold-smoked salmon	$4x10^4$ (strain NCDO2762)	$1x10^3$	28	$1.6x10^4$†	(Duffes *et al.*, 1999b)
Simulated cold-smoked fish system	$1.8x10^5$ cfu/ml	$2.2x10^3$ cfu/ml	18	<10 cfu/ml (DL)	(Duffes *et al.*, 1999c)
Vacuum-packaged cold-smoked salmon	$1.6x10^5$ (strain SF668)	$6.3x10^2$	28	$1.2x10^2$	(Duffes *et al.*, 1999c)
Vacuum-packaged cold smoked salmon	$1x10^5$ (strain SF668)	$1x10^3$	21	$3.5x10^3$ †	(Duffes *et al.*, 2000)
Cold-smoked salmon juice	~10^6 cfu/ml	$1x10^3$ cfu/ml	32	<10 cfu/ml (DL)	(Nilsson *et al.*, 1999)
Vacuum-packaged cold-smoked salmon	$2x10^6$	$2x10^2$	20	$2x10^3$†	(Nilsson *et al.*, 1999)
Cold-smoked salmon	$1x10^6$	$1x10^3$	7	<50 (DL)	(Yamazaki *et al.*, 2003)
Cold-smoked salmon	$1x10^4$	$1x10^3$	21	<50 (DL)	(Yamazaki *et al.*, 2003)
Frankfurters	$1x10^6$	$1x10^6$	29	$5x10^5$	(Buchanan and Klawitter, 1992b)
UHT milk	$1x10^4$ cfu/ml	$1x10^3$ cfu/ml	21	$5x10^3$ cfu/ml†	(Buchanan and Klawitter, 1992b)
Canned corn	$1x10^4$	$1x10^3$	19	$1x10^3$	(Buchanan and Klawitter, 1992b)
"All beef" dog food	$1x10^4$	$1x10^3$	12	<10 (DL)	(Buchanan and Klawitter, 1992b)
Vacuum-packaged processed chicken cubes	$1x10^4$ (strain DX)	$1x10^2$	28	$1x10^{5\%}$	Campos *et al.*, 1997
MRS broth	10^5 cfu/ml	$1x10^5$ cfu/ml	28	10^2 cfu/ml	(Amezquita and Brashears, 2002)

DL = Detection limit; * cfu/g of meat, unless otherwise noted; # *C. maltaromaticum* strain indicated when known; MRS = de Mann-Rogosa-Sharpe bacterial growth medium; UHT = Ultra High Temperature †Growth of *L. monocytogenes* was significantly less than control cultures; % = significantly suppressed from a maximal *L. monocytogenes* at $1x10^9$ cfu/g of meat.

000054

L. monocytogenes inhibition by *C. maltaromaticum* DX (a bacteriocin producer) or *C. maltaromaticum* 2818 (lacking bacteriocin production) was examined in vacuum-packed, minimally-processed chicken cubes with gravy at 4°C, 8°C and 15°C (Campos *et al.*, 1997). Alone, *L. monocytogenes* reached levels of 10^7 to 10^8 cfu/g (when initially inoculated at $1x10^2$ cfu/g) after 14 days at 4°C. *C. maltaromaticum* DX and *C. maltaromaticum* 2818 reached levels of approximately $1x10^8$ to $1x10^9$ cfu/g during the first week of storage at 4°C, 8°C or 15°C, when inoculated at $1x10^4$ cfu/g (Table 8). The growth of these strains was not influenced by the presence of *L. monocytogenes*. At 4°C and 8°C, growth of *C. maltaromaticum* DX inhibited *L. monocytogenes* by 3 log cycles during the second to third week of storage, while *C. maltaromaticum* 2818 suppressed *L. monocytogenes* growth to a lesser extent. No significant differences between the inhibition of *L. monocytogenes* by *C. maltaromaticum* DX and *C. maltaromaticum* 2818 were observed at 15°C (both *C. maltaromaticum* strains reduced *L. monocytogenes* growth by one log at 15°C). This study indicates that antagonistic actions by *C. maltaromaticum* strains against *L. monocytogenes* occur and are enhanced by low temperatures and the production of a bacteriocin.

Nilsson *et al.* (1999) reported that *C. maltaromaticum* strains A9a, A9b, A10a and A10b are able to inhibit the growth of *L. monocytogenes* strains 049, 050, 051, 052, 056, 057 and Scott A in an agar diffusion assay. The culturing of *L. monocytogenes* 057 alone on vacuum-packed, cold-smoked salmon stored at 5°C at an initial concentration of 2×10^2 cfu/g led to maximum cell numbers of $3x10^8$ cfu/g after 14 days of storage. Co-culture of *L. monocytogenes* with *C. maltaromaticum* led to relatively low maximum cell counts of $1x10^4$ and $2x10^3$ *L. monocytogenes* cfu/g were observed after 14 and 20 days of storage, respectively. *C. maltaromaticum* A9b and A10a grew rapidly at 5°C on cold-smoked salmon, increasing from an initial concentration of $7x10^6$ to approximately $4x10^8$ cfu/g after three days of storage, maintaining approximately $4x10^8$ cfu/g for the remainder of the study (Table 8).

Lewus *et al.* (1991) isolated two strains of *C. maltaromaticum* (GN and DX) from retail cuts of meat. Utilizing a bacterial overlay technique, the resulting zones of inhibition of growth of the pathogenic bacteria confirmed that these strains of *C. maltaromaticum* were antagonistic to four different strains of *L. monocytogenes* (Scott A, V7, 101m and 103m) and moderately antagonistic to *Aeromonas hydrophila* strains 7965 and K144.

An anti-listerial strain isolated from frozen surimi was identified as *C. maltaromaticum* CS526 and found to inhibit *L. monocytogenes* IID581 in cold-smoked salmon stored at refrigeration temperatures (Yamazaki *et al.*, 2003). In the absence of *C. maltaromaticum*, the *L. monocytogenes* grew from $1x10^6$ to approximately $1x10^9$ cfu/ml within three days in Trypticase Soy-Yeast Extract (TSYE) broth at 12°C. In contrast, *L. monocytogenes* viable counts declined to below the detection limit (10 cfu/ml) within one day and the organism was completely inhibited during incubation (seven days) in the presence of *C. maltaromaticum* CS526. Growth of *L. monocytogenes* in cold-smoked salmon in the absence of *C. maltaromaticum* increased from $1x10^3$ to $1x10^6$ cfu/g after 42 days at 4°C, 17 days at 12°C and 2 days at 20°C. Both *C. maltaromaticum* CS526 (a bacteriocin-producing strain) and *C. maltaromaticum* JCM5348 (which does not produce a bacteriocin) grew rapidly from initial inoculation levels of $1x10^6$ or $1x10^4$ cfu/g, respectively, to approximately $1x10^8$ to $1x10^9$ cfu/ml throughout the storage period. During co-culture with *C. maltaromaticum* CS526, at either 4°C or 12°C, *L. monocytogenes* populations decreased to below the detection limit (50 cfu/g of salmon) after seven days when

the initial inoculum levels of *C. maltaromaticum* CS526 were $1x10^6$ cfu/g of salmon. *L. monocytogenes* populations also decreased to below detection limits after 21 days at 4°C and 12 days at 12°C when the initial inoculum levels of *C. maltaromaticum* CS526 were $1x10^4$ cfu/g (Table 8). This work indicates that inhibition of *L. monocytogenes* is enhanced by increased concentrations of *C. maltaromaticum* and lowered incubation temperatures.

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15. Appendix D

Use of *C. maltaromaticum* as a Starter Culture for Commercial Fermented Sausage Production.

Lallemand S.A. of Toulouse, France, produces and markets a range of LAB for use in the French food industry, including sausage fermentation. *C. maltaromaticum* is among the bacterial species listed by this company for this food application. A memo from Institut Rosell (Lallemand) of Montreal, Canada, attesting to this fact, along with a page of a Lallemand S.A. product catalogue, attesting to the fact of use, have been included.

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INSTITUT ROSELL

Montreal. Quebec

January 25,2002

Senior Director of Development
Griffith Laboratories
757 Pharmacy Avenue
Toronto. ON M1L3JB

Dear Mr. Dariey,

This is to confirm that the bacteria (Carnobacterium piscicola) has been used in sausage fermentation in France for many years.

If you have any questions do not hesitate to contact me, by e-mail (ajoyeaux@lallemand.com).

Sincerely,

Dr. And re Joyeaux
Market Development Manager
Institut Rosell/ Lallemand

?480, boulevard SairK-Laurent Montreal (Quebec) Canada H2P 2Mb Telephone : 1514) 381-5631 • 1-800-452-4364
T^lpcopieur: (514) 383-4493 • Courriet coniact^rosell com

zoola Sffn ILLIdLH^) 88n88Z9TC XVj 11 1-1 ZOOZ/roT^T

LALLEMAND
TC50

Bacterial Strains : *Staphylococcus xylosus and Staphylococcus carnosus*

Lactobacillus sake and Carnobacterium piscicola

CHARACTERISTICS :

QUALITY	Mechanism of action	Lactobacilles Pediocoques	Microcoques Staphyloc
COLOR	reduction of nitrates	-	+++
	lowering of pH	+++	-
	consumption of O_2		++
	degradation of H_2O_2	-	±
AROMAS	Acid production	+++	-
	Protein hydrolyzation		++
	Lipid hydrolyzation		++
	Reduction in rancidite	-	++
TEXTURE FIRMNESS	Reduction in pH	+++	-
CONSERVATION	Suppression of the undesirable bacteria	++	-
	Reduction of nitrates	-	++

APPLICATIONS :

The mixture TC 50 east is intended for the support of dry products.

Pediococcus and *Lactobacillus*:

Provides regular acidification and prolonged time for a good connection of the meats.

Limitation of the development of the undesirable enterobacterias.

Staphylococcus:

Development of the coloring and the taste of dry sausages

Reduction in the residual amount of nitrates and nitrites.

16. Appendix E

Table 9. Foods selected for consumption analysis of *C. maltaromaticum* (ordered by food code).

FOOD CODE	DESCRIPTION	*C. maltaromaticum* ADDED (cfu/g of meat)	*Percent (%)* of total consumption of *C. maltaromaticum*[18]
23322100	Deer Bologna	10000	0.0328
25210110	Frankfurter wiener or hot dog NFS	10000	2.7263
25210150	Frankfurter or hot dog cheese-filled	10000	0.0505
25210210	Frankfurter or hot dog beef	10000	8.9948
25210220	Frankfurter or hot dog beef and pork	10000	3.0623
25210230	Frankfurter or hot dog beef and pork lowfat	10000	0.9155
25210250	Frankfurter or hot dog meat & poultry fat free	10000	0.2226
25210280	Frankfurter or hot dog meat & poultry	10000	1.3047
25210310	Frankfurter or hot dog chicken	10000	0.6235
25210410	Frankfurter or hot dog turkey	10000	1.2922
25210610	Frankfurter or hot dog beef lowfat	10000	0.1624
25210700	Frankfurter or hot dog meat & poultry lowfat	10000	0.0678
25220010	Cold cut NFS	10000	0.0205
25220100	Beef sausage NFS	10000	0.1552
25220110	Beef sausage brown & serve links cooked	5000	0.0826
25220120	Beef sausage smoked stick (include beef jerky)	10000	0.0612
25220130	Beef sausage smoked	10000	1.0471
25220140	Beef sausage fresh bulk patty or link cooked	5000	0.0632
25220350	Bratwurst cooked	5000	0.6471
25220390	Bologna beef low fat	10000	0.0551
25220400	Bologna pork and beef	10000	0.9724
25220410	Bologna NFS	10000	4.5450
25220420	Bologna Lebanon	10000	0.5302
25220430	Bologna beef	10000	1.0281
25220440	Bologna turkey	10000	0.4377
25220450	Bologna ring smoked	10000	0.0856
25220460	Bologna pork	10000	0.1059
25220470	Bologna beef lower sodium	10000	0.0287

[18] Consumption of each food was determined from an initial two-day intake survey acquired by the USDA's Continuing Survey of Food Intakes by Individuals and a weighted average of the amount (in grams) of each food eaten was extrapolated for the US population at the time of the survey. This gram amount of each food was then multiplied by the amount of *C. maltaromaticum* added *per* gram weight and divided by the total US population to obtain a percentage of the total amount of *C. maltaromaticum* ingested, when added to the foods specified.

FOOD CODE	DESCRIPTION	*C. maltaromaticum* ADDED (cfu/g of meat)	*Percent (%)* of total consumption of *C. maltaromaticum*[18]
25220480	Bologna chicken beef & pork	10000	0.1194
25220500	Bologna beef & pork lowfat	10000	0.2625
25220510	Capicola	10000	0.3115
25220710	Chorizos	10000	0.8460
25220910	Head cheese	10000	0.0307
25221210	Mortadella	10000	0.0643
25221250	Pepperoni	10000	0.7992
25221310	Polish sausage	10000	4.0309
25221350	Italian sausage	10000	1.7101
25221400	Sausage (not cold cut) NFS	10000	0.0559
25221410	Pork sausage fresh bulk patty or link cooked	5000	2.1181
25221420	Pork sausage brown & serve cooked	5000	0.4895
25221430	Pork sausage country style fresh cooked	5000	0.0203
25221460	Pork & beef sausage	10000	0.3050
25221470	Pork & beef sausage brown & serve cooked	5000	0.0921
25221480	Mettwurst	10000	0.2336
25221500	Salami NFS	10000	4.8150
25221520	Salami dry or hard	10000	0.3845
25221530	Salami Beef	10000	0.1184
25221610	Scrapple cooked	5000	0.1269
25221650	Smoked link sausage pork	10000	1.4479
25230310	Chicken/turkey loaf pre-pkg'd/deli luncheon meat	10000	9.6836
25230410	Ham loaf luncheon meat	10000	0.0694
25230430	Ham & cheese loaf	10000	0.1125
25230510	Ham lunch meat chop minced pressed minced not canned	10000	0.8951
25230520	Ham luncheon meat chopped spiced lowfat not can	10000	0.1244
25230530	Ham/pork luncheon meat chopped can (including spam)	10000	0.7590
25230560	Liverwurst	10000	0.9349
25230610	Luncheon loaf (olive pickle or pimiento)	10000	0.6365
25230710	Sandwich loaf luncheon meat	10000	0.3629
25230790	Turkey ham sliced extra lean pkg'd deli	10000	0.9747
25230800	Turkey ham	10000	0.7869
25230810	Veal loaf	10000	0.0931
25230820	Turkey pastrami	10000	0.1704
25230840	Turkey salami	10000	0.2609

000061

FOOD CODE	DESCRIPTION	*C. maltaromaticum* ADDED (cfu/g of meat)	*Percent (%)* of total consumption of *C. maltaromaticum*[18]
25230900	Turkey or chicken breast pkg'd/deli luncheon meat	10000	3.7329
25231110	Beef sliced prepackaged/deli luncheon meat	10000	2.6110
25231150	Corned Beef pressed	10000	0.4076
25240000	Meat spread or potted meat NFS	10000	0.0084
25240210	Ham deviled or potted	10000	0.1379
25240310	Roast beef spread	10000	0.0686

NFS = Not food specific

CURRICULUM VITAE

DOUGLAS L. ARCHER, PH.D.
PROFESSOR AND PAST CHAIR

Assistant Surgeon General, USPHS (ret)

I. PERSONAL DATA

Home Address:

Business Address: Food Science & Human Nutrition Department
University of Florida

Telephone:

Facsimile:

E-Mail:

II. EDUCATION

B.A. - Zoology (1968)
University of Maine, Orono, ME

M.S. - Bacteriology (1970)
University of Maine, Orono, ME

Ph.D. - Microbiology (1973)
University of Maryland, College Park, MD

III. SERVICE 12/73 to 12/93:

Commissioned Officer, U. S. Public Health Service - Retired 1/1/94: **Assistant Surgeon General** (Rear Admiral)

IV. EXPERIENCE

January 2, 2001 – Present:
Professor of Food Science and Human Nutrition, University of Florida.

January 3, 1994 – January 1, 2001:
Professor and Chair, Food Science and Human Nutrition Department, University of Florida, Institute of Food and Agricultural Sciences

November 6, 1992 - December 31, 1993:
Deputy Director for Programs, Center for Food Safety and Applied Nutrition, FDA.

December, 1989 - November 6, 1992
Deputy Director, Center for Food Safety and Applied Nutrition, FDA.

February, 1991 – July, 1991
Acting Director, Office of Seafood, Center for Food Safety and Applied Nutrition, FDA

February, 1986 - December, 1989:
Director, Division of Microbiology, Center for Food Safety and Applied Nutrition, FDA

1983 - 1986: Deputy Director, Division of Microbiology, Center for Food Safety and Applied Nutrition, FDA.

1975 - 1983: Research Team Leader, Immunobiology, Division of Microbiology, FDA, Cincinnati, Ohio.

1979 - 1983: Adjunct Assistant Professor of Toxicology, University of Cincinnati College of Medicine.

1973 - 1975: Research Microbiologist, Division of Microbiology, Bureau of Foods, Washington, DC.

1970 - 1973: Graduate Teaching Assistant, Department of Microbiology, University of Maryland, College Park, Maryland.

1968 - 1970: Graduate Teaching Assistant, Department of Microbiology, University of Maine, Orono, Maine.

V. AWARDS

2000: **Advancement of Industry Award**-American Meat Institute. "For outstanding service to the industry and consumers by defending sodium nitrite safety."

1993: **Public Service Fellow**, Woodrow Wilson National Foundation Fellowship, 1993 academic year – Rocky Mountain College, Billings, MT.

1993: **Distinguished Service Medal** for "Exceptional and Distinguished Career" in support of public health in the U.S.

1993: **U.S. Public Health Service Meritorious Service Medal** for "an extraordinary degree of commitment and dedication to food safety research, and to the development and implementation of public health and education programs."

1992: **J.C. Frazier Memorial Award**, Food Research Institute, "In recognition of outstanding achievements in the field of food Microbiology." University of Wisconsin, Madison.

1992: **U.S. Public Health Service Outstanding Unit Citation** for exemplary performance of duty - For leadership demonstrated during the implementation of the Nutrition Labeling and Education Act of 1990.

1991: **Appointed Assistant Surgeon General, United States Public Health Service.**

1989: **U.S. Public Health Service Citation** for "outstanding leadership of the microbiological programs of the Center for Food Safety and Applied Nutrition."

1989: **U.S. Public Health Service Meritorious Service Medal** for "sustained superior performance in the management and scientific leadership of FDA's research and regulatory efforts in the area of food safety."

1988: **Tanner Memorial Award** - Institute of Food Technologists - Outstanding Individual in Food Technology and Related Sciences. Award Lecture: "The True Health Impact of Foodborne Disease".

1987: **Commissioner's Special Citation** for "In recognition of sustained superior management performance and national and international recognition as an expert in the area of food safety and microbiology."

1986, 87, 88, 89: **U.S. Public Health Service Citation** for Outstanding Performance.

1984: **U.S. Public Health Service Meritorious Service Medal** for "sustained

high-quality leadership and professional accomplishments in microbiological toxicology research and providing research direction for the Division of Microbiology, FDA."

1979: **U.S. Public Health Service Commendation Medal** for "development of in vitro immunological systems."

VI. ORGANIZATIONS

International Association for Food Protection – 2001 – present

Florida Association for Food Protection – 2001 - present

National Meat Association, Member, 1997 – present.

American Frozen Foods Institute, Affiliate Member, 1997 - present

Food Allergy Network, February 1994 - Present.

Institute of Food Technologists (IFT), elected professional member, 1989 - Present.

Society for Applied Bacteriology, 1987 - 1990.

Society of Toxicology, 1978 - 1986.

American Society for Microbiology, 1970 - 1991.

Commissioned Officers Association, USPHS, 1973 - Present.

Society for Experimental Biology and Medicine, 1977 - 1990.

VII. PROFESSIONAL ACTIVITIES

International

U.S. Representative, WHO Expert Advisory Panel on Food Safety, January 1990 - February 2006.

U.S. Delegate to WHO Strategic Planning Meeting, March, 2001, Geneva, Switzerland

Advisor to Australian Government on formation of the Australian National Food Authority, April, 1991.

International Meat and Poultry HACCP Alliance, Scientific Advisory Committee Member, 1994 - Present.

Consultant to United Fresh Fruit and Vegetable Association, Produce Microbiology Committee; Allied Trade Associations, International Trade Council; 1994 – 1998.

Chairman, Codex Alimentarius Food Hygiene Committee, FAO/WHO, 1985 - 1994.

Consultant to WHO Committee on Listeria in Foods, 1988.

Chairman, ILSI Europe/WHO Workshop on a Scientific Basis for Regulations on Pathogenic Microorganisms in Foods. May 17-19, 1993, Brussels, Belgium.

Member and U.S. Delegation Chairman, 1986 -1990. U.S. (AOAC) Representative to International Dairy Federation meeting and Chairman, dilutions methodology, Helsinki, Finland, 1984.

U.S. (AOAC) Delegate to International Standards Organization, Helsinki, Finland, 1984.

Editorial Boards

Editorial Board Member, Food Control, 1989 - Present.

Contributing Editor, Food Technology, IFT, 2001 - 2003

North American Editor, Food Control, Elsevier, Ltd.
October 1994 – 1999.

Editorial Board Member, Journal of Natural Toxins, 1991 - 1995.

Advisory Board Member, Academic Press Nutrition and Food Science Publications, 1987 - 2000.

Member, Board of Publications of Fundamental and Applied Toxicology, Society of Toxicology, May 1983 - 1986.

Reviewer for Food Microbiology, Journal of Agricultural and Food Chemistry, Science of the Total Environment

Professional Society Related

IFT Committee on Mentoring, 2003

IFT Planning Committee for 3rd Food Safety and Quality Conference, 2003

IFT Committee on Codex Alimentarius (Global Regulations and Policy), 2003 - 2005

IFT, Elizabeth Stier Award jury member, 2003

IFT Task Force on Sound Science Advocacy, 2002 – Present

IFT Distinguished Lecturer, 2002

IFT/AAAS Congressional Science Fellow Selection Panel member, 2002.

IFT – Contributing Editor, Food Technology, 2001 – 2003

IFT Science, Communications and Government Relations Committee, Member, 2001-2002.

IFT Expert Panel on Emerging Pathogens, 2000

IFT Task Force Steering Committee on Food Safety Initiatives, October 1997 – Present

IFT Florida Section, Member, 1994 - Present.

IFT Committee on Education, September 1, 1994 - August 31, 1997; Appointed Secretary, March 1996 - 1997.

IFT Toxicology and Safety Evaluation Division, Appointed to the Executive Committee, August 1995 - August 1998.

IFT Florida Section, Elected Councilor, September 1995 - August 1997.

Domestic & Government-Related

International Food Information Council, Media Expert Resource, 2004-2006.

Board of Directors, National Institute for Agricultural Security, 2003 - present

Food Advisory Committee, US Food and Drug Administration, voting member of general committee, 2002 – present.

Chair, Subcommittee on Biotechnology and Member – FDA Food Advisory Committee, 2002 – present.

Member, Food Safety Advisory Council, American Meat Institute, 2000-2001

Board of Directors, International Life Sciences Research Office (LSRO) for the American Society for Nutritional Sciences, 1999 - 2003.

Coordinator, CSREES/USDA National Science and Education Conference "Toward Implementing the Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables," Orlando, FL, April 6-7, 1999.

Member, Cyclospora Advisory Panel, California Strawberry Commission, June 1996

Consultant to the FDA Food Advisory Committee on the Safety of Calgene Tomatoes, Washington, DC, April 7-9, 1994.

Consultant, Centers for Disease Control, Review of Draft Plan for the Detection, Prevention, and Control of New, Reemerging, and Drug Resistant Infectious Diseases, March 22-23, 1993.

Member, Advisory Committee to the Center for Veterinary Medicine, Safety of Sometribove (bovine somatotropin), March 31, 1993.

National Advisory Committee on Microbiological Criteria for Foods, Voting Member, 1989 - November 1994.

Chairman, National Advisory Committee on Microbiological Criteria for Foods, Subcommittee on Risk Assessment, January 1993 - November 1994.

Food Update Board of Governors, Food and Drug Law Institute Food Update, 1991 - 1993.

Public Health Service Overall Coordinator, L-Tryptophan/Eosinophilia-Myalgia Syndrome Crisis, October, 1989 - 1994.

Member, Government Steering Committee to Institute of Food Technology Research Committee, 1987 - 1994.

US Food and Drug Administration, Commissioner's Advisory Group on Women's Health Issues: Infectious Diseases, 1987 - 1988.

Consultant, Centers for Disease Control, writing of the "Recommendations for Preventing Transmission of Infection with HTLV-III/LAV in the Workplace, November 1985.

University of Florida (UF)
Institute of Food and Agricultural Sciences (IFAS),
and Academic Related Activities

Lead Person, UF participation in applying for the "Homeland Security Center for Post-Harvest Food Protection and Defense," a multi-institutional grant with UC Davis as lead institution, 2004

IFAS Genetics Institute Committee, 2004

Elected Member, IFAS Faculty Advisory Committee, 2003-2005

Co-Chair, IFAS Task Force for On-Campus Re-structuring, 2002.

Chair – IFAS Grievance Committee, 2002 - present

IFAS representative to Florida Food Safety and Food Security Advisory Council, 2002 - present

IFAS representative to UF Bioterrorism Task Force, 2001-Present

Co-Director (and establisher) of the UF/IFAS Center for Juice and Beverage Research, 2001

Chairperson, Plant Product Safety Center (elected), National Alliance for Food Safety, 2001

ESCOP/ECOP Food Safety Subcommittee, 2000 - present.

Screening Committee, position of Coordinator, Florida Sea Grant Extension.

Search and Screen Committee, Dean, College of Agriculture, Aug. 1998 – January 1999.

IFAS Long Range Planning Committee, Oct. 1998-Feb. 1999.

IFAS representative to National Alliance for Food Safety (NAFS) April 1998 – present.

IFAS representative to NAFS Operations Committee (elected) June 1998-June 2000, and July 2002-present.

IFAS Institutional Marketing Committee – May 1997-June 1998

Facilitator - UF's Workshop on Academic Management, Maximizing Your Leadership Potential, "Dealing with Institutional Conflict/Small Group Discussion", Camp Weed, FL, May 19, 1997.

Industry Representative - West Palm Beach Town Meeting, regarding Florida's needs of minimizing microbiological food safety risks for produce, West Palm Beach, FL, December 5, 1997.

Chair - Committee for the Aquatic Food Products Laboratory Building Dedication, August 1996 - April 1997.

Discussion Leader at the Institute for Academic Leadership, Department Chairs Workshop, Howey-in-the-Hills, FL, October 6-9, 1996.

Member - Search and Screen Committee for the UF Associate Director of Development and Alumni Affairs Position, August 1996.

Chair - Program Committee and Steering Committee for the October 28-30, 1996, UF/IFAS Statewide Faculty Meeting, March 8, 1996.

Member - IFAS Chairs' Subcommittee to Review the Task Force 1995 Recommendations for the Annual Faculty Achievement, Planning, and Evaluation Requirements and Forms, May 1995.

Member - UF, College of Veterinary Medicine, Center for Environmental and Human Toxicology (CEHT) Search Committee for the position of Director, CEHT, Member, October 1994.

Appointed to the UF University Senate, Member, 1994-96.

UF World Food Day Committee, Member, 1994.

Service to Other Schools

Review Team Member, CSREES Comprehensive Review, Department of Food Microbiology and Toxicology, University of Wisconsin, Sept. 2004.

Review Team Member, CSREES Comprehensive Review, Department of Nutrition and Food Science, University of Vermont, March, 2001.

In-depth Reviewer for University of California, Davis – Dr. Linda Harris, Extension Specialist. 2001

In-depth Reviewer for Rutgers University – Dr. Donald Schaffner, Extension Specialist. 2001

In-depth Reviewer for University of Wisconsin, Department of Food Microbiology & Toxicology, Dr. Charles Kasper, Professor. 2002

Review Team Member, CSREES Comprehensive Review, Department of Food Science and Human Nutrition, Michigan State University, December 1998.

Teaching Related

Primary Instructor – FOS 6915 - Research Planning (2 cr) – Required course for all incoming graduate students – 2003 – present.

Guest Lecturer in FOS 3042, Introduction to Food Science, "Food Safety Laws and Regulations," April 15 and 18, 1994.

Guest Lecturer in FOS 6915, Research Planning, "An Overview of Graduate Student Responsibilities and Expectations," September 6, 1994; August 29, 1995.

Team teach (33%) in Fall, Spring, and Summer terms, FOS 2001, Man's Food, 3 Credit Course, January 1995 – 2000.

Guest Lecturer in FOS 4731, Government Regulations and the Food Industry.

Guest Lecturer in AGG 4932/5932, Hazard Analysis Critical Control Point (HACCP) Systems, "Microbial Concerns in the Food Industry"

Instructor, US Army Veterinary Medical Command College, Fort Sam Houston, TX. Food safety – Presented 4 hours of lectures on risk assessment and food safety. 2001.

Student Related

Past Committees:

VIII. THESIS

M.S. Degree: A comparison of swarming and motility in Proteus mirabilis and a variant.

Ph.D. Degree: Control of interferon, virus capsid antigens, and migration inhibitory factor in Burkitt's Lymphoma-derived cell lines:Role of L-arginine.

IX. BIBLIOGRAPHY

A. BOOKS

Marshall, M. and Archer, D. Your Food and Health: A Study Guide for Man's Food. Second Edition. Kendall/Hunt Publishing Company, Dubuque, IA, 352 pages. Sixth Edition, 2004.

B. MONOGRAPHS

Archer, D.L. (co-author with 16 experts). 2002. Emerging Microbiological Food Safety Issues: Implications for Control in the 21st century. Institute of Food Tecnhologists, Chicago. 139 pages

C. REFEREED PUBLICATIONS

1. Archer, D. L. and Young, B. G. Control of interferon, migration inhibitory factory, and virus capsid antigens in Burkitt's Lymphoma-derived cell lines: Role of L-arginine. Infect. & Immun. 9:684, 1974.

2. Archer, D. L., Bukovic-Wess, J. A., and Smith, B. G. Gallic acid: A potential chemical tool for studying cellular interactions involved in the in vitro immune response. IRCS Med. Sci. 4:553, 1976.

3. Archer, D. L., Bukovic-Wess, J. A., and Smith, B.G. Inhibitory effect of an antioxidant, butylated hydroxyanisole, on the primary in vitro immune response. Proc. Soc. Exp. Biol. Med. 154:289, 1977.

4. Archer, D. L., Bukovic-Wess, J. A., and Smith, B.G. Suppression of macrophage-dependent T-lymphocyte functions by gallic acid, a food additive metabolite. Proc. Soc. Exp. Biol. Med. 156:465, 1977.

5. Archer, D. L., Smith, B.G. and Bukovic-Wess, J. A. Use of an in vitro antibody-producing system for recognizing potentially immunosuppressive compounds. Int. Arch. All. Appl. Immunol. 56:90, 1978.

6. Archer, D. L. and Johnson, H. M. Blockade of mitogen induction of the interferon lymphokine by a phenolic food additive metabolite. Proc. Soc. Exp. Biol. Med. 157:684, 1978.

7. Archer, D. L., Smith, B. G. and Wess, J. A. Evidence for helper T-cell participation in the antibody response to thymus-independent antigens. IRCS Med. Sci. 6:76, 1978.

8. Archer, D. L. Immunotoxicology of foodborne substances: An overview. J. Food Prot. 41:983-988, 1978.

9. Archer, D. L. and Wess, J. A. Chemical dissection of the primary and secondary in vitro antibody response with gallic acid and butylated hydroxyanisole. Drug and Chem. Toxicol. 2:155-166, 1979.

10. Archer, D. L., Smith, B. G., Ulrich, J. T. and Johnson, H. M. Immune interferon induction by T-cell mitogens involves different T-cell subpopulations. Cell Immunol. 48:420-426, 1979.

11. Archer, D. L. Antioxidants and the immune response. Proc. of the Fourth FDA Science Symposium: Inadvertent modification of the immune response. pp. 99-103, 1980.

12. Archer, D. L., Smith, B. G. and Johnson, H. M. Effects of toxicants on T-cell subpopulations as determined by lymphokine activity. Arch. Toxicol. Suppl. 4:138-142, 1980.

13. Chen, S. E., Tse, C. S., Bernstein, I. L. and Archer, D. L. In vivo suppression and enhancement of the murine homocytotropic antibody response by staphylococcal enterotoxin A. Int. Arch. Allergy Appl. Immunol. 63:470-472, 1980.

14. Stelma, G. N. and Archer, D. L. Detection of low levels of staphylococcal enterotoxin A in foods by mitogenesis. IRCS Med. Sci. 8:347, 1980.

15. Bernstein, I. L., Gallagher, J. S., Johnson, H. M., Archer, D. L. and Splansky, G. L. Evaluation of reactions to tartrazine. Proc. IV FDA Science Symposium: Inadvertent modification of the immune response. pp. 258-260, 1980.

16. Archer, D. L., Smith, G. G. and Peeler, J. T. Activation of murine T-lymphocyte DNA synthesis in vivo by staphylococcal enterotoxin A: Applicability to immunotoxicity testing. IRCS Med. Sci. 8:457, 1980.

17. Osborne, L. C. and Archer D. L. The antiviral effect of human fibroblast interferon is blocked by gallic acid. IRCS Med. Sci. 8:756, 1980.

18. Stelma, G. N., Bradshaw, J. G., Kauffman, P. E. and Archer, D. L. Thermal inactivation of mitogenic activities of staphy-lococcal enterotoxin A at 121~C. IRCS Med. Sci. 8:629, 1980.

19. Archer, D. L., Smith, B. V. and Johnson, H. M. Direct and induced suppression of in vitro antibody production by selected phenols: A comparison to virus-type interferon. In Biological Relevance of Immune Suppression (Dean, J. and Padarathsingh, M., eds.) Van Nostrand, NY, pp. 199-212, 1981.

20. Archer, D. L., Wess, J. A., and Johnson, H. M. Inverse relationship between immune interferon induction and mitogen effects on the maturation of the primary antibody response. Immunopharmacology 3:71-81, 1981.

21. Archer, D. L. and Smith, B. G. Immunotoxic and immunosuppressive effects of food chemicals: Focus on diethylstilbestrol. Proceedings of the 33rd Annual Reciprocal Meat Conference of the American Meat Science Association, pp. 143-146, 1981.

22. Luster, M. I., Dean, J. H., Boorman, G. A., Archer, D. L., Lauer, L., Lawson, L. D., and Wilson, R. A. The immunotoxicity of orthophenyphenol, Tris (2,3-dichloropropyl) phosphate and cyclophosphamide following acute exposure in mice. Toxicol. and Appl. Pharmacol. 58:252-261, 1981.

23. Blalock, J. E., Archer, D. L., and Johnson, H. M. Anticelluar and immunosuppressive activities of foodborne phenolic compounds. Proc. Soc. Exp. Biol. Med.

167:391-393, 1981.

24. Osborne, L. C., Peeler, J. T. and Archer, D. L. Reduction in the antiviral activity of human fibroblast interferon by gallic acid. Infect. & Immun. 33:769-774, 1981.

25. Archer, D. L., Wess, J. A. and Smith, B. G. Location of specific lesions in the immune response using in vitro tests. Proc. Soc. Toxicol. Can. pp. 137-149, 1981.

26. Wess, J. A. and Archer, D. L. Restoration by cyclic guanosine monophosphate and extracellular calcium of butylated hydroxyanisole-suppressed primary murine thymus-dependent antibody response. Immunopharmacology 3:71-81, 1981.

27. Buxser, S. E., Boventre, P. F. and Archer, D. L. Specific receptor binding of staphylococcal enterotoxin by murine splenic lymphocytes. Infect. & Immun. 33:827-833, 1981.

28. Blakley, B. and Archer, D. L. The effect of lead acetate on the immune response of BDF1 mice. Tox. and Appl. Pharm. 61:18-27, 1981.

29. Archer, D. L. and Smith, B. G. Immunotoxicity testing of food chemicals: Different results may be obtained with in vitro and in vivo exposure to gallic acid. Proc. AOAC Symposium on Analytical Methodology for Determining Immunotoxicity of Chemicals Including Pesticides, 1982.

30. Archer, D. L. New approaches to immunotoxicity testing. Env. Hlth. Persp. 43:109-113, 1982.

31. Blakley, B., Archer, D. L. and Osborne, L. C. The effect of lead on immune and viral interferon production. Canad. J. Comp. Med. 46:43-46, 1982.

32. Wess, J. A. and Archer, D. L. Disparate in vivo and in vitro immunomodulatory activities of Rhodamine B. Food and Chem. Toxicol. 20:9-14, 1982.

33. Blakley, B. R. and Archer, D. L. Mitogen stimulation of lymphocytes exposed to lead. Toxicol. and Appl. Pharmacol. 62:183-189, 1982.

34. Buxser, S. E., Bonventre, P. F. and Archer, D. L. Staphylococcal enterotoxin induced mitogenesis: Toxin binding and cell-cell interactions. Microbiologica 6:181-190, 1982.

35. Wess, J. A. and Archer, D. L. Evidence from in vitro murine immunologic assays that some phenolic food additives may function as antipromoters by lowering intracellular cyclic GMP levels. Proc. Soc. Exp. Biol. Med. 170:427-430, 1982.

36. Johnson, H. M., Archer, D. L. and Torres, B. A. Cyclic GMP as the second

messenger in helper cell requirement for gamma interferon production. J. Immunol. 129:2570-2572, 1982.

37. Archer, D. L. Assays for cell-mediated and humoral immunity. Proc. 13th Annual Conference on Environ. Toxicol. NTIS, 11-14, 1983.

38. Johnson, H. M., Torres, B. A. and Archer, D. L. Vasopressin and Phorbol Ester replacement of interleukin 2 requirement in gamma interferon production: Cyclic GMP as the common second messenger. Interleukins, Lymphokines and Cytokines. Proc. 3rd Intl. Workshop. (J. J. Oppenheim, S. Cohen and M. Landy, eds.) Academic Press, NY, 1:51-61, 1983.

39. Wess, J. A. and Archer, D. L. Differential effects of Nordihydroguaiaretic acid (NDGA) on B-cell subsets: Reversal of NDGA-induced antibody suppression by cyclic GMP is subset specific. Int. J. Immunopharmacol. 6:27-34, 1984.

40. Osborne, L. C., Peeler, J. T., and Archer, D. L. 1984. Effects of a strong and a weak carcinogen on murine alpha/beta interferon production in vivo. Fundamental and Appl. Toxicol. 4:210-215.

41. Archer, D. L. 1984. Diarrheal episodes and diarrheal disease: Acute disease with chronic implications. J. Food Prot. 47:321-327.

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43. Wentz, B. A., Duran, A. P., Swartzentruber, A., Lanier, J. M., Schwab, A. H., McClure, F. D., Archer, D. L. and Read, R. B. 1985. Microbiological quality of crabmeat during processing. J. Food Prot. 48:44-49.

44. Archer, D. L. and Wess, J. A. 1985. Cyclic nucleotides; in: Prostaglandins, Leukotrienes and Cancer, Vol. I. Basic Biochemical Processes. (Lands, W. E. M., ed.) Martinus Nijhoff, Boston, pp. 287-296.

45. Archer, D. L. and Glinsmann, W. H. 1985. Intestinal infection and malnutrition initiate acquired immune deficiency syndrome (AIDS). Nutr. Res. 5:9-19.

46. Archer, D. L. 1985. Enteric microorganisms in rheumatoid disease: Causative agents and possible mechanisms. J. Food Prot. 48:538-545.

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48. Archer, D. L. and Kvenberg, J. E. 1985. Incidence and cost of foodborne diarrheal

disease in the United States. J. Food Prot. 48:887-894.

49. Madden, J. M., McCardell, B. A., Archer, D. L. 1986. Virulence assessment of foodborne microbes. In (Pierson and Stern, Eds.) Foodborne Microorganisms and Their Toxins. Marcel Dekker, Inc., N.Y. pp. 291-315.

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53. Archer, D. L. 1988. Disease in day care. J. Env. Health. 51:143-147.

54. Bunning, V. K., Raybourne, R. R. and Archer, D. L. 1988. Foodborne enterobacterial pathogens and rheumatoid disease. Appl. Bacteriol. 65 (Suppl.): 87S-107S.

55. Archer, D. L. and J. E. Kvenberg. 1988. Regulatory significance of Aeromonas in foods. J. Food Safety. 9:53-58.

56. Garthright, W. E., Archer, D. L., and Kvenberg, J. E. 1988. Incidence of acute episodes of intestinal infectious diseases and costs of medical care and lost productivity. Public Health Service Reports 103:107-115.

57. Archer D. L., and Young F. E. 1988. Contemporary Issues: Diseases with a food vector. Clin. Microbiol. Rev. 1:377-398.

58. Archer, D. L. 1988. The true impact of foodborne infections. Food Technol. 42:53-58.

59. Bennett, R. W., Archer, D. L., and Lancette, G. 1988. Modified procedure to eliminate elution of food proteins during seroassay for staphylococcal enterotoxin. J. Food Safety 9:135-143.

60. Archer, D. L. 1989. Food counseling for persons infected with HIV-1: one strategy for defensive living. Public Health Rep. 104:196-198.

61. Archer, D. L. 1989. Listeria monocytogenes: what is its ecological niche? In: Foodborne Listeriosis. (Eds. Miller, A. J., Smith, J. L., and Somkuti, G. A. Society for Industrial Microbiology. pp. 5-8.

62. Archer, D. L. 1989. Food Safety: a scientific viewpoint. Proc. 37th Annual Meeting Agri. Res. Inst. p.77-82.

63. Archer, D. L. 1989. The new foodborne pathogens. J. Assoc. Food Drug Offic. 52:5-9.

64. Archer, D. L. 1989. Response to Listeria deserves a fair trial. Food Microbiol. 6:191-193.

65. Archer, D. L. 1990. Future challenges to the HACCP approach: the need for flexibility. Food Technol. 44:174-178.

66. Archer, D. L. 1990. Food counseling should be given to all persons infected with the human immunodeficiency virus (HIV). J. Infect. Dis. 161:358-9.

67. Klontz, K. C., Cawley, J. F., Archer, D. L., and White, G. L. 1990. L-tryptophan and the eosinophilia-myalgia syndrome: a new clinical entity. Physicians Assistant, July, 31-38.

68. Levine, W., Smart, J. F., Archer, D. L., Bean, N. H., and Tauxe, R. V. 1991. Foodborne disease outbreaks in nursing homes: 1975-1987. J. Amer. Med. Assoc., Vol. 266, No. 15:2105-2109.

69. Thompson, P., Salsbury, P. A., Adams, C. E., and Archer, D. L. 1991. U.S. Food Legislation. Lancet i:1557-1559.

70. Jackson, G. J., Langford, C. F., and Archer, D. L. 1991. Control of salmonellosis and similar foodborne infections. Food Control 2:26-34.

