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ORIGINAL SUBMISSION

PNT PEOPLE AND TECHNOLOGIES, LLC

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Division of Biotechnology and GRAS Notice Review

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December 3, 2009

Robert L. Martin, Ph.D
U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Div. of Biotechnology and GRAS Notice Review
HFS-255, 5100 Paint Branch Pkwy.
College Park, MD 20740-3835.

Re: GRAS Notice for black yeast beta-glucan(Polycan®)

Dear Dr. Robert L. Martin

Pursuant to proposed 21 CFR§170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the Federal Register [62 FR18938 (1 7 April 1997)] and on behalf of Glucan Corp. headquartered in Busan, S. Korea, , I am submitting in triplicate, as the notifier [PeopleAndTechnologies, LLC, 1424 W. Sam Houston Parkway, Suite 160, Houston, TX 77041], a Notice of the determination, on the basis of scientific procedures, that black yeast beta-glucan(Polycan®) produced from Aureobasidium pullulans SM-2001, produced by Glucan Corporation, Limited, as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the Federal, food, Drug and Cosmetic Act.

Information setting forth the basis for the GRAS determination, which includes a comprehensive summary of the data available and peer-reviewed journals in support of the safety of black yeast beta-glucan(Polycan®) under the intended conditions of use are enclosed.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process. All of the information that serves as the basis for this GRAS determination can be sent - upon request - to FDA and is available for review and copying at reasonable times.

Best regrads,

(b) (6)

Wonho Cho
PeopleAndTechnologies, LLC
US Sales Division of Glucan Co., Ltd
chowonho@gmail.com

BLACK YEAST BETA-GLUCAN (POLYCAN®) GRAS NOTICE

Prepared for: Robert L. Martin, 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

Prepared by: PeopleAndTechnologies, LLC
1424 W. Sam Houston Parkway
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Houston, TX 77043
U.S.A.

GRAS Notification for BLACK YEAST BETA-GLUCAN(POLYCAN®) derived from *Aureobasidium pullulans* SM-2001

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36 [6 2 FR 18938 (17 April 1997)] (U.S. FDA, 1997)

Glucan Corporation, Limited (Glucan) hereby notifies the Food and Drug Administration ("FDA") through its US Sales Division, PeopleAndTechnologies, LLC ("PNT") that its black yeast beta-glucan(Polycan®), which is the exopolymer produced through the fermentation, sterilization, filtration and freeze drying of *Aureobasidium pullulans* SM-2001, an UV induced mutant of *Aureobasidium pullulans* ATCC 42023 (a black yeast), is Generally Recognized as Safe (GRAS) for use as a food ingredient. This determination is based on scientific procedures, as described in the following sections, under the conditions of its intended use in foods. Therefore, consistent with Section 201 (s) of the *Federal Food, Drug, and Cosmetic Act,* the use of black yeast beta-glucan(Polycan®) in foods as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Wonho Cho
Chief Executive
PeopleAndTechnologies, LLC
US Sales Division of Glucan Corporation, Limited

Dec 03, 2009

Date:

B. Name and Address of Notifier

Glucan Corporation, Limited
Marine Bio Center 221, Silla University
Gwaebop-Dong, Sasang-Gu
Busan, S. Korea 617-736
Tel: +82-51-999-5886
Fax: +82-51-999-5887

C. Common Name of the Notified Substance

Black yeast beta-glucan(Polycan®)

D. Conditions of Intended Use

000005

Glucan intends to market black yeast beta-glucan(Polycan®) produced from *Aureobasidium pullulans* SM-2001 as a food ingredient in various traditional food products in which it serves as a source to improve the bone health by enhancing Osteoblast, improving mineral(Calcium) absorption and inhibiting Osteoclast as well as a source for the human immunomodulation.

The intended food uses include dairy products, baked goods and baking mixes, beverages and beverage bases, breakfast cereals, jams and jellies, processed fruits and fruit juices, gum and soft candy, soup bases and sauces and processed meat products. Black yeast beta-glucan(Polycan®) will be added to food products at use-levels ranging from 1 % to the maximum 10% (120mg~150mg)/serving, depending on the proposed food use.

