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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Display Date	4.22.99
Publication Date	4.23
Certifier	C. WMS-DAT

Food and Drug Administration

21 CFR Part 184

[Docket No. 84G-0257]

Carbohyrase and Protease Enzyme Preparations Derived From *Bacillus Subtilis* or *Bacillus Amyloliquefaciens*; Affirmation of GRAS Status as Direct Food Ingredients

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is affirming that carbohyrase enzyme preparations derived from either *Bacillus subtilis* or *B. amyloliquefaciens* and protease enzyme preparations derived from either *B. subtilis* or *B. amyloliquefaciens* are generally recognized as safe (GRAS) for use as direct food ingredients. This action is a partial response to a petition filed by the Ad Hoc Enzyme Technical Committee (now the Enzyme Technical Association).

DATES: The regulation is effective (*insert date of publication in the Federal Register*). The Director of the Office of the **Federal Register** approves the incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51 of certain publications listed in 21 CFR 184.1148 and 184.1150, effective (*insert date of publication in the Federal Register*).

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I. Introduction

In accordance with the procedures described in § 170.35 (21 CFR 170.35), the Ad Hoc Enzyme Technical Committee (now the Enzyme Technical Association), c/o Miles Laboratories, Inc., 1127 Myrtle St., Elkhart, IN 46514, submitted a petition (GRASP 3G0016) requesting that the following enzyme preparations be affirmed as GRAS for use in food: (1) Animal-derived enzyme preparations: Catalase (bovine liver); lipase, animal; pepsin; rennet; rennet, bovine; and trypsin; (2) plant-derived enzyme preparations: Bromelain; malt; and papain; (3) microbially-derived

enzyme preparations: Lipase, catalase, glucose oxidase, and carbohydrase from *Aspergillus niger*, var.; mixed carbohydrase and protease from *Bacillus subtilis*, var.; carbohydrase from *Rhizopus oryzae*; and carbohydrase from *Saccharomyces* species.

FDA published a notice of filing of this petition in the **Federal Register** of April 12, 1973 (38 FR 9256), and gave interested persons an opportunity to submit comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. The petition was amended by notices published in the **Federal Register** of June 12, 1973 (38 FR 15471), proposing affirmation that microbially-derived enzyme preparations (carbohydrase, lipase, and protease) from *A. oryzae* are GRAS for use in food; in the **Federal Register** of August 29, 1984 (49 FR 34305), proposing affirmation that the enzyme preparations ficin, obtained from species of the genus *Ficus* (fig tree), and pancreatin, obtained from bovine and porcine pancreas, are GRAS for use in food; in the **Federal Register** of June 23, 1987 (52 FR 23607), proposing affirmation that the protease enzyme preparation from *A. niger* is GRAS for use in food; and in the **Federal Register** of August 5, 1996 (61 FR 40648), proposing affirmation that carbohydrase and protease enzyme preparations from *B. amyloliquefaciens* are GRAS for use in food. In the June 23, 1987, notice, FDA also noted the petitioner's assertion that pectinase enzyme preparation from *A. niger* and lactase enzyme preparation from *A. niger* are included under carbohydrase enzyme preparation from *A. niger*, and that invertase enzyme preparation from *Saccharomyces cerevisiae* and lactase enzyme preparation from *Kluyveromyces marxianus* are both included under carbohydrase enzyme preparation from species of the genus *Saccharomyces*. The agency further noted that, therefore, pectinase enzyme preparation from *A. niger*, lactase enzyme preparation from *A. niger*, invertase enzyme preparation from *S. cerevisiae*, and lactase enzyme preparation from *K. marxianus* were to be considered part of the petition. Interested persons were given an opportunity to submit comments to the Dockets Management Branch (address above) on each amendment.

After the petition was filed, the agency published, as part of its comprehensive safety review of GRAS substances, two GRAS affirmation regulations that covered three of the enzyme preparations from animal and plant sources included in the petition. These two regulations are: (1) § 184.1685 *Rennet* (animal derived) (21 CFR 184.1685), which was published in the **Federal Register** of November 7, 1983 (48 FR 51151) and includes the petitioned enzyme preparations rennet and bovine rennet; and (2) § 184.1585 *Papain* (21 CFR 184.1585), which was published in the **Federal Register** of October 21, 1983 (48 FR 48805). Thus, rennet, bovine rennet, and papain are already affirmed as GRAS and need not be addressed further.

In a notice published in the **Federal Register** of September 20, 1993 (58 FR 48889), the agency announced that the petitioner had requested that the following enzyme preparations be withdrawn from the petition without prejudice to the filing of a future petition: (1) Pancreatin used for its lipase activity, (2) pancreatin used for its amylase activity, and (3) amylase derived from unmalted barley extract. In that notice, the agency stated that, in light of the petitioner's request, any future action by FDA on the petition would not include a determination of the GRAS status of these three enzyme preparations.

In a final rule published in the **Federal Register** of June 26, 1995 (60 FR 32904), the agency affirmed as GRAS the following enzyme preparations derived from animal sources: Catalase (bovine liver), animal lipase, pepsin, trypsin, and pancreatin (as a source of protease activity). In that same final rule, the agency also affirmed as GRAS the following enzyme preparations derived from plant sources: Bromelain, ficin, and malt.