71. Archer, Douglas L. 1995. Food Control - The Challenges Ahead From the Lessons of the Past. Food Control, Vol. 6, No. 2:69-70.

72. Archer, Douglas L. 1996. The Validation of Rapid Methods in Food Microbiology. Food Control, Vol. 7, No. 1:3-4.

73. Archer, Douglas L. 1996. Bad Bugs and Good Bugs: The Changing Perceptions. Food Control, Vol. 7, No. 2:61-62.

74. Archer, D.L. 1996. Preservation microbiology and Safety: Evidence that Stress Enhances Virulence and Triggers Adaptive Mutations. Trends in Food Science and Technolgy, Vol. 7, No. 3:91-95.

75. Archer, Douglas L. 1996. Listeria: The Science and the Politics. Food Control. Vol. 7, No. 4/5;181-182.

76. Bunning, V. K., Lindsay, J. A., Archer, D. L. 1997. Chronic Health Effects of

Microbial Foodborne Disease. World Health Statistics Quarterly 50:51-56

77. Archer, D.L. 2002. Evidence that nitrate and nitrite are beneficial to health. J. Food Prot. 65:872-875.

78. Archer, D.L., Schneider, K.R., and Schmidt, R.H. 2002. Can HACCP and other systems assure protection from terrorism against the food supply? AFDO J. 66(3):94-102.

79. Archer, D.L. 2004. Freezing: An underutilized food safety technology? International Journal of Food Microbiology 90:127-138

80. Fahey, J.W., Ourisson, P.J., Stephenson, K.K., Archer, D.L., and Degnan, F.H. 2004. Pathogen detection, control, testing, and elimination in fresh broccoli sprouts: A case study. J. Food Protection,submitted.

81. Felkey, K.D., Archer, D.L., Bartz, J.A., Goodrich, R., and Schneider, K.R. 2004. The efficacy of chlorine for sanitizing tomato surface wounds contaminated with Salmonella spp. J. Food Sci., submitted

82. Turner, R.E., Degnan, F.H., and Archer, D.L. 2005. Label claims for foods and supplements: a review of the regulations. Nutrition in Clinical Practice. Accepted.

D. MISCELLANEOUS PUBLICATION

1. Archer, D. 1994. Controlling Bacteria in Food Through Temperature, Fine Cooking Magazine, June/July Issue, 12-13.

2. Archer, D. 1996. Should All Juices be Pasteurized? Food Protection Inside Report, 2 pages.

3. Archer, D. 2000. E. coli O157:H7 – Searching for Solutions. Food Technology 54:142.

4. Archer, D. 2001. WHO Holds Food Safety Strategic Planning Meeting. Food Technology 55(5):22.

5. Archer, D. 2001. Nitrite and the Impact of Advisory Groups. Food Technology 55(3):26.

6. Archer, D. 2001. Can It Happen Again? Food Technology 55(9):28.

7. Archer, D. 2001. The Flap Over Functional Foods. Food Technology 55(8):32.

8. Archer, D.L., and Degnan, F.H. 2001. Impact of the bioterrorism threat. Food Technology 55(12):21.

9. Archer, D. 2002. Global foodborne disease surveillance system faces hurdles. Food Technology 56(2):24.

10. Archer, D. 2002. New technologies question the equality of pathogens. Food Technology 56(5):24.

E. ABSTRACTS (since 2001)

1. Archer, D.L., Schneider, K.R., and Goodrich, M. 2004. Guidance to expedite food product recalls to mitigate or contain purposeful contamination of commercially distributed food. Proceedings of the 68th Annual National Environmental Health Association Annual Educational Conference & Exhibition.

2. Felkey, K.D., Schneider, K.R., Archer, D.L., and Bartz, J.A. 2003. Optimization of chlorine treatments and the effects on survival of Salmonella spp. on tomato surfaces. Proceedings of the IAFP 90th Annual Meeting, New Orleans, LA

3. Archer, D.L. 2001. Microbiological importance of sodium nitrite in assuring cured meat safety. IFT annual meeting, New Orleans, LA.

4. Archer, D.L., and Clemens, R.A. 2001. Probiotics: regulatory and marketing claim issues. IFT annual meeting, New Orleans, LA.

5. Clemens, R.A., and Archer, D.L. 2001. Probiotics: safety and substantiation. IFT annual meeting, New Orleans, LA

F. POSTERS

1. Archer, D.L., Schneider, K.R., and Goodrich, R.M. 2004. Guidance to expedite food product recalls to mitigate or contain a purposeful contamination of commercially distributed food. National Environmental Health association 68th Annual Educational Conference and Exhibition, Anchorage, AK. May 9-12.

2. Felkey, K.D., Schneider, K.R., Archer, D.L., and Bartz, J.A. Aug. 2003, 90th International Association for Food Protection Mtg.

3. Warren, B.R., Parish, M.E., Archer, D.L., and Schneider, K.R. 2003. Detection of Shigella spp. on tomato surfaces by conventional culture methods and FTA Filtration/nested PCR. IFT International Food Safety & Quality Conference and Expo. Orlando, FL. Nov. 5-7.

4. Allen, R.L., Schneider, K.R., Archer, D.L., and Sargent, S.A. A recovery study of Salmonella spp. from the surfaces of tomatoes and packing line materials. 5th

Annual Foodborne Pathogen Analysis Conference, St. Petersburg, FL. July 28-31, 2002

X EXTRAMURAL FUNDING

Department of Homeland Security, Homeland Security National Center for Post-Harvest Food Protection and Defense. (10/05 – 10/09) University of Minnesota is Lead Institution. Role: Collaborating Researcher.

USDA – CSREES, Reducing microbial risks in fruits and vegetables. (1/01 – 12/02) Principle Investigator: R. Gravani, Cornell University. University of Florida Co-PIs: D.L. Archer, R.H. Schmidt
Role: Co-PI

USDA - IFAFS, Improving the Safety of Fruits and Vegetables: A Tri-state Consortium. (January 2001 – December 2005). Principle Investigators: E.A. Murano, D. Archer and L.J. Harris. Co-principle Investigators (University of Florida): K.R. Schneider and R. Goodrich. – This grant integrates teaching, research and Extension efforts to assure fresh fruit and vegetable safety and security from harmful human pathogenic bacteria.
Role: UF Project Director and PI

Department of Defense. Developing Guidance to Expedite Food Product Recalls to Mitigate or Contain a Purposeful Contamination of Commercially Distributed Food. (10/02 to 10/04) Principle Investigators: D.L. Archer, K.R. Schneider, W.S. Otwell, R.M. Goodrich, and R.H. Schmidt. This is an Extension grant. The goal is to train a cadre of trainers that can help small and medium sized food businesses to implement crisis management plans such that product recalls can be done very quickly, thus containing a purposeful contamination event.
Role: PI

USDA – NIFSI. Risk assessment of tanker truck sanitation as related to the secure transport of juice and dairy products. (09/03 to 09/06). This is a research/Extension grant. Principle Investigators: M.E. Parish, R.M. Goodrich, K.R. Schneider and R.H. Schmidt, DL Archer.
Role: Investigator

XI PRESENTATIONS

2004

Archer, D.L. Use of zero tolerance for Salmonella in seafoods. July 2004. IFT Annual Meeting and Expo. Las Vegas, NV. Invited

2003

000082

Archer, D. L.. *How can I make my farm more secure?*. Jan. 10-12, 2003. SE regional F&V Assn - Savannah, GA. Invited.

Archer, D. L.. *Homeland security and agriculture*. 1/29/03. SE Regional Partnership Conf. - Atlanta, GA. Invited.

Archer, D. L.. *Food safety on the farm*. 3/7/03. US Potato Board (CHIP) - Jacksonville, FL. Invited.

Archer, D. L.. *Introduction to bioterrorism and the threat to the food supply*. -1/1/03. Florida Environmental Health Association - Tallahassee, FL., Invited.

Archer, D. L.. *Bioterrorism and Agroterrorism: are we prepared?* 4/3/03. Florida Assn Food Protecction - Melbourne, FL. Invited.

Archer, D. L.. *Session II - Microbial Genetics*. Feb. 17-19, 2003. ABC Research 29th Technical Seminar - Orlando, FL. Descriptors: Moderator, Coordinated four speakers

Archer, D. L.. *Food Security Issues*. 7/21/03. Florida Pathogen Analysis Conf. (FL DACS) - St. Petersburg, FL. Invited.

2002

Archer, Douglas L.. *The politics and science of assuring biosecurity*. 3/12/02. St. Petersburg, FL. 2002 Division Meeting - North American Millers Association. Invited.

Archer, Douglas L.. *Can HACCP be a useful tool in assuring food security?*. 2/19/02. Gainesville, FL. ABC Research 28th Annual Technical Seminar. Invited.

Archer, Douglas L.. *Food Biosecurity*. -1/2/02. Coral Gables, FL.. Grocery Manufacturers of America. Invited.

Archer, Douglas L.. *General considerations about bio- and agri-terrorism*. 1/9/02. Coral Gables, FL. Farm Foundation -Bennett Agricultural Round Table. Invited.

Archer, Douglas L.. *Food Terrorism*. 2/21/02. Orlando, FL. IFT International Food Safety and Quality Conference. Invited.

2001

Archer, Douglas L.. *Microbiological importance of sodium nitrite in cured meat safety*. Jun, 2001. IFT Annual Meeting. New Orleans, LA. Invited.

Archer, Douglas L., Clemens, Roger A.. *Probiotics:regulatory and marketing claims issues*. Jun, 2001. IFT Annual Meeting. New Orleans, LA. Invited.

Archer, Douglas L.. *Food Safety Management and Regulation*. Jun, 2001. Presentation to the Chief Research Officers of the Institute of Food Technologists. IFT Annual Meeting, New Orleans, LA. Invited.

Over 150 PRESENTATIONS PRIOR TO 2000 (available on request)

Ver 7.1.2003

RESUME

Name: Todd Robert Klaenhammer

Birthdate:

Academic Rank: Distinguished University Professor, NC State University
William Neal Reynolds Distinguished Professor of Food Science and Microbiology, Director, Southeast Dairy Foods Research Center
NCSU Alumni Distinguished Professor; 93-94
Faculty appointments in:
- Food Science Department ,78 -
- Microbiology Department ,78-
- Genetics Department, 01-
- Biotechnology Faculty, 90 -
- Genomics Faculty, 01-

Professor Associe in Sciences et Technologie de Aliments, University of Laval, Canada, 93-94
Professor, Departments of Food Science & Microbiology, 88-92
Associate Professor, Departments of Food Science & Microbiology, 83-88
Assistant Professor, Departments of Food Science & Microbiology, 78-83

Distribution of Time: 80% Research, 20% Teaching

Education: 1973 - Bachelor of Science - Microbiology; University of Minnesota, Minneapolis, MN
1975 - Master of Science - Food Science (Food Microbiology), Minor - Biochemistry; University of Minnesota, St. Paul, MN
1978 - Doctor of Philosophy - Food Science (Food Microbiology), Minor - Genetics; University of Minnesota, St. Paul, MN

Academic Specialty: Genetics of Lactic Acid Bacteria, Food and Dairy Fermentations, Food Microbiology, Gene expression and Modification, Plasmid and Bacteriophage Genetics, Dairy Starter Cultures, Antimicrobial Activities and Bacteriocins.

Professional Activities and Awards:

A. **Member**:

American Association for the Advancement of Science
American Society for Microbiology
Institutes of Food Technologists
American Dairy Science Association
American Cultured Dairy Products Institute

Gamma Sigma Delta
Sigma Xi
North Carolina Society for Dairy Technology
Honorary Member - Fundacion Miguel Lillo, Republic Argentina
National Association of Colleges and Teachers of Agriculture
Council for Agricultural Science and Technology (CAST)
National Academy of Sciences

B. Activities:

Director, Southeast Dairy Foods Research Center, July 1, 1992 --

Technical Director of the Southeast Dairy Foods Research Center, North Carolina State University, 1989-1992

Foundation for Microbiology Lecturers, 1983-1984 & 1989-1990, American Society for Microbiology

Scientific Lecturer, Institute of Food Technologists, 1989-1990

IFT Professional Speaker, 1990 - 1996

Editorial Boards - Journal of Dairy Science (1981-1986)

Journal of Applied and Environmental Microbiology (1984-1992; three terms)

Journal of Food Protection (1982-1984)

Comprehensive Reviews of Food Science/Food Safety (CRFSFS), an electronic Journal (2000 - 2003)

Advisory Board Apen's Food Microbiology and Safety Series, (2000 - 2001)

Editorial Service
- Applied and Environmental Microbiology
- Molecular Microbiology
- Journal of Bacteriology
- Molecular Microbiology
- Plasmid
- Nature Bio/technology
- Molecular Biology
- Gene
- Biochemistry
- Microbiological Reviews
- Food Microbiology
- International Journal of Food Microbiology
- Canadian Journal of Microbiology
- Journal of Industrial Microbiology

Journal of Agricultural and Food Chemistry
Journal of Food Safety
Biochimie
Antimicrobial Agents and Chemotherapy
Le Lait
Food Biotechnology
Journal of Food Science
Journal of Dairy Research
Journal of Food Protection
Journal of General Microbiology
Journal of Food Science
Food Technology
Finish Journal of Dairy Science
Antonie van Leeuwenhoek

Ad Hoc Reviewer for National/International Grants and Projects

NRICGP/USDA Competitive grants
Animal Molecular Biology, 1985-1994
Processing & Value Added, 1992-2000
Food Safety, 1993-2000
Oregon State University. USDA-ARS Projects, 1982, 1993
University of Missouri, Research Assistance Act Steering Committee, 1984
USDA Small Business Innovative Research Program, 1985, 1987
USDA-ARS Southern Region, 1985
NC Biotechnology Center, competitive grants, 1985-1996 Delaware Sea Grant Program, 1986
Irish National Board for Science and Technology, 1987
National Science Foundation/Metabolic Biology Program, 1988
Center for Innovative Technology/Institute for Biotechnology at Virginia Commonwealth University, 1988
Australian Research Council, Research Grants Committee, National Board of Employment, Education, and Training, 1989
EOLAS, Irish Science and Technology Agency, Dublin, Ireland 1989
SC Agricultural Experiment Station, Clemson University, 1989
Natural Sciences and Engineering Research Council(NSERC), Competitive Strategic Grants of Canada, 1990-1998
USDA/South Carolina Agricultural Experiment Station Proposal, 1990
USDA/Competitive - NRICGP, Value Added Panel, 1995
Foundation for Research, Science & Technology, Public Good Science Fund, New Zealand, 92-96; Review Team for the New Zealand Dairy Research Institute Research Program on Dairy Lactic Acid Bacteria.
South African Foundation for Research Development (FRDSNO), Referee for Young Investigatory/Grant Program, 1992.
International Science Foundation, Long term research grants, 1993
National Science Foundation, Competitive/Cooperative, 1992
NCSU Review Team for University de Compiegne de Technologie, France, 1993

Institute of Food Technologists-Judge for Graduate Student Research Paper Competiton-1993,1994
The Welcome Trust, London, UK. Research Proposal Review, 1994 & 96.
Clemson University, *Food Safety Through Biotechnology Research Program*, 1994.

Scientific Advisory Committee, Dairy Research Foundation, 1986-1987
Scientific Advisory Committee, National Dairy Promotion and Research Board, 1987-1991
General Committee Vice Chairman, 1990
Chairman, Genetics and Biotechnology subcommittee, 1989-1991
Expert Advisory Committee on research needs related to acidophilus and bifidus milk, 1989

NDPRB Review Team for the Minnesota-South Dakota Dairy Center, 1990
Dairy Management, Inc., Research Operations and Planning Committee, 1997-2000

Tenure and Promotion Reviews
University of Illinois, Department of Food Science, 1987 & 1990
University of Delaware, Department of Food Science, 1988
University of Vermont, Department of Animal Science, 1988
University of Tennessee, Department of Food Science and Technology, 1988
Clemson University, Department of Food Science, 1989
University of Otago, Department of Microbiology, New Zealand, 1990
University of Minnesota, Department of Food Science, 1990,1999
Rutgers Cook College, 1990
Cornell University, Department of Food Science, 1990
University of Wisconsin, Department of Food Science,1993,1999
Oregon State University, Department of Microbiology, 1994, 1995, 1998
Rutgers University, 1998
Utah State University, 1997, 1998
California Polytechnic State University, 1998
University of Massachusetts, 2000

National Academy of Sciences, Member of Committee on Scientific Programs; 2003-
National Academy of Sciences, Chairman, German-American Frontiers Symposium, 2003-2006

Invited participant: NAS workshop on *Exploring a Vision: Integrating Knowledge for Food and Health.* June 9, 2003, Washington, D.C.

Science Advisor on Food and Agricultural Biotechnology for EINSHAC, Einstein Institute for Science and Health in the Courts. 2000 - 2004. Participated in the Chile Courst and EINSHAC Judicial Institute on Bioscience and Biotechnology, March 1-5, 2004.

C. **Awards**:

Pfizer Award: Research in Cheese and Cultured Dairy Products; American Dairy Science Association, June 1984

000088

Sigma Xi Research Award. North Carolina State University, 1986. Certificate of Distinguished Scholarly Achievement. NC State University, First Annual Honors Convocation, 1986

Nordica International Research Award. American Cultured Dairy Products Institute, 1989

Samuel Cate Prescott Research Award. Young Investigator Award for Research in Food Science. Institute of Food Technologists, 1989

NCSU Alumni Outstanding Research Award, North Carolina State University, 1990

Dairy Research Foundation Award. American Dairy Science Association, June 1990

William Neal Reynolds Professorship, College of Agriculture and Life Sciences, NCSU, 1992

NCSU Alumni Distinguished Professor, named for Graduate Teaching, 1993

The National Award for Agricultural Excellence, Carolina-Virginia Chapter, National Agricultural Marketing Association, 1994

Gamma Sigma Delta Certificate of Merit, 1995, North Carolina State University Chapter

Outstanding Instructor, Food Science Club, NCSU, 1996

Borden Award, Outstanding Research in Dairy Science, American Dairy Science Assn, 1996.

Research and Development Award, Institute of Food Technologists, 1998.

Educational Materials Certificate of Excellence, 1998. Outstanding agronomic educational publication (< 16 pages) on the subject of *Genetic Engineering of Food, Feed, and Fiber* - Authors: Spears, Klaenhammer, and Peters. Presented by the American Society of Agronomy.

Fellow - Institute of Food Technologists - 2000

Fellow - American Academy of Microbiology - 2001

Outstanding Achievement Award of the University of Minnesota, June 7, 2001

Elected to membership in the National Academy of Sciences, May 1, 2001.

Distinguished University Professorship, November 1, 2001, NC State University

Highly Cited Researcher, 2001. (http://www.isihighlycited.com) Institute for Scientific Information

William C. Frazier Memorial Award. May 29, 2002. The Department of Bacteriology, Food Science and Food Microbiology and Toxicology, Food Research Institute, the University of Wisconsin-Madison.

D. Professional Activities/Committees

National

Pfizer Award Selection Committee, American Dairy Science Association, 1984-1986; Chairman, 1987

USA National Committee of the International Dairy Federation, Group of experts on: Genetic manipulation of dairy cultures, 1985-
Roles of culture-containing dairy foods in health, 1992-

ESCOP Strategic Planning Workshop, Processing and Value Added Subcommittee. Co-author of Biotechnology/Molecular Biology Field of Science, 1989

Executive Committee, IFT Biotechnology Section, 1992-1993

Samual Cate Prescott Award Committee, 1996-1998

Nominator of the 1992 & 1997 Winners of the Marschall Rhone-Poulenc International Dairy Science Awards: Winner, Dr. Gerald Venema 1992; Dr. Willem deVos, 1997

Bordon Award Selection Committee, American Dairy Science Association, 1992-1994, 1997 -1999

American Dairy Science Association Research Committee, 1994-1998.

National Academy of Sciences, National Research Council, Committee on National Security Implications of Advances in Biotechnology: Threats to Plants and Animals, August, 1999.

IFT Task Force on Food Biotechnology: Expert Panel - 2000

Southeast Judicial Conference on Genetics in the Courtroom. September 17-20, 2000. Einstein Institute for Science, Health and the Courts (EINSHAC) and NIEHS. Faculty Science Advisor.

EINSHAC Chief Science Advisor, Agricultural and Food Biotechnology. 2001 to present.

IFT Award Jury, Morcel Loncin Research Prize - $50,000 given biannually for basic chemistry/physics/engineering research applied to food processing and improvement of food quality. 2003 - 2009.

University

Gamma Sigma Delta Publicity and Chapter Promotion, 1980, 1981
NSCU Biotechnology Policy Committee, 1981-1985
NCSU Biotechnology Subcommittee, Microbial Life, 1982
NCSU Steering Committee for Academic Efforts Related to Biotechnology, 1981, 1982, 1983
NCSU Academic Advisory Committee, 1984-
Gamma Sigma Delta - Liason Officer, 1984-1986
Sigma Xi, Program Committee, 1985-1986
Search Committee for the Associate Dean, Director of Research in the College of Agriculture, NCSU, 1987
Search Committee for a University Professor, Yeast-Fungal molecular biologists, 1987
NCSU Committee on Training Fellowships in Biotechnology, 1991
NCSU Alumni Award Selection Committee, 1991
Selection Committee for Biotechnology Teaching Coordinator, 1991
Biotechnology/Biological Sciences Focus Group, 1991-1992
Biotechnology Academic Advisory Committee, 1992-
Graduate Fellowships in Biotechnology, Chairman, 1992-
NCSU/CALS, Faculty Committee Representative for Provost Interviews, 1992
CALS Focus Group-Program Initiatives Committee: Biotechnology/ Biological Sciences, 1992
CALS - Faculty committee to review candidate for Director of Research, 1992
University Advisory Committee for BRITE (Biotechnological Research Initiative and Transition Enhancement)
NCSU/CALS, Faculty Committee Representative for Provost Forum - Vision for NCSU, November 1993.
CALS/FS, William Neal Reynolds Professorship Screening Committee, 1993.
NCSU, CALS Distinguished Alumnus Award Screening Committee, 1993.
CALS Strategic Planning Committee, 1996
CALS Genomic Research Laboratory, Advisory Board, 99-
Gamma Sigma Delta Awards Committee, 99-
Advisory Board, Genomic Sciences, NCSU 99-
Executive Advisory Board - NIH Biotechnology Fellowships/NCSU, 99-
CALS Biobased Products Committee - 99
GAANN-Biotechnology Fellowship Selection Committee: 1997 -
NIH Biotechnology Graduate Fellowship Selection & Executive Committee: 2000-
Genome Research Laboratory Board of Directors - 2000 -
Deans Faculty Advisory Group, CALS, 2002 -
Gamma Sigma Delta Honors and Awards Committee, 2002 - 2003

Departmental

Graduate Committee, 1980-1985, 1997-2000; Chairman, 1986-1989
Research and Graduate Program Spokesperson, 1987-2000
CSRS Review, coordination and research committees, 1987, 1999
Chairman and director for the committee which coauthored the $2,000,000 proposal for the Southeast Dairy Foods Research Center, 1987, 1997
USDA National Needs Graduate Fellowships in Food Science Committee and proposal co-author, 1987
Equipment Committee, Radiation Committee, Facilities Advisory Committees
Technical Advisory Committee of the Southeast Dairy Foods Research Center, Chairman, 1989-1992
Ph.D. Preliminary Exam Committee, Chairman 1989-199 , 1998-
University Advisory Committee for BRITE (Biotechnological Research Initiative and Transition Enhancement)
Computer committee, 1992, 1993
Promotion and Tenure Committee, Chairman, 1993
Teaching Task Force Committee, 1993-
Search Committees for Department Head and Food Microbiologist, 1994
Departmental Strategic Planning Committee, 1994-
Seminar Committee Chairman, 1995-1996
Teaching Peer Review, 1998 -
Awards Committee, 1998-
Departmental Executive Committee, 1999-
Leadership Review - Committee, 2000

Organizer/Chairman of National/International Symposia and Conferences

"Current Studies on the Intestinal Activities of the Lactobacilli," American Society for Microbiology, symposium convener and chairman, 1981

"Microbial Growth," Institute of Food Technologists, 1981

"Genetic Aspects of Dairy Cultures: Advances and Applications," American Dairy Science Association, symposium convener and chairman, 1982

"Plasmids and Behavior of Bacteria in Mammalian Cell Systems," symposium chairman, 1982

"Genetics of Food Fermentation Microorganisms," Gordon Research Conference on Microbial Safety of Foods, organizer and chairman, 1984

"Plasmids and genetics of lactic acid bacteria," American Society for Microbiology, symposium co-convener, 1986

USDA-UM-Dupont 1st International Symposium on Biotechnology and Food Quality, University of Maryland, College Park, Session Chairman for "Bioprocessing," 1988

"Development of Food Grade Vectors," Session convener and chairman, Third International Symposium on Lactic Acid Bacteria in Foods, 1990

Chairman and organizer of the First National Dairy Center Research Center Research Symposium, "The cutting edge of dairy research: a national dairy center research symposium." American Dairy Science Association, 1990.

Co-organizer of the SDFRC Workshop on "Designing new dairy foods: applications for biological technologies in the dairy food industry," SDFRC/ADSA, 1990.

Co-organizer of the Fourth International Conference on the Genetics of Streptococci, Enterococci, and Lactococci. Chairman and program coordinator for the program on lactococci, scheduled for May, 1994, Santa Fe, New Mexico.

Co-organizer of the Workshop on the *Bacteriocins of Lactic Acid Bacteria.* The Banff Centre, Alberta Canada, 1995.

Co-organizer of the USDA/Ireland/SDFRC International Symposium on Functional Foods: Designed Foods for the Future, 1998.

Organizing committee of the 6th International Symposium on Lactic Acid Bacteria: genetics, metabolism, and Applicaitions. September 19-23, 1999, Veldhoven, The Netherlands. First delegate to the committee ever appointed from outside Holland.

Co-organizer for the 6th International ASM Conference on Streptococcal Genetics, Asheville, NC, April 14-17, 2002.

Co-organizer for the 12th International Conference on Bacilli: Functional Genomics of Gram-positive Microorganisms, Baveno, Italy, June 22-27, 2003.

Experience in Teaching and Directing Theses and Dissertations:

A. **Dissertations Directed**:

Sanders, M.E. 1980. The role of restriction and modification on the origin of lytic bacteriophage against group N streptococci. M.S. Thesis. North Carolina State University, Raleigh.

Sutherland, S.M. 1980. Distribution of antibiotic resistance among the genus *Lactobacillus:* examination for plasmid DNA and resistance factor determinants. M.S. Thesis. North Carolina State University, Raleigh.

Wright, C.T. 1980. Factors affecting the resistance of *lactobacilli* to freezing. M.S. Thesis. North Carolina State University, Raleigh.

**#! Sanders, M.E. 1983. Mechanisms and plasmid linkage of bacteriophage resistance in group N streptococci. Ph.D. Thesis. North Carolina State University, Raleigh.

! Wright, C.T. 1983. Physiological, morphological, and environmental factors influencing the freezing death of Lactobacillus bulgaricus. Ph.D. Thesis. North Carolina State University, Raleigh.

! Barefoot, S.F. 1985. Bacteriocins produced by Lactobacillus acidophilus: characterization of Lactacin B. Ph.D. Thesis. North Carolina State University, Raleigh.

Joerger, M.C. 1985. Detection, purification and characterization of helveticin J: a bacteriocin produced by Lactobacillus helveticus-jugurti. M.S. Thesis. North Carolina State University, Raleigh.

Sing, W.D. 1985. Conjugal transfer of bacteriophage resistance determinants into Streptococcus cremoris species. M.S. Thesis. North Carolina State University, Raleigh.

** Steenson, L.R. 1986. Natural and acquired plasmid-encoded bacteriophage resistance and physical protection from lytic bacteriophage in group N streptococci. Ph.D. Thesis. North Carolina State University, Raleigh.

! Muriana, P.M. 1986. Bacteriocins produced by Lactobacillus acidophilus: physical evidence and conjugal transfer of plasmid-encoded determinants for lactacin F production and resistance. M.S. Thesis. North Carolina State University, Raleigh.

! Higgins, D.L. 1987. Characterization of conjugative plasmids encoding restriction and modification activities from Streptococcus lactis ME2. M.S. Thesis. North Carolina State University, Raleigh.

Sing, W.D. 1989. Mechanisms of phage resistance encoded by plasmids in lactococci. Ph.D. Thesis. North Carolina State University, Raleigh.

Joerger, M.J. 1990. Cloning, expression, and nucleotide sequence of the gene encoding helveticin J: a bacteriocin produced by *Lactobacillus helveticus* 481. Ph.D. Thesis. North Carolina State University, Raleigh.

**! Muriana, P.M. 1990. Lactacin F, a bacteriocin produced by *Lactobacillus acidophilus*: purification of the active sequence of the structural gene (laf). Ph.D. Thesis. North Carolina State University, Raleigh.

Raya, R.R. 1991. Genetic characterization of a temperate bacteriophage from *Lactobacillus gasseri* ADH. Ph.D. Thesis. North Carolina State University, Raleigh.

**# Romero, D.A. 1991. Lactococcal insertion sequences IS946: isolation, characterization, and development as an experimental tool. Ph.D. Thesis. North Carolina State University, Raleigh.

000094

! Harris, L.J. 1991. Development of a novel paired starter culture system for sauerkraut consisting of nisin-resistant Leuconostoc mesenteroides and nisin producing *Lactococcus lactis*. Ph.D. Thesis. North Carolina State University, Raleigh.

Durmaz, E. 1992. A fourth mechanism of bacteriophage resistance in *Lactococcus lactis* subsp. *lactis* ME2. M.S. Thesis. North Carolina State University, Raleigh.

Moineau, S.M. 1994. Controle des Bacteriophages de Lactocoques: Approaches. Moleculaires. Ph.D. Thesis. University of Lavel, Quebec, Canada.

D'Amelio, G.N. 1994. Genetic characterization of industrial lactococcal bacteriophages insnsitive to the phage defense mechanisms encoded by pTR2030. M.S. Thesis. North Carolina State University, Raleigh.

**! Dinsmore, P.K. 1996. Genetic and mutational studies of the abiA phage resistance gene in *Lactococcus* and characterization of phage mutants that are insensitive to abiA. Ph. D. Thesis. Food Science. North Carolina State University, Raleigh.

Evans, Scott, P. 1996. Heterologous expression of superoxide dismutase in lactic acid bacteria. M.S. thesis. Microbiology. North Carolina State University, Raleigh.

Djordjevic, G. 1997. Development of a triggered-suicide system for bacteriophage defense: Ph.D. Dissertation in Microbiology, NCSU.

Allison, G.E. 1997. Molecular analysis of lactacin F, A bacteriocin produced by *Lactobacillus johnsonii* and novel genetic applications in lactic acid bacteria. Ph.D. Dissertation in Microbiology, NCSU, Raleigh.

Jenny, P.A. 1998. Designing a triggered suicide system for phage defense of *Streptococcus thermophilus*. M.S. Thesis, Microbiology, NCSU, Raleigh.

Crowell, D.C. 1998. Microbial analysis of human intestinal flora after feeding *Lactobacillus acidophilus*. M.S. Thesis, Food Science, NCSU, Raleigh.

Walker, D.C. 1999. Localization of the IS-3 group insertion element IS*1223* at strategic positions in the *Lactobacillus* genome near genetic determinants for lactose utilization, conjugation and bacteriocin production. Ph.D. Thesis, Microbiology, NCSU Raleigh.

** ! Walker, S.A. 1999. Molecular characterization of the late promoter and surrounding region of the lytic, lactococcal bacteriophage 31 and its use in the development of expression systems for *Lactococcus lactis*. Ph.D. Thesis, Food Science, NCSU Raleigh.

Barrangou-Poueys, Rodolphe. 2000. *Leuconostoc fallax* strains and their bacteriophages from industrial sauerkraut fermentations. M.S. Thesis, Food Science, NCSU, Raleigh.

Girgis, Hany. 2000. Stress adaptation in lactic acid bacteria. M.S. Thesis, Food Science, NCSU, Raleigh.

Tuler, Tracy. 2001. The overexpression of lactococcal peptidases in the presence of a novle enzyme externalization system in *Lactococcus lactis*. M.S. Thesis, Food Science, NCSU, Raleigh.

Russell, W. Michael. 2001. Development of molecular tools for the study of intestinal and probiotic lactobacill. Ph.D. Thesis, Microbiology, NCSU.

Hawkins, R.H. 2001. Distribution and strain polymorphisms in probiotic lactobacilli. M.S. Thesis, Food Science, NCSU.

** Students (6) awarded the Kenneth R. Keller Research Award for the most outstanding Ph.D. dissertation in the College of Agriculture and Life Sciences, NCSU.

Theses (2) selected for the Richard M. Hoyt Award to recognize outstanding graduate student research in dairy science. American Dairy Science Association, 1983.

! Graduate students (8) awarded IFT Graduate Fellowships during their tenure at NCSU.

B. <u>Courses & Teaching</u>:

	FS(MB) 405	- Food Microbiology, 1978-1982, 1993, 1995, 1996, 1998-2003
	FS(MB) 506	- Advanced Food Microbiology, 1978-1983
	FS 499H	- Undergraduate Honors Research
	FS 491	- Special Topics in Food Science
	FS 680	- Seminar in Food Science, 1979-1990
	FS 699	- Research in Food Science, 1979-1996
*	FS 691A,B	- Fermentation Microbiology/Biotechnology, 1984
*	FS 691A	- Plasmid Biology of Food and Dairy Fermentations, 1986
	FS 691D	- Microbiology of Food and Dairy Fermentations, 1988, 1990
	MB 502B	- Virology/Bacteriophages, 1989
*	BT 695	- Gene Transfer and Cloning in Gram-Positive Bacteria, 1989
	ALS 499H	- Honors Research in Microbiology, 1988, 1996
	ALS 499H	- Honors Research in Biochemistry, 1989, 1990
	FS(MB) 525	- Fermentation Microbiology, 1992, 1994, 1996, 2000, 2002
	FS 591D	- Food Biotechnology, 1998
	FS(MB)725 -	- Fermentation Biotechnology, 1999

* Courses in the Biotechnology Curriculum

Teaching participation/invited guest lectures:

BIT666,	Core Technologies Module - "Gene transfer mechanisms, cloning and expression vectors for genetically-ill defined bacteria, 1992 - 2001
MB501,	Advanced Microbiology - "The lactic acid bacteria and their roles in industrial food fermentations," 1980
MB401,	General Microbiology - "Microbiology of Foods," 1981

GN666,	Molecular Genetics Laboratory - "Strategies for gene cloning and transfer in gram positive bacteria," 1995, 1996, 1997, 1998
FS405,	Food Microbiology - "Fermentations: new and old technologies in food bioprocessing," 1990
FS691F,	Muscle biology - "Strategies for molecular cloning of genes and gene systems," 1991
MB495,	Microbial Biotechnology - "Fermentation Biotechnology," 1991

Courses Taken:

GN666, Molecular Genetics Laboratory, 1985
1982 Bethesda Research Laboratories, DNA Sequencing Workshop, 1984

C. <u>Post Doctoral Students</u>

John B. Luchansky (87-88), Associate Professor, Food Research Institute, University of Wisconsin
Colin Hill (87-88), Senior Lecturer, University College Cork, Department of Microbiology, Ireland
Cheol Ahn (91-92), Head, Department of Applied Biology and Technology Kangwon National University, Korea.
Christophe Fremaux (93-93), Research Scientist, Texel and Institute de biologie moleculaire et d' ingenierie genetique, IBMIG, Faculte de Poitiers, UFR Sciences.
Karen Zagula (93-94), Faculty Member, Microbiology , Wake County Community College, Raleigh
Dan O'Sullivan (94-96), Assistant Professor, Department of Food Science and Nutrition, University of Minnesota, St. Paul.
Jeffery Greene (94-95), Medical Doctor of Pediatrics, The Ohio State University, Columbus
Ralf Zink (96-97). Director of Microbiology, Nestle, Ltd. Switzerland.
Koen Venema (97), Microbiologist, TNO Nutrition and Food Research Institute, The Netherlands.
David Mills (97-98), Assistant Professor, Dept.of Enology, Univ. of California, Davis.
John McCormick (97-98), Post-doctoral research associate, Department of Microbiology, University of Minnesota, Minneapolis
Martin Kullen (98-99) Coca-Cola - Industrial Microbiologist.
Mick Callanan (99- 2002) Dairy Products Research Center, Moorepark, Ireland
Eric Altermann (99-) Post-doctoral research associate, in residence, NCSU
Olivia McAulliffe (2000-2003) Dairy Products Research Center, Moorepark, Ireland
Andrea Azcarate (2001 -), post doctoral research associate, in residence, NCSU
Micheal Miller (2003 -), Post doctoral research associate, in residence, NCSU

D. <u>International Teaching/Research Activities</u>:

Latin America Professor Program. September 23 - October 7, 1985. Two week lecture and laboratory course on "Advances in the Molecular Biology of the Lactic Acid Bacteria." Lecture, 36 students; Laboratory, 19 students. Sponsored by the American Society for Microbiology. Hosted by the Centro de Referencia para Lactobacilos (CERELA), Tucuman, Argentina.

Instructor for the 5th National Conference for Food Technologists of the Argentina Dairy Industry, October 7, 1985. Subjects included current developments in starter culture and dairy processing technologies.

Instructor for "Recent Developments in the Molecular Biology of Lactic Acid Bacteria." A graduate course presented at University College, Cork, Ireland, for the Department of Dairy and Food Microbiology/Biotechnology. Six lectures and discussion over the period September 30 through October 2, 1987.

Invited Participant at the International Circle of Dairy Research Leaders, (ICODRL) Meeting on "Genetics of Lactic Acid Bacteria," Ede, The Netherlands, 1985.
FEMS Second International Symposium on Lactic Acid Bacteria: Genetics, Metabolism and Applications, Wageningen, The Netherlands, 1986. Invited speaker and participant.

Invited participant in the Annual Meeting of the Canadian Institute of Food Science and Technology. Symposium on Food Microbiology and Biotechnology, Hamilton Ontario, May 18, 1987.

Invited participant in the European Molecular Biology Organization Symposium on Genetic Manipulation of Lactic Acid Bacteria. Annecy France, April 1988.

Invited participant in the Australian Dairy Review Conference, New South Wales, May 1988.

Invited to the New Zealand Dairy Research Institute, Palmerston North, May 1988.

Invited participant to the 3rd International American Society for Microbiology Conference on Streptococcal Genetics, Minneapolis MN, June 1990. Subject, Molecular analysis of phage defense systems in lactococci.

Invited participant to the 6th International Symposium on the Genetics of Industrial Microorganisms, Strasbourg, France, 1990. Subject, antimicrobial interactions of lactic bacteria.

Invited participant in the Third International Symposium on Lactic Acid Bacteria, Genetics, Metabolism, and Applications. Session convener and speaker on the development of food grade cloning and expression vectors. September, 1990.

Invited speaker/participant in the Food Microbiology Symposium, the Royal Biochemical Society of England, April 1991. Development of bacteriophage resistant lactic acid bacteria.

Invited speaker in the EMBO-FEMS-NATO Workshop on *Bacterial Plasmid-Coded Toxins:* Bacteriocins, Microcins, and Lantibiotics. The Island of Bendor, France, September, 1991. Molecular Properties of Lactobacillus Bacteriocins.

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Invited Plenary Speaker at the 10th Australian Biotechnology Conference. February, 1992, Melbourne, Australia. Genetic strategies to develop bacteriophage-insensitive lactic acid bacteria for industrial fermentations.

Dairy Research and Development Corporation, Melbourne Australia, February 5, 1992. "The impact of microbial technologies on the cultured dairy product industries"

University of Melbourne, Russell Grimwade School of Biochemistry, Lactic Acid Bacteria Research Conference, February 3, 1992. "Development of integration and transposition systems for lactic acid bacteria".

Nordic Food Biotechnology Umbrella Program, Helsinki, Finland, September 2, 1992. "Genetics of bacteriocins produced by lactic acid bacteria"

Plenary Lecture on "Advances in Lactic Acid Bacterial Genetics" at Biotieteen Paivat-1992, Finnish Bioscience Days, Helsinki, Finland, September 3-4, 1992. "Development of gene transfer, integration, and expression systems for *Lactobacillus*".

Session Chairman on Bacteriophages at Biotieteen Paivat-1992, Finnish Bioscience Days, Helsinki, Finland, September 3-4, 1992.

Plenary Lecture presented to the NZ Society for Biochemistry and Molecular Biology and NZ Microbiological Society Joint Conference, May 17-20, 1993, Massey University, Palmerston North, New Zealand. "Biotechnology, Food Fights and Molecular Touché".

Presented Opening Address, "Properties and potential benefits of lactic acid bacteria", at the Lactic Acid Bacteria Research in Canada Workshop organized by Agriculture Canada in association with the University of Alberta, Edmonton, Canada. June 23-25, 1993. Supported by the Canadian Agricultural Research Council.

Plenary Lecture presented to the Fourth Symposium on Lactic Acid Bacteria, Genetics, Metabolism, and Applications, September 5-9, 1993, Noordwijkerhout, The Netherlands. "Peptide Bacteriocins Produced by Lactic Acid Bacteria: Genetic Aspects and Opportunities".

Invited symposium lecture at GIM94 - 7th International Symposium on the Genetics of Industrial Microorganisms. "Genetic strategies to construct phage-insensitive lactococci for dairy fermentations". Montreal Canada, June 26-July 1, 1994.

Invited Plenary Speaker on "Genetics of Intestinal Lactobacilli". International Dairy Lactic Acid Bacterial Conference, Palmerston North, New Zealand, February 22, 1995.

Invited Speaker on "Heterologous expression of bacteriocins from lactic acid bacteria. Workshop on the bacteriocins of lactic acid bacteria, applications and fundamentals. Banff Canada, April, 22, 1995.

Invited Symposium Speaker on "Recent genetic developments on lactic acid bacteria and their bacteriophages" Japanese Study Group for Lactic Acid Bacteria, Toyko University of Agriculture, Tokyo, Japan, July 28th, 1995.

Invited speaker on "The role of genetics in defining the intestinal roles of those species defined within the *Lactobacillus acidophilus* complex". Yakult Central Institute for Microbiological Research, Tokyo, Japan, July 31, 1995.

Invited Keynote speaker on "Combating competitors and parisites through advances in the genetics of lactic acid bacteria". Japanese Society for Bioscience, Biotechnology, and Agrochemistry. Sapporo, Japan, August 3, 1995.

Invited Participant to the European Commission Meeting on lactic acid bacteria, Biotechnology and Fair Programs , Cork Ireland, October 22-27, 1995.

"*In vitro adherence assays for studies of "probiotic" lactic acid bacteria; opportunities and limitations*". Fifth Symposium on Lactic Acid Bacteria: Genetics, Metabolism, and Applications. Veldhoven, The Netherlands, September 10, 1996.