E. Basis for the GRAS determination

Glucan's GRAS determination for black yeast beta-glucan(Polycan®) is based primarily on scientific procedures in accordance with 21 CFR§170.30. The GRAS of black yeast beta-glucan(Polycan®) has been determined on the basis of the view of experts qualified by scientific training and experience to evaluate the safety of black yeast beta-glucan (Polycan®) and the common knowledge about throughout the scientific community knowledgeable about the safety of black yeast beta-glucan(Polycan®). For a detailed discussion of the studies, peer-reviewed journals, data, materials and information that form this basis, please see Section V below and Appendices.

In addition, from the late 1990, in Japan and S. Korea, black yeast beta-glucan produced from *Aureobasidium pullulans* SM-2001, has been commonly called beta-glucan. It has been used as a natural source for the immunomodulation. Moreover, in S. Korea, black yeast beta-glucan(Polycan®) has been used as a food ingredient in dairy products, such as milk and yogurt, and processed meat products, such as ham and sausage, in which it serves as a source to improve bone health since 2004.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notice will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

PeopleAndTechnologies, LLC
5822 Santa Fe Springs Dr.
Houston, TX 77041

and

Glucan Corporation, Limited
Marine Bio Center 218, Silla University
Gwaebop-Dong, Sasang-Gu
Busan, S. Korea 617-736

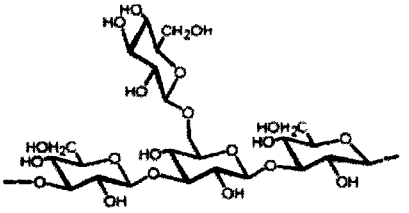
Should the FDA have any questions or additional information requests regarding this notification, PNT will supply these data and information.

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

A. Identity

Black yeast beta-glucan(Polycan®) is the exopolymer produced through the fermentation, sterilization, filtration and freeze drying of *Aureobasidium pullulans* SM-2001 which is an UV induced mutant of *Aureobasidium pullulans* ATCC 42023 (a black yeast). Black yeast beta-glucan(Polycan®) contains β-1,3/1,6-glucan. Fig. 1 shows that the ingredient contains mostly β-1,3/1,6-glucan and other organic materials, such amino acids, mono- or di-unsaturated fatty acids(linoleic and linolenic acids), and fibrous polysaccharide. In addition to trace amounts of protein and lipids, small amounts of β-1,4-glucan also are expected to be present in the final product.

(a) Typical β-1,3/1,6-glucan structure



(b) Schematic picture of the exopolymer containing β-1,3/1,6-glucan

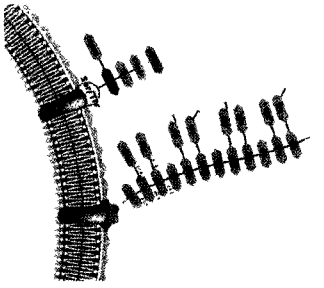


Fig. 1. Pictorial representation of β-1,3/1,6-glucan from *Aureobasidium pullulans* SM-2001

Common or Usual Name: Black yeast beta-glucan
Chemical Name: (1,3),(1,6)-β-D-glucan
Chemical Abstracts Service (CAS) Number: Not available
Empirical Formula and Formula Weight: Not available
Structure Formula: See Fig. 1.