This final rule addresses the following bacterially-derived enzyme preparations: (1) carbohydrase enzyme preparation from *B. subtilis*; (2) protease enzyme preparation from *B. subtilis*; (3) carbohydrase enzyme preparation from *B. amyloliquefaciens*; and (4) protease enzyme

preparation from *B. amyloliquefaciens*.¹ The other microbial enzyme preparations in the petition will be dealt with separately in a future issue of the **Federal Register**.

II. Standards for GRAS Affirmation

Under § 170.30 (21 CFR 170.30) and 21 U.S.C. 321(s), general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either scientific procedures or, in the case of a substance used in food prior to January 1, 1958, experience based on common use in food. General recognition of safety based upon scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive and ordinarily is based upon published studies, which may be corroborated by unpublished studies and other data and information (§ 170.30(b)). General recognition of safety through experience based on common use in food prior to January 1, 1958, may be determined without the quantity or quality of scientific procedures required for approval of a food additive, and ordinarily is based upon generally available data and information concerning the pre-1958 use of the substance (§ 170.30(c)).

For the enzyme preparations from *B. subtilis* and *B. amyloliquefaciens* that are the subject of this document, the Enzyme Technical Association bases its request for affirmation of GRAS status on a history of safe food use prior to 1958. As discussed in the preamble to the proposed

¹ Although the petition requested GRAS affirmation for mixed carbohydrase and protease enzyme preparation from *B. subtilis*, the petitioner subsequently agreed that this enzyme preparation be evaluated as two separate enzyme preparations, carbohydrase enzyme preparation from *B. subtilis* and protease enzyme preparation from *B. subtilis*. Enzyme preparations that contain mixtures of carbohydrases and proteases can be used either for their carbohydrase activity or for their protease activity, and they are usually sold according to their intended use. FDA requested the petitioner's agreement to this change to reflect the distinct uses of mixed carbohydrase and protease enzyme preparations in food depending on whether a particular preparation is being used for its carbohydrase activity or for its protease activity.

rule for the most recent amendment to § 170.30, general recognition of safety through experience based on common use in food requires a consensus on the safety of the substance among the community of experts who are qualified to evaluate the safety of food ingredients (50 FR 27294 at 27295, July 2, 1985).

III. Background

A. Identity and Technical Effect

Enzymes are proteins that originate from living cells and produce chemical change by catalytic action (*Random House Dictionary of the English Language*, 1987). Most enzymes are very specific in their ability to catalyze only certain chemical reactions; this high degree of specificity and strong catalytic activity are the most important functional properties of enzymes (Ref. 1).

Commercial enzyme preparations such as those that are the subject of this document usually contain several enzymes that have catalytic activities other than those for which they are sold—i.e., other than their characterizing enzyme activities. As discussed in more detail in section III.B of this document, the methods of manufacture for a specific commercial enzyme preparation are tailored to maximize the characterizing enzyme activity. The other enzymes that are present in the preparation generally are present at low levels.

Carbohydrases, which are also known as glycosidases, are enzymes whose catalytic activity is the hydrolysis (i.e., splitting) of *O*-glycosyl bonds in carbohydrates. The carbohydrase enzyme preparations that are the subject of this document each contain two or more carbohydrases, including: (1) α -amylase, which hydrolyzes α -1,4-glucan bonds in polysaccharides (e.g., starch) yielding monosaccharides, linear oligosaccharides and branched oligosaccharides (dextrins), and (2) β -glucanase, which hydrolyzes 1,3 and some 1,4 linkages in β -D-glucans (polysaccharides that are common in cereals such as oats, barley, and rye), yielding oligosaccharides and glucose (Refs. 2 and 3). Because the major carbohydrase in the carbohydrase enzyme preparations derived from *B. subtilis* or *B. amyloliquefaciens* is α -amylase, the primary use of these enzyme preparations

is the hydrolysis of starch in processes such as the preparation of starch syrups and the fermentation of beer (Refs. 3 through 5).

Proteases are enzymes whose catalytic activity is the hydrolysis of peptide bonds in proteins, yielding peptides and amino acids. The protease enzyme preparations that are the subject of this document each contain two or more proteases, including subtilisin and neutral proteinase (Refs. 2 and 3). The primary use of the protease enzyme preparations derived from *B. subtilis* or *B. amyloliquefaciens* is in the preparation of protein hydrolysates and the tenderizing of meat (Refs. 3 through 5).

Table 1 lists the characterizing enzyme activities and associated International Union of Biochemistry Enzyme Commission (EC) numbers of the carbohydrase and protease enzyme preparations derived from *B. subtilis* or *B. amyloliquefaciens*.

TABLE 1.—ENZYME ACTIVITIES AND EC NUMBERS ASSOCIATED WITH ENZYME PREPARATIONS DERIVED FROM *B. SUBTILIS* OR *B. AMYLOLIQUEFACIENS*

Enzyme Preparation	Characterizing Enzyme Activity	EC Number
Carbohydrase	α -Amylase	3.2.1.1
	β -Glucanase	3.2.1.6
Protease	Subtilisin	3.4.21.62
	Neutral Proteinase	3.4.24.28

B. Methods of Manufacture

All microbial strains, including bacterial strains, used to manufacture enzyme preparations are started from a pure laboratory culture and grown, or “fermented,” in a sterile liquid nutrient medium or sterile moistened semisolid medium. Accepted microbiological techniques are used to exclude contaminating organisms and to avoid development of substrains from within the culture itself (Ref. 6). Although specific conditions of fermentation vary from manufacturer to manufacturer, common fermentation procedures are: (1) The submerged culture method, which uses closed fermenters equipped with agitators, aeration devices, and jackets or coils for temperature control; and (2) the semisolid culture method, which uses horizontal rotating drums or large chambers fitted with trays (Refs. 5 and 6). During fermentation by either method, the pH, temperature, appearance or disappearance of certain ingredients, purity of culture, and level of

enzyme activity must be carefully controlled. The fermentation is harvested at the point where laboratory tests indicate that maximum production of enzyme activity has been attained.