"Phage-induced suicide and explosive expression systems for Lactococcus-new strategies for phage defense and gene expression". Fifth Symposium on Lactic Acid Bacteria: Genetics, Metabolism, and Applications. Veldhoven, The Netherlands, September 9, 1996.

"Novel approaches for the development of lactic acid bacteria resistant to phages". 20th Aniversary of CERELA, University of Tucuman, Argentina. October 11, 1996.

"Probiotic Roles of Lactobacilli: Genetic Imperatives & Opportunities" The 10th International Symposium on Lactic Acid Bacteria and Human Health. Seoul, Korea, August 22, 1997.

"Functional activities of Lactobacillus probiotics: genetic mandate. International conference on functional foods: designer foods for the future. US/Ireland Co-operation programme in agriculture & technology. Cork, Ireland, September 30 -October 2, 1997.

"Genetic strategies to control bacteriophages attacking dairy starter cultures". Keynote address at the European Dairy Experts Symposium - Dairying Behind the Dykes, September 17th, 1998, Arnhem, The Netherlands.

"Functional Food Science in the USA". Keynote address at the symposium *Functional Food Research in Europe*. Haikko Manor, Finland, Oct 1, 1998.

"Selection and design of probiotics"-Keynote Lecture - Food Microbiology 99, The Netherlands, 9/99.

LAB genome analysis and engineering - Tools Development and Exploitation. 6th Symposium on LAB special session, The Netherlands, 9/99.

"Interactions of bacteriophage with lactic acid bacteria" , Univ of Laval, Canada, 12/99.

"Functional genomics and rational selection of probiotics" Key Note Lecture in the La Fondation Des Governeurs, International Symposium on Probiotics and Health: The Intestinal Microflora. Montreal Canada, October 13, 2000.

"Genomics of Lactic Acid Bacteria". Keynote Invited Lecture at the Wageningen Centre for Food Sciences Food Summit 2000 on the Impact of Genomics on Food Sciences. November 28, 2000.

"Probiotic lactic acid bacteria: from Metchnikoff to genomics". Opening Lecture at the Danish Biotechnology Conference VII - Biotechnology in Food: Opportunities and Limits. Munkebjerg, Vejle, Denmark. May 31, 2001.

Research:

List of Publications

1. Klaenhammer, T.R. and L.L. McKay. 1976. Isolation and examination of transducing bacteriophage particles from *Streptococcus lactis* C2. J. Dairy Sci. 59:396-404.

2. Klaenhammer, T.R., L.L. McKay, and K.A. Baldwin. 1978. Improved lysis of group N streptococci for isolation and rapid characterization of plasmid deoxyribonucleic acid. Appl. Environ. Microbiol. 35:592-600.

3. Klaenhammer, T.R. and S.M. Sutherland. 1980. Detection of plasmid DNA in an isolate of *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 39:671-674.

4. Sanders, M.E. and T.R. Klaenhammer. 1980. Restriction and modification in the group N streptococci: the effect of heat on the development of modified lytic bacteriophage. Appl. Environ. Microbiol. 40: 500-506.

5. Klaenhammer, T.R. and E.G. Kleeman. 1981. Growth characteristics, bile sensitivity, and freeze damage in colonial variants of *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 41: 1461-1467.

6. Sanders, M.E., and T.R. Klaenhammer. 1981. Evidence for plasmid linkage of restriction and modification in *Streptococcus cremoris* KH. Appl. Environ. Microbiol. 42: 944-950.

7. Wright, C.T. and T.R. Klaenhammer. 1981. Calcium-induced alteration of cellular morphology affecting the resistance of *Lactobacillus acidophilus* to freezing. Appl Environ. Microbiol. 41:807-815.

8. Klaenhammer, T.R. 1982. Microbiological considerations in the selection and preparation of *Lactobacillus* strains for use as dietary adjuncts. J. Dairy Sci. 65:1339-1349.

9. Kleeman, E.G. and T.R. Klaenhammer. 1982. Adherence of *Lactobacillus* species to human fetal intestinal cells. J. Dairy Sci. 65:2063-2069.

10. Barefoot, S.F. and T.R. Klaenhammer. 1983. Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 45:1808-1815.

11. Wright, C.T. and T.R. Klaenhammer. 1983. Survival of *Lactobacillus bulgaricus* during freezing and freeze-drying after growth in the presence of calcium. J. Food Sci. 48:773-777.

12. Wright, C.T. and T.R. Klaenhammer. 1983. Influence of calcium and manganese on dechaining of *Lactobacillus bulgaricus*. Appl. Environ. Microbiol. 46:785-792.

13. Sanders, M.E. and T.R. Klaenhammer. 1983. Characterization of phage-sensitive mutants from a phage-insensitive strain of *Streptococcus lactis*: evidence for a plasmid determinant that prevents phage adsorption. Appl. Environ. Microbiol. 46: 1125-1133.

14. Wright, C.T. and T.R. Klaenhammer. 1984. Use of phosphated milk adversely affects the growth, cellular morphology and fermentative ability of *Lactobacillus bulgaricus*. J. Dairy Sci. 67:44-51.

15. Daeschel, M.A., R.F. McFeeters, H.P. Fleming, T.R. Klaenhammer, and R.B. Sanozky. 1984. Mutation and selection of *Lactobacillus plantarum* strains that do not produce carbon dioxide from malate. Appl. Environ. Microbiol. 47: 419-420.

16. Klaenhammer, T.R. 1984. A general method for plasmid isolation in lactobacilli. Current Microbiology 10: 23-28.

17. Sanders, M.E. and T.R. Klaenhammer. 1984. Phage resistance in a phage-insensitive strain of *Streptococcus lactis*: temperature dependent phage development and host-controlled phage replication. Appl. Environ. Microbiol. 47: 979-985.

18. Klaenhammer, T.R. 1984. "Interactions of bacteriophages with lactic streptococci." *Advances in Applied Microbiology*. Volume 30, pp 1-29. Allen I. Laskin (ed.) Academic Press, New York.

19. Barefoot, S.F. and T.R. Klaenhammer. 1984. Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. Antimicrobial Agents Chemother. 26:328-334.

20. Klaenhammer, T.R. and R.B. Sanozky. 1985. Conjugal transfer from *Streptococcus lactis* ME2 of plasmids encoding phage resistance, nisin resistance, and lactose-fermenting ability: evidence for a high-frequency conjugative plasmid responsible for abortive infection of virulent bacteriophage. J. Gen. Microbiol. 131:1531-1541.

21. Steenson, L.R. and T.R. Klaenhammer. 1985. *Streptococcus cremoris* M12R transconjugants carrying the conjugal plasmid pTR2030 are insensitive to attack by lytic bacteriophages. Appl. Environ. Microbiol. 50:851-858.

22. Daeschel, M.A. and T.R. Klaenhammer. 1985. Association of a 13.6 megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. Appl. Environ. Microbiol. 50:1538-1541.

23. Sing, W.D. and T.R. Klaenhammer. 1986. Conjugal transfer of bacteriophage-resistance determinants on pTR2030 into *Streptococcus cremoris* strains. Appl. Environ. Microbiol. 51:1264-1271.

24. Jarvis, A.W. and T.R. Klaenhammer. 1986. Phage resistance conferred on lactic streptococci by the conjugative plasmid pTR2030: effects on small isometric-, large isometric-, and prolate-headed phages. Appl. Environ. Microbiol. 51:1272-1277.

25. Joerger, M.C. and T.R. Klaenhammer. 1986. Characterization and purification of helveticin J: evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus-jugurt* 481. J. Bacteriol. 167:439-446.

26. Steenson, L.R. and T.R. Klaenhammer. 1986. Plasmid heterogeneity in *Streptococcus cremoris* M12R: effects on proteolytic activity and host-dependent phage replication. J. Dairy Sci. 69: 2227-2236.

27. Sanders, M.E., P.J. Leonhard, W.D. Sing, and T.R. Klaenhammer. 1986. Conjugal strategy for construction of fast-acid producing, bacteriophage resistant lactic streptococci for use in dairy fermentations. Appl. Environ. Microbiol. 52:1001-1007.

28. Jarvis, A.W. and T.R. Klaenhammer. 1987. The phage resistance plasmid pTR2030 inhibits lytic infection of R_1T temperate phage, but not induction of R_1T prophage in *Streptococcus cremoris* R1. Appl. Environ. Microbiol. 53:385-389.

29. Steenson, L.R. and T.R. Klaenhammer. 1987. Conjugal transfer of plasmid DNA between streptococci immobilized in calcium alginate gel beads. Appl.Environ. Microbiol. 53:898-900.

30. Muriana, P.M. and T.R. Klaenhammer. 1987. Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. Appl. Environ. Microbiol. 53:553-560.

31. Steenson, L.R., T.R. Klaenhammer, and H.E. Swaisgood. 1987. Calcium alginate-immobilized cultures of lactic streptococci are protected from attack by lytic bacteriophage. J. Dairy Sci. 70:1121-1127.

32. Klaenhammer, T.R. 1987. Plasmid-directed mechanisms for bacteriophage defense in lactic streptococci. FEMS Microbiol. Rev. 46:313-325.

33. Klaenhammer, T.R. 1988. Bacteriocins of lactic acid bacteria. Biochimie 70:337-349.

34. Higgins, D.L., R.B. Sanozky-Dawes, and T.R. Klaenhammer. 1988. Restriction and modification activities from *Streptococcus lactis* ME2 are encoded by a self-transmissible plasmid, pTN20, that forms cointegrates during mobilization of lactose-fermenting ability. J. Bacteriol. 170:3435-3442.

35. Luchansky, J.B., P.M. Muriana, and T.R. Klaenhammer. 1988. Application of electroporation for transfer of plasmid DNA to *Lactobacillus, Lactococcus, Leuconostoc, Listeria, Pediococcus, Bacillus, Staphylococcus, Enterococcus, and Propionibacterium*. Mol. Microbiol. 2:637-647.

36. Hill, C., D.A. Romero, D.S. McKenney, K.R. Finer, and T.R. Klaenhammer. 1989. Localization, cloning, and expression of genetic determinants for bacteriophage resistance (Hsp) from the conjugative plasmid pTR2030. Appl. Environ. Microbiol. 55:1684-1689.

37. Luchansky, J.B., E.G. Kleeman, R.R. Raya, and T.R. Klaenhammer. 1989. Genetic transfer systems for delivery of plasmid deoxyribonucleic acid to *Lactobacillus acidophilus* ADH: conjugation, electroporation, and transduction. J. Dairy Sci. 72:1408-1417.

38. Harris, L.J., M.A. Daeschel, M.E. Stiles, and T.R. Klaenhammer. 1989. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. J. Food Protect. 52:384-387.

39. Raya, R.R., E.G. Kleeman, J.B. Luchansky, and T.R. Klaenhammer. 1989. Characterization of the temperate bacteriophage adh and plasmid transduction in *Lactobacillus acidophilus* ADH. Appl. Environ. Microbiol. 55: 2206-2213.

40. Hill, C., K. Pierce, and T.R. Klaenhammer. 1989. The conjugative plasmid pTR2030 encodes two bacteriophage defense mechanisms in lactococci, restriction modification (R^+/M^+) and abortive infection (Hsp^+). Appl. Environ. Microbiol. 55:2416-2419.

41. Klaenhammer, T.R. 1989. Genetic characterization of multiple mechanisms of phage defense from a prototype phage-insensitive strain, *Lactococcus lactis* ME2. J. Dairy Sci. 72: 3429-3443.

42. Sing, W.D. and T.R. Klaenhammer. 1990. Plasmid-induced abortive infection in lactococci: A review. J. Dairy Sci. 73: 2239-2251.

43. Josephsen, J. and T.R. Klaenhammer. 1990. Stacking of three different restriction and modification systems in *Lactococcus lactis* by cotransformation. Plasmid 23:71-75.

44. Hill, C., L.A. Miller, and T.R. Klaenhammer. 1990. Nucleotide sequence and distribution of h pTR2030 resistance determinant *(hsp)* which aborts bacteriophage infection in lactococci. Appl Environ. Microbiol. 56:2255-2258.

45. Sing, W.D. and T.R. Klaenhammer. 1990. Characterization of phage abortion conferred in lactococci by the conjugal plasmid pTR2030. J. Gen. Microbiol. 136:1807-1815.

46. Romero, D.R. and T.R. Klaenhammer. 1990. Abortive phage infection and restriction/modification activities directed by pTR2030-determinants are enhanced by recombination with conjugal plasmids in lactococci. J. Gen. Microbiol. 136:1817-1824.

47. Joerger, M.C., and T.R. Klaenhammer. 1990. Cloning, expression, and nucleotide sequence of the *Lactobacillus helveticus* 481 gene encoding the bacteriocin helveticin J. J. Bacteriol. 172:6339-6347.

48. Romero, D.A., and T.R. Klaenhammer. 1990. Characterization of insertion sequence IS946, and iso-ISS1 element, isolated from the conjugative lactococcal plasmid pTR2030. J. Bacteriol. 172:4151-4160.

49. Hill, C., L.A. Miller, and T.R. Klaenhammer. 1990. Cloning, expression, and sequence determination of bacteriophage fragment encoding bacteriophage resistance in *Lactococcus lactis*. J. Bacteriol. 172: 6419-6426.

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50. Sing, W.D. and T.R. Klaenhammer. 1991. Characterization of restriction and modification plasmids from *Lactococus lactis* subsp. *cremoris* and their effects when combined with pTR2030. J. Dairy Sci. 74: 1133-1144.

51. Hill, C., L.A. Miller, and T.R. Klaenhammer. 1991. The bacteriophage resistance plasmid pTR2030 forms high molecular weight multimers in lactococci. Plasmid 25: 105-112.

52. Hill, C., I.J. Massey, and T.R. Klaenhammer. 1991. A rapid method to characterize lactococcal bacteriophage genomes. Appl. Environ. Microbiol. 57: 283-288.

53. Muriana, P.M., and T.R. Klaenhammer. 1991. Purification and partial characterization of lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088. Appl. Environ. Microbiol. 57: 114-121.

54. Muriana, P.M., and T.R. Klaenhammer. 1991. Cloning, phenotypic expression, and DNA sequence of the gene for lactacin F, an antimicrobial peptide produced by *Lactobacillus* spp. J. Bacteriol. 173: 1779-1788.

55. Alatossava, T., and T.R. Klaenhammer. 1991. Molecular characterization of three small isometric-headed bacteriophages which vary in their sensitivity to the lactococcal phage resistance plasmid, pTR2030. Appl. Environ. Microbiol. 57: 1346-1353.

56. Hill, C., L.M. Miller, and T.R. Klaenhammer. 1991. *In vivo* genetic exchange of a function domain from a type II-A-methylase between the lactococcal plasmid pTR2030 and a virulent bacteriophage. J. Bacteriol. 173: 4363-4370.

57. Harris, L.J., H.P. Fleming, and T.R. Klaenhammer. 1991. Sensitivity and resistance of *Listeria monocytogenes* ATCC 19115, Scott A, and UAL500 to nisin. J. Food Protection 54:836-840.

58. Klaenhammer, T. R. 1991. Antimicrobial and bacteriocin interactions of the lactic acid bacteria. Proceedings of the Sixth International Symposium on Genetics of Industrial Microorganisms, Vol I: 433-445.

59. Klaenhammer, T.R. 1991. Development of bacteriophage resistant strains of lactic acid bacteria. Biochemical Society Transactions 19: 675-681.

60. Klaenhammer, T.R., D. Romero, W. Sing, and C. Hill. 1991. Molecular analysis of pTR2030 gene systems that confer phage resistance to lactococci. In *Genetics and Molecular Biology of Streptococci, Lactococci, and Enterococci.* G. Dunny, P. Cleary, and L. McKay (ed). American Society for Microbiology, Washington DC. pp. 124-130.

61. Luchansky, J.B., M.C. Tennant, and T.R. Klaenhammer. 1991. Molecular cloning and DNA polymorphisms in *Lactobacillus acidophilus* and *Lactobacillus gasseri.* J. Dairy Sci. 74: 3293-3302.

62. Romero, D.A., and T.R. Klaenhammer. 1991. Construction of an IS946-based composite transposon in *Lactococcus lactis* subsp. *lactis.* J. Bacteriol. 173: 7599-7606.

63. Raya, R.R., and T.R. Klaenhammer. 1992. High frequency transduction by the *Lactobacillus* gasseri bacteriophage φadh. Appl. Environ. Microbiol. 58: 187-193.

64. Romero, D.A., and T.R. Klaenhammer. 1992. IS946-mediated integration of heterologous DNA into the genome of *Lactococcus lactis* subsp. *lactis*. Appl. Environ. Microbiol. 58: 699-702.

65. Stevens, K.A., B.W. Sheldon, N.A. Klapes, and T.R. Klaenhammer. 1992. Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. Appl. Environ. Microbiol. 57: 3613-3615.

66. Piard, J.-C., P.M. Muriana, M.J. Desmazeaud, and T.R. Klaenhammer. 1992. Purification and partial characterization of lacticin 481, a lanthionine-containing bacteriocin produced by *Lactococcus lactis* CNRZ 481. Appl. Environ. Microbiol. 58: 279-284.

67. Klaenhammer, T.R., C. Fremaux, C. Ahn, and K. Milton. 1992. Molecular Biology of bacteriocins produced by *Lactobacillus*. In Bacteriocins and Lactic Acid Bacteria, D. Hoover & L. Steenson (eds), Academic Press, NY, pp 151-180.

68. Foegeding, P.M., A.B. Thomas, D.H. Pilkington, and T.R. Klaenhammer. 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. 58: 884-890.

69. Klaenhammer, T.R. 1992. Genetic strategies to develop bacteriophage-insensitive lactic acid bacteria for industrial fermentations. Proceedings from the Tenth Australian Biotechnology Conference, Australian Biotechnology Association Ltd, Melbourne.

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Invited Seminars/Symposia/Lectures:

"Microbiological considerations in the selection and preparation of *Lactobacillus* strains for use as dietary adjuncts." Seminar, Annual Meeting of the American Society for Microbiology. Dallas, TX, 1981.

"Mechanisms of bacteriophage resistance in lactic streptococci: are plasmids important?" Miles Laboratories, Inc., Biotechnology Group Seminar, July 14, 1982.

"Microbiological characters that affect the proposed roles of *Lactobacillus acidophilus* in the intestinal tract of man and animals." Miles Laboratories, Inc., Biotechnology Group Seminar, July 14, 1982.

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"Selection and development of *Lactobacillus* strains for the food industry." Symposium, Recent Developments in Genetics for Food Application. Gordon Research Conference, Microbiological Safety of Foods. Plymouth, NH. 1982.

"Biotechnology in the food industry: advances in starter culture technology." Seminar, Annual Meeting of the American Society for Microbiology. New Orleans, LA, 1983.

"Resistance of group N streptococci to lytic bacteriophage: mechanisms and genetic determinants." Netherlands Society for Microbiology, International Symposium on lactic acid bacteria in foods. Wageningen, The Netherlands. September 8, 1983.

"Improvement of traditional food fermentations by genetic engineering of the lactic acid bacteria: fact or fantasy." Virginia Polytechnic Institute, Department of Food Science. Blacksburg, VA. April 9, 1984.

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"Mechanisms and genetics of bacteriophage resistance in lactic streptococci." Cornell University, Departments of Microbiology, Biotechnology, and Food Science. Ithaca, NY. April 19, 1984.

"Mechanisms and genetics of bacteriophage resistance in group N streptococci." Gordon Research Conference on Microbiological Safety of Foods. Plymouth, NH. July 18, 1984.

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"Fermentation Technology in Food Science." Department of Chemical Engineering. North Carolina State University. Raleigh, NC. April 3, 1985.

"Construction of Phage Resistant Lactic Streptococci." Department of Food Science and Microbiology. University of Alberta, Edmonton, Canada. November 7, 1985.

"Advances in the genetics of *Lactobacillus* species." Department of Food Science, University of Alberta, Edmonton, Canada, November 7, 1985.

"Will tomorrows dairy microbiologist be plagued by sexual mating, virulent viruses and antagonistic behavior." NCSU Microbiology Colloquium, December 2, 1985.

"Advances and applications of genetic technologies for the improvement of starter cultures for dairy fermentations." Symposium on Biotechnology in the Dairy Industry 81st Annual Meeting of the American Dairy Science Association. Davis, CA. June 24, 1986.

"Conjugal plasmids encoding phage resistance to lactic streptococci: genetic strategies for construction of phage insensitive starter cultures." Symposium on Recent Advances in the Genetics of Lactic Acid Bacteria. Annual Meeting the Society for Industrial Microbiology. San Francisco, CA. August 11, 1986.

"Applications of biotechnology in the construction of improved starter cultures for dairy fermentations." Symposium on Biotechnology in Agriculture and Food. 192nd Annual Meeting of the American Chemical Society Meeting, Anaheim, CA. September, 1986.

"Bacteriocin production and resistance in dairy lactobacilli: localization of genetic determinants and mechanisms for genetic transfer." Meeting of the International Circle of Dairy Research Leaders on Genetics of Lactic Acid Bacteria. Ede, The Netherlands. September 25-26, 1986.

"Conjugal plasmids directing phage resistance mechanisms in lactic streptococci: characterization of mechanisms, localization of genetic determinants, and mobilization strategies for construction of phage

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"Cheese, viruses and biotechnology." School of Agriculture and Life Sciences Spring Faculty Meeting. North Carolina State University. Raleigh, NC. May 7, 1986.

"Genetic strategies for construction of phage-insensitive dairy starter cultures." Institute National de la Recherche Agronomique Laboratoire de Microbiologie Laitiere'. Jouy-en-Josas, France. October 2, 1986.

"Characterization and use of conjugative plasmids that encode mechanisms of phage defense in lactic streptococci." Seminar, Department of Genetics, North Carolina State University. Raleigh, NC. October 7, 1986.

"Construction of bacteriophage-resistant starter cultures for dairy fermentations using conjugative plasmids that encode mechanisms for phage defense." Symposium on Agricultural and Industrial Microbial Genetics. 10th Annual Mid-Atlantic extrachromosomal Elements Meeting. Virginia Beach, VA. October 18, 1986.

"Biotechnological developments in dairy cultures." Symposium on Food Microbiology and Biotechnology. Canadian Institute of Food Science and Technology Annual Meeting. Hamilton, Ontario, Canada. May 18, 1987.
"Plasmid-directed mechanisms of bacteriophage defense in lactic streptococci." EMS Second International Symposium on Lactic Acid Bacteria Genetics, Metabolism and Applications. Wageningen, The Netherlands. September 24, 1987.

"Genetic strategies used in construction of phage-insensitive starter cultures for dairy fermentations." Nestle Research Centre, Vevey, Switzerland. September 28, 1987.

"Construction of phage-resistant dairy streptococci." Department of Food Science and Nutrition, University of Minnesota. St. Paul, MN. November 9, 1987.

"Bacteriocins." E.M.B.O. Symposium on Genetic Manipulation of Lactic Acid Bacteria: A Breakthrough in the Food Industry. Foundation Universitaire Des Sciences Et Techniques Du Vivant, Annecy, France. April 23-24, 1988.

"From Test Tubes to Table." Fermentation, safe packaging, additives, nutrition, diet and health. Agricultural Biotechnology and the Public. Raleigh, NC. February 22-24, 1988.

"Starter systems in the USA." Australian Dairy Culture Review Conference, New South Wales. May 4-6, 1988.

"Genetic engineering of phage resistance and other characteristics in starter cultures." New Zealand Dairy Research Institute, Palmerston, New Zealand. May 9, 1988.

"Production of novel antimicrobials through biotechnology". Institute of Food Technologists Symposium on Antimicrobials and their use in foods. 1988 Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 1988.

"Genetic characterization of multiple mechanisms of phage defense from a prototype phage insensitive strain, Lactococcus lactis ME2." International symposium on advances in the molecular biology of the lactic acid bacteria. ADSA, Edmonton, Canada, 1988.

"Genetic strategies to construct novel starter cultures for traditional food fermentations." Department of Food Technology, The Ohio State University. Columbus, OH. January 30, 1989.

"Improvement of food fermentations through application of molecular genetics." Symposium on biotechnology and ecology-key issues for the pickle industry, Pickle Packers International, Inc. Raleigh, NC. April 13, 1989.

"Potential for the development of bacteriophage resistant thermophilic cultures." 26th Annual Marschall Invitational Italian Cheese Seminar. Madison, WI. September 14, 1989.

"Genetic strategies to prevent phage disruption of industrial fermentations." ASM Foundation Lecture, Northern California Branch of the American Society for Microbiology. San Francisco, CA. October 7, 1989.

"Definition and use of gene transfer systems for molecular analysis of *Lactobacillus*". J.B. Luchansky, and T.R. Klaenhammer, Annual Meeting of the American Society for Microbiology. New Orleans, LA. May 1989.

"Phage resistance in starter cultures."Annual Meeting of the American Cultures Dairy Products Institute, New Orleans, LA, March 1990.

"Antimicrobial peptides and proteins: molecular approaches to safer foods." ABC Research and Food Processing Magazine symposium on "New Generation Foods Using Bioingredients." Chicago, IL, April 1990.

"Food science and biotechnology: scientific and public perceptions and the position of the emerging food scientist." NCSU Food Science Issues Forum, April 26, 1990.

"Molecular analysis of pTR2030 gene systems that confer phage resistance to lactococci." Invited seminar presented in the symposium on Lactococci: Molecular Biology and Biotechnology. Third International ASM Conference on Streptococcal Genetics, Minneapolis, MN, June, 1990.

"Past and future views on the application of molecular genetics to lactic acid bacteria." University of Helsinki, Finland, September, 1990.

"Molecular designs for antimicrobial and antiviral systems in dairy foods." Lacto-Labo, France, September, 1990.

"Development of Food Grade Vectors." Third International Symposium on Lactic Acid Bacteria in Foods, Wageningen, The Netherlands, September 1990.

"Molecular views of bacterial defenses and phage counterdefenses during industrial fermentations." University of Nebraska, Biotechnology Seminar Series, November, 1990.

"Molecular views of bacterial defenses and phage counterdefenses." Department of Biochemistry, North Carolina State University,December, 1990.

"Bacteriocin systems in foods: designing antimicrobial peptides." Research Branch Seminar, Agriculture Canada, Ottawa,March 1991.

"Designing antimicrobial proteins for use in foods." STELA - Universite Laval, Quebec City, Canada, March 1991.

"Development of bacteriophage resistant strains of lactic acid bacteria". Colloquium on Food Biotechnology, Royal Biochemical Society Meeting, University of Reading, UK, April 1991.

"Food Biotechnology --- Bridging the Gap." IFT-Scientific lectures presented at Texas A & M and the Northern Indiana sectiion of IFT, April 1991.

"Genetics and regulation of bacteriocin production by lactic acid bacteria." Symposium presentation at the 1991 Annual meeting of the Institute of Food Technologists, June, 1991.

"Molecular designs for anti-viral systems in dairy lactic acid bacteria." Seminar presentation at the Department of Food Science and Technology, University of California, Davis, July 1991.

"Molecular Properties of *Lactobacillus* Bacteriocins." EMBO-FEMS-NATO Workshop on Bacterial Plasmid-Coded Toxins: Bacteriocins, Microcins, and Lantibiotics. The Island of Bendor, France, September, 1991.

"Development of phage insensitive strains for industrial fermentations." Ecogene, Inc., Philadelphia, PA. December, 1991.

"Genetic strategies to develop bacteriophage-insensitive lactic acid bacteria for industrial fermentations." Plenary lecture, 10th Australian Biotechnology Conference. Melbourne, Australia, February, 1992.

"Molecular designs for antiviral systems in industrial fermentations". Center for Gene Research and Biotechnology Seminar Series, Oregon State University, May 18, 1992.

"Designing antiviral systems in bacteria". Department of Microbiology Seminar Series, The University of Oklahoma, November 9, 1992.

"Development of gene transfer, integration, and expression systems for *Lactobacillus*". Finish Biotieteenpaivat Proceedings, September 1992.

"Molecular organization of natural bacteriophage defense systems and genetic designs for phage-resistant culture used in dairy processing". N.C. Meeting of the American Society for Microbiology, April 1993.

"Development of integration and transposition systems for lactic acid bacteria". University of Melbourne, Russell Grimwade School of Biochemistry, Lactic Acid Bacteria Research Conference, February 3, 1992.

"Genetics of bacteriocins produced by lactic acid bacteria" Nordic Food Biotechnology Umbrella Program, Helsinki, Finland, September 2, 1992.

"Development of gene transfer, integration, and expression systems for *Lactobacillus*". Plenary Lecture on "Advances in Lactic Acid Bacterial Genetics" at Biotieteen Paivat-1992, Finnish Bioscience Days, Helsinki, Finland, September 3-4, 1992.

"Properties and potential benefits of lactic acid bacteria". Opening Address for the International Workshop on Lactic Acid Bacteria Research in Canada, University of Alberta, Canada, June 23, 1993.

"Biotechnology, Food Fights and Molecular Touche". Plenary Lecture for the "Microbes and Molecules", Joint Annual Meeting of the New Zealand Biochemistry and Molecular Biology Societies, May 17-20, 1993.

"Molecular organization of natural bacteriophage defense systems and genetic designs for phage-resistant cultures used in dairy bioprocessing". Symposium presentation at the N.C. Branch of the American Society for Microbiology Spring Meeting, April 23, 1993

"Gene Wars", NCSU Annual Biotechnology Program Retreat. February 14, 1994.

"Dairy biotechnology and molecular point-counterpoint". Michigan State University. Department of Food Science Seminar Series, April 27, 1994
"Biotechnology-The New Frontier, Dairy Biotechnology". Invited Speaker at the International Dairy Show, Minneapolis, Mn, October 5-8, 1994.

"Looking back at lactic acid bacteria and forward to applications in molecular genetics" Cornell University. Microbiology Seminar Series, November 11, 1994.

"Genetics of Intestinal Lactobacilli". International Dairy Lactic Acid Bacterial Conference, Palmerston North, New Zealand, February 22, 1995.

"Heterologous expression of bacteriocins from lactic acid bacteria. Workshop on the bacteriocins of lactic acid bacteria, applications and fundamentals. Banff Canada, April, 22, 1995.

"Molecular and functional diversity among bacteriocins produced by lactic acid bacteria". American Society for Microbiology Symposium on *Biodiversity: The Ecology of The Environment and Factors Controlling It.* May 21, 1995.

"Genetics and physiology of intestinal *Lactobacillus* species: program and research highlights" Presentation at the Dannon Yogurt Roundtable of Experts on the use of Live Cultures in Yogurt. San Antonio, TX, June 14, 1995.

"Recent genetic developments on lactic acid bacteria and their bacteriophages" Japanense Study Group for Lactic Acid Bacteria, Toyko University of Agriculture, Tokyo, Japan, July 28th, 1995.

"The role of genetics in defining the intestinal roles of those species defined within the *Lactobacillus acidophilus* complex". Yakult Central Institute for Microbiological Research, Tokyo, Japan, July 31, 1995.

"Combating competitors and parasites through advances in the genetics of lactic acid bacteria". Japanese Society for Bioscience, Biotechnology, and Agrochemistry. Sapporo, Japan, August 3, 1995.

" Novel genetic strategies for phage defense in lactic acid bacteria". In the IFT Symposium on *Recent Advances in Lactic Acid Bacteria.* New Orleans, LA. June 23, 1996.

"In vitro adherence assays for studies of "probiotic" lactic acid bacteria; opportunities and limitations. Fifth Symposium on Lactic Acid Bacteria: Genetics, Metabolism, and Applications. Veldhoven, The Netherlands, September 10, 1996.

"Phage-induced suicide and exploive expression systems for *Lactococcus*-new strategies for phage defense and gene expression. Fifth Symposium on Lactic Acid Bacteria: Genetics, Metabolism, and Applications. Veldhoven, The Netherlands, September 9, 1996.

"Novel approaches for the development of lactic acid bacteria resistant to phages. XX Aniversary of CERELA, University of Tucuman, Argentina. October 11, 1996.

"*Lactobacillus* probiotics: current developments" Nestle Research Center, New Milford, CT. October 21, 1996.

"Novel Approaches for phage defense of bioprocessing bacteria". Center for Microbial Physiology, Metabolic Engineering, Biodegradation, and BioInformation, Department of Microbiology, Universitiy of Minnesota. November 15, 1996.

"The *Lactobacillus* delivery system of the future". Embrex, Research Triangle Park Nov 6, 1996.

"Making the good guys better, FAVP biotechnology in lactic acid bacteria". 1997 James M. Craig Memorial Lecture in Applied and Environmental Microbiology. Oregon State University, March 3, 1997

"Designing genetic traps to kill bacterial viruses". 1997 James M. Craig Scientific Lecture in Applied and Environmental Microbiology. Oregon State University, March 4, 1997.

"Turning the table on bacterial viruses: novel strategies for phage protection of industrial starter cultures". University of Delaware, Department of Food Science, Graduate Student Invited Lecture, March 17, 1997.

"Advances in bacterial applications for biotechnology". Food Research Institute, University of Wisconsin, May 13, 1997.

"Designing genetic traps to kill bacterial viruses" Department of Bacteriology, University of Wisconsin, May 14, 1997.

"The in bacterial applications for biotechnology" Food Research Institute, University of Wisconsin, Madison, May 12, 1997.

"Current Research Developments on Lactic acid bacteria "Probiotics, Bacteriocin, and Bacteriophages." Rhone Poulenc, Madison, Wisconsin, May 16, 1997.

"Probiotic Roles of Lactobacilli: Genetic Imperatives & Opportunities" The 10th International Symposium on Lactic Acid Bacteria and Human Health. Seoul, Korea, August 22, 1997.

"Ace in the hole: current projects at Dairy Research Centers" International Dairy Foods Association, Worldwide Food Expo 97. Chicago IL, November 1, 1997.

"Functional activities of *Lactobacillus* probiotics: genetic mandate. International conference on functional foods: designer foods for the future. US/Ireland Co-operation programme in agriculture & technology. Cork, Ireland, September 30 - October 2, 1997.

"Future directions in isolation, culturing, and stabilization of probiotic strains". Food Microbiology - Mini Symposium, Nestle, Ltd. New Milford, CT. October 10th, 1997.

"A revolution in food microbiology: molecular facets". Divisional lecture in food microbiology a the annual meeting of the Institute of Food Technologists, June, 1998.

"Lethal genes and altruistic strategies for phage defense of lactococci and streptococci". Plenary lecture at the American Society for Microbiology Conference on Streptococcal Genetics, France, April, 1998.

"NCFM- Technical Review of *Lactobacillus acidophilus*. Rhodia NCFM Symposium. September 10th, 1998, Madison Wisconsin.
"Genetic strategies to control bacteriophages attacking dairy starter cultures". Keynote address at the European Dairy Experts Symposium - Dairying Behind the Dykes, September 17th, 1998, Arnhem, The Netherlands.

"Functional Food Science in the USA". Keynote address at the symposium *Functional Food Research in Europe*. Haikko Manor, Finland, Oct 1, 1998.

"Starters, curds, and whey: current research" North Central Cheese Industries Association - 1998 Annual Conference. Watertown, SD. October 14, 1998.

"Bacteriophage interactions with *Lactococcus*: molecular tools and practical controls. In the Symposium Session on Applications of Bacteriophages, presented at the 1999 Annual Meeting of the American Society for Microbiology. Chicago, IL, June 1, 1999.

"Probiotic Bacteria: today and tomorrow". Presentation at the 1999 Experimental Biology Meetings, Washington DC, in the symposium on *Probiotic Bacteria: Implications in Human Health*, April 18, 1999.

"Functional genomics and food science: forecasting your future". Symposium: Looking into the 21st Century: Futuristic Trends in Food Science. Annual Meeting of the Institute of Food Technology, Chicago IL, July 26th, 1999.

"Selection and design of probiotics". Plenary Lecture at Food Microbiology 99 - Ecology and Physiology of Food Related Microorganisms. Veldhoven, The Netherlands, September 16, 1999.

"Probiotic biotechnology: new opportunities for the dairy and food industries." Eastern Food Science Conference XI, on Foods for the Future. Hershey Lodge, PA, October 11, 1999

"Interactions of bacteriophage with lactic acid bacteria". Invited Seminar at University of Laval, Quebec City, Canada. December 10, 1999.

"Value of in vitro selection criteria in probiotics: what do we know about validations of the approaches used". Symposium Danone on Fermented Food, Fermentation and Intestinal Flora. New York Academy of Medicine. NY. May 25-26, 2000.

"Bacteriophage evolution in industrial bioprocessing and biotechnology". Invited seminar in the symposium program on Applied Evolution. Evolution 2000. Indiana University, June 27, 2000.

"Lactic acid bacteria and health". IFT 2000 Symposium on Dairy Food Trends: Past, Present, and Future. June 13, 2000.

"Biotechnology: what science is telling us about safety, labeling, and benefits and concerns" Presentation panelist for benefits and concerns. IFT 2000, June 13, 2000.

"Functional genomics and rational selection of probiotics" Key Note Lecture in the La Fondation Des Governeurs, International Symposium on Probiotics and Health: The Intestinal Microflora. Montreal Canada, October 13, 2000.

"Bacteriophage gymnastics and evolutionary tales in fermentation biotechnology" . Invited Seminar, Department of Genetics, NCSU, November 20, 2000.

"Genomics of Lactic Acid Bacteria". Keynote Invited Lecture at the Wageningen Centre for Food Sciences Food Summit 2000 on the Impact of Genomics on Food Sciences. November 28, 2000.

"College at Rutgers University Distinguished Speaker for the NJAES Distinguished Seminar Series on Food, Nutrition, and Human Health" April 20, 2001.

"Lactobacillus genome sequencing ", Lecture at Rhodia Global Probiotics Meeting, Chicago, IL, April 18, 2001.

"Probiotic lactic acid bacteria: from Metchnikoff to genomics". Opening Lecture at the Danish Biotechnology Conference VII - Biotechnology in Food: Opportunities and Limits. Munkebjerg, Vejle, Denmark. May 31, 2001.

"Tales of lactic acid bacteria: the journey from Louis Pasteur to genomics" Department of Food Science and Nutrition Special Seminar for presentation of the University of Minnesota Outstanding Achievement Award. June 7, 2001.

"Food Biotechnology and Genomics Tomorrow: Millions to Billions". NCSU Bridge Symposium, Sept 28, 2002.

"Practicing genomics in food microbiology". Albert L. Elder Lecture in Food Science, University of Illinois, Nov 8, 2001.

"Practicing genomics in lactic acid bacteria". Invited lecture, Dupont Experimental Station, Dec 5, 2001.

"The changing vistas of food microbiolology: from Pasteur to Genomics". John L. Etchells Memorial Lecture, Department of Food Science, NC State University. April 10,2002.

"Discovering lactic acid bacteria through genomics". Distinguished Speakers Seminar Series, University of Maryland. April 26, 2002.

"Traditions in Food Microbiology: From Pasteur to Genomics". William C. Frazier Memorial Lecture, University of Wisconsin, Food Research Institute, Department of Bacteriology, and Department of Food Science, May 19, 2002.

"Genomics of probiotic lactic acid bacteria: pathways for discovery". XIII International Symposium of the International Association of Gnotobiotics, NCSU College of Veterinary Medicin, July 15, 2002

"Antisense RNA-based bacteriophage defense strategies". Plasmid Biology. Pittsburgh, PA. Sturino, J. M., and T. R. Klaenhammer. July, 2002.

"Discovering lactic acid bacteria through genomics", 7th International Symposium on Lactic Acid Bacteria, Genetics, Metabolism, and Applications. Plenary Lecture, September 2, 2002.

"Milk and lactic acid bacteria: influence of the dairy environment on gene expression and culture functionality". Congrilait, International Dairy Congress, Paris, September 26, 2002.

"Lactic acid bacteria as bioprocessing and delivery vehicles: genomics perspectives", Tulane University Health Sciences Center, Department of Biochemistry, November 18, 2002.

"Influence of the dairy environment on gene expression of lactic acid bacteria". INRA Research Center, Rennes, France, February 26, 2002.

"Biotechnology and Food". NCSU Graduate School Board of Advisors, March 7, 2003

"Walking the line: Food Science to Genomics". E.A. Day Memorial Evening Lecture. Penn State University, April 2, 2003.

"The revolution in food microbiology: Pasteur to genomics". E.A. Day Memorial Scientific Seminar. Penn State University, April 3, 2003.

"Genome diversity among lactic acid bacteria", 2003 ASM Annual Meeting, Divisional Lecture in Food Microbiology.

"Influence of the dairy environment on gene expression and substrate utilization by lactic acid bacteria". International Dairy Federation Summit and Centenary, Bruges, Belgium; September 11, 2003.

"Role of scientific consensus reports in dispute resolution". UNIDO Global Technology Forum, Chile, March 4, 2004

"Bioconfinement of genetic engineered organisms: a scientific briefing". UNIDO Global Technology Forum, Chile, March 5, 2004

Grants/Gifts/Contracts

1. "Bacteriophage and antibiotic resistance in lactic streptococci: roles of plasmid DNA." NCSU Faculty Research and Development Grant. $2,500.00; July 1, 1979.

2. "Factors affecting the intestinal activity of the lactobacilli: bacteriocin production and adherence." Marschall Division of Miles Laboratories, Madison, WI. $10,000.00; November 1, 1980 - November 1, 1981.

3. "The lactobacilli: physiological, genetic and metabolic states." Research Fellowship Grant. $26,000.00; 1980-1984.

4. "Bacteriocin-mediated antagonism in the lactobacilli." Marschall Division of Miles Laboratories, Madison, WI. $13,500.00; April 1, 1982 - April 1, 1983.

5. "Mechanisms and genetics of bacteriophage resistance in phage- insensitive lactic streptococci." Miles Laboratories, Biotechnology Group, Elkhart, IN. $14,500.00; April 1, 1983 - April 1, 1984.

6. "Plasmid genetics and transfer in dairy lactobacilli." Dairy Research Foundation, Rosemont, IL. $92,481.43; January 1, 1984-January 1, 1987.

7. "Mechanisms and genetics of bacteriophage resistance in a prototype phage-insensitive strain." Miles Laboratories Biotechnology Group. Elkhart, IN. $9,530.00; April 1, 1984-April 1, 1985.

8. "Natural and acquired bacteriophage resistance in *Streptococcus cremoris*: interspecies genetic transfer and expression of prototype phage-insensitive phenotypes." Miles Laboratories Biotechnology Group, Elkhart, IN. $18,250.00; April 1, 1984 - September 1, 1985.

9. "Molecular biology of lactic acid bacteria." Grant in Aid, Stauffer Chemical Company Richmond, CA. $15,000.00; April, 1985.

10. "Transfer, Expression and cloning of phage resistance genes in lactic streptococci." USDA Competitive Grant-Biotechnology. $104,000.00; September 15, 1985 - September 30, 1987.

11. "Localization and cloning of genetic determinants encoding bacteriophage resistance in lactic streptococci: transfer, expression and characterization of defense mechanisms'." Miles Laboratories, Biotechnology Group. $119,350.00; September 1, 1985 - September 1, 1988.