B. Production Organism

Aureobasidium pullulans SM-2001, an UV induced mutant of *Aureobasidium pullulans* ATCC 42023 (a black yeast)

C. Physical Description

Reddish yellow powder

D. Method of Manufacture

Black yeast beta-glucan(Polycan®) is manufactured through the controlled fermentation, sterilization, filtration and freeze drying process of a selected strain of *Aureobasidium pullulans* SM-2001. *Aureobasidium pullulans* SM-2001, which externally secretes β-1,3/1,6-glucan, is obtained by mutating *Aureobasidium pullulans* ATCC 42023 (a black yeast) through the UV induction . The culture medium containing Vitamin C Ascorbic acid(a food

additive) 2g/L, yeast extract (a food additive) 2.5g/L is used for *Aureobasidium pullulans* SM-2001 and sucrose as the carbon source is dissolved into distilled water by 20%(w/v) and the sterilized sucrose solution is added to the culture medium by 0.5-2.0%(w/v). Then, *Aureobasidium pullulans* SM-2001 is pre-fermented in a 250 mL flask for 72 hours and in 100L tank for 1 day then the batch of 100L is fermented in 5 ton tank. After the fermentation, the batch is sterilized and black yeast beta-glucan(Polycan®) is separated from the production organism through the series of filter press. The final product is then spray-dried. The final product conforms to the contents of β-1,3/1,6-glucan by Megazyme kit(Cat. No. : K-YBGL). The diagram of manufacturing process is presented in Appendix 1. Glucan Corporation acquired ISO 9001 (URS) for its manufacturing process of black yeast beta-glucan(Polycan®).

E. Specifications

1. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical nature
Exopolysaccharides

2. HAZARDS IDENTIFICATION

Not a hazardous substance or preparation according to EC-directive 67/548/EEC or 1999/45/EC.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder
Color	reddish yellow
Odor	slight

Safety data

pH	5-8 (20℃) (20 % solution)
Bulk density	150 - 450 Kg/m^3
Water solubility	very high solubility
Viscosity, dynamic	400-900 cp (18s spindle, 3 rpm, 25℃) (20 % solution)

4. STABILTY AND REACTIVITY

Conditions to avoid None known

5. TOXICOLOGICAL INFORMATION

Skin irritation	No irritating effects
Eye irritation	No eye irritation
Further information	The product contains no substances known to be hazardous to health in concentrations which need to

be taken into account.

III.Self-Limiting Levels of Use

A. Dry Food Products

Not Applicable

B. Dairy Products and beverages

The use of black yeast beta-glucan(Polycan®) for beverages, such as milk, yogurt and fruit juices, is self-limiting because the solubility of black yeast beta-glucan(Polycan®) is decreased and the aggregation is started with concentrations above 5%. This aggregation in products causes a slimy or tacky mouthfeel, which limits the use of black yeast beta-glucan(Polycan®) as an ingredient for beverages to levels below 5% of the food weight.

IV.Intended Technical Effect

Black yeast beta-glucan(Polycan®) possesses several beneficial characteristics when consumed as an ingredient in food products. One of the beneficial characteristics is to contribute to the improvement of bone health by enhancing osteoblast, increasing mineral (calcium) absorption, and inhibiting osteoclast. Another beneficial effect, inflammation inhibition, was also observed in animal studies. Below are the summaries of the animal studies of black yeast beta-glucan(Polycan®)'s anti-osteoporotic effect as well as inflammation inhibition effect. The full version of published journals are presented in Appendix 2-6 in the same order of 1-5 below.

1. Investigated the anti-osteoporotic effects of black yeast beta-glucan(Polycan®) in ovariectomized mice at doses of 31.25, 62.5 and 125mg/kg. Black yeast beta-glucan(Polycan®) was administered orally once a day for 28 day to bilateral ovariectomized mice, beginning 4 weeks after surgery. Changes in body weight, bone weight, bone mineral contents, bone mineral density, failure load, histologic profiles, and histomorphometric analyses were determined, in addition to serum osteocalcin, calcium, and phosphorus levels. Black yeast beta-glucan(Polycan®) significantly and dose-dependently suppressed decreases in bone weight, bone mineral contents, failure load, bone mineral density, and serum calcium and phosphorus levels and the increase in serum osteocalcin levels. In addition, black yeast beta-glucan(Polycan®) significantly suppressed decreases in histomorphometric parameters such as volume, length, and thickness of trabecular bone and thickness of cortical bone and the increase in osteoclast cells in the femur and tibia. Although the effects of black yeast beta-glucan(Polycan®) were generally modest and smaller than those of alendronate, the effects on cortical bone thickness were more favorable for black yeast beta-glucan(Polycan®) than for alendronate. In addition, black yeast beta-glucan(Polycan®) exhibited favorable effects on ovariectomy-induced osteoporosis. [shin HD et al. nutrition. 2007. 23: 853-860]