In practice, the processes by which microbial-derived enzyme preparations are produced vary widely. Each single strain of microorganism produces a large number of enzymes (Ref. 5). The absolute and relative amounts of various individual enzymes produced vary markedly among species and even among strains of the same species. They also vary depending upon the composition of medium on which the microorganism grows, and upon the fermentation conditions. The petitioner states that for a specific enzyme preparation the production strain, medium composition, and fermentation conditions are optimized to maximize the desired enzyme activity (Refs. 7 and 8).

The carbohydrase and protease enzymes from *B. subtilis* and *B. amyloliquefaciens* are excreted into the fermentation medium (Refs. 9 through 11). In the semisolid culture method, an enzyme that is present in the fermentation medium is extracted either directly from the moist material, or later after the culture mass has been dried. In the submerged culture method, the microorganisms and other insolubles are removed from the fermentation medium by decanting, filtering, or centrifuging, and therefore an extraction step is not required. In either method, further processing steps may involve clarification, evaporation, precipitation, drying, and grinding (Refs. 6 and 9 through 12).

IV. Safety Evaluation

A. Pre-1958 History of Use in Food

Enzyme preparations have been safely used for many years in the production and processing of food, for example, in the baking, dairy, and brewing industries (e.g., see Refs. 1, 4, and 13).

1. Bacillus Subtilis

The petitioner has provided generally available information, including published reviews, showing that carbohydrase and protease enzyme preparations derived from *B. subtilis* were

commonly used in food prior to 1958 (Refs. 4 and 5). This information is summarized in Table 2.

TABLE 2.—APPLICATIONS OF BACTERIAL CARBOHYDRASE AND PROTEASE ENZYME PREPARATIONS IN FOOD PRIOR TO 1958

Enzyme preparation	Food categories	Technical effect or industry application	References
Carbohydrase	Beer	Mashing ¹	4 and 5
	Syrup for cocoa and chocolate	Reduction of viscosity	4 and 5
	Sugar	Recovery from scrap candy	4 and 5
	Distilled beverages	Mashing	4 and 5
	Precooked cereals	Modification of cereal starches to improve characteristics	4
Protease	Beer	Chillproofing	4
	Condiments	Not reported	5
	Milk	Protein hydrolysis	5

¹ Mashing is the conversion of starch to sugars.

In the published article by Underkofler et al. (Ref. 5), the authors use the general terms “bacterial amylase” and “bacterial protease” to refer to bacterially-derived carbohydrase and protease enzyme preparations used in food at the time of the article. However, the article also includes a table in which the source bacterium for bacterially-derived enzyme preparations is identified as *B. subtilis*.

In the published article by Underkofler and Ferracone (Ref. 4), the authors use the general terms “bacterial carbohydrase” and “bacterial protease” to refer to bacterially-derived carbohydrase and protease enzyme preparations used in food at the time of the article. Unlike the Underkofler et al. article, however, the Underkofler and Ferracone article does not identify the source bacterium for these enzyme preparations. Although it is not possible to determine conclusively whether the descriptor “bacterial” in the Underkofler and Ferracone article refers to *B. subtilis*, the use of this term by the same principal author in two scientific articles published in consecutive years to describe the source of protease and carbohydrase enzyme preparations used in the food industry, coupled with the identification of the source bacterium for these enzyme preparations as *B. subtilis* in the Underkofler et al. article, makes it likely that the source bacterium referred to by Underkofler and Ferracone was in fact *B. subtilis*.

The food uses shown in Table 2, using terminology from the cited reference(s), were documented in articles that were published before or during 1958; the cited references demonstrate

that the use of these enzyme preparations in a variety of foods was widely recognized by 1958. Therefore, the agency concludes that carbohydrase and protease enzyme preparations derived from *B. subtilis* were in common use in food prior to January 1, 1958.

2. *Bacillus Amyloliquefaciens*

According to the petitioner (Refs. 8 and 14 through 16), the species *B. amyloliquefaciens* was not classified under the name *B. amyloliquefaciens* until it was taxonomically separated from the species *B. subtilis* in the late 1980's (Refs. 17 and 18). Therefore, the petitioner asserts, references in contemporaneous scientific literature to pre-1958 food use of enzyme preparations from *B. amyloliquefaciens* occur under the name *B. subtilis*.

With respect to carbohydrase components of the petitioned enzyme preparations, the petitioner cites scientific literature describing a distinctive group of bacteria, within the group originally considered to be *B. subtilis*, that are known to possess a high level of α -amylase activity and are currently designated as *B. amyloliquefaciens* (Refs. 19 through 22). The petitioner also cites a scientific review article (Ref. 23) that states that the source organism for commercial preparations of α -amylase from *B. amyloliquefaciens* was called *B. subtilis* prior to its current designation as *B. amyloliquefaciens*. With respect to the protease components of the petitioned enzyme preparations, the petitioner cites a statement in the same scientific review article (Ref. 23) that most bacterial protease preparations produced before 1960 were derived from *B. amyloliquefaciens*.