12. "Gene transfer and cloning systems for lactobacilli." UDIA Dairy Research Foundation. $172,244.00; January 1, 1987 - December 31, 1989.

13. "Molecular characterization of pTR2030-directed phage resistance in lactic streptococci." USDA-Competitive Grants/Molecular Biology. #NC09350. $190,000.00; September 1, 1987 - August 31, 1990.

14. "Inhibition of Listeria monocytogenes in fermented muscle foods by bacteriocin producing lactic acid starter cultures". National Live Stock and Meat Board. $26,000. D. Pilkington, P.M. Foegeding, and T.R. Klaenhammer.

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15. "Research center for innovative biological, thermal, and chemical processing of Dairy Foods." National Dairy Research and Promotion Board. $2,000,000.00; July 1, 1988 to June 30, 1992. Southeast Dairy Foods Research Center. T.R. Klaenhammer and H.E. Swaisgood, co-PI's.

16. "Genetic characterization, gene transfer, and cloning in *Lactobacillus acidophilus* ADH." Southeast Dairy Foods Research Center. $153,963.00; July 1, 1988 - June 30, 1991. J.B. Luchansky and T.R. Klaenhammer, co-PI's.

17. "Molecular characterization of phage defenses in lactococci: expression, regulation, and manipulation of genes, products, and multiple gene systems." Miles, Inc., Biotechnology Products Division. $245,448.00; January 1, 1989 - December 32, 1991.

18. "Cloning and expression of antioxidant enzymes in dairy lactic acid bacteria." Southeast Dairy Foods Research Center. $80,234.00; July 1, 1989 - June 30, 1991. H.M. Hassan and T. R. Klaenhammer, co-PI's.

19. "Gene expression of bactericidal proteins in lactic acid bacteria." Nestle, Ltd., Vevey, Switzerland. $72,836.72; January 1, 1991 - December 31, 1991, with 2nd year option.

20. "Bactericidal proteins and peptides in dairy lactobacilli: molecular applications in food safety and biotechnology". National Dairy Promotion and Research Board. $144,727. January 1, 1991 - December 31, 1993.

21. "Cellular mechanisms of resistance to cytolytic peptides". North Carolina Dairy Foundation. $36,000. July 1, 1991 - June 30, 1994.

22. "Genetic characterization, gene transfer, and cloning in *Lactobacillus acidophilus* ADH." Southeast Dairy Foods Research Center. $4,500. January 1, 1992 - April 1, 1992.

23. "Genetic designs and combinations for use in a single strain starter culture rotation program." Marschall Products-Rhone Poulenc. $15,000. July 1, 1992 - June 30, 1993.

24. "Bacteriophage expression signals." Marschall Products - Rhone-Poulenc. $15,000. August 1, 1992 - July 30, 1993.

25. "Molecular characterization of a *Lactobacillus* adherence-bridging protein." National Dairy Promotion and Research Board. $110,000. July 1, 1992 - June 30, 1994.

26. "Bacteriophage-specific suicide systems for industrial fermentations." USDA-NRICGP 92-37500-8018. $189,000. October 1, 1992 - September 30, 1995.

27. "Genetic modifications of lactococci to promote industrial longevity of dairy starter cultures." Marschall Products, Rhone-Poulenc, Inc. $15,000. December 1, 1993 - November 30, 1994.

28. "Multicomponent phage defense mechanisms in lactococci". Marschall Products, Rhone-Poulenc, Inc. $15,000. December 1, 1993 - November 30, 1994.

29. "Explosive plasmid phage expression systems". North Carolina Biotechnology Center-Research Initiation Grant. $40,000. July 1, 1993 - December 31, 1994.

30. "Evaluation of commercial strains for development of a multifunctional fluid-milk product containing *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bifidobacterium* species. T.R. Klaenhammer and C. Walker. $32,303. California Dairy Research Foundation, December 1, 1992 - January 30, 1994.

31. "Proposal for continuation of the Southeast Dairy Foods Research Center for application of new technologies to dairy products and processing. $1,600,000. National Dairy Promotion and Research Board. July 1, 1994 - June 30, 1998.

32. "Fourth International Conference on Streptococcal Genetics. $5,507. USDA-National Research Initiative Competitive Grants Program. May 15, 1994- May 18, 1994.

33. "Genetic modification of lactococci to prevent the appearance of new bacteriophages". Marschall Products, Rhone Poulenc, Inc. $15,000. January 1, 1995 - December 31, 1995

34. "Multiple phage resistance mechanisms in lactococci". Marschall Products, Rhone Poulenc, Inc. $15,000. January 1, 1995-December 31, 1995.

35. "Use of genetic engineering to improve the biochemical/technological properties of dairy lactic acid bacteria: cloning and expression of superoxide dismutase". Southeast Dairy Foods Research Center. $108,025. July 1, 1994 - June 30, 1996. Co-PI with H. Hassan, Microbiology.

36. 'Plasmid-phage expression systems for lactic acid bacteria" Southeast Dairy Foods Research Center. $87,207. July 1, 1995 - June 30, 1998.

37. "*In ovo* immunization of chicks with live vaccines presented by lactobacilli" Embrex, Inc.- Kenan Institute - North Carolina Biotechnology Center, Collaborative Funding Assistance Program Grant. $60,000. January 1, 1996 - December 31, 1997.

38. "Microbial analysis of human small intestinal flora after feeding *Lactobacillus acidophilus*. Abbott Laboratories, Ross Products Division - $11,322 & North Carolina Dairy Foundation - $16,000. January 1, 1996 - December 31, 1996.

39. "Targeting transcription regulators as a general strategy for phage defense of *Lactococcus* species" Southeast Dairy Foods Research Center. $49,059. July 1, 1996 - June 30, 1998 .

40. "Designing novel phage defense systems for mesophilic and thermophilic dairy starter cultures". Marschall products, Rhone-Poulenc, Inc. $15,000. January 1, 1996 - December 31, 1996.

41. "Microbial analysis of human small intestinal flora after feeding *Lactobacillus acidophilus*. Abbott Laboratories, Ross Products Division - $15,000. January 1, 1997 - December 31, 1997.

42. "Identification of five *Lactobacillus* strains provided by northeast nutraceuticals." $3,000. October 1, 1996 - December 1, 1996.

43. "Genetic analysis and modification of lactococcal chromosomal sequences contributing to phage evolution". Marschall products, Rhone-Poulenc, Inc. $15,000. January 1, 1996 - December 31, 1996.

44. "In ovo applications for lactobacilli producing antimicrobial peptides against enteric pathogens." North Carolina Biotechnology Center. $40,000. January 1, 1997- December 31, 1997.

45. "Genetic analysis of lactococcal chromosomal sequences contributing to the evolution of bacteriophages." Rhone-Poulenc, Inc. $15,000, Madison, WI.. January 1, 1997- December 31, 1997.

46. "Designing phage-triggered defense systems for dairy starter cultures." Rhone-Poulenc, Inc. $15,000, Madison, WI.. January 1, 1997- December 31, 1997.

47. "Triggered suicide systems for bacteriophage defense and gene containment. USDA-NRICGP $210,000. 1997-2000.

48. "Factors affecting gene expression in *Lactobacillus acidophilus*: development of expression vectors and definition of transcriptional and translational controls." SDFRC/DMI $241,319. 1997-2000.

49. "Functional foods: designer foods for the future" UDSA. $20,000 to support USDA/Ireland International Conference, September 30-October 2, 1997.

50. "Lactase overexpression systems for lactic acid bacteria". SDFRC Industrial Enhancement Project/The Protein Group. $60,000. Jan 1, 1998 - Dec 31, 1999

51. "Designing phage-triggered defense systems for dairy starter cultures. Rhodia, Inc. $15,000, July 1, 1998 - June 30, 1999.

52. "Evaluation of a novel genetic strategy to interfere with the developmental cycles of bacteriophages attacking dairy starter cultures. Rhodia, Inc. $15,000, July 1, 1998 - June 30, 1999.

52. Southeast Dairy Foods Research Center. $483,443, Dairy Management, Inc. 1999.

53. Sixth Symposium on Lactic Acid Bacteria: $8,000. Support for US participants and invited speakers. USDA/NRICGP. 9/15/99 - 9/30/2000.

54. "Leaky lactic acid bacteria: applications for accelerated cheese ripening" $163,553. Southeast Dairy Foods Research Center. 1/2000 to 12/2002.

55. Southeast Dairy Foods Research Center. $680,401. Dairy Management, Inc. 2000 (*Total funding from DMI for support of SDFRC from 1988 through 2000 is $6,085,844)*

56. *Lactobacillus acidophilus* genome sequencing and functional analysis. $368,885. Rhodia, Inc. Collaborative project with NCSU-Klaenhammer and CalPoly-Cano. 1/2000 - 12/2001.

57. Improvement of *Lactobacillus acidophilus* stress tolerance by altering membrane fatty acid composition. Courtney (Ohio State Univ) and Klaenhammer (NCSU), MAFMA Industry Match, $15,000.

58. "Phage defense systems for dairy starter cultures. $15,000. Rhodia, Inc. January 2001 - December 2001.

59. "Molecular decoys that disrupt the development cycles of bacteriophages attacking lactic acid bacteria" Rhodia, Inc. January 2001 - December 2001.

60. Southeast Dairy Foods Research Center. $564,585. Dairy Management, Inc. 2001 (*Total funding from DMI for support of SDFRC from 1988 through 2001 is $6,650,429).*

61. "Utilization of fructooligosaccharides by probiotic bacteria". Hutkins, R. and Klaenhammer, T.R. Dairy management, Inc. January 1, 2001 - December 31, 2002. $169,972.

62. "NIH Biotechnology training program at North Carolina State University" $1,013,376. 2001-2005. Dr. Robert Kelly Coordinator. TRK is a member of executive committee that co-authored the proposal and operates the program.

63. "Functional genomic analysis of probiotic lactobacilli".$189,065. Dairy Management, Inc. & Southeast Dairy Foods Research Center. January 1, 2002 - December 31, 2004.

64. "New Phage Defenses for Lactococcus Starter Cultures ",$20,000, Rhodia Inc. July 2002 - June 2003.

65. Targeting bacteriophages with molecular decoys and poisons", $20,000, Rhodia, Inc., July 2002, to June 2003.

66. Isolation and characterization of bacteriophages associated with a large scale fermentation facility", $100,000, Cargill Dow. March 1, 2002 - February 28th, 2003.

67. Southeast Dairy Foods Research Center. $546,913. Dairy Management, Inc. 2002 (*Total funding from DMI for support of SDFRC from 1988 through 2001 is $8,296,719).*

68. Assessment of changes in bacterial communities using terminal restriction fragment length polymorphisms: the effect of probiotics on fecal flora and symptoms of human subjects with IBS, IBD, and undergoing antibiotic therapy. $62,278. Southeast Dairy Foods Research Center/NC Dairy Foundation. January 1, 2003 - December 31, 2003.

69. Engineering lactic acid bacteria for delivery of antioxidants into dairy products. H.M. Hassan and T.R. Klaenhammer, NC Dairy Foundation and SDFRC. $62,134.

70. Functional and comparative genomics on Lactobacillus acidophilus NCFM and other probiotic lactic acid bacteria. Rhodia, Inc., Klaenhammer & Altermann. SDFRC enhancement project. $87,655. July 1, 2003 - June 30, 2004.

71. Control of bacteriophages and contaminants in a large scale lactate fermentation facility. Cargill/Dow, Minnetonka, MN. $100,000. July 1, 2003 to June 31, 2004.

Anderson SL, Q.J., Rubin BY. (2003) EGCG corrects aberrant splicing of IKAP mRNA in cells from patients with familial dysautonomia. *Biochem Biophys Res Commun* 310:627-633. (MENTHONE/EGCG/RI/0820/Burdock/75037)

Arimoto-Kobayashi S, I.N., Sato Y, Sugiyama C, Okamoto K, Hayatsu H, Negishi T. (2003) Inhibitory effects of (-)-epigallocatechin gallate on the mutation, DNA strand cleavage, and DNA adduct formation by heterocyclic amines. *J Agric Food Chem* 51:5150-5153. (MENTHONE/EGCG/RI/0820/Burdock/75042)

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Chang, P., Mirsalis, J., Riccio, E., Bakke, Lee, Shimon, J., Phillips, S., Fairchild, D., Hara, Y. and Crowell, J. (2003) Genotoxicity and toxicity of the potential cancer-preventive agent polyphenon E. *Environ Mol Mutagen* 41:43-54. (MENTHONE/EGCG/RI/0820/Burdock/75038)

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Teel, R. and Castonquay, A. (1992) Antimutagenic effects of polyphenolic compounds. *Cancer Lett* 66:107-113. (MENTHONE/EGCG/RI/0820/Burdock/75053)

Yen, G.C. and Chen, H. (1996) Relationship between antimutagenic activity and major components of various teas. *Mutagenesis* 11:37-41. (MENTHONE/EGCG/RI/0820/Burdock/75052)

Yoda, Hu, Zhao, W. and T. (2004) Different susceptibilities of Staphylococcus and Gram-negative rods to epigallocatechin gallate. *J Infect Chemother* 10:55-58. (MENTHONE/EGCG/RI/0820/Burdock/75045)

Mary Ellen Sanders, Ph.D.
Consultant
Dairy and Food Culture Technologies

CURRICULUM VITAE

EMPLOYMENT

Feb. 2003 to present. Partner in a collaborative business relationship, FunctionalBiotics LLC (www.functionalbiotics.com), with two experts in regulatory affairs and toxicology (Drs. James Heimbach and George Burdock) to address industry needs such as safety, efficacy, labeling and GRAS determination, related to the introduction of probiotic products to the US market.

Jan. 1990 to present. Consultant, Dairy and Food Culture Technologies, Centennial, CO. Developed consulting business, Dairy and Food Culture Technologies (www.mesanders.com), and currently operate as an independent consultant in the areas of probiotics, dairy and food starter culture technology and bacteriophage. Activities include all aspects of technical project management, clinical trial coordination, consumer and health care professional orientation and public speaking. Clients have included food, supplement, and pharmaceutical companies, industry support groups and non-profit commodity groups. Participate as an invited speaker at numerous scientific forums worldwide. Frequently contacted by media for technical perspective on probiotic-containing foods and supplements.

November 1996 to 2002. Adjunct Research Faculty, Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.

July 1994 to Sept. 1995. Director of Product Development, Leprino Foods Company, Denver, CO

Sept. 1983 - Jan. 1990. Different positions within research group at Miles Inc, Elkhart IN as follows (descriptions reflect promotions and corporate restructuring):

- May 1989 - Jan. 1990. Head of Research and Development, Marschall Dairy Business
- March 1988 - May 1989. Supervisor, Dairy Culture Research, Biotechnology Group
- Sept. 1983 - March 1988. Research Scientist, Dairy & Agricultural Fermentation Product Research, Biotechnology Group

EDUCATION

- **Ph. D.**, Food Science, Microbiology minor, August, 1983, North Carolina State University, Raleigh, Major Advisor: Dr. T. R. Klaenhammer.
- **M. S.**, Food Science, Microbiology minor, 1980, North Carolina State University, Raleigh, Major Advisor: Dr. T. R. Klaenhammer. Thesis title: The role of restriction and modification on the origin of lytic bacteriophage against the group N streptococci.
- **B.S.**, Food Science, 1977, University of California, Davis.

PROFESSIONAL ORGANIZATIONS AND ACTIVITIES

- Member of Joint Action Team of the International Dairy Federation, "Methods to determine certain functional and safety properties of probiotics in food". May 2004 - present. Program Chair for "Physiological Properties of Probiotics".
- International Scientific Association for Probiotics and Prebiotics, Inc., Co-Founder and President. ISAPP was founded in 2001 as an internation non-profit organization promoting the advancement of science in the area of probiotics and prebiotics. (www.isapp.net)
- Scientific Advisory Committee for International Dairy Federation World Dairy Congress, September 2002.
- Member of FAO/WHO Working Group charged with establishing guidelines for the evaluation of probiotics in foods (ftp://ftp.fao.org/es/esn/food/wgreport2.pdf)
- Addressed the Food Advisory Committee to the FDA, September 26-27, 2000 on the topic of probiotic-containing foods and participate in discussions on FDA approach to safety and efficacy of probiotic products.
- Scientific Advisory Program Committee for Danone Symposium "Fermented Food, Fermentation and Intestinal Flora", 2000.
- Institute of Food Technologist Scientific Lecturer, 1998-2001.
- Editor, "Dairy Beat", Quarterly newsletter of California Dairy Foods Research Center, 1996-1998.
- Editor, Dairy Technology Division of IFT Newsletter, 1996-1997.
- Editorial Review Board for Journal of Dairy Science, 1993-2001.
- Ad hoc review of manuscripts for International Dairy Journal, Anaerobe, Journal of Dairy Research, Journal of Clinical Nutrition, Journal of Food Science, Radiation Research and Applied and Environmental Microbiology.
- Reviewer for research grants for PARC program, NIH Small Business Technology Transfer Grant program and Dairy Management Inc. U.S. Dairy Center grants.
- Committee to Update Research Needs, Food Biotechnology, IFT, 1991.
- Director, American Dairy Science Association, 1993-1996.
- General Chair for 1990 ADSA Meeting.
- Chair, Dairy Foods Division of ADSA, 1989.
- Executive Committee of the Biotechnology Division of IFT, 1988, 1992.
- Chair, Rocky Mountain Section of IFT, 1993-95.
- Newsletter editor, Rocky Mountain Section of IFT, 1995-1997.
- Executive Committee of Dairy Foods Division of IFT, 1995-1998.
- Professional Member of: American Society for Microbiology
  American Dairy Science Association
  Institute of Food Technologists

TEACHING EXPERIENCE

- Guest Lecturer, Dairy Microbiology, Agricultural Biotechnology, California Polytechnic State University
- Feb. 11-13, 1997.
  Cheese II Short Course, California Polytechnic State University, Dairy Products Technology Center, "Starter Culture Microbiology ".
- Sept. 1979 - Dec. 1979; Oct. 1983 - Dec. 1983.

000149

Laboratory instructor for senior-level food microbiology course and NCSU. Solely responsible for one hour background lecture per week, writing and grading lab exams, organizing laboratory exercises, and writing new lab exercises.

AWARDS AND HONORS

- Recipient of Science and Technology Award, $1,500, 1986 from Miles Laboratories for research and development accomplishments on genetic systems for lactic acid bacteria.
- Recipient of the 1984 National Milk Producers Federation Award (R. M. Hoyt Memorial Award). $1,000. This yearly award is sponsored through the American Dairy Science Association and is awarded to one student ADSA member per year for outstanding graduate research.
- Recipient of 1983 Kenneth R. Keller Research Award from NCSU. One thesis among those submitted in 1983 to the College of Agriculture and Life Science at NCSU was selected as the award winner. Selection was on the basis of thesis quality and significance of research. $1,400.
- Recipient of Outstanding Teaching Assistant Award, 1982, Department of Food Science, NCSU. Sponsored jointly be N. C. Academy of Outstanding Teachers, NCSU Alumni Association and NCSU Graduate Student Association.
- Institute of Food Technologists Graduate Fellowship, $6,000, 1982-83 academic year, sponsored by General Foods.
- Institute of Food Technologists Graduate Fellowship, $2,500, 1981-82 academic year, sponsored by Grocery Manufacturers of America.
- Graduate Student Paper Competition, First Place, $100, 76th Annual Meeting of the American Dairy Science Association, Louisiana State University, Baton Rouge. 1981.

Publications (http://www.mesanders.com/Publications.htm)

Presentations (http://www.mesanders.com/Presentations.htm)

000150

dairy & food culture technologies

Mary Ellen Sanders, Ph.D.

Dairy and Food Culture Technologies

BIO SKETCH

Dr. Mary Ellen Sanders is an internationally recognized consultant in the area of probiotic microbiology. She helps food and supplement companies develop new probiotic products and provides technical support for enhancing existing probiotic product lines. She has extensively reviewed the technical literature on probiotics, published on the science and marketing of probiotic bacteria, coordinated clinical studies to validate probiotic efficacy, updated the FDA on the topic of probiotics, participated in a working group convened by the FAO/WHO to make recommendations to Codex for guidelines for use of probiotics in foods and assisted numerous U.S. companies in evaluating probiotics as food or dietary supplement ingredients. Dr. Sanders joined the Joint Action Team of the International Dairy Federation that has taken on the task of establishing methods to determine certain functional and safety properties of probiotics in food. Dr. Sanders formed a collaborative business relationship, FunctionalBiotics LLC, with two experts in regulatory affairs and toxicology (Drs. James Heimbach and George Burdock) to address industry needs such as safety, efficacy, labeling and GRAS determination, related to the introduction of probiotic products to the US market.

In 2002, Dr. Sanders' peers elected her the first President and Chairman of the Board of the International Scientific Association for Probiotics and Prebiotics (ISAPP). This new, international, non-profit association of scientists is dedicated to advancing the fundamental and applied science of probiotics and prebiotics (www.isapp.net)

Dr. Sanders' past research efforts have focused on strain development of lactic acid bacteria with a primary emphasis on genetic improvement of starter cultures as a scientist in the Biotechnology Group of Miles Laboratories and *in vitro* characterization and identification of probiotic lactobacilli and bifidobacteria for commercial applications, speciation of lactobacilli and bifidobacteria, evaluation of the effect of probiotics on fecal microecology and tracking specific probiotics through the gastrointestinal tract as a visiting Research Scientist at the Dairy Products Technology Center at California Polytechnic State University, San Luis Obispo.

000151

ABSTRACTS AND PUBLIC-FORUM PAPERS PRESENTED

1. *Upcoming*: Sanders, M.E. 2004. Probiotics in the control of allergy: clinical support and product development. Institute of Food Technologist Annual Meeting, July 12-16, Las Vegas, NV.
2. Sanders, M. E. and J. Heimbach. 2004. Probiotic and prebiotic standards for yogurt and yogurt drinks. IDFA Cultured Dairy Products Conference, May 18-19, Minneapolis, MN.
3. Sanders, M.E. 2004. Probiotic bacteria in yogurt: how much is enough? Nutracon, March 4, 2004, Anaheim, CA.
4. Sanders, M. E. 2003. Building a probiotic dairy product: a case study. Worldwide Food Expo 2003, October 30, 2003, Chicago, IL.
5. Sanders, M. E. 2003. Probiotic dairy products: marketing challenges. 40th Annual Marschall Cheese & Dairy Expo, September 17-18, 2003, Visalia, CA.
6. Sanders, M. E. 2003. Probiotics: What's New? 40th Annual Marschall Cheese & Dairy Expo, September 17-18, 2003, Visalia, CA.
7. Sanders, M. E. 2003. Update on probiotics – new research. Cultured Dairy Products Conference, May 20-21, Milwaukee, WI.
8. Sanders, M. E. 2003. Probiotics: definition, importance to microbiology and health, and optimal standards for human use. American Society for Microbiology, May 18 – 23, Washington DC.
9. Sanders, M. E. 2003. The role of probiotics in human health. Wisconsin Dietetics Association 2003 Annual Conference, April 10, Madison, WI.
10. Sanders, M. E. 2003. The science and marketing of probiotics: emphasis on opportunity for dairy products. 2003 All Star Convention, March 12-16, La Jolla, CA.
11. Sanders, M.E. 2003. *L. acidophilus* NCFM®: functional properties of a unique probiotic strain. Expo West, March 7, Anaheim, CA.
12. Sanders, M. E. 2003. Probiotics: scientific basis and clinical applications. American Society of Parenteral and Enteral Nutrition, National Nutrition Week, January 20-24, San Antonio, TX.
13. Sanders, M. E. 2002. Safety of oral probiotics in human health: what is known? Montreal International Symposium Probiotics and Health: Biofunctional Perspectives. October 24-25, 2002, Montreal, Quebec.
14. Sanders, M. E. 2002. Probiotics: what are they and how are they used? American College of Nutrition 43rd Annual Symposium on Advances in Clinical Nutrition, October 3-6, 2002, San Antonio, TX.
15. Sanders, M. E. and F. Guarner. 2002. Convene round table on: Yogurts and Fermented Milks. Benefits of Live Cultures. September 25, 2002. World Dairy Congress, Paris.
16. Sanders, M. E. 2002. *L. acidophilus* NCFM®: functional properties of a unique probiotic strain. Vitafoods International 2000 Exhibition and Conference, Geneva, Switzerland.
17. Sanders, M. E. 2002. Probiotics for humans: status and future of the science and marketplace. FDA National Center for Toxicological Research, Jefferson, AK.
18. Sanders, M. E. 2002. The scientific basis and clinical effects of probiotics. Michigan State University Malcolm Trout Lecture Series, E. Lansing, MI.
19. Sanders, M. E. 2002. Marketing opportunities for nutraceuticals and probiotics. SmartMarketing 2002 (sponsored by IDFA), San Diego, CA.
20. Sanders, M. E. 2001. Probiotic delivered through foods. Probiotics, Prebiotics and New Foods Conference. Rome, Italy.
21. Sanders, M. E. 2001. Regulatory aspects of health claims associated with probiotics. International Dairy Federation Nutrition Conference. Auckland, New Zealand.

000152

22. Sanders, M.E. 2001. Probiotics: new strains and strain specific research. Nutricon 2001, San Diego, CA.
23. Sanders, M. E. 2001. Probiotics in the human diet: what is their role? American Dietetic Association Annual Meeting, St. Louis, MO.
24. Sanders, M. E. 2001. Probiotics: efficacy and applications to dairy products. 2001 Annual Meeting of Institute of Food Technologist, New Orleans, LA.
25. Sanders, M. E. 2001. Regulatory considerations for labeling foods and supplements with health statements in the U.S. First International Bio-Minerals Symposium: Trace Elements in Nutrition, Health and Disease. Salt Lake City, UT.
26. Sanders, M. E. 2001. Dairy foods and those friendly bacteria important to your health. California Dairy Industry Conference, Pomona, CA.
27. Sanders, M. E. 2001. The emerging role of probiotics in health. Food 3000, Rome, Italy.
28. Sanders, M. E. 2000. Functional foods in the USA: can probiotics and prebiotics play a role? American Association of Candy Technologists, Section Meeting, Chicago, IL.
29. Sanders, M. E. 2000. Probiotics: foods in the marketplace. Food Advisory Committee to FDA, meeting on probiotics, Arlington, VA.
30. Sanders, M. E. 2000. Probiotic theory and application in foods. General Mills Technical Seminar Series, Minneapolis, MN.
31. Sanders, M. E. 2000. Probiotics/prebiotics. Cultured Dairy Products Conference, Las Vegas, NV.
32. Sanders, M. E. 2000. The perfect partnership: milk and probiotics. Fluid Milk Forum, Dairy Management Inc., Rosemont, IL.
33. Sanders, M. E. 2000. Probiotic products in the U.S.: current status and future challenges. California Institute for Agricultural Research Conference XIII, Sacramento, CA.
34. Sanders, M. E. 2000. Better health and better foods: the role of probiotics. East Coast Section of IFT, Philadelphia, PA.
35. Sanders, M. E. 2000. Probiotics. Oregon Dairy Industries 2000 Conference. Eugene, OR.
36. Sanders, M.E. 2000. USA perspectives on probiotics. PROBDEMO Meeting, Rovaniemi, Finland.
37. Sanders, M. E. 1999. Current market and potential for probiotic-containing products in the U.S. IFT Scientific Lecturer. Ohio IFT Division, Cleveland, OH.
38. Sanders, M. E. 1999. Microbiological issues in marketing probiotic products. International Workshop on Dairy Products and Colon Cancer Risk Reduction, Utrecht, The Netherlands.
39. Sanders, M. E. 1999. Bringing a probiotic-containing functional food product to market: microbiological, product, regulatory and labeling issues. Sixth Symposium on Lactic Acid Bacteria, Veldhoven, The Netherlands.
40. Sanders, M. E. 1999. Approaches and limitations of in vitro characterization of probiotic bacteria. Submitted to 1999 Annual Meeting of the American Dairy Science Association, Memphis, TN.
41. Yeung, P. S. M., R. Cano, C. L. Kitts, M. E. Sanders and P. S. Tong. 1999. Gene-based techniques for species identification and strain relatedness of commercial probiotic strains. 1999 IFT Annual Meeting, Abstract 37D-27.
42. Yeung, P.S.M., R. Cano, P. S. Tong, and M. E. Sanders. 1999. Comparison of API, 16S rDNA sequencing and fatty acid analysis as methods to speciate commercial probiotic bacteria. 1999 Annual Meeting of the American Dairy Science Association, Memphis, TN.

43. Iturriria-Laverty, K., P. S. Tong, and M. E. Sanders. 1999. Microbiological stability of probiotic and starter bacteria in commercial yogurt and cottage cheese. 1999 Annual Meeting of the American Dairy Science Association, Memphis, TN.
44. Sanders, M. E. 1999. Functional properties of yogurt. Dannon International Research Center Symposium: "Yogurt: Eighty Years of Active Research for Health", Barcelona, Spain.
45. Sanders, M. E. 1999. Probiotics and health. IRTU Conference on "Dairy Products and Health", Ulster, Northern Ireland.
46. Sanders, M. E. 1999. Considerations for use of probiotic bacteria to modulate human health. 1999 Experimental Biology annual meeting, Washington, D.C.
47. Sanders, M. E., R. Cano and T. R. Klaenhammer. 1998. Lactic acid bacteria – they can be "healthy" for the consumer and you. California Milk Producer Research Conference: Report on Producers' Investments, Visalia, CA.
48. Sanders, M. E. 1998. Current market and potential for probiotic-containing products in the U.S. Northern California Section IFT, San Jose, CA.
49. Sanders, M. E. 1998. Overview of functional foods with an emphasis on probiotic bacteria. Washington DC Section IFT, October 22, Washington DC.
50. Sanders, M. E. 1998. Current market and potential for probiotic-containing products in the U.S., Bonneville Section IFT, September 18, Park City, UT.
51. Sanders, M. E. 1998. Scientific basis of probiotic functionality. Brigham Young University, Departmental Seminar Series, Provo, UT.
52. Sanders, M. E. 1998. Current market and potential for probiotic-containing products in the U.S. Cultured Dairy Products Symposium, Milwaukee, WI.
53. Sanders, M. E. 1997. Overview of functional foods. US/Ireland Co-operation Programme in Agricultural Science and Technology, Conference on Functional Foods, Cork, Ireland.
54. Sanders, M. E. 1997. Probiotics: theory, potential and use. Nutracon 97 Conference, Las Vegas, NV.
55. Sanders, M. E. 1997. Development of consumer probiotics in the US market. Institute of Food Technologists Annual Convention, Orlando, FL.
56. Sanders, M. E. 1996. Probiotic cultures and human health. XII International Symposium on Gnotobiology (ISG), Honolulu, HI.
57. Sanders, M. E. 1996. Probiotic cultures for milk products: positive human health effects and microbiological challenges. Departmental Seminar, Dairy Products Technology Center, Cal Poly State University, San Luis Obispo, CA.
58. Sanders, M. E. 1996. Overview of probiotics: their effect on human health. 16th Symposium on Nutrition and Food Technology, Chicago, IL.
59. Sanders, M. E. 1995. Probiotics: from all things to all people, to snake oil. World Congress on Anaerobic Bacteria and Infections, San Juan, Puerto Rico.
60. Sanders, M. E. 1995. Probiotics: definition and range of applications. Institute of Food Technologists Annual Meeting, Anaheim, CA.
61. Sanders, M. E. 1994. Lactic acid bacteria and human health. Old Herborn University Seminar, Herborn, Germany.
62. Sanders, M. E. 1994. The effect of lactic acid bacteria on human health. Univ. Minnesota Departmental Seminar, St. Paul, MN.
63. Sanders, M. E. 1994. The next generation of fluid milks with added lactic cultures. Spring Dairy Industry Conference, Sacramento, CA.
64. Sanders, M. E. 1994. Culture containing milks for the California market: project update. California Cheese Symposium, San Francisco, CA.
65. Sanders, M. E. 1993. Health attributes of cultured dairy foods. Annual Meeting of American Cultured Dairy Products Institute, Las Vegas, NV.

000154

66. Sanders, M. E. 1992. Rapid Microbiological Methods. Rocky Mountain Section of IFT Suppliers Night, Denver, CO.
67. Sanders, M. E. 1991. HACCP. Rocky Mountain Section of IFT, Denver, CO.
68. Sanders, M. E. 1990. Genetic approaches for the improvement of strains for Italian cheese manufacture. Marschall Italian Cheese Seminar, Madison, WI.
69. Sanders, M. E. 1990. Genetic approaches for improving food starter cultures. ASM Conference on Biotechnology, Chicago, IL.
70. Sanders, M. E. 1989. Bacteriophage resistance and its applications to yogurt flora. International Conference on Health Attributes of Live Active Culture Yogurt, New York.
71. Sanders, M. E. 1989. Phage-resistance in cheese-making bacteria. Western Michigan University, Kalamazoo, MI.
72. Sanders, M. E. 1988. Phage resistant starters. E.M.B.O. Workshop on Genetic Manipulation of Lactic Acid Bacteria: A Breakthrough in the Food Industry, Annecy, France.
73. Sanders, M. E., P.J. Leonhard, and T.R. Klaenhammer. 1986. The use of pTR2030 for the conjugal construction of a fast acid-producing, phage-resistant strain of *Streptococcus lactis*. American Dairy Science Association Annual Meeting, Davis, CA.
74. Sanders, M. E. 1986. Genetic characterization of lactic acid bacteria. Western Food Industry Conference, Davis, CA.
75. Sanders, M. E. 1985. Advances in starter culture technology. X Food Microbiology Research Conference, Chicago, IL.
76. Sanders, M. E. 1985. Protoplast transformation of *Streptococcus cremoris*. Wind River Conference on Genetic Exchange, Estes Park, CO.
77. Sanders, M. E. and T. R. Klaenhammer. 1984. Temperature-sensitive phage resistance in a phage-insensitive strain of *Streptococcus lactis*. 84th Annual Meeting of the American Society of Microbiology, St. Louis, MO.
78. Klaenhammer, T.R. and M. E. Sanders. 1983. Resistance of group N streptococci to lytic bacteriophage: mechanisms and genetic determinants. International Symposium on Lactic Acid Bacteria in Foods: Genetics, Metabolism and Applications. Netherlands Society for Microbiology, Wageningen, The Netherlands.
79. Sanders, M. E. and T. R. Klaenhammer. 1983. Phage resistance in a phage insensitive strain of *Streptococcus lactis*: involvement of plasmid DNA in genetically determining phage adsorption. 77th Annual Meeting of the American Dairy Science Association, University of Wisconsin, Madison, WI.
80. Sanders, M. E. 1982. Involvement of plasmid DNA in the bacteriophage resistance of the lactic streptococci. Carolina - Virginia Section Meeting of Institute of Food Technologists, Winston-Salem, NC.
81. Sanders, M. E. and T. R. Klaenhammer, 1981. Plasmid composition of mutants deficient in restriction and modification and proteolytic capacities in *Streptococcus cremoris* KH. American Society for Microbiology International Conference on Streptococcal Genetics, Sarasota, FL.
82. Sanders, M. E. and T. R. Klaenhammer, 1981. Evidence for plasmid linkage of restriction and modification systems in *Streptococcus cremoris*. 76th Annual Meeting of the American Dairy Science Association, Louisiana State University, Baton Rouge, LA.
83. Sanders, M. E. and T. R. Klaenhammer, 1980. Restriction and modification in the lactic streptococci: the effect of heat on the development of modified, lytic bacteriophage. 75th Annual Meeting of the American Dairy Science Association, Virginia Polytechnic Institute, Blacksburg, VA.
84. Sanders, M. E. and T. R. Klaenhammer, 1979. Host restriction and modification in the lactic streptococci: effect of environmental stress. North Carolina Branch of the American Society for Microbiology, Raleigh, NC.

000155

PUBLICATIONS

1. Rastall, R., Gibson, G., Gill, H., Guarner, F., Klaenhammer, T., Pot, B., Reid, G., Rowland, I., Sanders, M.E. Probiotics, prebiotics and synbiotics to enhance human health: an overview of enabling science and potential applications. Appl. Environ. Microbiol. Submitted.
2. Sanders, M.E., T. Tompkins, J. Heimbach, S. Kolida. Weight of evidence needed to substantiate a health effect for probiotics and prebiotics: scientific and regulatory considerations in Canada, EU, and U.S. Eur J Nutr. In Press.
3. Yeung, P. S. M., M. E. Sanders, C. L. Kitts, R. Cano, P. S. Tong. Application of genomic and phenotypic analyses to commercial probiotic strain identity and relatedness. J. Appl. Microbiol. In Press.
4. Reid, G, M. E. Sanders, H. R. Gaskins, G. Gibson, A. Mercenier, R. Rastall, M. Roberfroid, I. Rowland, C. Cherbut, and T. R. Klaenhammer. 2003. New scientific paradigms for probiotics and prebiotics. J. Clin. Gastroenterol. 37:105-118.
5. Sanders, M. E., L. Morelli and T. Tompkins. 2003. Sporeformers as human probiotics: *Bacillus*, *Sporolactobacillus* and *Brevibacillus*. Comp. Rev. Food Sci. Food Safety. 2:101-110.
6. Sanders, M. E. 2003. Probiotics: considerations for human health. Nutr. Rev. 61:91-99.
7. Sanders, M. E. 2002. Probiotics: State of the art in the USA. Microecol. Therapy 29:1-5.
8. Sanders, M. E. 2002. Market and regulatory challenges for functional dairy foods: communicating health benefits. Bulletin of the International Dairy Federation 375:17-19.
9. Yeung, P. S. M., M. E. Sanders, C. L. Kitts, R. Cano and P. S. Tong. 2002. Species-specific identification of commercial probiotic strains. J. Dairy Sci. 85:1039-1051.
10. Sanders, M. E., L. Morelli and S. Bush. 2001. "Lactobacillus sporogenes" is not a *Lactobacillus* probiotic. ASM News 67:385-386.
11. Yeung, P. S. M. and M. E. Sanders. 2001. Probiotic Products: Considerations in Development and Labeling. Food Technology International 2001:37-41.
12. Kaplan, C. W., J. C. Astaire, M. E. Sanders, B. S. Reddy and C. L. Kitts. 2001. Terminal restriction fragment pattern analysis of changing bacterial assemblages in rat feces during ingestion of *Lactobacillus acidophilus* NCFM®. Appl. Environ. Microbiol. 67:1935-1939.
13. Sanders, M. E. and T. R. Klaenhammer. 2001. The scientific basis of *Lactobacillus acidophilus* NCFM® functionality as a probiotic. J. Dairy Sci. 84:319-331.
14. Sanders, M. E. 2000. Dairy products. In J. Lederberg (Ed.) Encyclopedia of Microbiology, Second Edition, Academic Press, Orlando, FL. Vol. 2, p. 1-8.
15. Sanders, M.E. 2000. Considerations for use of probiotic bacteria to modulate human health. J. Nutr. 130:384S-390S.
16. Sanders, M. E. 1999. Proboitics. Food Technology 53:67-77.
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000156

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SUBMISSION END

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FOOD ADDITIVE SAFETY

January 27, 2005

Dr Robert Merker
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Pain Branch Parkway
College Park, MD 20740-3835

RE: GRN Notice #000159 – Clarification amendment

Dear Dr. Merker,

This letter is to amend the current GRAS dossier entitled "DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SPECIFIC STRAINS OF *Carnobacterium maltaromaticum* AS A FOOD INGREDIENT," which requests the addition of *C. maltaromaticum* strains CB1, CB2, CB3, LV17, UAL26, ATCC 35586 and ATCC 43225 to ready-to-eat meat products and fresh comminuted, processed meat products (GRN No. 000159). Several points have been raised concerning this GRAS dossier, and based on our conversation of January 25, 2005, we submit the following amendments to the pending GRAS Notification.

The FDA has submitted the GRAS Notification and dossier to the USDA, as the GRAS Notification requests the addition of *C. maltaromaticum* to meat.[1] The USDA indicated that it could not support the addition of *C. maltaromaticum* to "fresh comminuted, processed meat products." The USDA's Food Safety and Inspection Service has issued statements regarding how

[1] US Department of Agriculture. Food Safety and Inspection Service. Labeling and Consumer Protection. Memorandum of Understanding (http://www.fsis.usda.gov/OPPDE/larc/Ingredients/MOU.htm); site visited 13JAN05.

the term "fresh" may or may not be used to define meat. In the USDA's statement, the word "fresh" may not be used to describe any meat treated with an antimicrobial substance.[2] Therefore, the Notifier will forgo any addition of *C. maltaromaticum* to meat considered "fresh," in this context, and any reference to "fresh comminuted meat products" in the Notification should be disregarded.

Second, a question was raised concerning the composition of the delivery system that the lyophilized *C. maltaromaticum* is contained within, and is consequently sprayed onto the ready-to-eat meat products. In addition to lyophilized *C. maltaromaticum*, the delivery system is composed of dextrose, maltodextrin, anhydrous disodium phosphate, hydrolyzed soy protein, and a yeast extract. The Client confirmed that the delivery system is composed of food ingredients or substances of food grade quality. The Client has indicated that the information pertaining to the composition of the delivery system be kept as "Business Confidential."

Third, a question was raised concerning the exposure assessment of *C. maltaromaticum* when added to ready-to-eat meats. As *C. maltaromaticum* is a viable organism, the addition of 1 x 10^4 cfu *C. maltaromaticum*/g of meat would result in progressively higher levels of the bacteria upon storage of the meat. Based on studies done by Paludan-Muller *et al.* (1998),[3] the growth of *C. maltaromaticum* on meat was found to plateau at a maximal concentration of 1 x 10^9 cfu/g of meat. Multiplying this maximal concentration of *C. maltaromaticum per* gram of meat with the average amount of meat eaten by the US consumer *per* day, 141 g/day *per* person[4] (based on figures from the USDA Economic Research Service's determination of the *per capita* consumption of 114 pounds of total meat for the year 2002[5]), will yield a maximal consumption of *C. maltaromaticum* of 1.41 x 10^{11} cfu/day. This is not believed to significantly add to the consumption of bacteria by the US population.

Fourth, clarification was requested concerning the ability of the non-genetically modified *C. maltaromaticum* used in this GRAS dossier (strains CB1, CB2, CB3, LV17, UAL26, ATCC

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[2] US Department of Agriculture. Food Safety and Inspection Service. Labeling and Consumer Protection (http://www.fsis.usda.gov/OPPDE/larc/Claims/Fresh.htm); site visited 12JAN05.
[3] Please refer to the attached bibliography. All articles cited in this letter were previously cited in GRN 000159, and are available on request.
[4] Multiply 114 pounds of total meat by the conversion factor of 0.4535 to yield kilograms, multiplied by 1000 to yield grams, divided by 365 days *per* year for the resulting grams of meat consumed *per* person *per* day.
[5] Economic Research Service/United States Department of Agriculture (Red meat, poultry, and fish (boneless, trimmed equivalent): *per capita* consumption (http://www.ers.usda.gov.data.foodconsumption/spreadsheets.asp)); site visited 25JAN05.