2. Treatment with black yeast beta-glucan(Polycan®) inhibited osteoclastogenesis of bone marrow stem cells in a co-culture system with calvariae osteoblastic cells. In addition, the treatment increased mineral deposition in osteoblastic cells. These two observations prompted us to evaluate whether the exopolymer could be used as an anti-osteoprotic agent, and efficacy of the exopolymers to prevent bone loss was compared with alendronate, a bisphosphonate, in ovariectomized mice prone to osteoporosis. Administration of the exopolymer to the ovariectomized mice resulted in improved effects on femur weight and histomorphometric changes of femur such as trabecular bone volume, trabecular bone thickness, and cortical bone thickness. In conclusion, the exopolymer treatment inhibited bone loss from osteoporosis induced by ovariectomy, and the effect was comparable to alendronate administration[song HB et al. J. microbial. Biotechnol. 2006. 16(1): 37-45].

3. The effects of black yeast beta-glucan(Polycan®) containing ß-1,3/1,6-glucan were observed on the rat rib fracture healing. 31.25, 62.5 and 125mg/kg of black yeast beta-glucan(Polycan®) were orally administered once a day for 13 days to rib fractured rats from 6 hours after operation, and changes on the volume, mineral density, strength and histopathology of callus were observed. The effects of black yeast beta-glucan(Polycan®) were compared with 10μg/kg of human parathyroid hormone 1-34(hPTH) subcutaneously treated group. Treatment of black yeast beta-glucan(Polycan®) induced dose dependent increases on the callus mineral density and strength with dramatical decreases on the callus volume. In addition, more active cartilaginous ossifications were observed in black yeast beta-glucan(Polycan®) treated group as decreased, and consequently, osteoid tissues in callus were dramatically increased at histopathological observation. It is, therefore, concluded that black yeast beta-glucan(Polycan®) used in the present study accelerated the fracture healing, especially on the ossification. 125mg/kg of black yeast beta-glucan(Polycan®) shows similar or slight lower effects on accelerating fracture healing as compared with hPTH 10μg/kg. [Lee HS et al. Lab Anim. Res. 2008. 24(1): 39-44]

4. The effects of black yeast beta-glucan(Polycan®) containing ß-1,3/1,6-glucan were observed on acute xylene induce inflammation. Black yeast beta-glucan (Polycan®) at a dose of 62.5, 125 or 250 mg/kg were administered once orally to xylene-treated mice (0.3ml of xylene was applied on the anterior surface of the right ear to induce inflammation), and the body weight change, ear weight, histological profiles and histomorphometrical analyses of ear were conducted upon sacrifice. The xylene was topically applied 30 min after dosing with ß-glucan. The results were compared to those of diclofenac, indomethacin and dexamethasone (15mg/kg injected once intaperitoneally). All animals were sacrificed 2 hours after xylene application, xylene application resulted in marked increases in induced ear weights compared to that of intact control ear, hence, the differences between intact and induced ear were also significantly increased. The histological characteristics of acute inflammation, such as severe vasodilatation, edematous changes of skin and infiltration of inflammatory cells, were detected in xylene-treated control ears with marked increase in the thickness of the ear tissues. However, these xylene-induced acute inflammatory changes were significantly and dose-dependently decreased by black yeast beta-glucan(Polycan®) treatment. We conclude that black yeast beta-glucan(Polycan®) has a somewhat favorable effect in the reduction of the acute inflammatory responses induced by xylene application in mice. [Kim HD et al.