As FDA noted in the preamble to another final rule affirming an enzyme preparation as GRAS (58 FR 27197 at 27199, May 7, 1993), the taxonomic placement and name of an organism may change as a result of scientific advances. If internationally accepted rules of nomenclature are observed, references to a particular organism can be followed historically in the scientific literature. Thus, changes in the taxonomic placement of an organism should not affect the ability to identify scientific references to the organism, including scientific references to its toxigenicity, pathogenicity, or use in the production of food or enzymes. In reviewing the petition, FDA has evaluated whether the scientific information documenting pre-1958 food use of bacterially-derived

carbohydrase and protease enzyme preparations pertains to carbohydrase and protease enzyme preparations from *B. amyloliquefaciens*. Although it is not possible to determine conclusively whether any one reference to *B. subtilis* in the scientific literature refers to the species now referred to as *B. amyloliquefaciens*, the totality of the scientific evidence supports a determination that some carbohydrase and some protease enzyme preparations that were described in scientific literature documenting their common use in food before 1958 as derived from *B. subtilis* were in fact derived from *B. amyloliquefaciens*. Therefore, the agency concludes that carbohydrase and protease enzyme preparations derived from *B. amyloliquefaciens* were in common use in food prior to January 1, 1958.

B. Corroborating Evidence of Safety

Because enzymes are highly efficient catalysts, they are needed in only minute quantities to perform their function. When used in accordance with current good manufacturing practice (CGMP), the amounts added to food represent only a minute fraction of the total food mass. FDA estimates dietary exposure to enzyme preparations derived from *B. subtilis* or *B. amyloliquefaciens* at 200 mg/person/day (Ref. 24). This estimate is exaggerated because the agency used the total consumption of microbially-derived enzyme preparations in food as an approximation for the consumption of enzyme preparations derived from *B. subtilis* or *B. amyloliquefaciens*. Thus, the estimate relies on the worst-case assumption that all microbially-derived enzyme preparations that are consumed in food are derived from *B. subtilis* or *B. amyloliquefaciens*. This assumption is extremely conservative because there are numerous microbially-derived enzyme preparations that are GRAS for use in food (see, e.g., 21 CFR 184.1012, 184.1027, 184.1387, 184.1388, 184.1924, and 184.1985).

1. The Enzyme Components

Enzymes, including carbohydrase and protease enzymes in the enzyme preparations that are the subject of this document, are naturally occurring proteins that are ubiquitous in living organisms.

A wide variety of enzymes has always been present in human food. Many naturally occurring enzymes remain active in unprocessed food and therefore are consumed as active enzymes. For example, active enzymes are present in fresh fruits and vegetables and are not inactivated unless the fruits or vegetables are cooked (Refs. 1 and 25).

Enzymes derived from microorganisms have been used as components of foods that have been safely consumed as part of the diet throughout human history (Ref. 26). For example, such common foods as bread and yogurt are produced using enzymes derived from microorganisms (Refs. 26 and 27).

The carbohydrase and protease enzymes in the enzyme preparations that are the subject of this document are substantially equivalent² to carbohydrase and protease enzymes from other microorganisms that FDA has evaluated and found to be safe and that are routinely consumed as part of a normal diet in the United States. For example, FDA has affirmed the use of a mixed carbohydrase and protease enzyme preparation derived from *Bacillus licheniformis* is GRAS (see 21 CFR 184.1027). In addition, carbohydrases derived from various fungi (e.g., *Rhizopus niveus*,

² A 1996 report of the joint Food and Agriculture Organization and World Health Organization (FAO/WHO) consultation group (Ref. 28) stated that “[s]ubstantial equivalence embodies the concept that if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (i.e. the food or food component can be concluded to be as safe as the conventional food or food component). Account should be taken of any processing that the food or food component may undergo as well as the intended use and the intake by the population.” As discussed more fully in FDA’s proposal to amend the agency’s regulations pertaining to substances that are generally recognized as safe (62 FR 18938 at 18944, April 17, 1997), international expert groups such as the FAO/WHO consultation group and the Organization for Economic Co-operation and Development (OECD) consultation group have recommended that the concept of “substantial equivalence” be applied to the safety assessment of foods and substances intentionally added to food.

Rhizopus oryzae, and *A. niger*) are approved for use as secondary direct food additives (see 21 CFR 173.110, 173.130, and 173.120, respectively).

In general, issues relevant to a safety evaluation of proteins such as the enzyme component of an enzyme preparation are potential toxicity and allergenicity. Pariza and Foster (Ref. 1) note that very few toxic agents have enzymatic properties, and those that do (e.g., diphtheria toxin and certain enzymes in the venom of poisonous snakes) catalyze unusual reactions that are not related to the types of catalysis that are common in food processing and that are the subject of this document. Further, as the agency has noted in the context of guidance to industry regarding the safety assessment of new plant varieties, enzymes do not generally raise safety concerns (57 FR 22984 at 23000, May 29, 1992). Exceptions include enzymes that catalyze the formation of toxic substances or substances that are not ordinarily digested and metabolized. The catalytic activities of the enzymes that are the subject of this document are well known; they split proteins or carbohydrates into smaller subunits that are readily metabolized by the human body and that do not have toxic properties.

According to Pariza and Foster (Ref. 1), there have been no confirmed reports of allergies or primary irritations in consumers caused by enzymes used in food processing. There have been, however, some reports of allergies and primary irritations from skin contact with enzymes or inhalation of dust from concentrated enzymes (for example, proteases used in the manufacture of laundry detergents) (Refs. 29 through 31). These reports relate primarily to workers in production plants (Ref. 30) and are not relevant to an evaluation of the safety of ingestion of such enzymes in food.