35586 and ATCC 43225) to transfer antibiotic resistance genes to other bacteria. An extensive review of the literature failed to find information concerning an enhanced ability of *C. maltaromaticum* to promote antibiotic resistance gene transfer *via* (1) transformation (*i.e.*, the uptake of free DNA), or (2) conjugation, with the subsequent transfer of plasmids to other bacteria found in meat. *C. maltaromaticum* has been found to be naturally present on retail, chilled and ready-to-eat meat products, indicating that humans have been consuming *C. maltaromaticum* bacteria as a natural constituent on meat for decades, with no indication of adverse reactions (Amezquita and Brashears, 2002; Kelly *et al.*, 1996; Milliere and Lefebvre, 1994; Schobitz *et al.*, 1999).

In addition, while there is literature indicating that strains of *C. maltaromaticum* contain plasmids containing bacteriocins[6] (Ahn and Stiles, 1990; Quadri *et al.*, 1994; Schobitz *et al.*, 1999), there has been no mention in the literature, or biological plausibility for, non-genetically modified *C. maltaromaticum* bacteria having the ability to promote genetic transfer of antibiotic resistance genes into other bacteria. The articles cited above did not report any plasmid-derived antibiotic resistance genes. As mentioned previously, the *C. maltaromaticum* strains identified in this GRAS dossier are non-genetically modified bacteria that were isolated from normally consumed fish or meat sources. To summarize, it is highly unlikely that the *C. maltaromaticum* strains identified in this GRAS dossier would have the ability to transfer antibiotic resistance to other bacteria.

In summary, any reference to the addition of *C. maltaromaticum* to a meat category described using the term "fresh comminuted meat products" should be disregarded in the GRAS dossier, as the USDA has indicated that the term "fresh" cannot be used to define meat that contains antimicrobial agents; the Notifier foregoes the use of *C. maltaromaticum* in these products. In addition, the delivery system for the lyophilized *C. maltaromaticum* that is to be sprayed on the ready-to-eat meat products are food ingredients or substances of food grade quality, and consist of dextrose, maltodextrin, anhydrous disodium phosphate, hydrolyzed soy protein, and a yeast extract.

A revised exposure assessment of the quantity of *C. maltaromaticum* that will be consumed has been based on the maximal number of *C. maltaromaticum* that has been reported

[6] Ribosomally synthesized, low molecular weight antibacterial proteinaceous materials that inhibit the growth of adjacent *C. maltaromaticum*, other lactic acid-producing bacteria, and *Listeria monogenes*.

to grow on meat (to a maximal concentration of 1 x10^9 cfu/g). Multiplying this number by the average amount of meat eaten *per* person *per* day (141 g/person/day), yields a maximal consumption of *C. maltaromaticum* of 1.41 x 10^{11} cfu/person/day.

Finally, a review of the literature failed to find any indication that *C. maltaromaticum* bacteria naturally possess, or would be expected to possess, an enhanced ability to promote the transfer of antibiotic resistance genes, *via* transformation or conjugation. Any published studies discussing plasmids contained within *C. maltaromaticum* bacteria fail to report antibiotic resistance genes contained within a plasmid. It has been estimated that *C. maltaromaticum* has been consumed on meat for decades, with no reported adverse effect from this ingestion.

If you have any further questions regarding these or any other topics concerning the GRAS dossier of the addition of *Carnobacterium maltaromaticum* to ready-to-eat meat products, please contact me.

Sincerely,

George A. Burdock, Ph.D.
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RE: GRN Notice #000159 – Clarification Amendment

Dear Sirs,

This letter is to amend the current GRAS dossier[1] entitled "DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SPECIFIC STRAINS OF *Carnobacterium maltaromaticum* AS A FOOD INGREDIENT," which requests the addition of *C. maltaromaticum* strains CB1, CB2, CB3, LV17, UAL26, ATCC 35586 and ATCC 43225 to ready-to-eat (RTE) meat products and fresh comminuted, processed meat products. Several

[1] GRAS Notification Letter dated October 5, 2004.

points have been raised concerning this GRAS dossier, and based on the conversation of February 17, 2005,[2] we submit the following amendments to the pending GRAS Notification (GRN Notice #000159).

This clarification addresses three issues:

1. Addition to "fresh" comminuted, processed meat products.
2. The effect of *C. maltaromaticum* on normal signs and rate of meat spoilage, with the concurrent inhibition of *Listeria monocytogenes*.
3. Labeling of the ingredients contained within the matrix in which *C. maltaromaticum* is delivered to the RTE meat products.

1. Addition to fresh comminuted, processed meat products.

The FDA has submitted the GRAS Notification and dossier to the USDA, as the GRAS Notification requests the addition of *C. maltaromaticum* to meat.[3] The USDA indicated that it could not support the addition of *C. maltaromaticum* to "fresh comminuted, processed meat products." The USDA's Food Safety and Inspection Service has issued statements regarding how the term "fresh" may or may not be used to define meat. In the USDA's statement, the word "fresh" may not be used to describe any meat treated with an antimicrobial substance.[4] In response, the Notifier will forgo any addition of *C. maltaromaticum* to meat considered "fresh," in this context, and any reference to "fresh comminuted, processed meat products" in the Notification should be disregarded.

2. The effect of *C. maltaromaticum* on normal signs and rate of meat spoilage, with the concurrent inhibition of *Listeria monocytogenes*.

Questions were raised concerning the sensory perceptions of foods that contain *C. maltaromaticum*, the maximal growth levels of *C. maltaromaticum,* and the efficacy of *C. maltaromaticum* to inhibit the growth of *L. monocytogenes*. The USDA has requested data to substantiate the statement that the addition of *C. maltaromaticum* to RTE meat products does not alter normal signs of spoilage. The documentation found in the attached Appendix A (*i.e.*, summaries of four efficacy studies, A. "Growth Characteristics of *C. piscicola* on Vacuum

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[2] Teleconference between Dr. Robert Merker of the FDA, Mr. Jeffrey Canavan of FSIS, Dr. George Burdock and Dr. Ray Matulka of Burdock Group.

[3] US Department of Agriculture. Food Safety and Inspection Service. Labeling and Consumer Protection. Memorandum of Understanding (http://www.fsis.usda.gov/OPPDE/larc/Ingredients/MOU.htm); site visited 13JAN05.

[4] US Department of Agriculture. Food Safety and Inspection Service. Labeling and Consumer Protection (http://www.fsis.usda.gov/OPPDE/larc/Claims/Fresh.htm); site visited 12JAN05.

Packaged Wieners," B. "Sausage Inoculation Trials," C. "Challenge Study: *Listeria monocytogenes* on a Ready-to-eat Meat Product," and D. "Inhibition of *Listeria monocytogenes* by *Carnobacterium piscicola*") addresses these issues. This documentation includes sensory data, indicating that the presence of *C. maltaromaticum* does not increase the rate of spoilage of ready-to-eat meat products, growth curve data indicating that the addition of *C. maltaromaticum* does not increase the overall number of lactic acid bacteria, and competition data indicating that *C. maltaromaticum* significantly inhibits the growth of *L. monocytogenes*. This data has been prepared for Griffith Laboratories (the Notifier), who wishes this material to be maintained as "Business Confidential." Below is a summary of the data.

A. Growth Characteristics of *C. piscicola* on Vacuum Packaged Wieners:

In regard to the sensory data available, the report entitled "Growth Characteristics of *C. piscicola* on Vacuum Packaged Wieners" utilized a trained sensory panel to determine the acceptability of wieners inoculated (*via* dipping) with an inoculum level of 2.5×10^6 cfu/ml of *C. maltaromaticum* (previously named *C. piscicola*) UAL26 or LV17 (also known as UAL8), when stored at 4°C for up to 12 weeks (Appendix A).

Prior to sensory evaluation by a trained panel, wieners were cooked by placing in a saucepan containing two liters of boiling tap water. Wieners were cut into 1.27 cm pieces and placed in coded foil-covered jars, and just prior to evaluation, heated for 15 min in a 200°F (94°C) oven, to obtain an internal temperature of approximately 66°C).

The sensory evaluation was conducted by a group of nine panelists trained over a three-month period. The samples were evaluated for overall aroma intensity, meat flavor intensity, seasoned flavor, smoke intensity, sourness/acidity, off flavor, and overall acceptability using a 15 cm unstructured line scale, with 0 = none (or very bland) and 15 = extreme (or very strong). Between samples, palates were cleansed with crackers and a 1:1 dilution of 7-Up®.

The results indicate that the growth of LAB on the wieners inoculated with *C. maltaromaticum* strains UAL8 or LV17 (*i.e.*, UAL26) reached maximum anaerobic lactic acid bacteria counts of 6.44 and 5.08 LOG cfu/cm^2 after 7-8 weeks of cold storage, while there was only a small decrease in the overall pH of the wiener samples.

The sensory evaluation found that after 12 weeks of cold storage, wieners inoculated with *C. maltaromaticum* UAL8 and UAL26 were similar to control and reference samples for all characteristics (please see Appendix A). Samples inoculated with strain UAL8 had slightly higher off-flavor scores at Week 12 than samples inoculated with UAL26, but were not significantly different from control samples.

B. Sausage Inoculation Trials:

The report entitled "Sausage Inoculation Trials" utilized both a consumer panel and a trained sensory panel to determine the acceptability of sausages inoculated with varying concentrations ($10^3 - 10^5$ cfu/g of meat) of *C. maltaromaticum* CB1, when stored at 4°C for up to 20 days.

On Day 5 of the consumer panel study, the acceptability of raw appearance of the sausages with the 10^3 cfu *C. maltaromaticum*/g inoculum was significantly better than the control samples. Raw odor freshness of the 10^4 cfu/g inoculum was significantly less than the control. By Day 10, no significant differences were observed in any of the attributes noted. On Day 15, the 10^3 cfu/g inoculum had significantly better raw odor freshness than the control samples, while by Day 20 none of the control or test samples inoculated with *C. maltaromaticum* were considered suitable for evaluation based on a pre-panel assessment.

The consumer panel results indicate that inoculation of sausage with *C. maltaromaticum* CB1 at 10^3 cfu/g resulted in a product that performed better or equivalent to the non-inoculated control samples.

The results from the study that utilized a trained sensory panel indicated that two attributes showed significant differences across all samples: Dairy and Bloom.[5] On Days 0 and 5, the test samples (*C. maltaromaticum*-inoculated sausages) were higher in Bloom. By Day 10, the Control and both (2) test samples increased in Dairy intensity and decreased in Bloom. Overall, samples (both controls and *C. maltaromaticum*-treated samples) decreased in Bloom (in intensity) over time. For the control samples, a slight decrease in Sweet and a slight increase in Sour occurred, while the Dairy intensity more than doubled and the decrease in Bloom almost doubled, at Day 15. For the samples containing *C. maltaromaticum* (*i.e.*, test material), a slight

[5] Dairy is a term related to the specific taste characteristic of the product. Bloom is a term for the ability of meat to stay red and fresh looking.

decrease in Sweet was noted, as Sour began to increase by Day 15, while the Dairy characteristic increased and Bloom decreased. Overall, the control and test samples (containing inoculums of *C. maltaromaticum*) did not vary significantly, based on the responses made by the trained sensory panel.

In addition, plate counts (on both MRS and APT agar)[6] revealed that the total number of *Lactobacillus* bacteria or heterofermentative lactic acid bacteria growing on APT and MRS agar was similar on the un-inoculated control and the test products (inoculated with *C. maltaromaticum* strain CB1 at 10^3 or 10^4 cfu/g of meat), as indicated in Figures 2, 3, and 4 of the Sausage Inoculation Trial data set (Appendix A). Plating of the sausages inoculated with *C. maltaromaticum* CB1 on agar-plated lawns of *L. monocytogenes* resulted in significant formation of zones of inhibition, indicating the inhibition of growth of *L. monocytogenes* when in the presence of *C. maltaromaticum* CB1, as indicated in Figure 6 ("Indirect method of bacteriocin detection in sausages") of the Sausage Inoculation Trial data set (Appendix A).

In summary, these data indicate that inoculation of sausages with *C. maltaromaticum* CB1, at 10^3 or 10^4 cfu/g, did not significantly alter the sensory perception of these sausages, when compared to controls, when evaluated by both a consumer panel and a trained sensory panel, for up to 20 days. In addition, the total number of lactic acid and heterofermentitive bacteria growing on the samples inoculated with *C. maltaromaticum* did not increase over control values, while plating of the sausages with *C. maltaromaticum* significantly inhibited *L. monocytogenes* growth.

C. Challenge Study: *Listeria monocytogenes* on a Ready-to-eat Meat Product:

The Challenge Study results indicate that lactic acid bacteria count on inoculated wieners (when stored at 5°C for up to 84 days, then enumerated on APT agar plates incubated anaerobically at 25°C), increased to a maximal level of 6 – 7 log cfu/cm^2 at Day 42 (Figure 1 of the Challenge study), and maintained that level until the end of the study (Appendix A). This bacterial growth was not significantly different from control samples. *C. maltaromaticum* CB1 also significantly inhibited a cocktail of four serovars (*i.e.*, strains) of *L. monocytogenes* that have been associated with foodborne illness [HPB 642 (1/2a), FS 15 (1/2b), NTCC 5105.3 (3a)

[6] MRS agar is utilized for the enrichment, cultivation and isolation of all species of *Lactobacillus*. APT agar is utilized for the enumeration and cultivation of heterofermentative lactic acid bacteria.

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Table 1. Amount of liquid matrix applied to RTE foods during the addition of *C. maltaromaticum*

Matrix composition	CB1 Medium	Amount in	Micocin II*	Reconstituted	Micocin II as applied to a

Summary

In summary, any reference to the addition of *C. maltaromaticum* to a meat category described using the term "fresh comminuted, processed meat products" should be disregarded in the GRAS dossier, as the USDA has indicated that the term "fresh" cannot be used to define meat that contains antimicrobial agents; the Notifier foregoes the use of *C. maltaromaticum* in these products. The Notifier also submits efficacy data indicating that the addition of *C. maltaromaticum* does not alter the sensory qualities of the RTE meat products, does not increase the overall level of lactic acid bacteria growing on RTE meat products, and significantly inhibits the growth of *L. monocytogenes*. In addition, the delivery system for the lyophilized *C. maltaromaticum* that is to be applied to the RTE meat products are food grade ingredients, and consist of

, which are of food grade quality and are applied to the RTE meat products at an insignificant quantity. Therefore, these substances are incidental ingredients for the processing and application of *Carnobacterium maltaromaticum,* and are not required to be named on the RTE meat product label.

If you have any further questions regarding these or any other topics concerning the GRAS dossier of the addition of *Carnobacterium maltaromaticum* to ready-to-eat meat products, please contact me.

Sincerely,

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

Appendix A

The following pages contain information regarding the studies entitled: "Growth Characteristics of *C. piscicola* on Vacuum Packaged Wieners," "Sausage Inoculation Trials," "Challenge Study: *Listeria monocytogenes* on a Ready-to-eat Meat Product," and "Inhibition of *L. monocytogenes* by *C. piscicola*."

Growth Characteristics of *C. piscicola* on Vacuum Packaged Wieners

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4.3. Reconstitution of Starter Culture

A 10g sachet of lyophilized *C. piscicola* culture is dissolved in 6.6 liters of sterilized distilled water so that the bacterial cell concentration is between 4.54×10^5 and 2.27×10^6 colony forming units (CFU) per ml.

4.4. Proposed Use

It is proposed that living *Carnobacterium piscicola* will be added to vacuum or modified atmosphere packaged ready-to-eat (RTE) meat products as another means of mitigating the effects of contamination caused by human pathogenic bacteria, such as *Listeria monocytogenes*.

During packaging of RTE meat products, such as wieners, it is proposed that a dose (~ 1 ml) of reconstituted *C. piscicola* will be applied to each 454 g (1 lb) package via a series 1 micro meter "airless" liquid applicator (Spray Dynamics). The spray head controller will be sequenced with a Curwood Pathfinder or Multivac packaging machine so than only packages receiving the spray application will be indexed by the packager.

Based on the cell concentration of the reconstituted culture (4.54×10^5 – 2.27×10^6 CFU/ml), it is estimated that each package of product will be inoculated with between 1×10^3 and 5×10^3 viable *C. piscicola* cells per gram of product.

4.5. Growth Characteristics of *C. piscicola* on Vacuum Packaged Wieners

The following laboratory-scale study was designed to investigate the growth characteristics of *C. piscicola* on vacuum packaged wieners inoculated with *C. piscicola* under conditions comparable to those proposed for commercial production. In addition, the effects on sensory properties, such as aroma and flavour profile, were investigated.

4.5.1. Materials and Methods

4.5.1.1. Bacterial Cultures and Inoculate

Two strains of *C. piscicola* were chosen for study, strain UAL 8 (synonym LV17) was originally isolated from vacuum packaged, refrigerated fresh pork and described by Shaw and Harding (1984), and strain UAL 26, which was isolated from vacuum packaged beef (M. Stiles, University of Alberta). All cultures were maintained in 50% glycerol with medium at -80°C.

For the preparation of inocula, bacteria from frozen culture were subcultured twice in APT broth (Difco) over 24 hours at room temperature. Cultures were centrifuged (10,000 rpm x 10 min, 7°C) and pelleted cells were resuspended with 0.85% sterile saline and washed three times by centrifugation. The final cell pellet was resuspended with 10 ml sterile 0.85% saline to a final concentration of 1×10^9 CFU per ml.

4.5.1.2. *Inoculation, Packaging and Sampling of Wieners*

Prior to dipping, each 10 ml aliquot of washed bacterial cells was diluted into 4L of sterile 0.85% saline to provide an inoculum level of 2.5 x 10^6 CFU per ml. Individual wieners were dipped into the inoculum suspension for 1 minute, drain dried, and vacuum packaged in groups of 5 wieners per bag (high barrier, low O_2 VP bags). For control samples, wieners were dipped in 0.85% sterile saline without bacterial inoculum.

Treated and control samples were placed into refrigerated 4°C storage (monitored with a Delphi temperature recorder) for up to 12 weeks. Sampling of wieners for microbiological analyses and sensory evaluation was performed on day 0 and after 2, 4, 6, 7, 8, 10, and 12 weeks of storage.

4.5.1.3. *Microbiological Analysis*

Samples were prepared for microbiological analysis by excising 1.8 cm length pieces of wiener (equivalent to 10 cm^2) into a sterile Stomacher bag and homogenizing for 2 min in 90 ml of sterile 0.1% peptone water using a Colworth Stomacher (Baxter Diagnostics Corp., Canlab Division).

Bacterial enumerations were conducted by standard dilution and plating techniques, and included:

1. Total aerobic plate count on Plate Count agar (PCA; Difco) incubated aerobically at 25°C, 48 hr;

2. Lactic acid bacteria on APT agar (Difco) incubated anaerobically (BBL Anaerobic System with 10% CO2) at 25°C, 48hr; and

3. Enterobacteriaceae on Violet Red Bile Agar with 1% added glucose (VRBG, Difco) incubated at 35°C, 4-18 hrs.

Bacterial concentrations were reported as logarithms (log_{10}) colony forming units (CFU) per cm^2 of tissue (LOG CFU/cm^2).

4.5.1.4. *Sensory Evaluation*

Prior to sensory evaluation by a trained panel, wieners (4-5) were cooked by placing in a saucepan containing 2 l boiling tap water, which was immediately removed from the heat element and allowed to stand for 5 min (internal temperature approx. 83°C). Wieners were cut into 1.27 cm (0.5 inch) pieces and placed in coded foil-covered jars, and just prior to evaluation, heated for 15 min in a 200°F (94°C) oven (internal temperature approx. 66°C).

Sensory evaluation was conducted by a group of 9 panelists trained over a three-month period, and was performed in sensory booths under dim red lighting using a computerized Compusense program. Samples were evaluated for overall aroma intensity, meat flavour intensity, seasoned flavour, smoke intensity, sourness/acidity, off flavour, and

overall acceptability using a 15 cm unstructured line scale with 0 = none (or very bland) and 15 = extreme (or very strong). Between samples, palates were cleansed with crackers and a 1:1 dilution of 7-Up.

All collected data were analyzed using the GLM of SAS version 6.12 (SAS Institute, 1996) and the Student Newman Keul's multiple range test was used to test for significant differences among treatments and storage times.

4.5.2. Results

4.5.2.1. Growth of LAB and pH of Vacuum Packaged Wieners

Samples of packaged wieners that had been inoculated with *C. piscicola* strains UAL 8 or UAL 26 reached maximum anaerobic lactic acid bacteria (LAB) counts of 6.44 and 5.08 LOG CFU/cm² after 7 or 8 weeks of cold storage, respectively (Fig. 4-2).

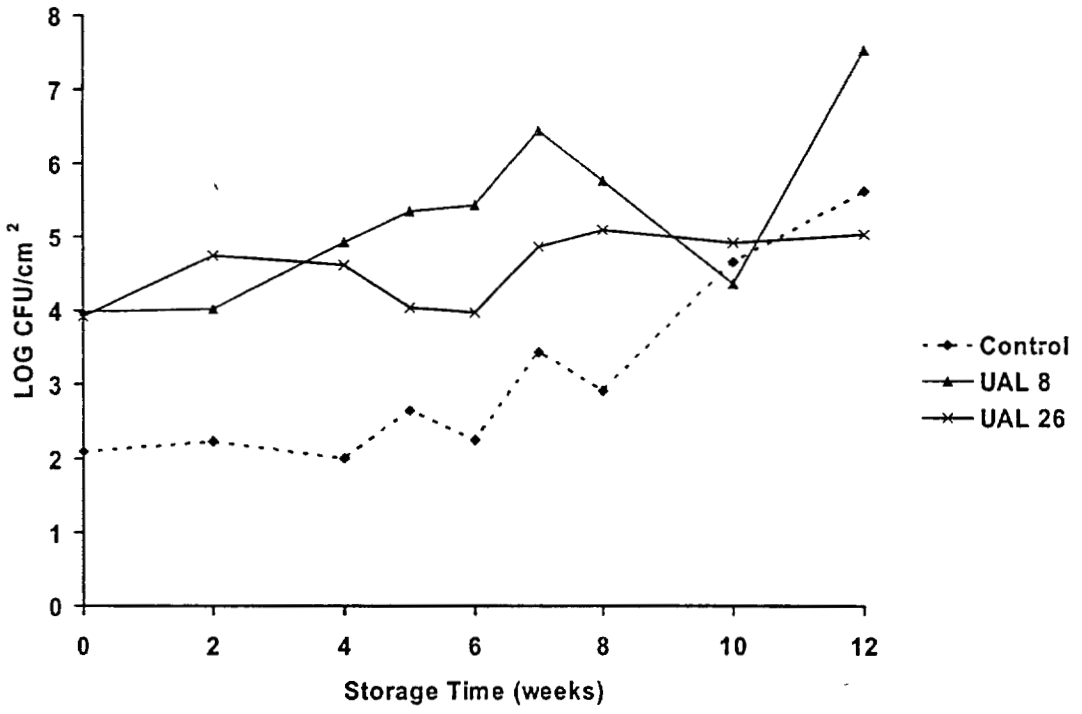

Fig. 4-2. Anaerobic lactic acid bacteria (LAB) counts on dipped wieners. Wieners were inoculated with *C. piscicola* strains UAL 8 or UAL 26 by dipping (2.5 x 10^6 CFU/ml), vacuum packaged in groups of five, and placed into cold storage (4°C) for periods of up to 12 weeks. Samples were removed on day 0 and at various weeks during storage and the levels of anaerobic bacteria were determined by standard dilution and plating techniques. Control wieners were dipped in sterile 0.85% saline only.

The slow rate of growth of *C. piscicola* on vacuum packaged wieners stored under these conditions was accompanied by a relatively small decrease in surface pH during storage (Fig. 4-3). These data confirm that Carnobacteria are not active fermenters, relative to

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other LAB. In a separate experiment investigating the growth characteristics of a strain of *Leuconostoc gelidium* on vacuum packaged wieners, it was found that anaerobic bacterial counts increased up to 7.5 LOG CFU/cm² over 8 weeks, with a concomitant pH decrease from 6.21 to 5.44 over the same time period.

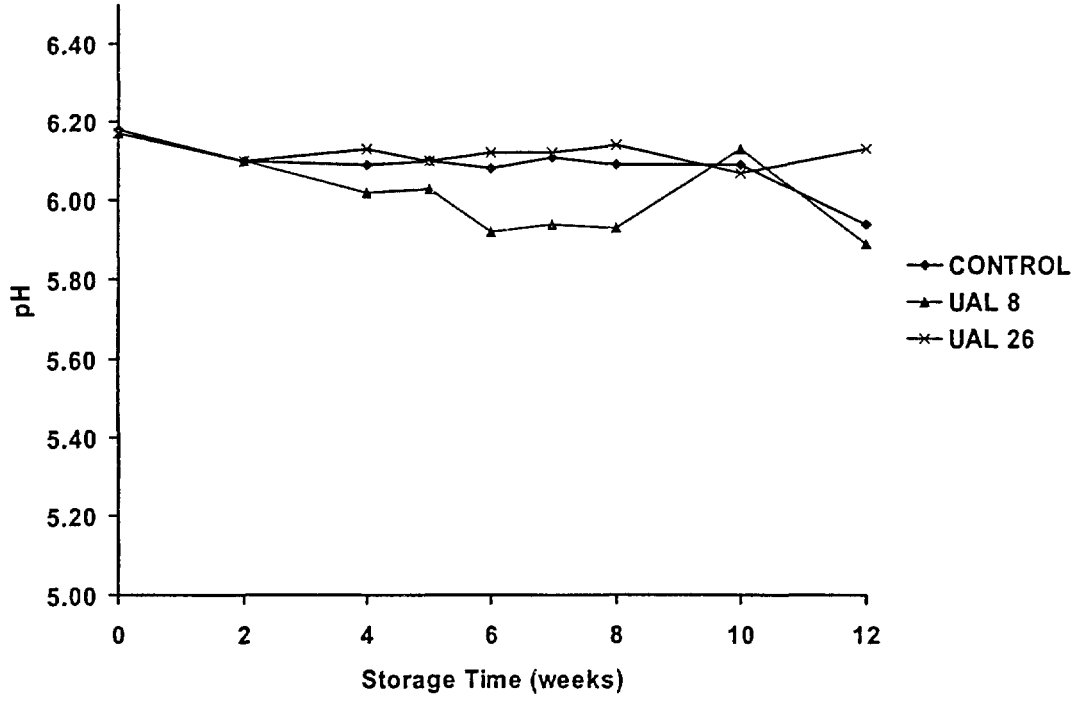

Fig. 4-3. Surface pH of wieners at various times during storage following inoculation with *C. piscicola* strains UAL 8 and UAL 26. At each time-point, two pH readings for each treatment were measured using a hand-held pH meter. Control wieners were dipped in sterile 0.85% saline only.

4.5.2.2. ***Sensory Evaluation***

After 12 weeks of cold storage, wieners inoculated with *C. piscicola* UAL 8 and UAL 26 were similar to control and reference samples for all characteristics (Fig. 4-4). Samples inoculated with strain UAL 8 had slightly higher off-flavour scores (4.51) at week 12 than samples inoculated with UAL 26 (2.6). In a separate experiment with *L. gelidium*, wieners were evaluated to be unacceptable or spoiled by week 7 following inoculation and the samples were found to be significantly different than control samples in aroma, meaty, seasoning, smoky, sour and off-flavours.

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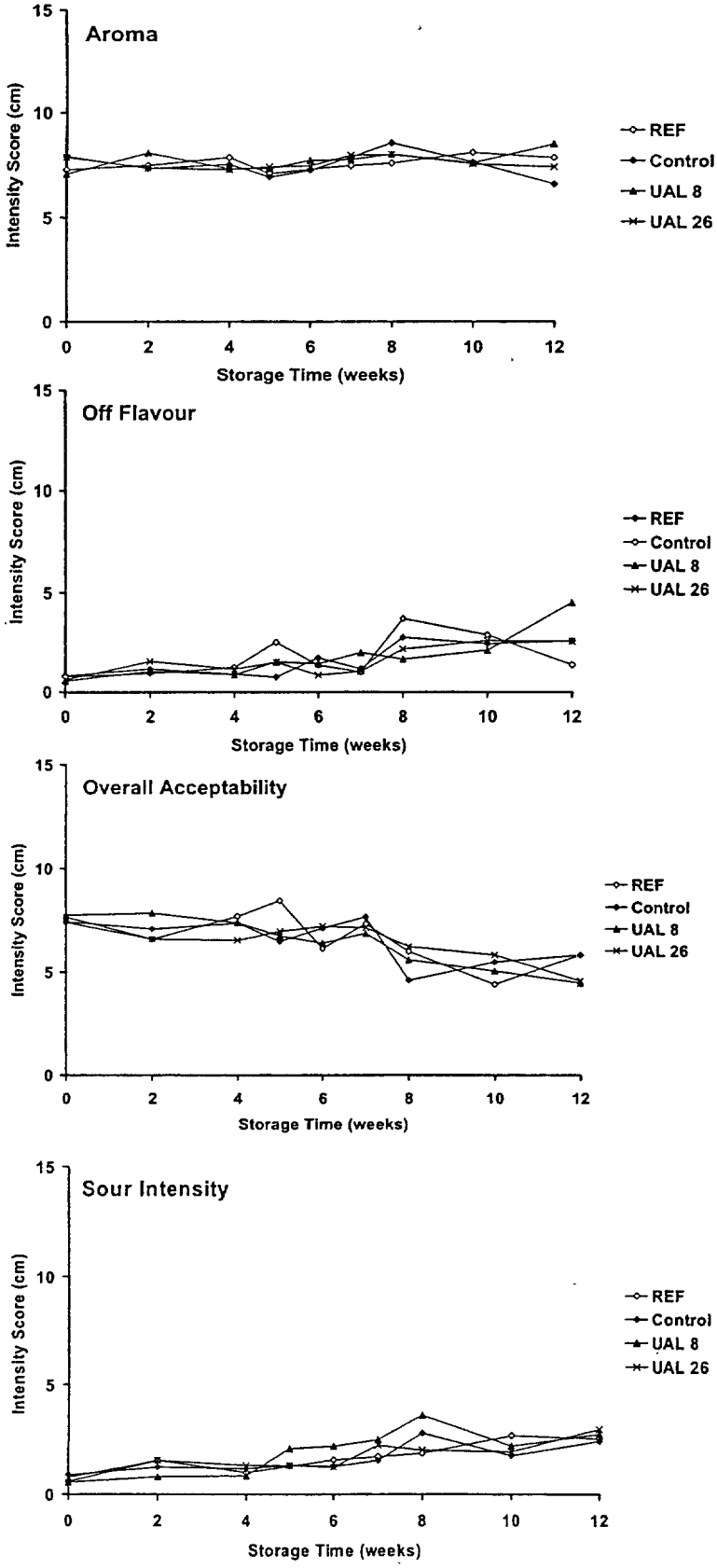

Fig. 4-4. Sensory analysis of wieners at various times during storage following inoculation with *C. piscicola* strains UAL 8 and UAL 26. At each time-point, samples were removed and subjected to evaluation for overall aroma intensity, off-flavour, sourness/acidity, and overall acceptability by nine panellists. Results were scored using a 15 cm unstructured line scale where 0 is bland or none and 15 is extreme or very strong. Control samples were dipped in sterile 0.85% saline only and hidden reference samples (REF) were wieners that had not been subjected to any dipping treatment.

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4.5.3. Conclusions

In comparison with other lactic acid bacteria, such as *Leuconostoc gelidum*, *C. piscicola* is a slow-growing species when inoculated onto vacuum packaged wieners. The levels of *C. piscicola* reached a maximum of 7.5 LOG CFU/cm^2 after 12 weeks of cold storage. Based on sensory evaluations using a trained nine-member panel over the 12 week storage period, there were no significant adverse effects on aroma, off-flavours, sour intensity, or overall acceptability resulting from inoculation with *C. piscicola*.

4.6. References

4. Shaw, B.C. & Harding, C.D. (1984). A numerical taxonomic study of lactic acid bacteria from vacuum-packed beef, pork, lamb and bacon. *Journal of Applied Bacteriology* **56**, 25-40. [01-305-A011.pdf].

Report

Sausage Inoculation Trials

Objectives

To evaluate the use of *Carnobacterium piscicola* CB1 as a preservative culture in breakfast sausage produced at the Food Processing Development Centre, Agriculture, Food and Rural Development, Leduc, Alberta, Canada. This facility is under CFIA (Canadian Food Inspection Agency) and is a certified processing facility.

The objectives include:
[1] to show that the shelf-life of fresh breakfast sausage is not compromised when manufactured with an inoculum of *Carnobacterium piscicola* CB1 culture.
[2] to show that flavor and texture of the breakfast sausage are not compromised when manufactured with an inoculum of *C. piscicola* CB1.
[3] to show that the dominant spoilage population of inoculated breakfast sausage is *C. piscicola* CB1.
[4] to show that a bacteriocin is produced during storage-life of the breakfast sausage.

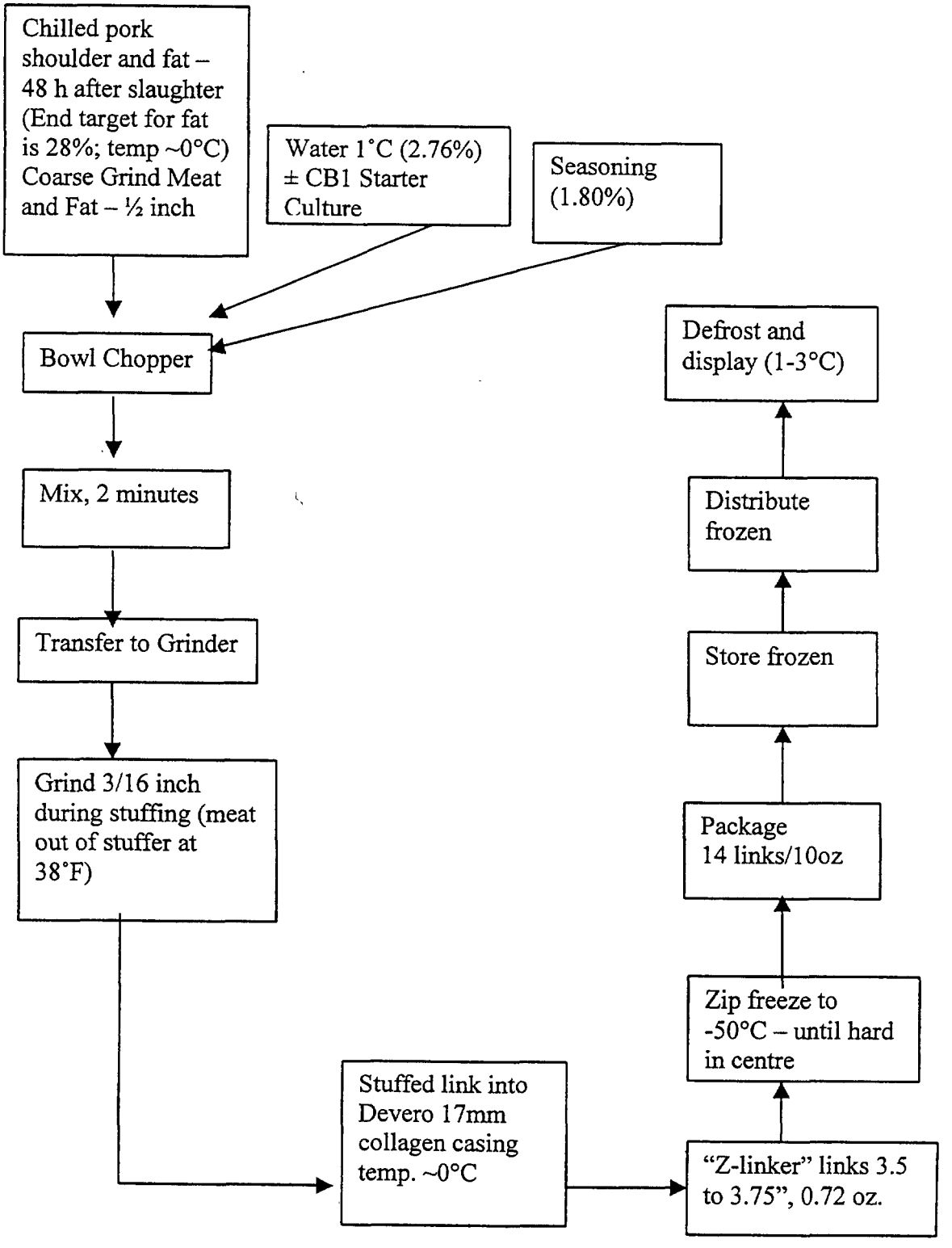
Leduc: To compare, utilizing a panel of consumers, the odor and flavor attributes and appearance acceptability of pork sausages inoculated with two levels of bacterial starter culture to an non inoculated control after storage at 4 °C for five storage times.

A. Methods

B.1. Production Methods

The product was manufactured at the Food Processing Development Centre on three trial dates: Trial 1 (January 16, 2003), Trial 2 (March 27, 2003), and Trial 3 (April 10, 2003). The manufacturing flow diagram is shown in Figure 1. Production records for each trial are detailed in Appendix Tables 1 to 3. Chilled pork shoulder (19.56 kg) and pork fat (4.3 kg) were weighed to give 28 ± 5% target fat percentage and course ground (13 mm) in a cutter and grinder (Model AW114, K & G Wetter, Breidenstein, Germany). The coarse ground product was divided into four batches of 23.86 kg to which 690 mL of water (2.76%) and 450 g of seasoning (1.8%) were added. In the test products (batches 3 and 4)

Figure 1: Process Flow Diagram (modified from Testing Protocol: Evaluation of *C. piscicola* CB1 starter culture in commercially produced breakfast sausage, Griffith Laboratories)



C. piscicola CB1 was added to give 10^3 cfu/g for batch 3 and 10^4 cfu/g for batch 4 (10^5 cfu/g for batch 4, trial 1) The ground meat and ingredients were mixed for 2 min in a bowl chopper (Model K64, Seydelmann, Stuttgart, Germany) and fine ground (4 mm) in a cutter and grinder (Model AW114, K & G Wetter, Breidenstein, Germany). The product was transferred to a sausage stuffer (Handtmann VF80, Germany) and an attachment was used to stuff the meat into a Devero 17 mm collagen casing (UniPac, Edmonton). The stuffed sausage was cut into links 3.5 to 3.75" in a "Z-linker" (Devro; Model Link 001) to give sausages that were approximately 0.72 oz (20.4 g) per sausage. The individual sausage links were placed on aluminum trays that were lined with parchment paper for rapid freezing in a -50°C Zip freezer (Model LR38729-1, Canadian Liquid Air Ltd., Canada) for approximately 35 minutes, until the product was frozen in the centre. The frozen sausages were packaged on styrofoam trays (14 sausages, approx. 10 oz per pack) and wrapped in plastic wrap. The plastic wrap was pulled air-tight around the packages and sealed by the Royal Deluxe Heat Sealer (Model 1238, Fran-Esse Equipment, Montreal, Canada). The packages were stored at -20°C until defrosted for sensory and microbial analysis.

Samples were taken after course and fine grinding of the meat and before stuffing of the sausage link. These samples were analyzed for fat percentage by the Mojonnier method of fat analysis in food products (Agriculture, Food and Rural Development, Food Safety Division; Test Procedure – FC-0244). The results are shown in Table 1.

Table 1: Percentage of fat in fine ground pork samples as determined by the Mojonnier Method of Fat analysis in Food Products (Test Procedure: FC-0244; Alberta Agriculture, Food and Rural Development, Food Safety Division)

Sample Description	Percent Fat (%)
Trial #1 – Control 1	27.5
Trial #1 – Control 2	26.0
Trial #1 – CB1 x 10^3	27.4
Trial #1 – CB1 x 10^5	26.8
Trial #2 – Control 1	30.6
Trial #2 – Control 2	31.7
Trial #2 – CB1 x 10^3	30.9
Trial #2 – CB1 x 10^4	29.0
Trial #3 – Control 1	26.0
Trial #3 – Control 2	25.2
Trial #3 – CB1 x 10^3	25.6
Trial #3 – CB1 x 10^4	29.1

B.2A. Sensory Methods for Consumer Evaluation (Food Processing Development Centre – Leduc, Alberta)

Consumer panels were conducted on samples that had been thawed and stored at 4°C for 0, 5, 10, 15 and 20 days. Samples from one storage time were tested on a specified day. The same 25 consumers were used to evaluate each of the samples in a trial. Sausage samples were placed in aluminum foil pans (22 cm x 15 cm and 5 cm deep.) The pans were covered with aluminum foil and placed in a convection oven at 163°C (325°F) for 13 min. The foil cover was removed and the samples were replaced in the oven for an additional 3 min. When the cooking was complete, the interior sausage temperatures were checked to ensure that they had reached above 80°C. The samples were cut in half. Each portion was placed in a separate 4 oz Styrofoam cup, immediately covered and served.

Each panelist was given a tray containing water, crackers, a coded sample cup and a sample cup containing an identified control treatment. Respondents were instructed to cleanse their palates with the water and crackers before proceeding with the evaluation and between samples. The coded cups were presented one at a time. A hidden control samples was included in the coded samples. After the completing the sample evaluation, panelists were asked to assess the visual acceptability of the raw sausages. These were displayed in their original packaging under fluorescent light. To maintain the sample integrity, they were kept cool by placing them on ice packs.

The final sample evaluation involved assessment of the raw sausage odor. Raw samples were presented in the same covered Styrofoam containers as the cooked samples. Panelists compared attributes of the coded samples with the control.

B.2B. Sensory Methods for Trained Panel (Compusense, Inc.)

Products:

Name	Date Produced
Control 1	March 27, 2003
Batch 3 CB1 (10^3)	March 27, 2003
Batch 4 CB1 (10^4)	March 27, 2003
Control 1	April 10, 2003
Batch 3 CB1 (10^3)	April 10, 2003
Batch 4 CB1 (10^4)	April 10, 2003
Reference (Control 2)	March 27, 2003

* All products were provided frozen by CanBiocin Inc.

Product training, using reference standards and universal scale frames of reference, was conducted prior to testing with current trained panelists. Ten trained panelists participated in the testing at the Compusense Research Facility in Guelph Ontario. Testing was conducted from May 12, 2003 until May 15, 2003.

The test products were stored in the freezer at -12°C (10°F) and were removed on specific days prior to the test to ensure product was 15, 10, 5 and 0 days old. Test products removed from the freezer were stored in the refridgerator at 4°C (39°F) to defrost and were cooked on the day of the test. Eight sausages of each test product were prepared at one time. For each product, a General Electric electric skillet was sprayed with PAM pure Canola oil cooking spray and the test products were cooked for 6 minutes, (and flipped every minute). The internal temperature of the sausages were taken using a digital thermometer and all exceeded 177°C (350°F). The sausages were cut into 1 cm slices and transferred into 120 mL (3.5 oz.) foam containers labeled with three-digit blinding codes, with three slices per sample. Cups were covered with tight-fitting lids and served immediately.