Arch Pharm Res. 2007. 30(3): 323-328]

5. The effects of black yeast beta-glucan(Polycan®) on formalin induced chronic inflammation were observed. Doses of 62.5, 125, and 250 mg/kg of black yeast beta-glucan(Polycan®) were orally administered once ad day for 10 days to formalin-induced chronic inflammatory mice (0.02ml of 3.75$ formalin was subaponeurotically injected in to the left hind paw), and then the bilateral hind-paw thickness and volume were measured daily, while the paw wet-weight, histological profiles, and histomorphometircal analyses were conducted at termination. The results were compared with those for diclofenac, indomethacin, and dexamethasone (intraperitoneally injected) 15 mg/kg-dosed group. All the animals were sacrificed 10 days after dosing. As a result of the formalin injection, a marked increase in the difference between the intact and formalin-induced paw thickness and volume was detected in the formalin-injected control compared with that in the intact control with time, plus at the time of sacrifice, the difference in the paw wet-weights was also dramatically increased. In a histological and histomorphometrical analysis, severe histological profiles of chronic inflammation were detected in the formalin-injected control with a marked increase in the thickness of the skin of the dorsum pedis. However, these formalin-induced chronic inflammatory changes were significantly and dose-dependently decreased by black yeast beta-glucan(Polycan®) treatment. In conclusion, black yeast beta-glucan(Polycan®) treatment inhibited the chronic inflammatory response induced by formalin injection in the mice. However, somewhat low efficacies were detected compared with those for the diclofenac-, indomethacin-, and dexamethasone-treated groups. [Kim HD et al. J microbial biotechnol. 2006. 16(12): 1954-1960]

V. Detailed Summary of Basis for Notifier's GRAS Determination

Glucan's GRAS determination is based upon a series of toxicological studies of black yeast beta-glucan(Polycan®), a human clinical study, and the substantial equivalence in the composition with Biothera's bakers yeast beta-glucan. These animal toxicological studies include a GLP toxicity (4 week gavage study with rats), genotoxicity studies, and pathogenicity and toxigenicity study of the production using rat or mice. Published journals describing these studies in detail are presented as Appendix 7-10 respectively. The human clinical study is Randomized, Double-Blind, Clinical Trials of Immunomodulating Polycan® of Black Yeast for Its Efficacy and Safety presented in detail as Appendix 11.

A. 4 weeks rat gavage study

Four groups of 11 male and 11 female Sprague-Dawley rats, 5 weeks of age at the initiation of dosing, orally received black yeast beta-glucan(Polycan®) 2 times/day for 4 weeks by gavage at levels of 0 (control group), 250, 500, 1000 mg/kg body weight

No deaths occurred during the administration or recovery periods, and there were no clinical signs or ophthalmological abnormalities. Mean food consumption and body weights did not differ significantly from the controls. There were no treatment-related changes in hematology and urinalysis parameters relative to the controls. In addition, no

significant changes in histopathology parameters were observed in the group of 1000 mg/kg dosage. It was concluded that the no-observed-adverse-effect-level (NOAEL) of was at least 1000 mg/kg body weight, the highest dose level.

B. Genotoxicity study

Micronucleus test

In the research the genotoxic effect of black yeast beta-glucan(Polycan®) containing ß-1,3/1,6-glucan originated from *Aureobasidium pullulans* SM-2001, was evaluated using the mouse micronucleus test. Black yeast beta-glucan(Polycan®) was administered once a day for 2 days by oral gavage to male ICR mice at doses of 1000, 500 and 250mg/kg. cyclophosphamide was used as a known genotoxic agent in a positive control group. The appearance of a micronucleus is used as an index for genotoxic potential. The results obtained indicated that black yeast beta-glucan(Polycan®) shows no genotoxicity effect up to 1000mg/kg dosing levels. In addition, it is also considered that there were no problems from cytotoxicity of black yeast beta-glucan(Polycan®) tested in this study because the polychromatic erythrocyte ratio was detected as >0.47 in all tested groups [Lee HS et al. Toxicol. Res. 2008. 24(1): 11-15]