The 1977 report of the Select Committee on GRAS substances concerning the plant enzyme papain (Ref. 29) supports the view that the ingestion of an active protease at levels found in food products is not likely to affect the human gastrointestinal tract, where many proteases already exist at levels adequate to digest food:

In common with other proteolytic enzymes, papain digests the mucosa and musculature of tissues in contact with the active enzyme for an appreciable period. Because there is no food use of papain that could result in the enzyme preparation occurring in sufficient amount in foods to produce these effects, this property does not pose a dietary hazard.

FDA concludes that generally available and accepted data and information corroborate the safety of the enzyme components of the enzyme preparations that are the subject of this document by establishing that these enzyme components are identical or substantially equivalent to enzymes that are known to have been safely consumed in the diet for many years. FDA also concludes that generally available and accepted data and information corroborate that the enzyme components of the enzyme preparations that are the subject of this document are nontoxigenic and nonallergenic when ingested.

2. Enzyme Sources, Manufacturing Methods, and Processing Aids

Enzyme preparations used in food processing are usually not chemically pure; in addition to the enzyme component(s), they may contain other components derived from the production organism and the fermentation medium, residual amounts of processing aids, and substances added as stabilizers, preservatives, or diluents. The agency has concluded that the enzyme components of the carbohydrase and protease enzyme preparations derived from *B. subtilis* or *B. amyloliquefaciens* do not raise safety concerns; therefore, the remaining safety issue is whether other components of the enzyme preparations are toxic or raise other safety concerns.

a. *Antibiotics.* Some microorganisms are capable of producing antibiotics, which are a special class of metabolites that can inhibit the growth of, or kill, other microorganisms. Some microorganisms have genetic traits that make them resistant to one or more antibiotics such as penicillin, tetracycline, and kanamycin. These traits or markers are often located on plasmids (extrachromosomal pieces of deoxyribonucleic acid (DNA) that are easily transferred to other microorganisms in the environment (e.g., in the gastrointestinal tract). The presence of antibiotics in the food supply would be expected to favor the growth of microorganisms resistant to the

antibiotic, and thus could accelerate the spread of antibiotic resistance among microorganisms, including human pathogens, rendering them resistant to therapy with antibiotic drugs. Therefore, experts have recommended that microbial-derived enzyme preparations that are intended for food use not contain clinically important antibiotics (Refs. 1 and 32).

Accordingly, FDA has evaluated the potential for carbohydrase or protease enzyme preparations derived from *B. subtilis* or *B. amyloliquefaciens* to contain antibiotics as contaminants derived from the bacterial source. Although *Bacillus* species are capable of producing a number of linear or cyclic polypeptide antibiotics following the exponential phase of growth as part of the process of spore formation (Ref. 33), the production of antibiotics can be repressed by selection of strains that produce low or undetectable levels of antibiotics as well as by strict control of the growth conditions. In addition, the enzyme preparations can be tested for the presence of antibiotic activity by routine methods (Ref. 34) to ensure that they do not contain antibiotics. Because of safety concerns about the presence of antibiotics in substances added to food, a condition of agency affirmation of GRAS status for the enzyme preparations that are the subject of this document is that the enzyme preparations not contain antibiotics.

b. *Toxicity and pathogenicity.* A published scientific review article (Ref. 23) states that *Bacillus* species, with the exception of the *B. cereus* group (which does not include *B. subtilis* or *B. amyloliquefaciens*) do not produce toxins. Another published scientific review article on the safety of *B. subtilis* and *B. amyloliquefaciens* (Ref. 35) notes that *B. subtilis* is consumed in large quantities in the Japanese food natto. Further, according to a monograph on microbial enzymes that was prepared under the auspices of the agency-initiated review of GRAS substances conducted during the 1970's, there had been no reported problems of pathogenicity or toxicity with enzyme preparations derived from *B. subtilis* for use in food as of the time of that review (Ref. 12).

More recently, de Boer and Diderichsen (Ref. 35) searched the scientific literature for references that might implicate *B. subtilis* or *B. amyloliquefaciens* as a cause of human disease. These authors characterized *B. subtilis* as an opportunistic microorganism with no pathogenic

potential to humans. Although they reported that cultures from some patients with opportunistic infections have revealed the presence of *B. subtilis* along with other microorganisms, they attributed the presence of *B. subtilis* in these cultures to the virtual ubiquity of this microorganism in the environment (e.g., *B. subtilis* commonly occurs in the soil and can be isolated in the home environment from sites such as the kitchen and bathroom). De Boer and Diderichsen also noted that only patients treated with immunosuppressive drugs appeared to be susceptible to such infections. Moreover, viable cells, which are not present in finished enzyme preparations, would be a prerequisite for any opportunistic infection in an immunocompromised patient. De Boer and Diderichsen also reported that their search for references on *B. amyloliquefaciens* infections revealed no such cases. As discussed in section IV.A.2 of this document, any references to *B. amyloliquefaciens* prior to the late 1980's would be expected to occur under the name *B. subtilis*.