Prior to each testing session, panelists received the Reference sample (Control #2, March 27th, Day 0) for calibration purposes. After evaluating the Reference sample, panelists

proceeded to evaluate each of the test samples. Panelists evaluated eighteen samples for each test day. To avoid fatigue related to testing many samples, the panelists were given 60 second breaks between samples, and also a 2 minute break after every third sample. Panelists were instructed to drink warm temperature distilled water (44°C / 112°F) and eat unsalted soda crackers between the samples to cleanse their palates. During the 2 minute break, panelists were also instructed to cleanse their palate with peeled English cucumber slices. Spit cups were provided and panelists were instructed not to swallow the samples. All samples were evaluated under white lights.

B.3. Microbiological Methods

Bacteriological sampling was done on Day 0 (24 h thaw after frozen storage) and repeated every fifth day for 20 days. Duplicate samples were excised in 10 g portions and placed into sterile filtered stomacher bags (VWR International) and blended with 90 mL of sterile 0.1% peptone water in a Stomacher (Lab Blender 400) for 2 min. Appropriate serial dilutions in 0.1% peptone water were streaked onto prepoured APT (Difco, All Purpose Tween) agar and Lactobacilli MRS agar (Difco) plates. The plates were incubated at 30°C for 48 h in anaerobic jars (BBL Anaerobic System; Becton and Dickinson Co., Cockeysville, Maryland) filled with a gas mixture containing 10% CO_2 and 90% N_2. Duplicate counts (log cfu/g) were recorded for each sample (two 10 g samples were taken from each package at each sampling time). Samples were also plated on Violet Red Bile Agar (VRBA, Difco) and Violet Red Bile Glucose Agar (VRBG) to determine coliform and Enterobacteriaceae counts, respectively. The pH of the blended sausage samples was determined (Accumet® pH meter 915, Fisher Scientific Inc., Canada).

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B.4. Bacteriocin Assays

The samples from Trial 3 were assayed at Day 0, 5, 10, 15 and 20 by indirect and direct bacteriocin assays. In the indirect method, a 0.5 cm portion of sausage was excised and heat treated in a microwave oven for 2 min to kill the producer organism. The heated sausage segments were embedded in APT agar and held at 25°C for 4 hours. The plates were overlayered with 0.75% soft APT agar inoculated with 1% *Listeria monocytogenes* CDC 7762 (serotype 4b) as the indicator organism. In the direct method, 5 ml of sterile 0.85% saline was added to a sausage link and blended for 2 min in a Stomacher (Lab Blender 400). The free moisture in the filtered stomacher bag was removed (approx. 1 mL), centrifuged 12,000 x g for 5 min, the supernatant was removed and heat treated at 60°C for 30 min. The heat treated samples were centrifuged again and 10 μL of the supernatant was spotted onto APT agar plates, allowed to dry and overlayered with the *L. monocytogenes* indicator organism. The overlayered plated from both assays were incubated at 37°C for 24 hours and evaluated for inhibitory activity.

C. Results

C.2A. Sensory Results from Consumer Panel

Notes

- In the following tables of results, means within a row followed by the same superscript letter are not significantly different ($p>0.05$) from each other.
- Odor Intensity and Freshness attributes for cooked and raw sausages, and Flavor Intensity and Freshness attributes for cooked sausages were rated on a 21-point line scale anchored in the center by the reference (control) sample. The control sample was given a value of '0'. Descriptors of "much more" were placed at the right endpoint and given a value of '10'. Conversely, descriptors of "much less" were placed on the left endpoint and assigned a value of '-10' (see Appendix A).
- The Raw Appearance attribute was rated on an eight-point acceptability scale with '1' labeled "extremely unacceptable" and '8' labeled "extremely acceptable."
- Pre-panel assessments were done by the panel leaders to determine whether the product was fit to be assessed by the panelists. Therefore, Samples that were rejected in the pre-panel assessment and are denoted "N/T" (Not Tested) in the tables of results.
- Inoculum levels in replicate 1 were 10^3 and 10^5 cells of the test organism per gram of meat. Inoculum levels for Trialss 2 and 3 were 10^3 and 10^4 cells per gram of meat

Summary of Difference Among Treatments for Each Replicate

- Data for samples inoculated at 10^5 cfu/g (trial 1) not included in the interpretation.
- Blank cells in the table indicate no significant differences between treatments.
- Text in the Table in blue indicates favorable results for inoculated samples.

Day 0 No differences between products except
Rep 2, appearance acceptability: 10^4 more acceptable than control and 10^3
Rep 3, odor freshness: 10^4 slightly less intense than control, not different from 10^3

Day 5

	Rep 1	Rep 2	Rep 3
Cooked			
Odor intensity	10^3 less intense than control		
Odor freshness			
Flavor intensity			
Flavor freshness			
Raw			

Appearance acceptability		10^3 more acceptable than control, neither 10^3 nor control was diff from 10^4	10^3 more acceptable than control, neither 10^3 nor control was diff from 10^4
Odor intensity			
Odor freshness		10^4 less fresh than control, neither 10^4 nor control was diff from 10^3	

Day 10

	Rep 1	Rep 2	Rep 3
Cooked			
Odor intensity	Control less intense than 10^3		
Odor freshness			
Flavor intensity	10^3 more intense than control		
Flavor freshness			10^3 more fresh than control, but neither 10^3 nor control was diff from 10^4
Raw			
Appearance acceptability	10^3 less acceptable than control		10^4 less acceptable than control. 10^3 same as control and 10^4
Odor intensity	10^3 more intense than control		
Odor freshness	10^3 less fresh than control		

Day 15

	Rep 1	Rep 2	Rep 3
Cooked			
Odor intensity			
Odor freshness			
Flavor intensity			
Flavor freshness			
Raw			
Appearance acceptability			10^4 less acceptable than control, neither 10^3 same as control and 10^4
Odor intensity			
Odor freshness		10^3 more fresh than	

		control, but neither 10^3 nor control was diff from 10^4	

Day 20

	Rep 1	Rep 2	Rep 3
Cooked			
Odor intensity			10^4 more intense odor than control and 10^3, which were the same
Odor freshness		10^3 more fresh than 10^4, but neither 10^3 nor 10^4 was diff from control	10^4 less fresh than control and 10^3, which were the same
Flavor intensity			
Flavor freshness			
Raw			
Appearance acceptability			
Odor intensity			10^4 stronger odor than control and 10^3, which were the same
Odor freshness		10^3 more fresh than 10^4, neither 10^3 nor 10^4 was diff from control	10^4 less fresh than control and 10^3, which were the same

Results: Panels conducted February 3-7, 2003 (Trial 1).

Attribute Mean Scores and Tukey's Analysis

Day 0

Attribute	Control	Level 1 (10^3)	Level 2 (10^5)
Cooked			
Odor Intensity	-0.15^a	0.09^a	0.69^a
Odor Freshness	-0.09^a	0.11^a	0.18^a
Flavor Intensity	0.52^a	0.77^a	1.18^a
Flavor Freshness	0.19^a	1.24^a	0.48^a
Raw			
Appearance Acceptability	5.28^a	5.28^a	5.12^a
Odor Intensity	0.01^a	0.33^a	0.26^a
Odor Freshness	0.29^a	0.84^a	0.63^a

Day 5

Attribute	Control	Level 1 (10^3)	Level 2 (10^5)
Cooked			
Odor Intensity	0.62^a	-1.19^b	-0.19^{ab}
Odor Freshness	0.07^a	-0.94^a	-0.81^a
Flavor Intensity	0.61^a	0.05^a	0.71^a
Flavor Freshness	0.65^a	0.04^a	0.43^a
Raw			
Appearance Acceptability	5.44^a	5.00^a	5.44^a
Odor Intensity	0.01^a	0.19^a	0.58^a
Odor Freshness	-0.26^a	0.12^a	0.04^a

Day 10

Attribute	Control	Level 1 (10^3)	Level 2 (10^5)
Cooked			
Odor Intensity	-0.55^b	2.03^a	1.60^a
Odor Freshness	0.31^a	-1.08^a	-0.20^a
Flavor Intensity	0.21^b	3.06^a	1.47^{ab}
Flavor Freshness	0.54^a	-1.74^a	-0.33^a
Raw			
Appearance Acceptability	5.44^a	4.36^b	4.40^b
Odor Intensity	0.42^b	3.88^a	0.28^b
Odor Freshness	0.45^a	-3.42^b	1.28^a

Day 15

Attribute	Control	Level 1 (10^3)	Level 2 (10^5)
Cooked			
Odor Intensity	0.39^b	-0.84^b	3.30^a
Odor Freshness	0.08^a	0.16^a	-4.17^b
Flavor Intensity	1.08^a	0.86^a	N/T
Flavor Freshness	0.48^a	0.36^a	N/T
Raw			
Appearance Acceptability	5.76^a	4.64^b	3.72^c
Odor Intensity	0.03^b	0.95^b	5.20^a
Odor Freshness	0.27^a	-0.66^a	-4.78^b

Day 20

Attribute	Control	Level 1 (10^3)	Level 2 (10^5)
Cooked			
Odor Intensity	-0.34^b	0.73^b	3.06^a
Odor Freshness	-0.32^a	-2.31^{ab}	-3.83^b
Flavor Intensity	N/T	N/T	N/T
Flavor Freshness	N/T	N/T	N/T
Raw			
Appearance Acceptability	5.20^a	4.88^a	4.16^b
Odor Intensity	0.28^b	1.98^{ab}	3.28^a
Odor Freshness	-0.54^a	-1.72^a	-3.66^b

Summary of Observations:

- Day 0, no significant differences were observed in any of the attributes tested.
- Day 5, the 10^3 inoculated sausages had a lower Cooked Odor Intensity than the unidentified control sausage.
- Day 10, both inoculum levels elicited significantly more intense Cooked Odor and less acceptable appearance scores. The lower level inoculated sausages showed significantly higher Cooked Flavor Intensity and Raw Odor Intensity and lower Raw Odor Freshness scores than the unidentified control samples.
- Day 15, the 10^5 inoculated sausages only showed differences from the identified control in the appearance of the raw product; whereas at the 10^5 inoculum level the sausages differed significantly from the controls for all attributes.
- Day 20, the 10^3 inoculum level did not result in significant differences from the unidentified control for any of the attributes; the 10^5 inoculated sausages were significantly different from the unidentified control for all attributes.

Results: Panels conducted April 11-17, 2003 (Trial 2).

Summary of Means and Tukey's Analysis

Day 0

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	0.2^a	-0.7^a	-0.3^a
Odor Freshness	-0.3^a	0.8^a	0.7^a
Flavor Intensity	1.1^a	1.1^a	0.5^a
Flavor Freshness	0.4^a	1.1^a	0.3^a
Raw			
Appearance Acceptability	5.3^b	5.4^b	6.1^a
Odor Intensity	-0.7^a	-1.0^a	-0.5^a
Odor Freshness	0.6^a	1.0^a	0.7^a

Day 5

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	-0.3^a	1.0^a	-0.1^a
Odor Freshness	0.3^a	-0.1^a	-0.8^a
Flavor Intensity	-0.3^a	0.9^a	-0.3^a
Flavor Freshness	0.6^a	0.9^a	-0.2^a
Raw			
Appearance Acceptability	5.1^b	5.7^a	5.5^{ab}
Odor Intensity	0.4^a	-0.5^a	-0.9^a
Odor Freshness	0.5^a	-0.1^{ab}	-0.4^b

Day 10

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	-0.3^a	0.6^a	-1.0^a
Odor Freshness	0.8^a	1.3^a	0.3^a
Flavor Intensity	1.6^a	1.9^a	0.9^a
Flavor Freshness	1.7^a	1.6^a	1.0^a
Raw			
Appearance Acceptability	5.5^a	5.8^a	5.4^a
Odor Intensity	-0.4^a	0.3^a	-0.5^a

Odor Freshness	0.5[a]	0.1[a]	0.6[a]

Day 15

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	0.1[a]	-0.1[a]	0.5[a]
Odor Freshness	0.6[a]	0.3[a]	-0.6[a]
Flavor Intensity	0.9[a]	0.6[a]	-0.2[a]
Flavor Freshness	1.6[a]	1.5[a]	1.4[a]
Raw			
Appearance Acceptability	5.0[a]	5.3[a]	4.8[a]
Odor Intensity	0.3[a]	-1.1[a]	-0.9[a]
Odor Freshness	0.4[b]	1.6[a]	1.0[ab]

Day 20

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	-1.1[a]	-0.2[a]	0.6[a]
Odor Freshness	-0.2[ab]	2.3[a]	-1.8[b]
Flavor Intensity	N/T	N/T	N/T
Flavor Freshness	N/T	N/T	N/T
Raw			
Appearance Acceptability	5.0[a]	4.8[a]	4.1[a]
Odor Intensity	-0.0[a]	-0.1[a]	0.3[a]
Odor Freshness	0.4[ab]	1.0[a]	-0.6[b]

Summary of Observations:

- Day 0, the sausages with the 10^4 inoculum had a significantly higher Acceptability for Raw Appearance than the control.
- Day 5, the Acceptability of Raw Appearance of the sausages with the 10^3 inoculum was significantly better than the Control. Raw Odor Freshness of the 10^4 inoculum product was significantly less than the Control.
- Day 10, no significant differences were observed in any of the attributes.
- Day 15, the 10^3 inoculum had significantly better Raw Odor Freshness than the Control.

- Day 20, the products were not considered suitable for evaluation based on pre-panel assessment.

Results: Panels conducted April 28 – May 2, 2003 (Trial 3).

Summary of Means and Tukey's Analysis

Day 0

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	0.5^a	-0.4^a	0.3^a
Odor Freshness	0.0^a	-0.5^{ab}	-0.7^b
Flavor Intensity	0.5^a	0.4^a	1.0^a
Flavor Freshness	0.6^a	-0.5^a	0.5^a
Raw			
Appearance Acceptability	5.6^a	5.8^a	5.5^a
Odor Intensity	0.4^a	0.3^a	0.3^a
Odor Freshness	0.3^a	0.4^a	0.2^a

Day 5

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	0.2^a	0.6^a	-0.1^a
Odor Freshness	0.2^a	0.2^a	0.3^a
Flavor Intensity	1.0^a	1.0^a	1.2^a
Flavor Freshness	1.3^a	1.3^a	0.7^a
Raw			
Appearance Acceptability	5.4^b	5.9^a	5.5^{ab}
Odor Intensity	-0.4^a	0.3^a	-0.1^a
Odor Freshness	0.2^a	0.5^a	0.5^a

Day 10

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	0.2^a	0.4^a	-0.1^a
Odor Freshness	-0.4^a	0.0^a	-0.4^a
Flavor Intensity	0.2^a	0.9^a	0.6^a
Flavor Freshness	-0.2^b	0.6^a	0.3^{ab}

Raw			
Appearance Acceptability	5.8^{a}	5.5^{ab}	5.2^{b}
Odor Intensity	0.5^{a}	0.5^{a}	0.8^{a}
Odor Freshness	0.3^{a}	0.1^{a}	-0.1^{a}

Day 15

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	0.9^{a}	-0.1^{a}	1.0^{a}
Odor Freshness	0.0^{a}	0.3^{a}	-0.1^{a}
Flavor Intensity	0.8^{a}	0.9^{a}	0.8^{a}
Flavor Freshness	0.2^{a}	0.5^{a}	0.0^{a}
Raw			
Appearance Acceptability	5.5^{ab}	6.0^{a}	5.1^{b}
Odor Intensity	0.7^{a}	0.2^{a}	1.0^{a}
Odor Freshness	0.2^{a}	0.4^{a}	-0.5^{a}

Day 20

Attribute	Control	Level 1 (10^3)	Level 2 (10^4)
Cooked			
Odor Intensity	0.3^{b}	0.2^{b}	1.9^{a}
Odor Freshness	0.3^{a}	0.5^{a}	-3.7^{b}
Flavor Intensity	N/T	N/T	N/T
Flavor Freshness	N/T	N/T	N/T
Raw			
Appearance Acceptability	5.1^{a}	5.4^{a}	5.0^{a}
Odor Intensity	0.2^{b}	0.2^{b}	3.7^{a}
Odor Freshness	-0.5^{a}	-0.0^{a}	-4.4^{b}

Summary of Observations:

- Day 0, there was a significant difference in lower Cooked Odor Freshness between the 10^4 inoculum and the Control.
- Day 5, the product with the 10^3 inoculum was significantly more acceptable than the Control for Raw Appearance Acceptability.
- Day 10, the Cooked Flavor Intensity of the 10^3 inoculum product and the Cooked Flavor Freshness of the 10^4 inoculum product were had significantly better than

the Control. However, neither of the inoculated products differed from each other in either attribute.

- Day 15, the Raw Appearance of the 10^3 inoculum product was significantly more acceptable than the 10^4 inoculum product, but neither was significantly different from the control.
- Day 20, the products were not considered suitable for evaluation based on pre-panel assessment.

C.2B. Sensory Results from Trained Panel

Sensory Project Test Date: June 17, 2003

Data Analysis ***Test Location: Griffith Labs, Canada***

Project Number: SD 0307026.00 ***Lead Scientist: Lorraine Tam***

Project Objective: See project code name Lifeguard.

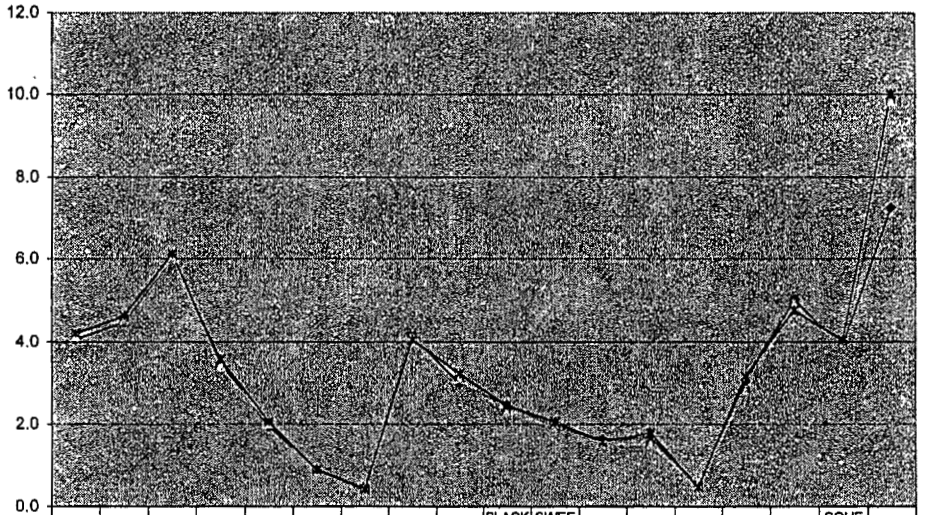
Sensory Objective:

1. Determine if there is a significant difference between samples over time (Day 0, 5, 10 & 15).
2. Determine if the test sample attributes change at the same time intervals as the control.

Results:
Samples by Day

Day 0: The test samples were higher in Bloom.

Day 0



	FIRMSK	FIRMCROSS	OFLAVINT	SALT	SWEET	SOUR	BITTER	PORK_FAT	PORK_LEAN	BLACK_PEPPER	SWEET_AROM	SAGE	HOT_SPICE	DAIRY	HEAT	JUICY	COHESIVENESS	BLOOM
Control D-0	4.1	4.5	6.0	3.5	1.9	0.9	0.4	4.1	3.0	2.5	1.9	1.6	1.7	0.5	3.1	5.0	3.9	7.3
T1 - Ma-Do	4.1	4.5	6.0	3.4	2.0	0.8	0.5	4.1	3.1	2.3	2.0	1.5	1.5	0.5	2.8	4.9	3.9	9.8
T1 - Ma-Do	4.1	4.5	6.0	3.4	2.0	0.8	0.5	4.1	3.1	2.3	2.0	1.5	1.5	0.5	2.8	4.9	3.9	9.8
T2 - Ma-D0	4.3	4.5	6.1	3.6	2.0	0.9	0.5	4.3	3.0	2.5	1.9	1.5	1.7	0.5	3.1	5.1	3.9	8.9
T2 - Ap-D0	4.2	4.6	6.1	3.6	2.0	0.9	0.4	4.1	3.2	2.4	2.1	1.8	1.8	0.5	3.2	4.8	4.1	10.0

Day 5: The test samples were higher in Bloom.

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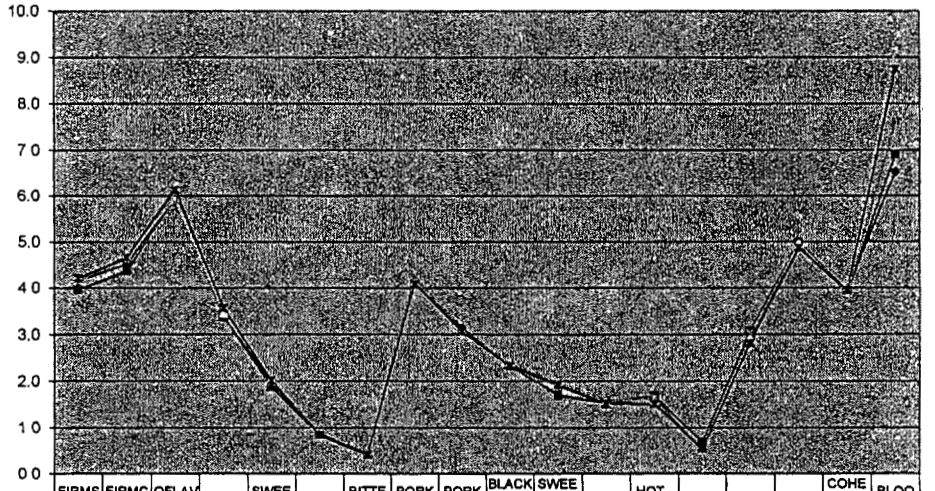
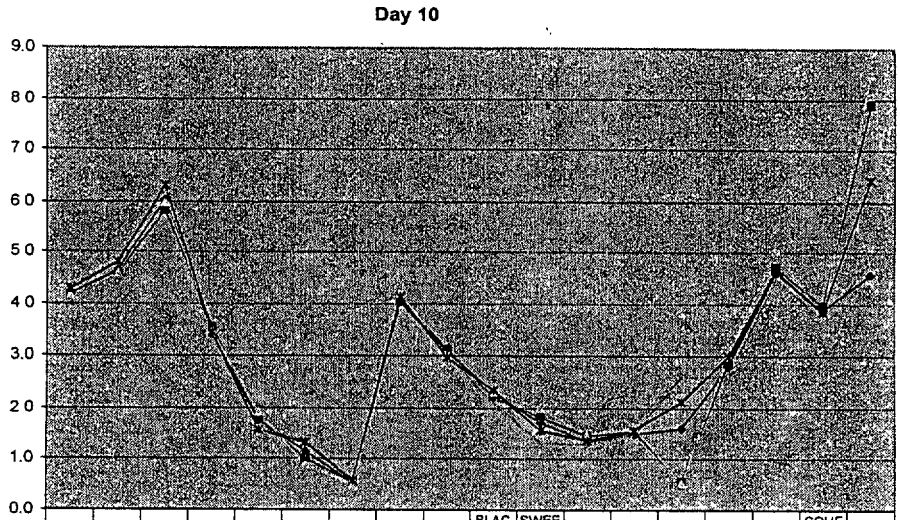
Day 5



	FIRMSK	FIRMCROSS	OFLAVINT	SALT	SWEET	SOUR	BITTER	PORK_FAT	PORK_LEAN	BLACK_PEPPER	SWEET_AROM	SAGE	HOT_SPICE	DAIRY	HEAT	JUICY	COHESIVENESS	BLOOM
Control D-5	4.1	4.5	5.9	3.4	2.0	0.9	0.4	4.1	3.1	2.4	1.9	1.5	1.6	0.5	2.9	4.9	3.9	8.5
T1 - Ma-D5	4.0	4.4	6.0	3.4	1.9	0.8	0.4	4.1	3.1	2.3	1.7	1.5	1.7	0.7	3.1	5.0	3.8	6.9
T1 - Ap-D5	4.2	4.7	5.9	3.4	2.0	0.8	0.4	4.1	3.1	2.4	2.0	1.6	1.6	0.4	2.9	5.0	3.9	9.2
T2 - Ma-D5	4.1	4.7	6.0	3.6	2.0	0.9	0.4	4.2	3.1	2.4	2.0	1.6	1.6	0.4	3.0	4.9	3.9	7.6
T2 - Ap-D5	4.2	4.7	6.1	3.6	2.0	0.8	0.4	4.1	3.1	2.3	1.9	1.5	1.5	0.5	2.8	4.9	3.9	8.7

Day 10: Control and both test 2 samples increased in Dairy intensity, and decreased in Bloom.



	FIRMSK	FIRMCROSS	OFLAVINT	SALT	SWEET	SOUR	BITTER	PORK_FAT	PORK_LEAN	BLACK_PEPPER	SWEET_AROM	SAGE	HOT_SPICE	DAIRY	HEAT	JUICY	COHESIVENESS	BLOOM
Control D-10	4.2	4.6	6.0	3.4	1.7	1.1	0.5	4.0	3.1	2.3	1.7	1.3	1.5	1.6	2.8	4.6	3.9	4.6
T1 - Ma-10	4.2	4.6	5.8	3.6	1.8	1.0	0.5	4.1	3.1	2.2	1.8	1.4	1.5	0.6	2.9	4.7	4.0	7.9
T1 - Ap-D10	4.2	4.6	6.0	3.5	1.9	0.9	0.5	4.2	3.0	2.3	2.0	1.5	1.6	0.5	3.0	5.0	3.8	8.5
T2 - Ma-D10	4.3	4.8	6.2	3.4	1.6	1.3	0.6	4.1	3.0	2.1	1.3	1.3	1.5	2.6	2.6	4.5	3.8	4.6
T2 - Ap-D10	4.3	4.8	6.3	3.5	1.6	1.3	0.6	4.1	3.0	2.3	1.6	1.4	1.6	2.1	3.0	4.7	3.9	6.4

Day 15: Test 1 Ap. was lower than all samples for Dairy. All test samples were higher than the control for Bloom.

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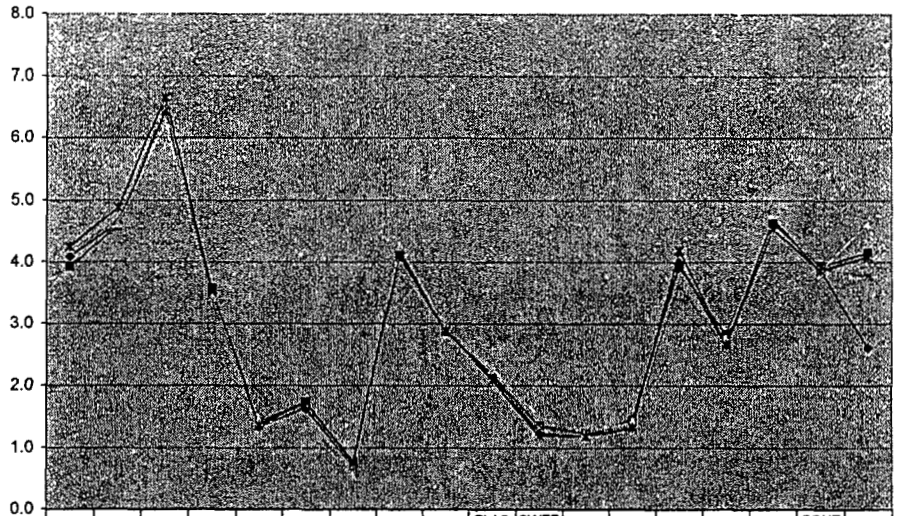

	FIRMSK	FIRMCROSS	OFLAVINT	SALT	SWEET	SOUR	BITTER	PORK_FAT	PORK_LEAN	BLACK_PEPPER	SWEET_AROM	SAGE	HOT_SPICE	DAIRY	HEAT	JUICY	COHESIVENESS	BLOOM
Control D15	4.1	4.8	6.5	3.4	1.3	1.6	0.7	4.1	2.9	2.2	1.2	1.2	1.5	4.0	2.6	4.7	3.9	2.6
T1 - Ma-15	3.9	4.6	6.4	3.6	1.4	1.7	0.7	4.2	2.9	2.2	1.4	1.2	1.4	3.9	2.8	4.6	3.9	4.2
T1 - Ap-D15	4.2	4.6	6.2	3.4	1.4	1.4	0.5	4.3	2.9	2.3	1.5	1.3	1.4	2.7	2.8	4.8	3.9	4.6
T2 - Ma-D15	4.2	4.7	6.5	3.4	1.4	1.7	0.7	4.0	3.0	2.1	1.3	1.3	1.3	3.6	2.6	4.5	3.9	3.8
T2 - Ap-D15	4.2	4.9	6.7	3.6	1.4	1.7	0.8	4.1	2.9	2.1	1.2	1.2	1.3	4.2	2.7	4.5	3.9	4.1

Results

By Sample: Two attributes showed significant differences across all samples; Dairy and Bloom.

- Dairy- Control changed at Day 10 as did both Test 2 samples. Test 1 samples changed at Day 15.
- Bloom- In general all samples decreased in intensity over time. However, Test 1 AP did not show a significant drop until Day 15

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Control: Day 5, bloom decreases

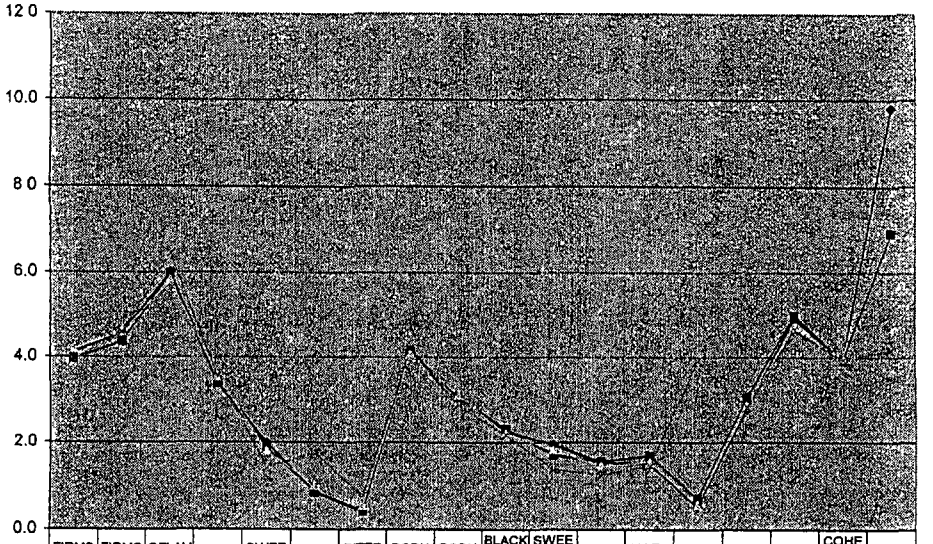
At day 10 Dairy intensity increases and bloom begins to decrease.

At Day15, slight decrease in Sweet and slight increase in Sour; Dairy intensity more than doubles and the decrease in bloom is almost double.

Test 1 Ma: Day 5, bloom decreases.

At Day 10 bloom increases.

At Day15, Sour begins to increase, Dairy increases significantly and Bloom decreases.



	FIRMSK	FIRMCROSS	OFLAVINT	SALT	SWEET	SOUR	BITTER	PORK_FAT	PORK_LEAN	BLACK_PEPPER	SWEET_AROM	SAGE	HOT_SPICE	DAIRY	HEAT	JUICY	COHESIVENESS	BLOOM
T1 - Ma-Do	4.1	4.5	6.0	3.4	2.0	0.8	0.5	4.1	3.1	2.3	2.0	1.5	1.5	0.5	2.8	4.9	3.9	9.8
T1 - Ma-D5	4.0	4.4	6.0	3.4	1.9	0.8	0.4	4.1	3.1	2.3	1.7	1.5	1.7	0.7	3.1	5.0	3.9	6.9
T1 - Ma-10	4.2	4.6	5.8	3.6	1.8	1.0	0.5	4.1	3.1	2.2	1.8	1.4	1.5	0.6	2.9	4.7	4.0	7.9
T1 - Ma-15	3.9	4.6	6.4	3.6	1.4	1.7	0.7	4.2	2.9	2.2	1.4	1.2	1.4	3.9	2.8	4.8	3.9	4.2

Test 1 Ap. At day 10 bloom decreases.

At Day 15, slight decrease in Sweet as Sour begins to increase, Dairy increases significantly and bloom decreases significantly.

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Test 1 April

	FIRMSK	FIRMCROSS	OFLAVINT	SALT	SWEET	SOUR	BITTER	PORK_FAT	PORK_LEAN	BLACK_PEPPER	SWEET_AROM	SAGE	HOT_SPICE	DAIRY	HEAT	JUICY	COHESIVENESS	BLOOM
T1 - Ma-Do	4.1	4.5	6.0	3.4	2.0	0.8	0.5	4.1	3.1	2.3	2.0	1.5	1.5	0.5	2.8	4.9	3.9	9.8
T1 - Ap-D5	4.2	4.7	5.9	3.4	2.0	0.8	0.4	4.1	3.1	2.4	2.0	1.6	1.6	0.4	2.9	5.0	3.9	9.2
T1 - Ap-D10	4.2	4.6	6.0	3.5	1.9	0.9	0.5	4.2	3.0	2.3	2.0	1.5	1.6	0.5	3.0	5.0	3.8	8.5
T1 - Ap-D15	4.2	4.6	6.2	3.4	1.4	1.4	0.5	4.3	2.9	2.3	1.5	1.3	1.4	2.7	2.8	4.8	3.9	4.6

Test 2 Ma: Day 5, bloom decreases.

At day 10 bloom decreases.

At Day15, slight decrease in Sweet as Sour begins to increase, Dairy increases and bloom decreases.

Test 2 AP: Day 5, bloom decreases.

At Day 10, slight decrease in Sweet as Sour begins to increase, bloom decreases.

At Day 15, Sour slight increase, Dairy intensity doubles, and bloom decreases.

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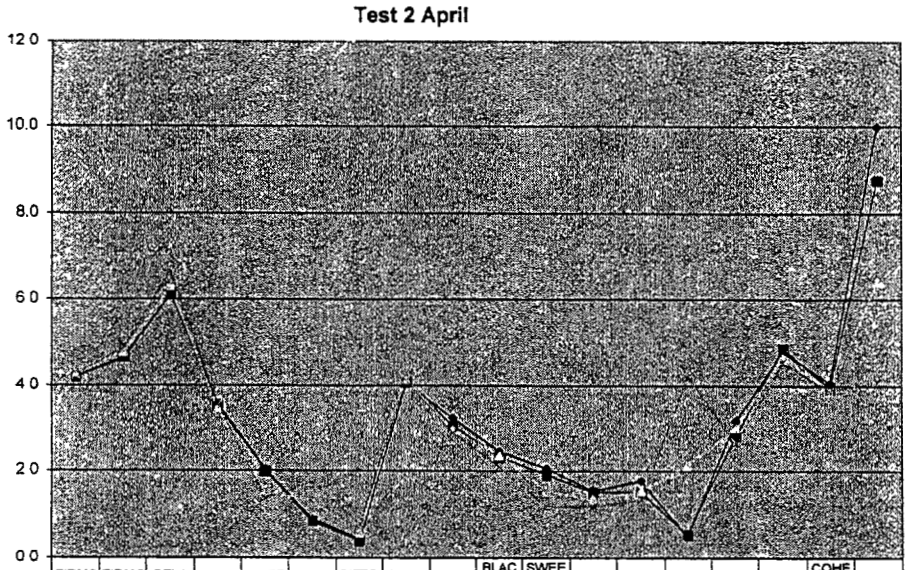

	FIRMSK	FIRMCROSS	OFLAVINT	SALT	SWEET	SOUR	BITTER	PORK_FAT	PORK_LEAN	BLACK_PEPPER	SWEET_AROM	SAGE	HOT_SPICE	DAIRY	HEAT	JUICY	COHESIVENESS	BLOOM
T2 - Ap-D0	4 2	4.6	6.1	3 8	2 0	0.9	0 4	4 1	3.2	2.4	2.1	1.6	1.8	0.5	3.2	4.8	4 1	10 0
T2 - Ap-D5	4.2	4 7	6.1	3.6	2 0	0 8	0.4	4.1	3.1	2 3	1.9	1 5	1.5	0 5	2 8	4.9	3 9	8 7
T2 - Ap-D10	4 3	4 8	6.3	3.5	1.6	1.3	0.6	4.1	3.0	2.3	1 6	1.4	1 6	2 1	3.0	4.7	3 9	6.4
T2 - Ap-D15	4.2	4.9	6.7	3.6	1.4	1 7	0.8	4.1	2.9	2.1	1.2	1.2	1 3	4 2	2.7	4 5	3.9	4 1

C.3. Microbiological Results

The results from the microbial sampling are reported in Figure 2 (Trial 1), Figure 3 (Trial 2) and Figure 4 (Trial 3). The pH data are shown in Figure 5.

C.4. Bacteriocin Assays

The activity test results from Trial 3 are illustrated in Figures 5 and 6. In both assays bacteriocin production was detected at Day 10, 15 and 20; however, in Trial 3 it should be noted that there was a lag phase in the growth of the *C. piscicola* CB1 inoculum that was not observed in Trials 1 and 2. These data are shown in Figure 4. The lag phase in Trial 3 was confirmed at the time of activity testing.