C. Pathogenicity study

6. Oral

To obtain the acute information of the oral dose toxicity of black yeast beta-glucan(Polycan®) originated from *Aureobasidium pullulans* SM-2001, a UV induced mutant of *Aureobasidium pullulans*, having various pharmacological effects, in male and female mice. In order to calculate 50% lethal dose (LD_{50}), approximate LD and target organs, test article was administered twice by oral gavage to male and female ICR mice at total 1000, 500 and 250mg/kg. The mortality and changes on body weight, clinical signs and gross observation were monitored during 14 days after dosing. As the results, we could not find any mortalities, clinical signs, changes in the body weight and gross findings. The results obtained in this study suggest that black yeast beta-glucan(Polycan®) is non-toxic in mice and is therefore likely to be safe for clinical use. The LD_{50} and approximate LD in mice after single oral dose of black yeast beta-glucan(Polycan®) were considered over 1000mg.kg, respectively [Lee HS et al. J. toxicol. Pub. Health. 2005. 21(4): 361-365]

7. Subcutaneous

To obtain the acute information of the subcutaneous dose toxicity of black yeast beta-glucan(Polycan®) originated from *Aureobasidium pullulans* SM-2001, having various pharmacological effects, in mice. Test articles were once subcutaneously administered to mice at dose levels of 250, 125 and 62.5 mg/kg. The mortality and changes on body weight, clinical signs and gross observation were monitored during 14 days after dosing. As the results, we could not find mortalities, clinical signs and changes in the body weight except for residuals of test articles and related skin ulcer at injection sites. Hypertrophy of spleen related to the immune modulate effect of black yeast beta-glucan(Polycan®) was

dose-dependently detected in all dosing groups tested as gross findings, and hyperplasia of lymphatic follicles and increase of number of megakaryocytes in red pulps are dose-dependently observed at histopathological observation of spleen. In addition, infiltration of inflammatory cells in residuals of test articles in injection sites. The skin ulcer detected in the present study was considered to be biting wounds. The results obtained in this study suggest that black yeast beta-glucan(Polycan®) have no systemic toxicity in mice and is therefore likely to be safe for clinical use. The median lethal dose(LD_{50}) and approximate lethal dose in mice after single subcutaneous dose of black yeast beta-glucan(Polycan®) were considered over 250mg/kg, the highest dissolved concentration in distilled water respectively.[Lee JH et al. Lab anim. Res. 2005. 21(4): 299-305]

D. safety determination

In Animal toxicological studies (maximum 1000mg/kg) including a GLP toxicity, genotoxicity studies, and pathogenicity and toxigenicity study using rat or mice in detail as Appendix 7-10, no compound-related toxicity was observed. Furthermore, in a human clinical study[400mg/day/human (60kg)] presented in detail as Appendix 11, Randomized, Double-Blind, Clinical Trials of Immunomodulating Polycan® of Black Yeast for Its Efficacy and Safety, no adverse effects have been associated with the repeated consumption of black yeast beta-glucan(Polycan®). Applying the maximum dose (1000mg/kg) of the animal toxicity studies to human (60kg) by the body surface area conversion, it allows a conclusion that 166mg/kg body weight for human, which corresponds to 9960 mg/person/day for a 60kg adult, should not result in any adverse effect.

The safety of black yeast beta-glucan(Polycan®) is also supported by the substantial equivalence in the composition of the ingredients with that of Biothera's bakers yeast beta-glucan (GRAS Notice No. GRN 000239). As stated in Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety held at Rome, Italy in 1996, " 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)" (FAO/WHO, 1996; U.S. FDA, 1997). In addition, in the Code of Federal Regulations (CFR), it is stated that "the FDA believes that in certain instances the concept of equivalence may have applicability to the technical element of a GRAS determination" (US.FDA, 1997).