A few reports have implicated *B. subtilis* as a potential source of food poisoning when present as a contaminant in food (Refs. 36 and 37). However, a particular strain of virtually any microorganism may, under certain circumstances, mutate to become an opportunistic pathogen. Therefore, FDA considered these reports in the context of: (1) The information summarized in the monograph on microbial enzymes (Ref. 12); (2) the scientific review article describing *Bacillus* species other than those in the *B. cereus* group as nontoxigenic (Ref. 23); (3) the documented consumption of *B. subtilis* bacteria in the Japanese food natto (Ref. 35); and (4) the characterization by de Boer and Diderichsen of *B. subtilis* as an opportunistic microorganism with no pathogenic potential to humans (Ref. 36). Based on this information, FDA concludes that nontoxigenic and nonpathogenic strains of *B. subtilis* are widely available and have been safely used in a variety of food applications. Because an enzyme preparation derived from a toxigenic or pathogenic source would not be GRAS, a condition of agency affirmation of GRAS status for the enzyme preparations that are the subject of this document is that the bacterial strains used as a source of these enzyme preparations be nontoxigenic and nonpathogenic.

c. Manufacturing methods and processing aids. Enzyme preparations that are manufactured in accordance with CGMP using the methods described in section III.B of this document meet the general requirements and additional requirements in the monograph on enzyme preparations in the Food Chemicals Codex, 4th ed. (Ref. 3). Such enzyme preparations are produced using substances that are acceptable for use in foods and under culture conditions that ensure a controlled fermentation, thus preventing the introduction of extraneous microorganisms that could be the source of toxic materials and other toxic substances (Ref. 3).

FDA concludes that generally available and accepted data and information corroborate the safety of carbohydrase and protease enzyme preparations derived from nontoxigenic and nonpathogenic strains of *B. subtilis* or *B. amyloliquefaciens* and manufactured in accordance with CGMP by establishing that any added substances or impurities derived from the enzyme source or introduced during the manufacturing of such enzyme preparations would not be expected to present health concerns.

V. Comments

FDA received seven comments in response to the filing notice and none in response to the amendment notices. Of these, FDA received two comments from food manufacturers, two from trade associations, one from a manufacturer of enzymes for use in animal feed, one from a pharmaceutical manufacturer, and one from a consumer group. Six comments supported the petition for GRAS affirmation, stating that the enzyme preparations included in the petition have a long history of use in foods such as cheese, bread, and corn syrup.

One comment stated that *B. subtilis* has a history of use in animal feed and requested GRAS affirmation for this use. However, the petition is for the use of certain enzyme preparations in human food, and not in animal feed. Therefore, the agency finds that this comment is not relevant to the petition.

One comment asserted that enzyme preparations should not be considered GRAS. The comment further asserted that the use of enzyme preparations should be declared on the label

of foods and that consumers should be warned about hazards inherent in their use. The comment stated that enzyme preparations are rarely purified to any significant degree and contain a variety of cellular constituents and metabolic debris. The comment further argued that, although enzyme preparations are used at low levels and are inactivated after the treatment of food, they may elicit allergic reactions and other biological activities which could be detrimental to human health. In support of this statement, the comment cited a published scientific article (Ref. 38) that reported that enzyme preparations from *B. subtilis* caused temporary weight loss and aggravated infection in mice when injected into the abdominal cavity and caused hemolysis and hemagglutination of sheep erythrocytes in in vitro studies.

FDA has evaluated the comment and the article it cited. For the following two reasons, FDA concludes that the study cited by the comment is not relevant to food uses of the bacterial enzyme preparations that are the subject of this document.

First, the paper did not identify the composition of the *B. subtilis* enzyme preparations tested. The preparations were intended for use in laundry detergents; such nonfood grade enzyme preparations need not conform to specifications for enzyme preparations used in food processing. For example, nonfood grade enzyme preparations may include processing aids that are not acceptable for food use. Because of such differences, the results from the testing of laundry cleaning enzyme preparations have little value in the safety assessment of food-processing enzyme preparations.

Second, in the cited study, adverse effects were observed in mice after the intraperitoneal administration of *B. subtilis* autolysates. However, exposure to enzyme preparations in food occurs by ingestion and not by injection. The difference in the route of exposure is particularly significant for assessing the significance of immunological effects. With intraperitoneal administration, the components of the immune system are directly exposed to a high level of the test compound. This contrasts with exposure to enzyme preparations in food, whereby low levels of the enzyme preparations are ingested and undergo hydrolysis by digestive enzymes before any interaction with

the immune system. Pariza and Foster (Ref. 1) note that there are no confirmed reports of allergic reactions in consumers caused by enzymes used in food processing.

Moreover, a report of the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) corroborates the safety of food uses of enzyme preparations from *B. subtilis* (Ref. 39). This report concluded that results from a 90-day feeding study in rats showed no adverse effects. The test diet was meat protein-based and supplemented with a protease enzyme preparation from *B. subtilis* at a 1-percent level (equivalent to approximately 1 gram of enzyme preparation per kilogram of body weight per day). This level is more than 300 times greater than the highest level that would be expected in the human diet (200 mg/person/day, or 3.3 mg/kg body weight per day for a 60 kg person), as estimated in section IV.B of this document.