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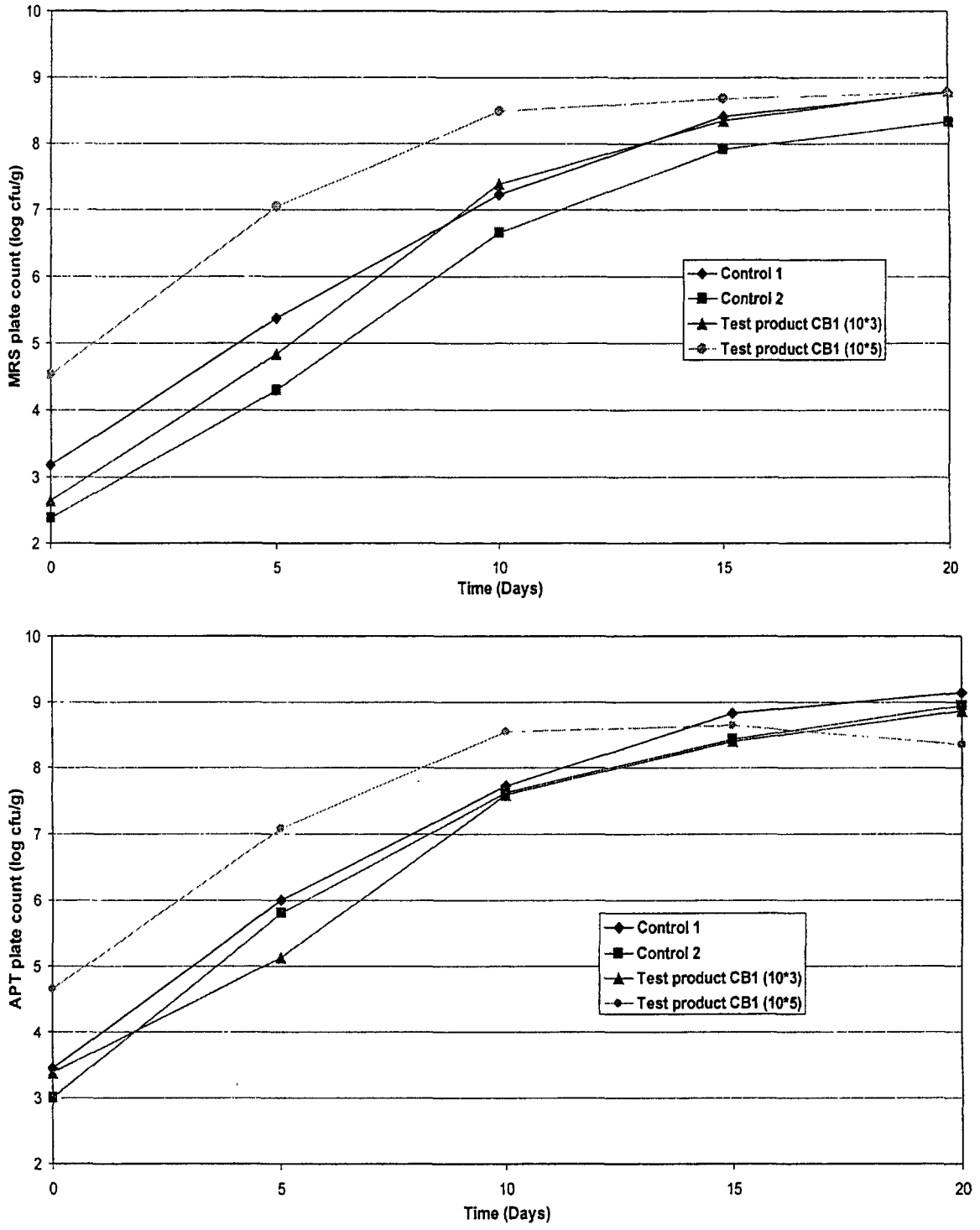

Figure 2: Plate counts represented by mean (log cfu/g) of two samples from Trial 1

A) Growth of microorganisms on MRS agar

B) Growth of microorganisms on APT agar

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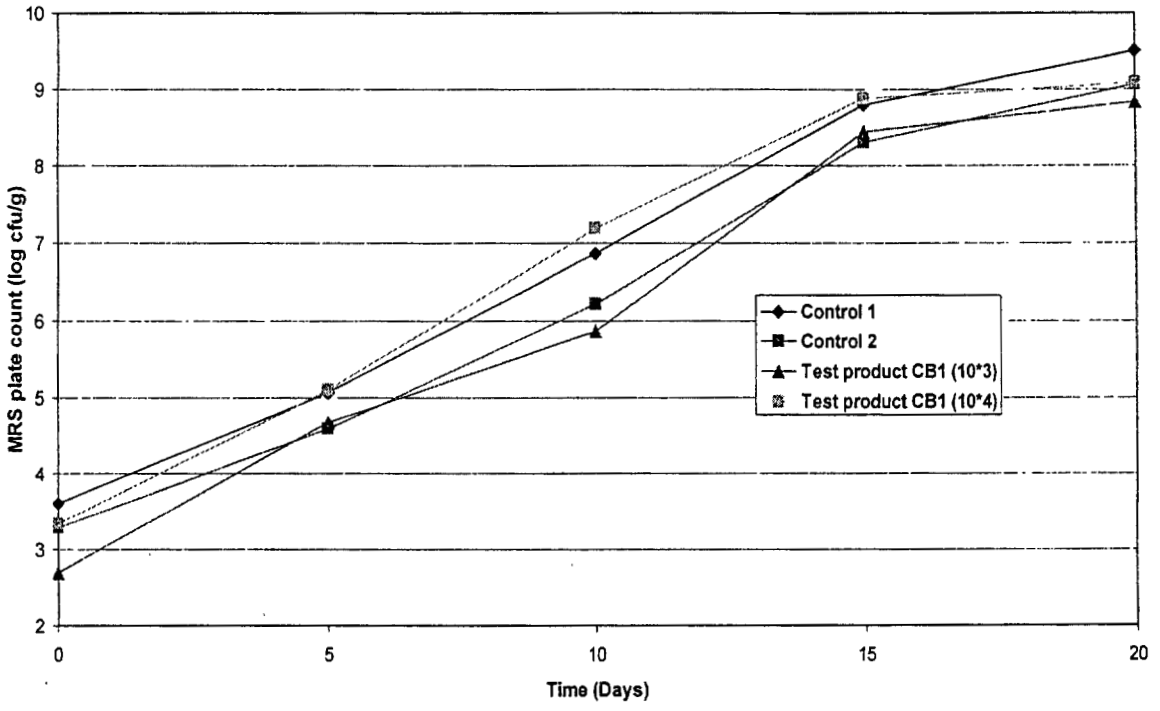


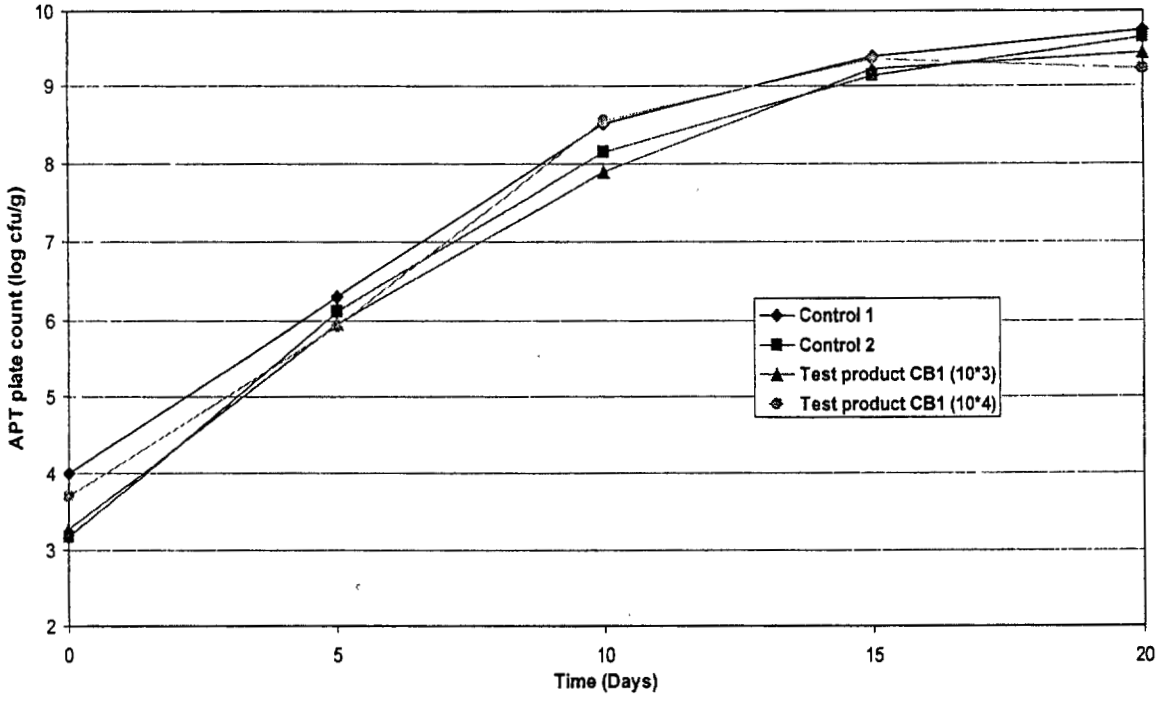
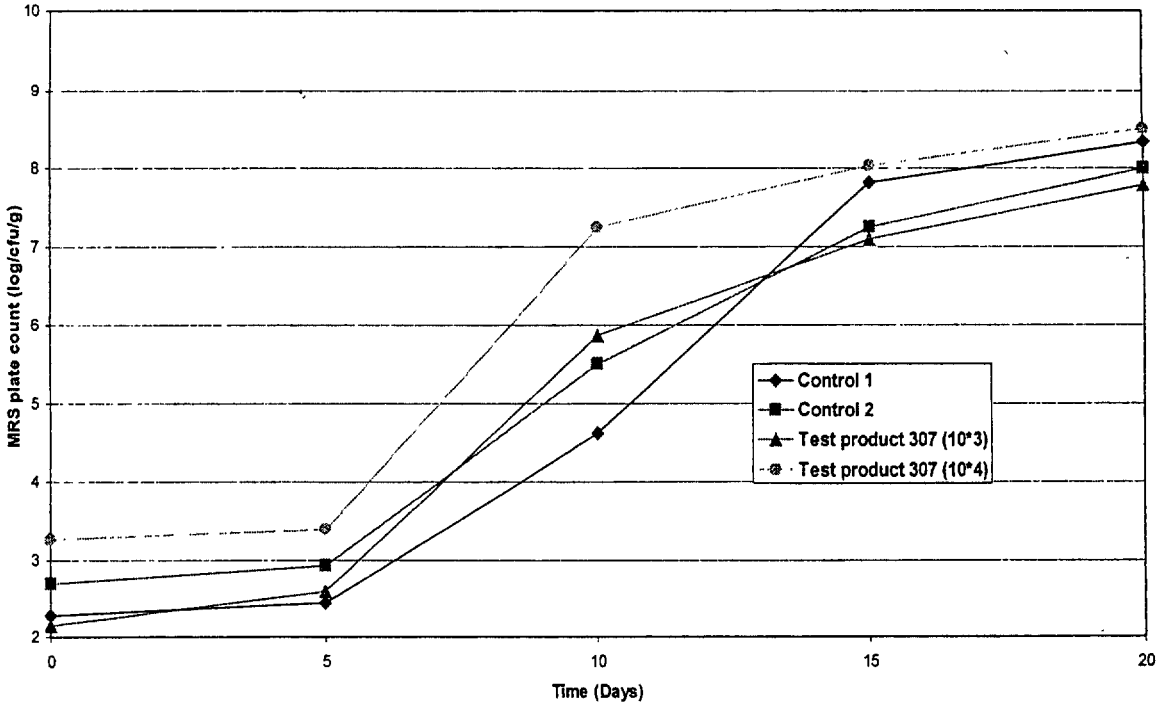


Figure 3: Plate counts represented by mean (log cfu/g) of two samples from Trial 2

A) Growth of microorganisms on MRS agar

B) Growth of microorganisms on APT agar



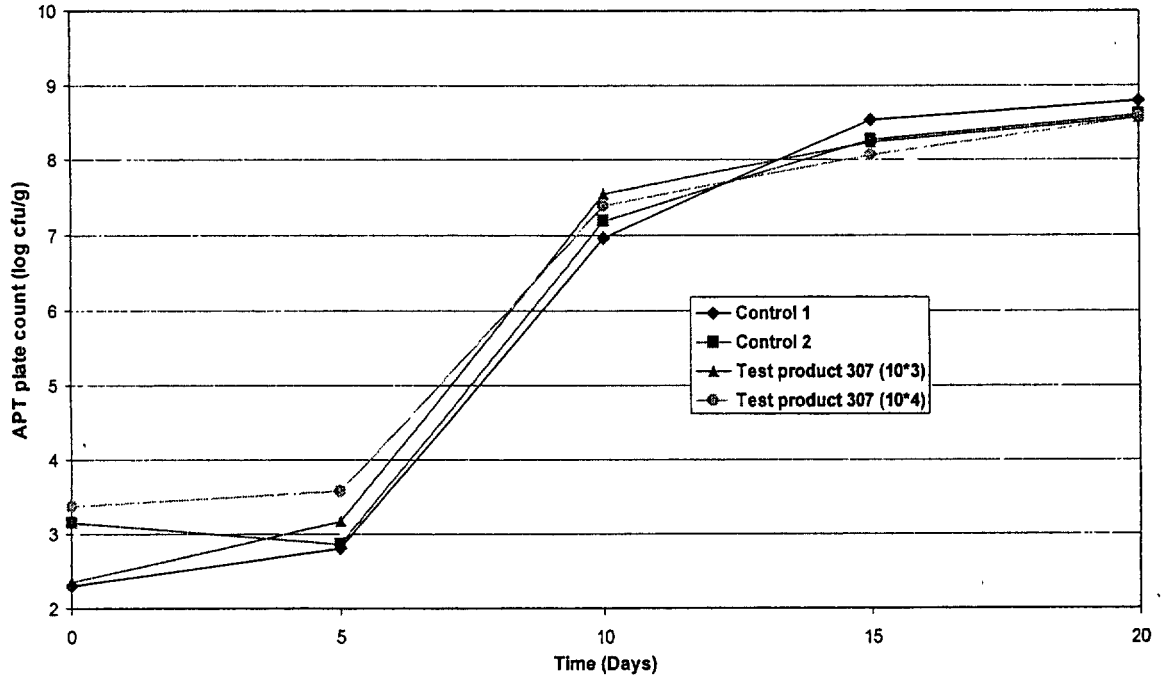


Figure 4: Plate counts represented by mean (log cfu/g) of two samples from Trial 3

A) Growth of microorganisms on MRS agar

B) Growth of microorganisms on APT agar

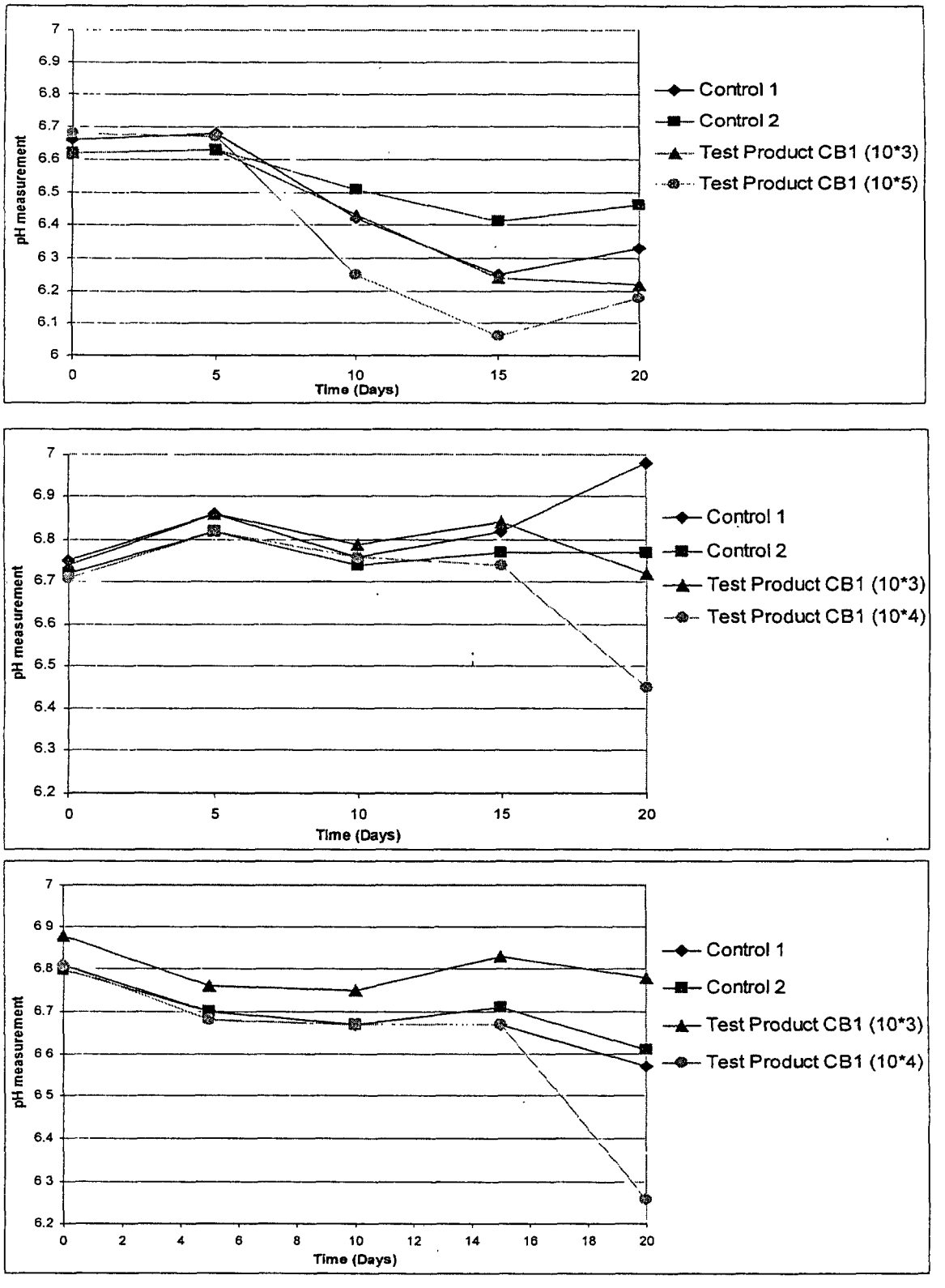


Figure 5: pH data from Trials 1 (Figure 5A), Trial 2 (Figure 5B) and Trial 3 (Figure 5C)

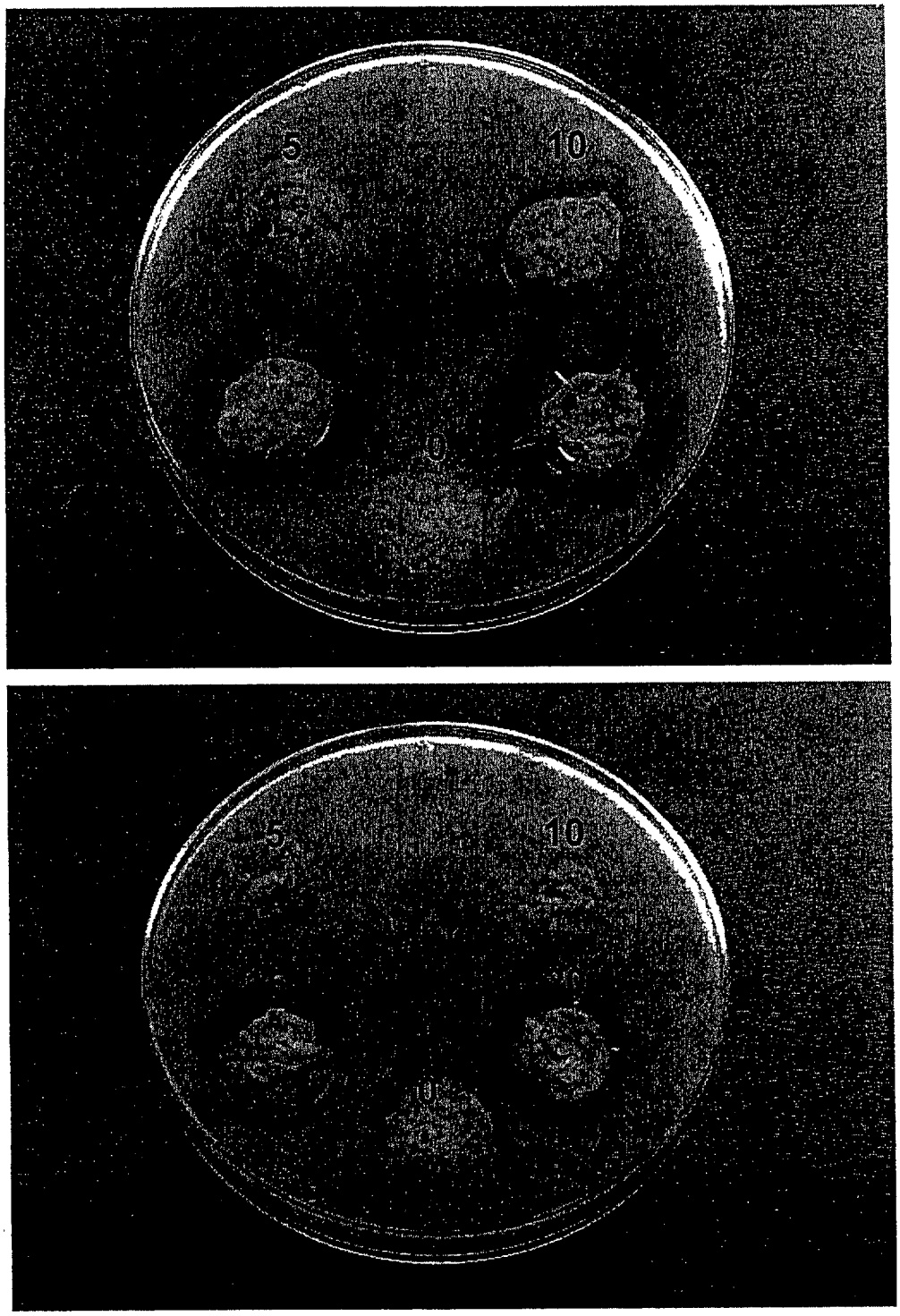


Figure 6: Indirect method of bacteriocin detection in sausages
A) Indicator organism *L. monocytogenes* CDC 7762
B) Indicator organism *C. divergens* UAL 9

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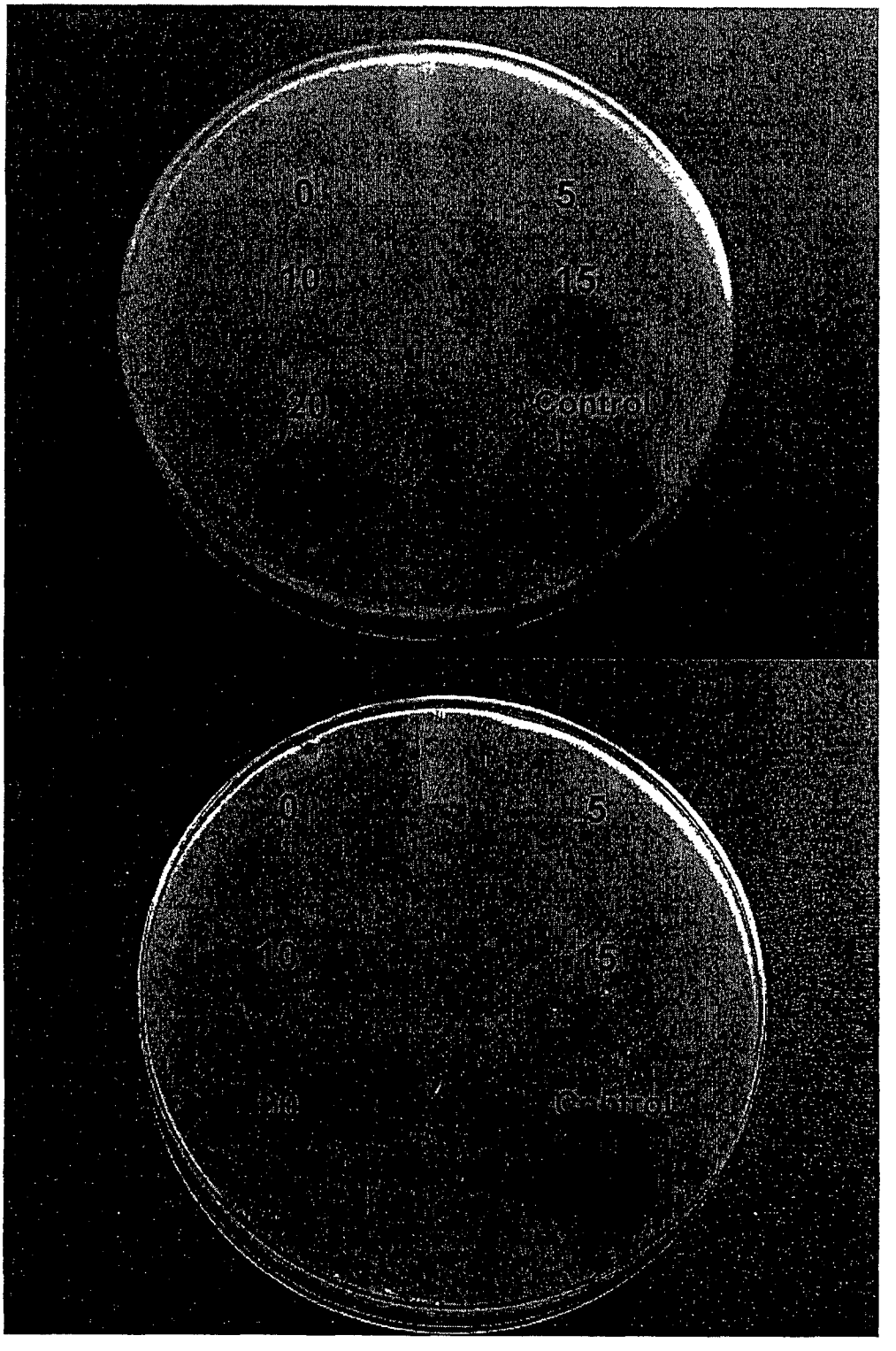

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Figure 7: Direct method of bacteriocin detection in sausages
A) Indicator organism *L. monocytogenes* CDC 7762
B) Indicator organism *C. divergens* UAL 9

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Challenge Study:

***Listeria monocytogenes* on a Ready-to-Eat Meat Product.**

Note: In order to protect proprietary rites, Griffith Laboratories has renamed the *Carnobacterium maltaromaticum* (previously named *Carnobacterium piscicola*) strain stated in the following data ("UAL307") to the new designation of "CB1." Therefore, the *C. maltaromaticum* strain UAL307 is synonymous with *C. maltaromaticum* strain CB1. The term "UAL307" is Business Confidential information, and should be maintained as such.

CanBiocin Inc.

Challenge Study:

Listeria monocytogenes on a Ready-to-eat Meat Product.

Summary

1. The experimental protocol included three replicates of a *Listeria monocytogenes* challenge study on wieners with six selected strains of *Carnobacterium piscicola*.
2. The *Listeria* cocktail differed from that used in previous studies. It was comprised of four serovars of *L. monocytogenes* that have been associated with foodborne illness. The *Listeria* strains were tested for their compatibility to ensure that they would grow in the presence of one another.
3. The wieners were inoculated by dipping them into saline solution containing the inoculum. The inoculated wieners were air dried, vacuum packaged and stored at 4°C for 84 days (12 weeks).
4. Wieners were sampled at weekly or biweekly intervals during storage. LAB (Lactic Acid Bacteria) were enumerated on APT agar and *L. monocytogenes* were enumerated PALCAM agar.
5. The background microflora of the wieners was exceptionally low throughout the study The pH decreased from 6.8 to approximately 6.0 over the course of the study.
6. The LAB grew as expected on the wieners. With an inoculum of 10^4 per cm^2 there was no lag phase detected at 7 days and samples reached maximum population within 35 days.
7. *C. piscicola* UAL8 and UAL26 did not give the favorable results observed in earlier studies.
8. The genetically enhanced strain UAL26/8A gave excellent inhibition of the *Listeria* cocktail, confirming the synergistic effect of these two bacteriocins working together.
9. The two new natural strains UAL307 and UAL682 gave excellent results, equivalent to UAL26/8A. They inhibited growth and decreased the number of viable *L. monocytogenes* on the wieners.
10. *C. piscicola* UAL307 is recommended for further testing because of its marginally better results compared with UAL682 and the greater knowledge of its characteristics.

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Introduction

Listeria monocytogenes is a major concern for the processed meat industry because of the potential for this organism to grow on refrigerated, vacuum packaged meat products and to cause infections in people that consume contaminated products.

CanBiocin has licensed technology developed at the University of Alberta by Drs. McMullen and Stiles in which selected lactic acid bacteria are inoculated onto wieners at time of packaging as a biopreservative with the objective of inhibiting the growth of *L. monocytogenes*.

Earlier challenge studies done at the University of Alberta and by CanBiocin Inc revealed that the *L. monocytogenes* cocktail used in the previous studies was inhibited on wieners stored at 5°C for 60 days. The *L. monocytogenes* cocktail used in the initial study was based on strain compatibility. Serotypes were not specifically known. Based on these earlier data, *Carnobacterium piscicola* UAL8 was the organism of choice for biopreservation.

The current study was planned in consultation with Griffith Laboratories (Toronto). A different range of *Listeria* was selected. The selection was based on serotypes that have been associated with foodborne human disease, including 1/2a, 1/2b, 3a and 4b.

The object of the current challenge study was to determine the efficacy of *C. piscicola* strains UAL8 and UAL26 against the *Listeria* cocktail inoculated onto vacuum packaged wieners stored at 5°C. The genetically modified strain UAL26/8A was also tested because of its enhanced (synergistic) antilisterial activity as well as two new, promising, bacteriocin-producing meat isolates UAL307 and UAL682.

The trials were conducted in our laboratory at the University of Alberta between May and October , 2002

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Strains of *Carnobacterium piscicola* selected for this challenge study

UAL8 is a well-characterized strain that has been used successfully as a biopreservative in other studies. The strain was originally isolated by B. Shaw (Bristol) from vacuum packaged fresh pork. It has been deposited in the National Collection of Food Bacteria as strain NCIMB 702852. UAL8 is synonymous with strain LV17 (Shaw)]

UAL8C2 is the isogenic, nonbacteriocinogenic variant of UAL8

UAL26 was isolated in the Food Microbiology Laboratory at the University of Alberta. It produces a bacteriocin equivalent to piscicolin 126.

UAL26/8A is a genetically enhanced strain of UAL26. The gene for carnobacteriocin A was cloned into UAL26 and yielded a strain with greater antibacterial activity, indicating a synergistic interaction between the two bacteriocins.

UAL307 and UAL682 are promising new isolates obtained from meats in the Food Microbiology Laboratory.

Preliminary studies

The four strains of *L. monocytogenes* used for the *Listeria* cocktail in the wiener experiment were selected based on the following criteria:

1. The *Listeria* strains selected for the cocktail do not inhibit each other.

2. *Listeria* strains selected are inhibited by the Lactic Acid Bacteria (LAB) used in the experiment.

~~3. *Listeria* strains include one of each of the following serotypes: 1/2ab, 3a and 4b.~~

4. Strains can be differentiated by a genetic typing technique.

Results indicated that the four strains selected for the cocktail met these criteria, these results are as follows:

1. The strains CDC 7762, HPB 642, FS 15 and NTCC 5105.3 did not inhibit each other in deferred inhibition assays at 25°C.

2. The four *Listeria* strains were shown to be inhibited by deferred inhibition by the selected LAB, at 25°C and at 4 and 8°C. Greater inhibition was observed at the lower temperatures.

3. LAB also produced zones of inhibition using spot on lawn tests against these strains of *L. monocytogenes* at 30°C and 10°C. Larger zones of inhibition were observed at lower incubation temperatures.

4. The Random Amplification of Polymorphic DNA (RAPD) molecular genetic technique can be used as a method of strain identification among the *Listeria* in the cocktail.

5. Serotyping showed that the four *Listeria* strains were the desired serotypes. Three of the four strains were food isolates:

HPB 642 (1/2a)	pâté (Health Protection Branch)
FS 15 (1/2b)	beef wieners; Meat Safety Project (Lynn McMullen)
NTCC 5105.3 (3a)	clinical isolate (National Type Culture Collection)
CDC 7762 (4b)	processed meat (Center for Disease Control and Prevention)

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Experimental Protocol

1. Growth of strains for experimental use

- Resuscitate frozen (-70°C glycerol stocks) strains of *L. monocytogenes* in 5 mL of TSBYE (tryptic soy broth with 0.6% yeast extract, Difco). Incubate at 37°C for 24 h.

- Resuscitate frozen (-70°C glycerol stocks) strains of LAB in 5 mL of APT broth (All Purpose Tween, Difco). Incubate strains at 25°C for 24 h.

- Subculture (0.1% inoculum) separately each *L. monocytogenes* strain in 10 mL of TSBYE. Incubate tubes at 25°C for 24 h.

- Subculture (0.1% inoculum) separately each LAB strain in 10 mL of APT. Incubate tubes at 15°C for 48 h.

2. Strain preparation for product inoculation

- Rep. 1

Centrifuge 10 mL of LAB and *Listeria* spp. (15 mL NTCC 5105.3, due to less growth) at 10,000 x g for 8 min. Wash cells twice with sterile 0.85% saline and resuspend in 10 mL of sterile 0.85% saline.

Add 100 µL of each *Listeria* strain to 7 sterile microcentrifuge tubes (resulting in 7 tubes with 400 µL of *Listeria* cocktail).

Add 4 mL of each LAB culture to separate sterile microcentrifuge tubes (resulting in 12 x 4 mL tubes for each culture).

- Reps 2 & 3

Centrifuge 20 mL of LAB and 20 mL of *Listeria* spp. at 10,000 x g for 8 min. Wash cells twice with sterile 0.85% saline and resuspend in 20 mL of sterile 0.85% saline.

Add 500 µL of each *Listeria* strains to 7 sterile microcentrifuge tubes (resulting in 7 x 2 mL tubes of *Listeria* cocktail).

Add 10 mL of the required LAB culture to each tube (resulting in 12 tubes with 10 mL).

3. Inoculation of wieners and description of treatments for each replicate

[1] Add 4 L sterile 0.85% saline to a sterile 12 qt bowl. Take a 1.1 mL sample to check for contaminants in the inoculation liquid. Add 13 wieners to a sterile, stainless steel fry strainer and completely immerse the wieners in the saline for 2 min. Place the inoculated wieners on a sterile rack in a clean air bench and air dry the wieners for 5 min (rotate after approximately 2.5 min). Repeat the dipping and drying procedure to obtain the required number of inoculated wieners.

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Aseptically transfer two inoculated wieners to a vacuum package bag (high oxygen barrier film) and package with a CVP Fresh Vac A-300 vacuum packaging machine. Repeat until all wieners are packaged. Store all packages in a 5°C walk-in cooler. Monitor temperature with a temperature logger (Delphi Inc.). Print reports weekly to obtain average cooler temperature and to monitor any fluctuations in storage conditions.

[2] Same as treatment 1 except that 400 µL (Rep. 1) or 5 mL (Reps. 2 and 3) of *Listeria* cocktail was added to the 4 L sterile saline dip and mixed for 2 min prior to the dipping of the wieners. A 1.1 mL sample was taken from the inoculated saline dip to check for the inoculum level and for any potential contaminants.

[3 to 8] Same as treatment 1 except that 4 mL (Rep. 1) and 10 mL (Reps. 2 and 3) of *C. piscicola* UAL8, UAL8C2, UAL26, UAL26/8A, UAL307, UAL682, separately, was added to the 4 L sterile saline dip and mixed for 2 min prior to the dipping of the wieners. A 1.1 mL sample was taken from each inoculated saline dip to check for inoculum level and any potential contaminants.

[9 to 14] Same as treatments 3 to 8 except that 400 µL (Rep. 1) or 2 mL (Reps. 2 and 3) of *L. monocytogenes* cocktail was added to the 4 L sterile saline dip and mixed for 2 min prior to the dipping of the wieners. A 1.1 mL sample was taken from the inoculated saline dip to check for inoculum level and any potential contaminants.

4. Sampling and Plating

- Vacuum packages were opened by wiping the outside of the bag with 70% ethanol and opening the bag with flame-sterilized scalpels. A wiener sample (10 cm^2 in surface area, based on measured diameter of the wiener as 1.8 cm) was placed in a sterile, "filter"-stomacher bag and blended for 2 min with 90 mL of sterile 0.1% peptone. Serial dilutions in sterile 0.1% peptone were spread-plated on APT agar and PALCAM selective agar plates.
- APT plates were incubated in anaerobic jars (10% CO_2) at 25°C for 48 h. PALCAM plates were incubated, aerobically at 37°C for 48 h. All colonies on PALCAM plates with a black circumference were counted as presumptive *L. monocytogenes*. Each wiener in the package was sampled, resulting in duplicate counts (wiener A and B) for each sampling time within each replicate.
- Sampling schedule included the initial production day (week 0), weeks 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12. After bacterial sampling the pH of the blended samples was measured.

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Results

An indigenous background microflora was not detected on the uninoculated (control) wieners. The initial pH of the wiener samples was 6.8. It decreased to about 6.0 over the 84-day course of the study.

Figure 1. – Growth of lactic acid bacteria on wieners for each of three replicates.
The data show reasonable similarity between replicates. The difference in inoculum level between rep. 1 and reps. 2 and 3 resulted from a lower than expected inoculum level in rep 1 and a planned modification of the experimental protocol to achieve the higher inoculum levels in reps. 2 and 3. Strain UAL8 in rep. 2 did not grow as well as the other strains but this was not the cause of the poor performance of UAL8 in these trials.

The LAB followed the expected pattern of growth on the product. There was a lag phase of one week for LAB in rep. 1 but no lag phase beyond the first week of the study for reps. 2 and 3. Cultures reached maximum population within 42 to 49 days (rep. 1) and 35 days (reps. 2 and 3). The study was continued for 84 days. There was no decrease in the LAB counts by the end of the study and there was no detectable overt spoilage (colour or odour).

Figures 2 to 4 – Growth of L. monocytogenes on the wieners compared with growth of the Listeria control.
The data are separated into three graphs for ease of comparison against the *Listeria* control: [1] UAL8 and UAL8C2; [2] UAL26 and UAL26/8A; [3] UAL307 and UAL682

[1] Data for the growth of *L. monocytogenes* in the presence of *C. piscicola* UAL8 and UAL8C2 (the nonbacteriocin producing variant of UAL8) are shown in Figure 2. Strain UAL8 failed to inhibit the *Listeria* except in rep. 3. In fact there was only a minor difference in the growth of the *Listeria* in the presence of UAL8 compared with UAL8C2.

[2] Data for the growth of the *L. monocytogenes* cocktail in the presence of *C. piscicola* UAL26 and UAL26/8A (the genetically improved strain combining UAL26 and carnobacteriocin A from UAL8) are shown in Figure 3. UAL26 failed to inhibit the *Listeria* in rep. 1 but growth was limited in the presence of UAL26 in reps. 2 and 3. The genetically modified variant UAL26/8A inhibited the growth of *L. monocytogenes* and, over time, it resulted in a notable decrease in the count of *L. monocytogenes* on the wieners.

[3] Data for the growth of the *L. monocytogenes* in the presence of *C. piscicola* UAL307 and UAL682 are shown in Figure 4. UAL307 gave excellent control of *L. monocytogenes* in all three replicates. UAL682 gave good results but not as impressive as strain 307. The data in Figure 4 indicate that an inoculum of 10^4 LAB per cm^2 could be important. Differences between rep. 1 and reps. 2 and 3 indicate that the difference in inoculum level could be a factor in the rate of die-off of the *Listeria*.

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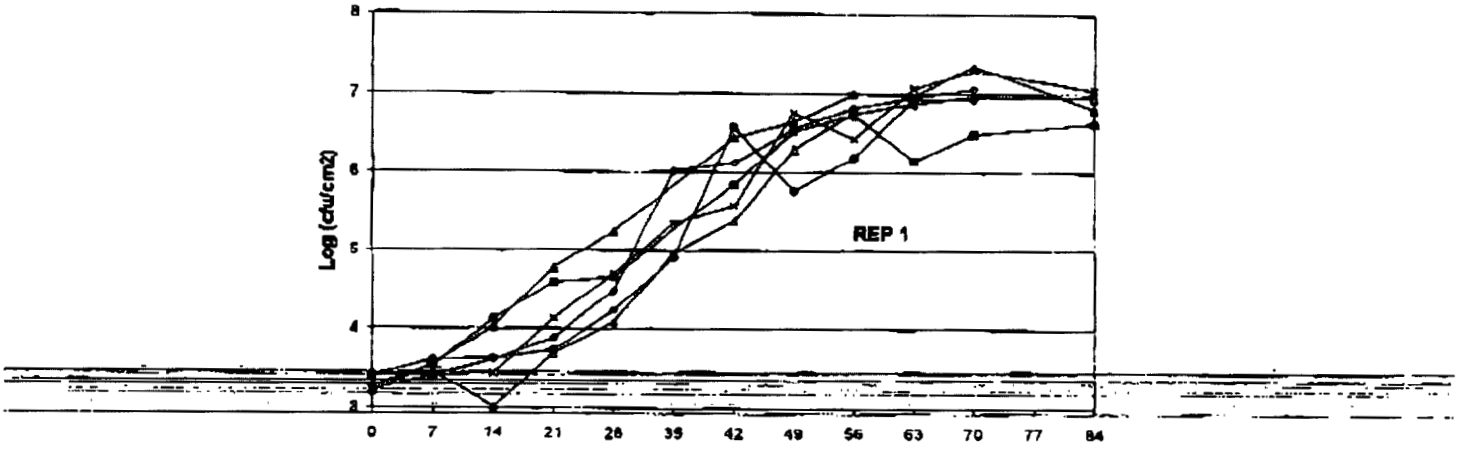

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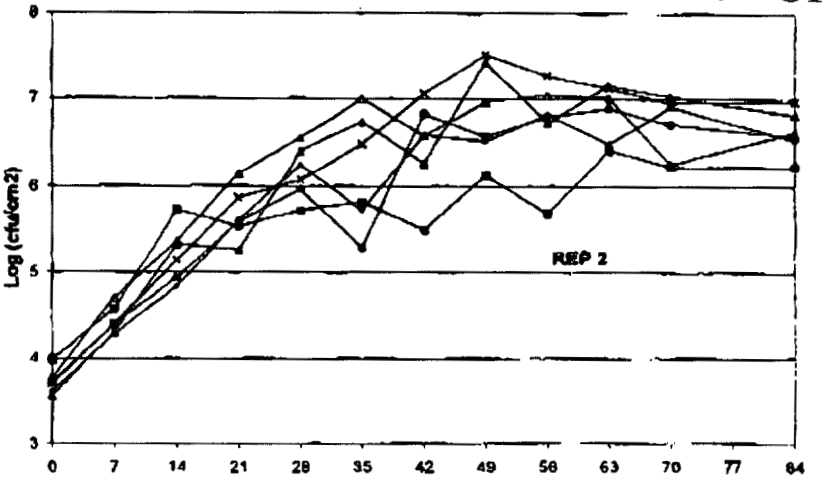


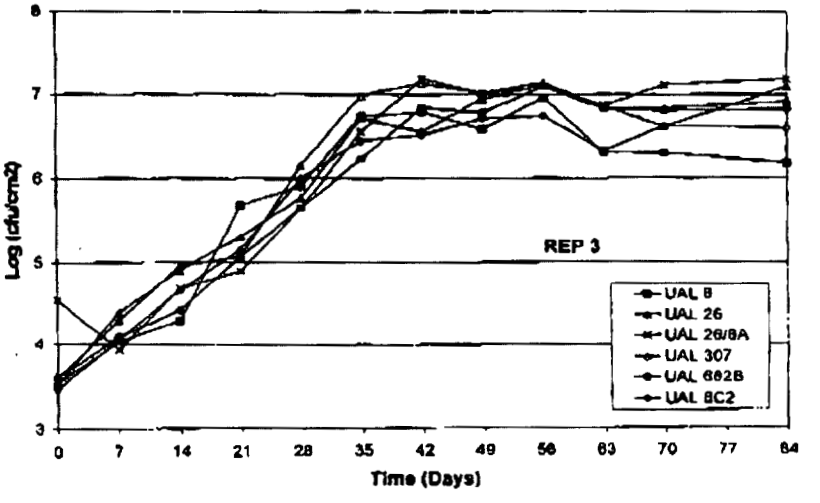
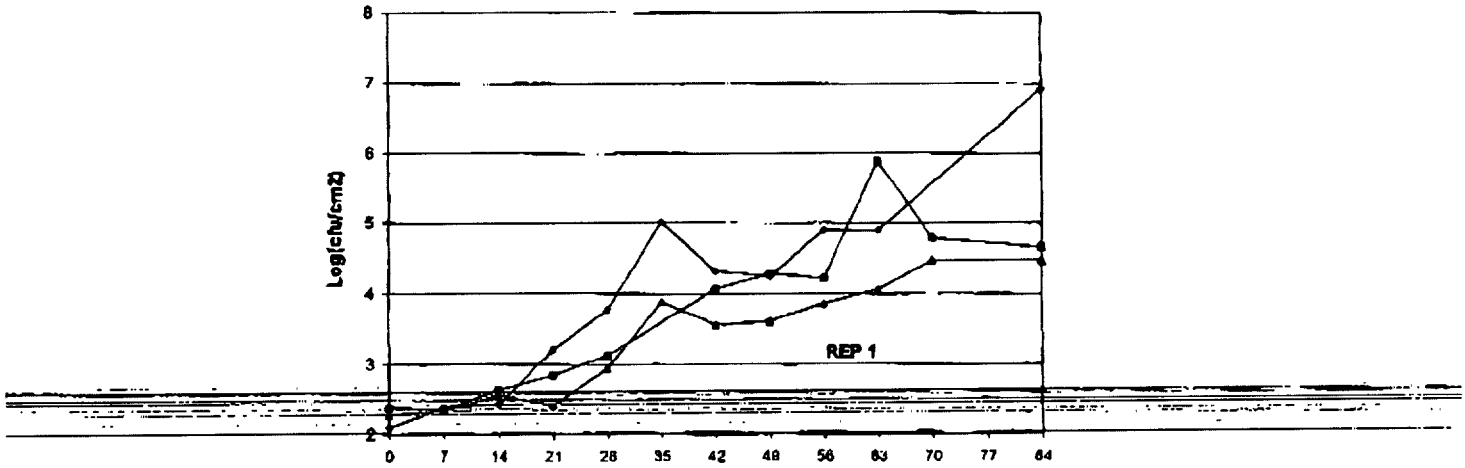
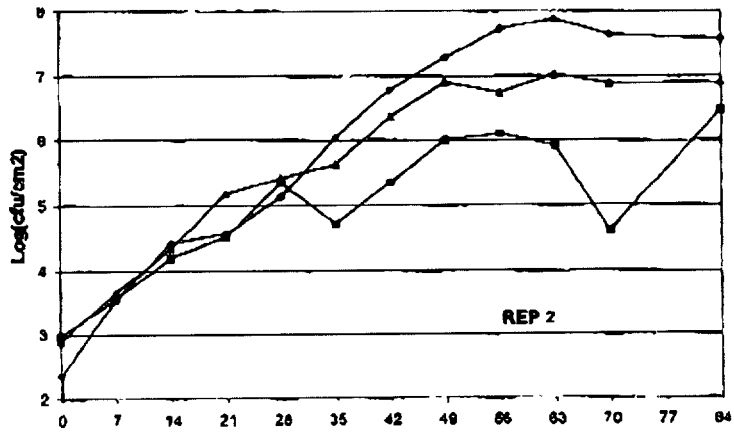


Figure 1: Lactic acid bacteria count of wieners stored at 5°C determined on APT agar plates incubated anaerobically at 25°C.