With respect to the comment's assertion that enzyme preparations should be declared on the label of foods in which they are used, the agency notes that under certain circumstances, applicable regulations already require use of an enzyme preparation in a food to be declared on the label, depending upon the nature of the enzyme preparation's use and technical effect in the food. Section 403(i)(2) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 343(i)(2)) requires that all ingredients of multi-ingredient foods be listed on the label of the food. By regulation, FDA has exempted certain ingredients that are used only as processing aids from this requirement. Section 101.100(a)(3)(ii)(a) and (a)(3)(ii)(c) (21 CFR 101.100(a)(3)(ii)(a) and (a)(3)(ii)(c)) provides an exemption from the ingredient listing requirement for processing aids that are added to a food for their technical or functional effect during processing, but are either removed from the food before packaging or are present in the finished food at insignificant levels and do not have any technical or functional effect in the finished food. Although many enzyme preparations are used as processing aids in food (e.g., amylase preparations used in the manufacture of glucose syrup and protease preparations used in the manufacture of protein hydrolysates), other enzyme preparations that are added during processing (e.g., protease preparations used in tenderizing meat)

are not processing aids as defined in § 101.100(a)(3)(ii) because they remain active in the finished food product. For example, enzymes used in the manufacture of swiss and cheddar cheese remain active in the finished cheese, enhancing body, flavor, and aroma (49 FR 29242, July 19, 1984). Because such effects in the finished food remove the enzymes from the ingredient listing exemption for processing aids in § 101.100(a)(3)(ii)(c), the use of such enzymes must be declared on the label. Therefore, whether a label declaration is needed for the use of an enzyme preparation in a food will depend upon its function and effect in the food.

VI. Conclusions

The petitioner has provided generally available evidence demonstrating that carbohydrase and protease enzyme preparations from *B. subtilis* were in common use in food prior to 1958. FDA has determined, under § 170.30(a) and (c)(1), that this information provides an adequate basis upon which to conclude that the safety of these enzyme preparations for use in food is generally recognized among the community of experts qualified by scientific training and experience to evaluate the safety of food ingredients.

The petitioner has also provided generally available evidence demonstrating that the bacterium now known as *B. amyloliquefaciens* was formerly included within the *B. subtilis* classification. Based on its analysis of the data submitted, the agency concludes that the evidence of common use in food pertains to carbohydrase and protease enzyme preparations from the bacterium now known as *B. amyloliquefaciens* as well as to carbohydrase and protease enzyme preparations from *B. subtilis*.

This evidence of common use in food prior to 1958 is corroborated by information that the enzymes themselves and the sources from which they are derived are nontoxic and nontoxicogenic, and that manufacturing will not introduce impurities that would adversely affect the safety of the finished enzyme preparations. Moreover, the carbohydrase and protease enzyme preparations from *B. subtilis* and *B. amyloliquefaciens* are substantially equivalent to enzymes naturally present in foods that have been safely consumed in the human diet for many years.

Having evaluated the information in the petition, along with other available information related to the use of these enzyme preparations, the agency concludes that carbohydrase enzyme preparation and protease enzyme preparation derived from either *B. subtilis* or *B. amyloliquefaciens* are GRAS under conditions of use consistent with CGMP. The agency is basing its conclusion on evidence of a substantial history of safe consumption of the enzyme preparations in food by a significant number of consumers prior to 1958, corroborated by the other evidence summarized in section IV.B of this document.

FDA is affirming that the use of these bacterially-derived carbohydrase and protease enzyme preparations in food is GRAS with no limits other than CGMP (21 CFR 184.1(b)(1)). To clarify the identity of each enzyme preparation, the agency is including in §§ 184.1148(a) and 184.1150(a) the EC numbers of the enzymes that supply the characterizing enzyme activities of each preparation. In order to make clear that the affirmation of the GRAS status of these enzyme preparations is based on the evaluation of specific uses, the agency is including in §§ 184.1148(c) and 184.1150(c) the technical effect and the specific substances on which each enzyme preparation acts, although the data show no basis for a potential risk from any foreseeable use of these enzyme preparations.

For simplicity, FDA is affirming the GRAS status of both carbohydrase enzyme preparations in a single combined regulation that describes the source of the enzyme as *B. subtilis* or *B. amyloliquefaciens*, rather than affirming the GRAS status of carbohydrase derived from *B. subtilis* separately from that of carbohydrase derived from *B. amyloliquefaciens*. Likewise, FDA is affirming the GRAS status of both protease enzyme preparations in a single combined regulation that describes the source of the enzyme as *B. subtilis* or *B. amyloliquefaciens*.

To ensure that the enzyme preparations are of suitable purity for use in food, FDA is including in the regulations the general requirements and additional requirements for enzyme preparations in the monograph “Enzyme Preparations” in the Food Chemicals Codex, 4th ed. (1996) as general specifications for these enzyme preparations. Furthermore, to ensure that the use of these enzyme preparations does not promote the development of antibiotic resistance, the agency is specifying

that the enzyme preparations must be free of antibiotic activity as determined by a suitable method (e.g., the method described in Ref. 34).

VII. Environmental Considerations

The agency has determined under 21 CFR 25.32(f) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

VIII. Analysis for Executive Order 12866

FDA has examined the impacts of this final rule under Executive Order 12866. Executive Order 12866 directs Federal agencies to assess the costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety effects; distributive impacts; and equity). According to Executive Order 12866, a regulatory action is significant if it meets any one of a number of specified conditions, including having an annual effect on the economy of \$100 million, adversely affecting in a material way a sector of the economy, competition, or jobs, or raising novel legal or policy issues. FDA finds that this final rule is not a significant regulatory action as defined by Executive Order 12866. In addition, the agency has determined that this final rule is not a major rule for the purpose of Congressional review.

The primary benefit of this action is to remove uncertainty about the regulatory status of the petitioned substances. No compliance costs are associated with this final rule because no new activity is required and no current or future activity is prohibited by this rule.