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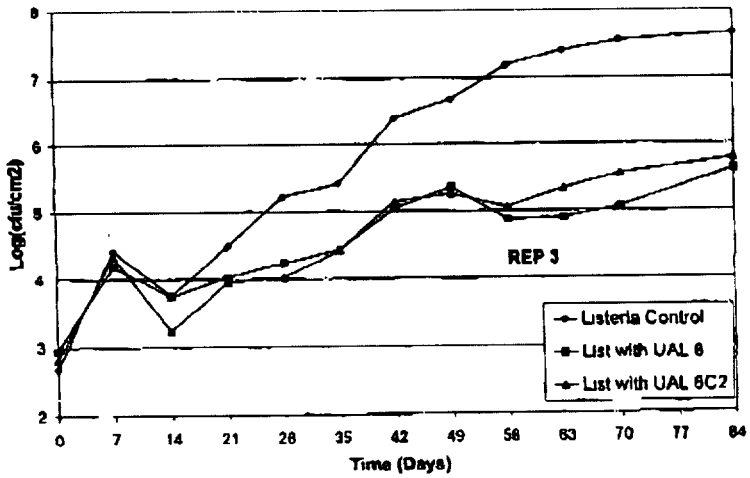
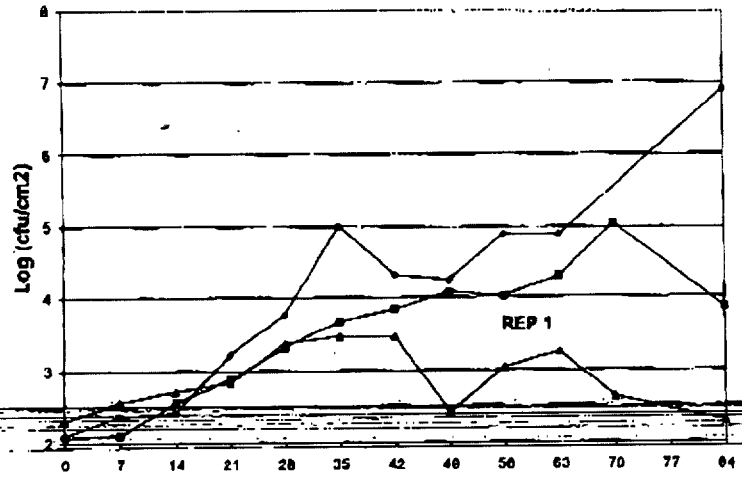
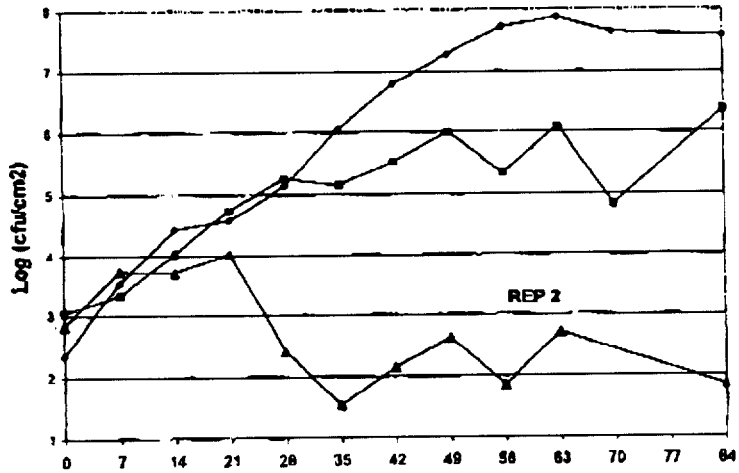


Figure 2: Growth/Survival of *Listeria monocytogenes* determined on PALCAM in the presence of *Carnobacterium piscicola* UAL8 and UAL8C2





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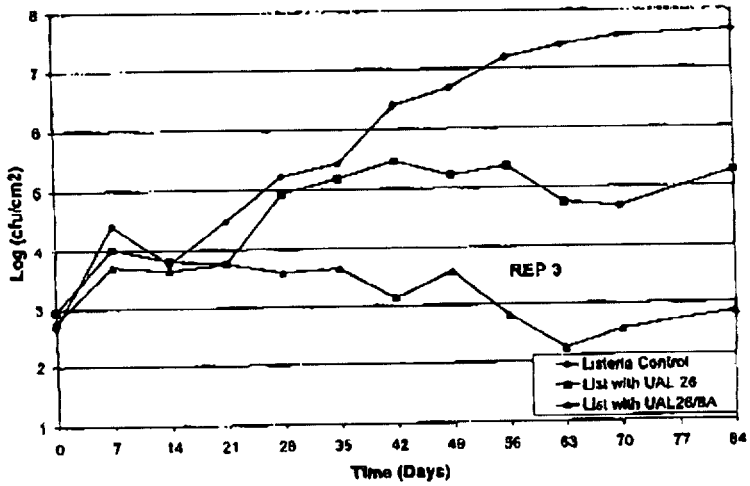

Figure 3: Growth/Survival of *L. monocytogenes* determined on PALCAM in the presence of *C. piscicola* UAL26 and UAL26/8A

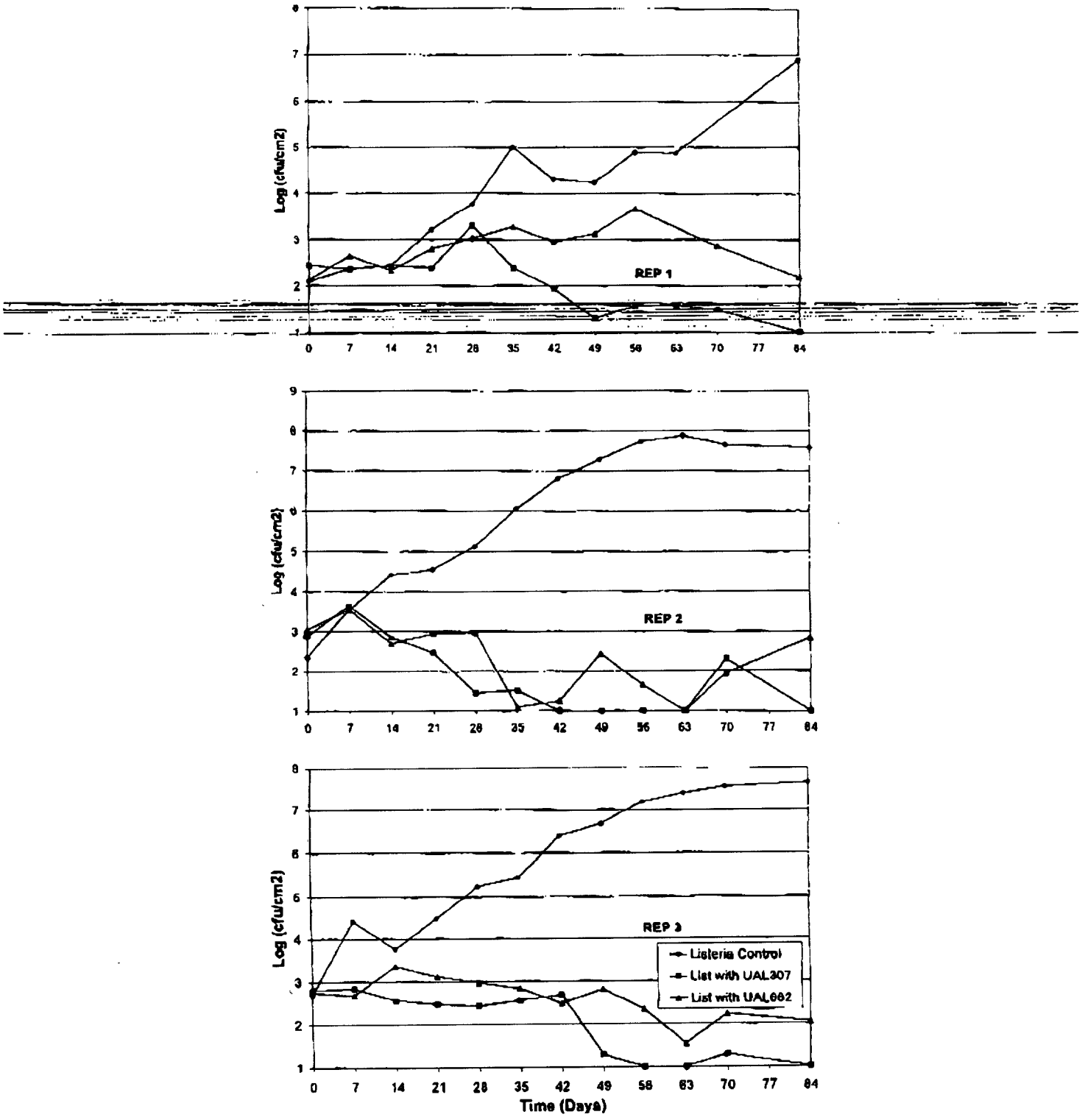

Figure 4: Growth/Survival of *L. monocytogenes* determined on PALCAM in the presence of *C. piscicola* UAL307 and UAL682B

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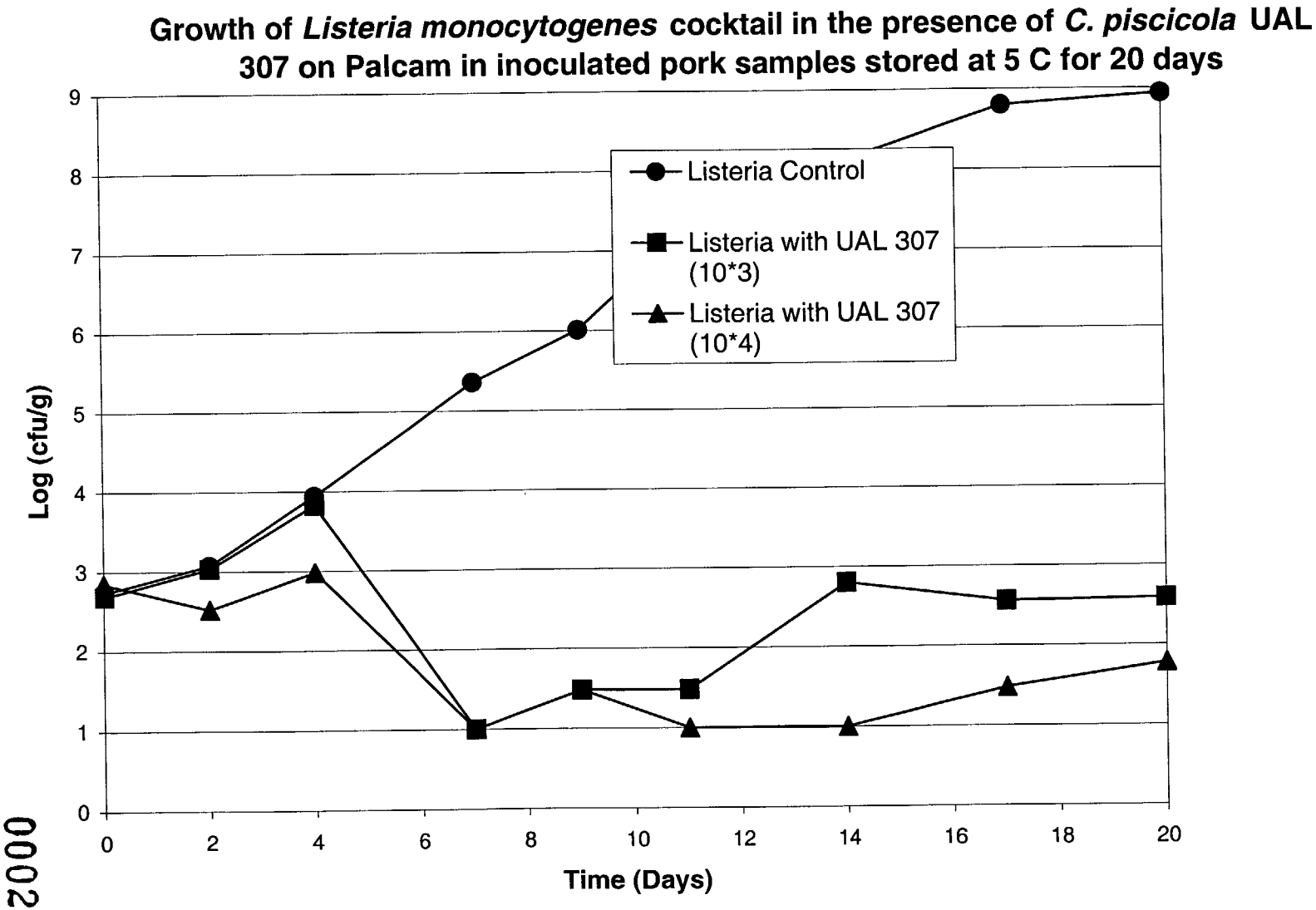
Inhibition of *Listeria monocytogenes* by *Carnobacterium piscicola*

Note: In order to protect proprietary rites, Griffith Laboratories has renamed the *Carnobacterium maltaromaticum* (previously named *Carnobacterium piscicola*) strain stated in the following data ("UAL307") to the new designation "CB1." Therefore, the *C. maltaromaticum* strain UAL307 is synonymous with *C. maltaromaticum* CB1. The term "UAL307" is Business Confidential information, and should be maintained as such.

Inhibition of *Listeria monocytogenes* by *Carnobacterium piscicola*



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Appendix B

The following pages contain specification sheets for dextrose monosaccharide (Cerelose® Dextrose 020010), maltodextrin (Star-Dri® 100 Maltodextrin), anhydrous disodium phosphate, hydrolyzed soy protein (Vegamine #28), and Maxarome® autolyzed yeast extract powder.

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PRODUCT SPECIFICATION

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CANADA COLORS & CHEMICALS LTD.
60 SCARSDALE ROAD
DON MILLS, ONTARIO
M3B 2R7

Maxarome® Powder

Product Code: 5085

55 Pound Poly-lined Box

Product Description: Maxarome® Powder is a unique autolyzed yeast extract designed specifically for flavor enhancement. Maxarome contains 2% naturally occurring active flavor enhancing nucleotides. Maxarome imparts a sweeter, meatier flavor that will not interfere with other delicate flavor systems. Maxarome can replace MSG at a ratio of 3:1 (MSG:Maxarome).

Appearance: Free flowing powder.

Analytical Information

Chemical	Average
Total Solids	97.5%
pH (2% solution)	5.5
NaCl	38.0%

Microbiological	Maximum
Total Plate Count	10^4/g
Yeast and Molds	100/g
Coliform	10/g
E. coli	1/g
Salmonella	Negative by test

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Kosher Status: Certified by the Organized Kashruth Laboratories.

Storage: Store in cool dry place.

Shelf Life: One year from date of delivery when kept in above mentioned conditions.

Giat-brocades Savory Ingredients, Inc.
2009 Renaissance Blvd. • Suite B
King of Prussia, PA 19406
610-272-4040
800-662-4478
FAX: 610-272-2696

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APPENDIX A-1: RAW MATERIAL SPECIFICATIONS FOR MICOCIN II™ MANUFACTURE

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Date: 5/1/00
Supersedes: 12/5/98

product data

Cerelose® Dextrose 020010

Cerelose Dextrose, 020010, is a general purpose product for most food, beverage, and industrial uses.

Chemical and Physical Properties	Typical
Moisture, %	8.5
Dextrose Equivalent (D.E.)	>99.5
Dextrose (as D-glucose), % db	>99.2
Color	0.5
Color, ICUSMA	7.5
Clarity	>92.0
SO_2, ppm	<10
Typical Screen Test	
% on U.S.S. #20 (841 micron)	<2%

Sensory Data

Odor	No detectable foreign odor
Flavor	Clean, sweet taste

Microbiological Standards	Typical
Bacteria/g	<200
Yeast/g	<20
Mold/g	<20
Coliforms/g	<10
E. coli/30g	negative
Salmonella sp/100g	negative

Nutritional Data/100g	Typical
Calories	366
Total Solids, g	91.5
Total Carbohydrate, g	91.5
Simple sugars, g	91.5
Other Carbohydrate, g	0
Ash	Trace

There are no fat, protein, fiber, vitamins, or minerals (including sodium) of dietary significance.

Certification

Kosher pareve

Packaging and Storage

50 lb (22.7 kg) bags
100 lb (45.4 kg) bags
25 kg (55 lb) bags
Drum
Bulk Bags
Bulk Car
Bulk Truck

Dextrose in bags will keep indefinitely in a clean, dry area when not exposed to high (>90°F/32°C), prolonged temperature.

Regulatory Data

CAS no. 14431-43-7

United States	
Standard of Identity	21 CFR 168.111
GRAS Affirmation	21 CFR 184.1857
USDA	9 CFR 318.7 & 381.147
Labeling	Dextrose Dextrose monohydrate

Canada	
Standard Food	CFDA Regulation B.18.015
Labeling	Dextrose Dextrose monohydrate

Features and Benefits

Dry crystalline powder
Free flowing
> 99.2 % monosaccharide as alpha-d-glucose
Consistent particle size
High sweetening
Flavor enhancement; non-masking
Bulking
Carrying
Browning
Highly fermentable
High osmolarity
High reducing capacity

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CornProducts INTERNATIONAL
6500 South Archer Ave.
Bedford Park, IL 60501-1933
United States
(708) 563-2400

CASCO
401 The West Mall, Suite 400
Etobicoke, Ontario M9C 5P7
Canada
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APR. 24. 2001 8:45AM A. E. STALEY/TLNA C. S. NO. 4077 P. 4/4

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A.E. Staley Manufacturing Company 2200 E. Eldorado Street Decatur, Illinois 62521 Telephone 217/423-4411

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STAR-DRI® 100 MALTODEXTRIN

Routine Tests	Specifications	Test Numbers
Moisture	6.0% max.	46550
Dextrose Equivalent	9-12%	25617
Flavor	7 - 10	31071
PH	4.0 - 4.7	60550
Foreign Matter	5 ppm max.	32557
Haze	7.5 max.	37505
Color	10 max.	22568
Total Bacteria Count	1000 max/gram	10560
Yeast	50 max/gram	97010
Mold	50 max/gram	47010
Salmonella	Negative	10547
Foam	Pass/Fail	32005
Screen Analysis (10 Min. Ro-Tap):		
On U.S. #60	10% max.	69528
Through U.S. #100	80 – 100%	69550
Through U.S. #325	Record	69560

FDA Status
Complies with FDA Regulation 21 CRF 184.1444 as Maltodextrin - Generally recognized as safe.

Recommended Storage Conditions
Store at ambient temperature and humidities. Storage in a cool, dry area will increase shelf life.

Shelf Life
Twelve months.

1/9/2001

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DISODIUM PHOSPHATE, ANHYDROUS (DSPA/A), POWDER FCC GRADE

FCC Grade Disodium Phosphate, Anhydrous complies with the specifications of the current Food Chemicals Codex for Sodium Phosphate, Dibasic.

Key Properties:

- Buffering Agent
- Chelating Agent

Applications:

Buffer for processed foods.
Stabilizer for milk and dairy products during processing.
Prevent color loss during heat treatment
Produces texture in processed cheese.
Animal Feed.
pH adjustment for pasta and cereal processing.

Generally Recognized as Safe (GRAS) when added directly to human food under 21 CFR 182.1778, 182.5778, and 182.8778. Food grade sodium Phosphate (mono-, di-, tri-) is generally recognized as safe when used in accordance with good manufacturing practices.

FOR MORE COMPLETE INFORMATION ON PROPERTIES AND SAFE HANDLING OF THIS MATERIAL, SEE THE MATERIAL SAFETY DATA SHEET (MSDS).

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PRAYON, INC

Corporate Office:

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P.O. Box 1473
Augusta, GA 30903
Phone 706-798-4346
Fax 706-798-0015

Shipping Location:

Prayon, Inc
1610 Marvin Griffin Road
Augusta, GA 30906
Phone 706-771-9320
Fax 706-771-9390

For order assistance, please call our Customer Service Department Toll Free: 1-800-477-2966

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INFORMATION

PRAYON Inc.
P.O. BOX 1473
AUGUSTA, GEORGIA 30903
PHONE: (706) 771-9320
FAX: (706) 771-9390

PRAYON

PRODUCT DATA SHEET

9731 6491
104 200

GRIFFITH LABORATORIES

Disodium Phosphate, Anhydrous, Powder

(DSP/A)

GRADE	CODE NUMBER	DATE EFFECTIVE
FCC	118	August 3, 2001

GENERAL DESCRIPTION
White, free-flowing, powder product

FORMULA	MOLECULAR WEIGHT	CAS NUMBER
Na_2HPO_4	142	7558-79-4

Chemical and Physical Properties	Specifications
Assay, % Na_2HPO_4	98.0% Minimum
P_2O_5	49.0% – 51.0%
pH (1% solution)	8.5 – 9.5
Loss on Drying	5.0% Maximum
Insoluble Matter	0.2% Maximum
Arsenic (as As), ppm	3 Maximum
Fluoride, ppm	50 Maximum
Heavy Metals (as Pb), ppm	10 Maximum
Sizing, USSS:	
Cumulative Retained on	
20 Mesh (840 µm)	0.2 % Maximum
100 Mesh (149µm)	10 % Maximum
200 Mesh (74µm)	40 % Minimum

Shelf Life: 24 months

NOTE: Specification Limits are subject to change. Please contact Prayon for the most current data sheet.

Packaging: 50 lb. multiwall bags; 2000 lb supersack
Labeling Requirements: Product Label required by law.
Shipping Classification: Sodium Phosphate
Handling Precautions: No precautionary statement required on label.

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Handle in accordance with good industrial hygiene and safety practices. These include avoiding unnecessary exposure and removal of material from eyes, skin, and clothing.

This Product Data Sheet is subject to the terms and conditions on the reverse side hereof.

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8243701

INNOVATION IN TASTE
INNOVA
A Griffith Laboratories Company

Effective Date: August 8, 2001
Supercedes: February 16, 2000

Scanned Sept 7/01

SPECIFICATION

Product Name: Vegamine 28D
Product Number: 020-0029
Prepared by: Susan Vaught, Manager, Regulatory Compliance
Authorized by: Paul A. Traci, Vice President Research & Development/Quality Assurance

Legislation: I certify that the Innova/Griffith Laboratories product listed below contains the ingredients stated. All ingredients contained in this product are approved for use in a regulation of the U.S. Food and Drug Administration.

Ingredient Statement: Hydrolyzed Soy Protein with a maximum of 2% Partially Hydrogenated Soybean Oil. Kosher/Pareve certified.

Product Properties (*Critical Properties):

Description	Target ± Tolerance	Test Method
*Physical Properties		
Appearance	Dark tan powder	GL QA 2
Chemical Properties		
Ash	56.0 ± 2.0	AOAC 32.1.05 (17th Ed.)
Organic Solids	42.0 ± 2.0	Calculation
*Total Chlorides (as Sodium Chloride)	44.0 ± 2.0	AOAC 42.1.15 (17th Ed.)
Protein (N x 6.25)	30.0 ± 3.0	AACC 39.1.15 (17th Ed.)
*pH (1% Solution)	5.5% ± 0.3	AOAC 42.1.04 (17th Ed.)
*Moisture	4.0% maximum	AOAC 44..2.03 (17th Ed.)
Granulation (U.S. Series) thru #16	95% minimum	GL QA/RM/FP 10/ASTA 10
Microbiological Properties		
*Total Plate Count	10,000 cfu/g maximum	BAM, App. 1.10, (8th Ed.)
*Coliforms	50 cfu/g maximum	BAM, 4.11 (8th Ed.)
*E.Coli	Negative	BAM, 4.11 (8th Ed.)
*Yeast & Mold	50 cfu/g maximum	AOAC17.2.08 (17th Ed.)
*Salmonella	Negative	BAM, App. 1.06 (8th Ed.)

Note: * Critical Property: Only these are analyzed per lot, and appear on a COA.

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Product Name: Vegamine 28D
Product Number: 020-0029
Page 2

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Packaging Properties:

Description	Target ± Tolerance	Test Method
Liner Construction		
Polyethylene -	4 mil	
LDPE and/or LLDPE	29 x 49"	
Case Construction		
Flute	BC	
Style	RSC	
Case Dimension	15-3/8 x 12¾ x 12¼"	
Case Cube	1.57 ft^3	Measurement
Pallet Stack		
Cases/Layer	9	Count
Layers/Load	4	Count
Cases/Load	36	Count
Case Pack		
Packaged	50 lb carton	Count
Net Weight	50 lb ± 8 oz	Count
Packaged	25 kilograms (55#)	
Net Weight	25 kilograms ± 0.23 kg	

Storage / Shipping: Product should be held at cool (60-70°F / <60% R.H.), clean/dry conditions during both storage and shipment. All transoceanic shipments shall be appropriately blocked and braced at the fill door to prevent product shifting and damage.

Shelf-Life: Up to 360 days when held at recommended conditions.

Codes/Labeling: Chicago Plant Coding
Lot = 4 Digit Lot Number/Month/Day/Year/4 digit carton number
Example: Lot 0016 Jan 01 97 0001

Nutritional (based/100 grams): Nutritional data available upon request.

Manufacturing Plant Location: Chicago, IL

Paul A. Traci, Ph.D.
Vice President
Research & Development/
Quality Assurance

000252

BURDOCK GROUP
Consultants

2001 9th Ave – Suite 301
Vero Beach, FL 32960
Telephone: 772-562-3900
Facsimile: 772-562-3908

888 – 17th Street, N.W. – Suite 810
Washington, DC 20006
Telephone: 202-785-8200
Facsimile: 202-785-8666
gburdock@burdockgroup.com

AM

April 8, 2005

Dr Robert Merker
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RECEIVED
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OFFICE OF
FOOD ADDITIVE SAFETY

Jeffrey W. Canavan, RD, LD
Labeling and Consumer Protection Staff
USDA, FSIS, OPPED, LCPS
1400 Independence Avenue
Room 614 Annex Building
Washington, DC 20250-3700

RE: GRN Notice #000159 – Clarification Letter

Dear Sirs,

This letter is to amend the current GRAS dossier[1] entitled "DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SPECIFIC STRAINS OF *Carnobacterium maltaromaticum* AS A FOOD INGREDIENT," which requests the addition of *C. maltaromaticum* strains CB1, CB2, CB3, LV17, UAL26, ATCC 35586 and ATCC 43225 to ready-to-eat (RTE) meat products and fresh comminuted, processed meat products. Several

[1] GRAS Notification Letter dated October 5, 2004.

points have been raised concerning this GRAS dossier, and based on the conversation of April 5, 2005,[2] we submit the following clarification amendments to the pending GRAS Notification (GRN Notice #000159).

This clarification addresses two issues that were presented by the FDA and the USDA:

1. The FDA has submitted the GRAS Notification and dossier to the USDA, as the GRAS Notification requests the addition of *C. maltaromaticum* to meat.[3] Representatives of the USDA's Food Safety Inspection Service (FSIS) stated that there is not adequate information on the suitability of use of all the strains indicated in the current GRAS dossier. The USDA stated that there is enough information on the suitability of use for strain CB1. However, suitability documentation would be needed on the other strains, in order to maintain them on this GRAS dossier.
2. The FDA has indicated that clarification is needed to determine distinct identity of each of the strains indicated in this GRAS dossier. Representatives of the FDA indicated that the GRAS Notice did not give enough information that distinguishes these strains from other strains of *Carnobacterium maltaromaticum*.

1. Suitability of the use of distinct strains of *Carnobacterium maltaromaticum* for use in meat products.

The USDA and FDA have already received information concerning the efficacious use of *Carnobacterium maltaromaticum* in ready-to-eat (RTE) meat products (facsimile dated 23FEB05), which was incorporated into an Amendment Letter dated 06APR05. This Clarification Amendment Letter included summaries of four efficacy studies, A. "Growth Characteristics of *C. piscicola* on Vacuum Packaged Wieners," B. "Sausage Inoculation Trials," C. "Challenge Study: *Listeria monocytogenes* on a Ready-to-eat Meat Product," and D. "Inhibition of *Listeria monocytogenes* by *Carnobacterium piscicola.*" This documentation included sensory data, indicating that the presence of *C. maltaromaticum* CB1, LV17 or UAL 26

[2] Teleconference between members of the FDA, USDA/FSIS, and the Burdock Group.
[3] US Department of Agriculture. Food Safety and Inspection Service. Labeling and Consumer Protection. Memorandum of Understanding (http://www.fsis.usda.gov/OPPDE/larc/Ingredients/MOU.htm); site visited 13JAN05.

do not increase the rate of spoilage of ready-to-eat meat products. Growth curve data was also presented, indicating that the addition of *C. maltaromaticum* CB1, LV17 or UAL 26 do not increase the overall number of lactic acid bacteria. In addition, competition data was presented, indicating that *C. maltaromaticum* CB1, LV17 or UAL 26 inhibits the growth of *L. monocytogenes*. This data has been prepared for Griffith Laboratories (the Notifier), who wishes this material to be maintained as "Business Confidential."

2.Confirmation of the distinct identities of the selected strains of *Carnobacterium maltaromaticum*.

Questions were raised by the members of the FDA concerning the ability to discriminate between the specific *C. maltaromaticum* strains that have been presented in this GRAS dossier. An FDA representative (Dr. Merker) indicated that the GRAS Notice (GRN No. 159) has not given enough information that distinguishes the strains in this GRAS Notice from other strains of *C. maltaromaticum*. Dr. Merker indicated that as two of the strains indicated in the GRAS (*i.e.*, ATCC 35586 and ATCC 43225) have been deposited in an internationally recognized repository of bacterial strains (American Type Culture Collection),[4] these strains have been acknowledged as being distinct entities, by virtue of having been deposited in this repository (Appendix I). A distinguishing characteristic for each strain deposited in the ATCC database is the source of the bacteria (*e.g.*, origin of the bacterial isolate). For example, ATCC strain 35586 was isolated from the kidney of an adult cutthroat trout (*Salmo clarki*), and was deposited by SF Hiu (Appendix I). ATCC strain 43225 was isolated from vacuum-packed lamb, and was deposited by BG Shaw and CD Harding (Appendix I). LV17 has also been deposited in a bacterial repository, the National Collection of Industrial and Marine Bacteria (currently known as the United Kingdom National Culture Collection), under the strain number 702852.[5] This strain was deposited by BG Shaw, and was isolated from vacuum-packed pork (Appendix I).

C. maltaromaticum strains CB1, CB2, and CB3 have also been deposited in the ATCC, under the "Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure" (Appendix I). The confidential information that was

[4] American Type Culture Collection; www.atcc.org; site visited 07APR05.
[5] United Kingdom National Culture Collection; www.ukncc.co.uk/databases/Data.asp?DATA=201842 ; site visited 07APR05

submitted, and accepted by ATCC, stated that *C. maltaromaticum* strain CB1 was isolated from fresh pork, while strain CB2 was isolated from fresh pork sausage, while strain CB3 had been isolated from fresh chicken (Appendix I). This information (confidential, for patent purposes) indicates that an internationally recognized bacterial repository recognizes these strains as being distinct from other *C. maltaromaticum* strains already on deposit at the ATCC.

LV17 has been reported to produce carnobacteriocins A, BM1 and B2 (Allison *et al.*, 1995). As indicated by a table produced for Griffith Laboratories (Table 1), *C. maltaromaticum* strains CB1 and CB3 do not contain the genes necessary to produce carnobacteriocin B2, and therefore CB1 and CB3 are distinctly different from LV17 ("Business Confidential").

Table 1. Detection of genes for bacteriocin production in different strains of *Carnobacteria spp.*

Strain	CbnA	CbnB2	CbnBM1	Pis126	Unknown
LV17 (synonym UAL8)	+	+	+		
UAL26	--		+	+	
JG126 (Australian source			+		
A9b (Nilsson *et al*, 1999)					
CB1		--	+	+	+
CB2			+	+	+
CB3		--	+	+	

Certain *Carnobacterium* strains have been probed, and the gene for bacteriocin production was detected (+), or was not (--). Blank spaces denote that we (or others, to the best of our knowledge) have not tested for the presence of the gene encoding bacteriocin production. We cannot say with absolute certainty that it is negative, unless a "-" is noted.

In addition, randomly amplified polymorphic DNA (RAPD) profile analysis[6] has indicated that CB1, CB2 and CB3 have different chromosomal separation points (Figure 1 and Figure 2, Appendix II), further indicating that these strains are distinct from one another, and distinct from other bacteria (*e.g.*, LV17). RAPD analysis has been utilized for the characterization of many different bacterial species and sub-species, including strain-specific

[6] In RAPD analysis, patterns are generated by the amplification of random DNA segments with single 10-mer primers. The primer is not targeted to amplify any specific bacterial sequences and will hybridize at multiple random chromosome locations and initiate DNA synthesis. After agarose gel electrophoresis separation of the amplified DNA fragments, a pattern of bands will be produced, which is characteristic of that particular bacterial strain (Morea *et al.*, 1999).

differentiation of Lactococci in mixed starter cultures (Baruzzi *et al.*, 2002; Erlandson and Blatt, 1997). In addition, this data has been reviewed by Dr. Douglas L. Archer of the University of Florida, Department of Food Science and Human Nutrition, who agrees with the conclusion that CB1, CB2, and CB3 are uniquely characterized strains of *Carnobacterium maltaromaticum* (Appendix III). Griffith Laboratories requests that the technical information regarding these strains be kept "Business Confidential."

Summary

In summary, the previously submitted efficacy data indicate that the addition of *C. maltaromaticum* strain CB1 does not alter the sensory qualities of the RTE meat products, does not increase the overall level of lactic acid bacteria growing on RTE meat products, and inhibits the growth of *L. monocytogenes*. In addition, strains CB1, CB2 and CB3 have been deposited to the ATCC, and have been indicated as being isolated from unique food sources. In addition, the detection of genetic material for the production of specific carnobacteriocins indicates that *C. maltaromaticum* strains CB1, CB2 and CB3 differ from strain LV17 in their ability to produce various carnobacteriocins. Finally, chromosomal digestion analysis indicates that *C. maltaromaticum* strains CB1, CB2, and CB3 differ in their digestion points.

Overall, this data indicates that there is efficacy data for CB1, as well as sufficient data to characterize this bacterium as a specific strain entity. Therefore, we request that *C. maltaromaticum* strains CB2, CB3, UAL 26, LV17, ATCC 43225 and ATCC 35586 be deleted from this GRAS Notice.

If you have any further questions regarding these or any other topics concerning the GRAS dossier of the addition of *Carnobacterium maltaromaticum* to ready-to-eat meat products, please contact me.

Sincerely,

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

Appendix I

The following pages contain information regarding the deposition of various strains of *Carnobacterium maltaromaticum* into internationally recognized culture depository organizations.

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Customers in Europe, Australia, Japan, Hong Kong, Korea, New Zealand, Singapore and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Bacteria

ATCC® Number:	**43225™** Order this item	**Price:**	**$198.00**
Organism:	*Carnobacterium maltaromaticum* (Miller et al.) Mora et al.; deposited as *Lactobacillus carnis* Shaw and Harding		
Designations:	LV61 [DSM 20722; JCM 9135; LMG 9840; NCDO 2764]	**Isolation:**	vacuum-packed lamb
Depositors:	BG Shaw		
Biosafety Level:	1	**Shipped:**	freeze-dried
Growth Conditions:	ATCC medium 44: Brain heart infusion agar or brain heart infusion Temperature: 26.0C		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

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Related Products

Comments: taxonomy [8789]

References:

6042: Validation list no. 20. Int. J. Syst. Bacteriol. 36: 354-356, 1986.
7792: Shaw BG, Harding CD . Atypical Lactobacilli from vacuum-packaged meats: Comparison by DNA hybridization, cell composition and biochemical tests with a description of Lactobacillus carnis sp.nov.. Syst. Appl. Microbiol. 6: 291-297, 1985.
8789: Collins MD, et al. Classification of Lactobacillus divergens, Lactobacillus piscicola, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, Carnobacterium. Int. J. Syst. Bacteriol. 37: 310-316, 1987.
88827: Mora D, et al. Reclassification of Lactobacillus maltaromicus (Miller et al. 1974) DSM 20342(T) and DSM 20344 and Carnobacterium piscicola (Collins et al. 1987) DSM 20730(T) and DSM 20722 as Carnobacterium maltaromaticum comb. nov.. Int. J. Syst. Evol. Microbiol. 53: 675-678, 2003. PubMed: 12807185

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All prices are listed in U.S. dollars and are subject to change without notice. A discount off the current list price will be applied to most cultures for nonprofit institutions in the United States and Canada. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee. Fees for permits, shipping, and handling may apply.

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Carnobacterium & maltaromaticu Word Search Clear Search

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Product Description

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Bacteria

ATCC® Number:	**35586™** Order this item	**Price:**	**$198.00**
Organism:	*Carnobacterium maltaromaticum* (Miller et al.) Mora et al.; deposited as *Lactobacillus piscicola* Hiu et al.		
Designations:	B270 [DSM 20730; NCDO 2762]	**Isolation:**	kidney of adult cutthroat trout, Salmo clarki, Oregon
Depositors:	SF Hiu	**History:**	ATCC<<--SF Hiu <<--R.A. Holt
Biosafety Level:	1	**Shipped:**	freeze-dried
Growth Conditions:	ATCC medium 416: Lactobacilli MRS broth Temperature: 30.0C		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Products

References:

8657: Hiu SF, et al. Lactobacillus piscicola, a new species from salmonid fish. Int. J. Syst. Bacteriol. 34: 393-400, 1984.
8789: Collins MD, et al. Classification of Lactobacillus divergens, Lactobacillus piscicola, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, Carnobacterium. Int. J. Syst. Bacteriol. 37: 310-316, 1987.
88827: Mora D, et al. Reclassification of Lactobacillus maltaromicus (Miller et al. 1974) DSM 20342(T) and DSM 20344 and Carnobacterium piscicola (Collins et al. 1987) DSM 20730(T) and DSM 20722 as Carnobacterium maltaromaticum comb. nov.. Int. J. Syst. Evol. Microbiol. 53: 675-678, 2003. PubMed: 12807185

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UKNCC Data Sheet

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Carnobacterium piscicola

COLLECTION: NCIMB - National Collection of Industrial and Marine Bacteria
STRAIN NUMBER: 702852
STRAIN_TYPE: Bacterium
STRAIN_NAME: MR 371, LV 17, UP 204
EQUIVALENT_STRAINS: NCFB2852
DEPOSITOR: Gordon Shaw IFR, Bristol
ISOLATED_FROM: vacuum packed pork
REFERENCE: Shaw & Harding 1984
MEDIUM: yglpb medium; 22

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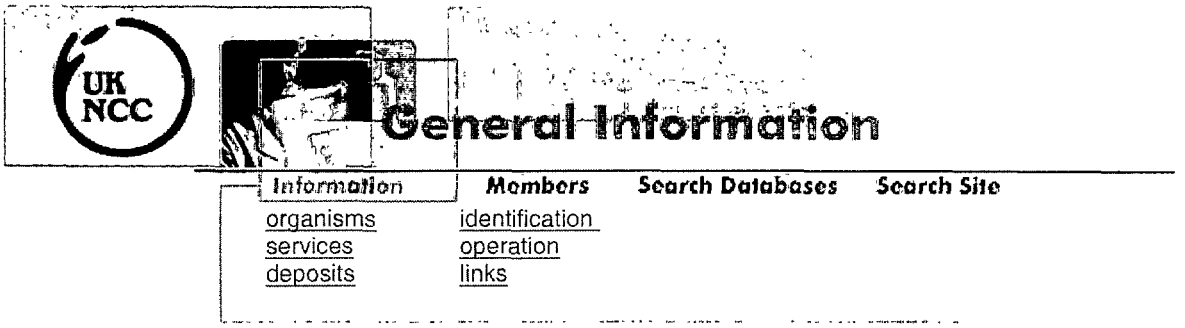

General Introduction

NEW - Catalogues and Publications from the UKNCC

NEW - UKNCC Research Projects

The United Kingdom National Culture Collection (UKNCC) co-ordinates the activities, marketing and research of the UK national service collections of microbial organisms. Information on UKNCC services and on other collection activities may be found through the menu items above.

UKNCC maintains a database of the combined strains available in each member collection. If you want to find a strain then you should search the UKNCC strain database. You should also look at the conditions relating to supply of strains and related information before placing an order. A number of UKNCC members also maintain ther own on-line catalogues which are available through their own websites. Links to members websites can be found through the links above.

Please fill in our questionnaire for more information about the activities of the UKNCC.

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Appendix II

The following pages contain confidential information, produced on behalf of Griffith Laboratories, concerning the characterization of the various strains of *Carnobacterium maltaromaticum*, for use in food production, as well as cited reference materials. Griffith Laboratories requests that this information be kept "Business Confidential."

Figure 1:

Figu

1 – 1
2 – (
3 – (
4 – (
5 – *L*
6 – (
7 - *L*
8 – *E*

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APPENDIX 2:

Figure 2:

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Appendix III

The following page contains a statement made by Dr. D.L. Archer, concerning the characterization of the *Carnobacterium maltaromaticum* strains CB1, CB2, and CB3.

UNIVERSITY OF FLORIDA

Institute of Food and Agricultural Sciences
Food Science and Human Nutrition Department

PO Box 110370
Gainesville, FL 32611-0370 USA
Telephone: (352) 392-1991
Fax: (352) 392-9467
E-mail: fos@mail.ifas.ufl.edu

April 8, 2005

Dr. Ray Matulka
The Burdock Group
2001 9th Ave., Suite 301
Vero Beach, FL 32960-6416

Dear Ray:

I reviewed the data you sent to me on several strains of *Carnobacterium maltaromaticum*, and based on the data as I see and interpret it, I agree with your conclusion that strains CB1, CB2, and CB3 are all *Carnobacterium maltaromaticum*, but each is a differentiable strain by several parameters, including RAPD analysis.

I further note that each of these strains has been deposited with the American Type Culture Collection as differentiable by source of the isolate, as well.

Please let me know if I can be of further assistance.

Sincerely,

Douglas L. Archer
Professor

Equal Opportunity/Affirmative Action Institution

Reference List for Industry Submission, GRN 000159

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000275 - 000281	Allison, G.E.; Worobo, R.W.; Stiles, M.E.; Klaenhammer, T.R.	Heterologous Expression of the Lactacin F Peptides by Carnobacterium piscicola LV17	April 1995	Applied and Environmental Microbiology	Volume 61, Number 4, pgs 1371-1377
000282 - 000289	Baruzzi, F.; Matarante, A.; Morea, M.; Cocconcelli, P.S.	Microbial Community Dynamics during the Scamorza Altamurana Cheese Natural Fermentation	2002	Journal of Dairy Science	Volume 85, Number 6, pgs 1390-1397
000290 - 000295	Erlandson, Karn; Batt, Carl A.	Strain-Specific Differentiation of Lactococci in Mixed Starter Culture Populations Using Randomly Amplified Polymorphic DNA-Derived Probes	July 1997	Applied and Environmental Microbiology	Volume 63, Number 7, pgs 2702-2707
000296 - 000304	Morea, M.; Baruzzi, F.; Cocconcelli, P.S.	Molecular and physiological characterization of dominant bacterial populations in traditional Mozzarella cheese processing	1999	Journal of Applied Microbiology	Volume 87, pgs 574-582

NA- Not applicable