IX. Regulatory Flexibility Analysis

FDA has examined the impacts of this final rule under the Regulatory Flexibility Act. The Regulatory Flexibility Act (5 U.S.C. 601–612) requires agencies to consider alternatives that would minimize the economic impact of their regulations on small entities. No compliance costs are associated with this final rule because no new activity is required and no current or future activity

is prohibited. Accordingly, under the Regulatory Flexibility Act (5 U.S.C. 605(b)), the agency certifies that this final rule will not have a significant economic impact on a substantial number of small entities.

X. Paperwork Reduction Act of 1995

This final rule contains no collections of information. Therefore, clearance by the Office of Management and Budget under the Paperwork Reduction Act of 1995 is not required.

XI. Effective Date

As this rule recognizes an exemption from the food additive definition in the Federal Food, Drug, and Cosmetic Act, and from the approval requirements applicable to food additives, no delay in effective date is required by the Administrative Procedure Act, 5 U.S.C. 553(d). The rule will therefore be effective immediately (5 U.S.C. 553(d)(1)).

XII. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Pariza, M. W., and E. M. Foster, "Determining the Safety of Enzymes Used in Food Processing," *Journal of Food Protection*, vol. 46, pp. 453–468, 1983.
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4. Underkofler, L. A., and W. J. Ferracone, "Commercial Enzymes—Potent Catalysts that Promote Quality," *Food Engineering*, vol. 29, pp. 123, 125–126, 130, and 133, 1957.
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14. Response of the Enzyme Technical Association to the letter dated June 26, 1986, of Lawrence J. Lin regarding GRASP 3G0016, received with a letter dated October 3, 1986, from Roger D. Middlekauff of the Enzyme Technical Association, to Lawrence J. Lin, FDA.
15. Letter dated August 17, 1995, from Gary L. Yingling, Enzyme Technical Association, to Alan M. Rulis, FDA.
16. Letter dated April 16, 1996, from Alice J. Caddow, Enzyme Technical Association, to Linda Kahl, FDA.
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List of Subjects in 21 CFR Part 184

Food additives, Food ingredients, Incorporation by reference.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, and redelegated to the Director, Center for Food Safety and Applied Nutrition, 21 CFR part 184 is amended as follows:

PART 184—DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE

1. The authority citation for 21 CFR part 184 continues to read as follows:

Authority: 21 U.S.C. 321, 342, 348, 371.

2. Section 184.1148 is added to subpart B to read as follows:

§ 184.1148 Bacterially derived carbohydrase enzyme preparation.

(a) Bacterially derived carbohydrase enzyme preparation is obtained from the culture filtrate resulting from a pure culture fermentation of a nonpathogenic and nontoxigenic strain of *Bacillus subtilis* or *B. amyloliquefaciens*. The preparation is characterized by the presence of the enzymes α -amylase (EC 3.2.1.1) and β -glucanase (EC 3.2.1.6), which catalyze the hydrolysis of *O*-glycosyl bonds in carbohydrates.

(b) The ingredient meets the general requirements and additional requirements in the monograph on enzyme preparations in the Food Chemicals Codex, 4th ed. (1996), pp. 128–135, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 N. Capitol St. NW., suite 700, Washington, DC. In addition, antibiotic activity is absent in the enzyme preparation when determined by an appropriate validated method such as the method "Determination of antibiotic activity" in the Compendium of Food Additive Specifications, vol.

2, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Organization of the United Nations, Rome, 1992. Copies are available from Bernan Associates, 4611-F Assembly Dr., Lanham, MD 20706, or from The United Nations Bookshop, General Assembly Bldg., rm. 32, New York, NY 10017, or by inquiries sent to “<http://www.fao.org>”. Copies may be examined at the Center for Food Safety and Applied Nutrition’s Library, 200 C St. SW., rm. 3321, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter to hydrolyze polysaccharides (e.g., starch).

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

3. Section 184.1150 is added to subpart B to read as follows:

§ 184.1150 Bacterially-derived protease enzyme preparation.

(a) Bacterially derived protease enzyme preparation is obtained from the culture filtrate resulting from a pure culture fermentation of a nonpathogenic and nontoxigenic strain of *Bacillus subtilis* or *B. amyloliquefaciens*. The preparation is characterized by the presence of the enzymes subtilisin (EC 3.4.21.62) and neutral proteinase (EC 3.4.24.28), which catalyze the hydrolysis of peptide bonds in proteins.

(b) The ingredient meets the general requirements and additional requirements in the monograph on enzyme preparations in the Food Chemicals Codex, 4th ed. (1996), pp. 128–135, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Center for Food Safety and Applied Nutrition’s Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 N. Capitol

St. NW., suite 700, Washington, DC. In addition, antibiotic activity is absent in the enzyme preparation when determined by an appropriate validated method such as the method “Determination of antibiotic activity” in the Compendium of Food Additive Specifications, vol. 2, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Organization of the United Nations, Rome, 1992. Copies are available from Bernan Associates, 4611–F Assembly Dr., Lanham, MD 20706, or from The United Nations Bookshop, General Assembly Bldg., rm. 32, New York, NY 10017, or by inquiries sent to “<http://www.fao.org>”. Copies may be examined at the Center for Food Safety and Applied Nutrition’s Library, 200 C St. SW., rm. 3321, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter to hydrolyze proteins or polypeptides.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

Dated: 3/26/99
March 26, 1999



L. Robert Lake
Director
Office of Policy, Planning and Strategic Initiatives
Center for Food Safety and Applied Nutrition

[FR Doc. 99-???? Filed ??-??-99; 8:45 am]

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