Although Glucan's black yeast beta-glucan and Biothera's bakers yeast beta-glucan have different originations (*A. Pullulans* and *S. cerevisiae),* both beta-glucans have significant amount of β-1,3/1,6-glucan and both are 80% pure carbohydrate. Other chemical and microbiological parameters are also in the similar range of limits. The primary difference between the two products relates to their solubility. Due to the characteristic of being externally formed to the cell, black yeast beta-glucan is soluble while Biothera's bakers yeast beta-glucan is insoluble. Other than solubility, black yeast beta-glucan and Biothera's bakers yeast beta-glucan are substantially equivalent in their composition. A comparison of the specifications for black yeast beta-glucan and bakers yeast beta-glucan is shown in Table 1.

Table 1 Comparison of black Yeast Beta-Glucan with bakers yeast Beta-Glucan

Specification	black yeast beta-glucan	bakers yeast beta-glucan
Description	The exopolymer of *Aureobasidium pullulans SM-2001, an UV induced mutant of A. Pullulans ATCC 42023 (black yeast)*	Fine Beige/tan powder
Organism	*A. pullulans*	*S. cerevisiae*
Total Carbohydrate (%)	At least 82	At least 80
Beta-glucan (%)	At least 40	At least 70
Protein (%)	<10	<10
Fat (%)	<5	<20
Ash (%)	<10	<5
Moisture (%)	<6	<8
Lead (ppm)	ND	<0.5
Mercury (ppm)	ND	<0.05
Arsenic (ppm)	ND	<0.1[a]
Selenium (ppm)	ND	N/A
Cadmium	ND	<1[a]
Zinc (ppm)	N/A	N/A
Aerobic Plate Count (CFU/g)	<3,000	<20,000
Yeast and Mold (CFU/g)	≤20	≤25
Coliforms (CFU/g)	<10	<10
Escherichia coli	Negative	Negative
Salmonella sp	Negative	Negative
Coagulase positive *Staphylococci*, *Clostriduim perfringens*, *Clostridium botulnum* or any other recognized pathogen or hamful microbial toxin	N/A	N/A

CFU = colony forming units, ND = the concentration is below the detection limit or is non-detected.

N/A = not analyzed for by Glucan, na = not available per 21 CFR § 172.898

[a] Derived from certificates of analysis for various batches of Biothera's yeast beta-glucan

Furthermore, because the maximum use level, 150mg/serving of black yeast beta-glucan is 3/4 of the use level, 200mg/serving of Biothera's bakers yeast beta-glucan (GRAS Notice No. GRN 000239), all the major parameters of black yeast beta-glucan(Polycan®) including the total population all-user mean intake, an estimated heavy consumer (90th percentile) all-user intake, the greatest mean and percentile all-user intakes on an absolute basis, and the greatest mean and 90th percentile all-user intakes on a body weight basis are lower than those of Biothera's bakers yeast beta-glucan. Those major parameters of black yeast beta-glucan(Polycan®) are in the section of Intake Estimate below. Moreover, none of the components of black yeast beta-glucan is new to human metabolic processes.

In addition to all these scientific procedures of supporting the safety of black yeast beta-glucan(Polycan®), 5 metric ton/ year of black yeast beta-glucan(Polycan®) has been widely used in dairy products, processed meat products, and dietary supplements in S. Korea.
In conclusion, the intended use level of the 120-150 mg/serving should not pose any safety risk to any such consumer.

E. Intake Estimate

The various food products including baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, diary product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, soft candy, and soup and soup mixes, meat products are intended to market by Glucan Corporation for its Black yeast beta-glucan(Polycan®). Its intended use is at a level of 150 mg/serving shown in Table 2. The use of black yeast beta-glucan(Polycan®) for dairy products and beverages, such as milk, yogurt, cheese, and fruit juices, is self-limiting because the solubility of Black yeast beta-glucan(Polycan®) is decreased and the aggregation starts with concentrations above 5%. This aggregation in products causes a slimy or tacky mouth feel, which limits the use of black yeast beta-glucan(Polycan®) as an ingredient to level below 5% of the food weight.

Table 2. Proposed food uses and use levels for black yeast beta-glucan(Polycan®)

Food category	Proposed food-use	RACC[a] (g or ml)	Black Yeast beta-glucan (Polycan®) levels	
			(mg/serving)	(%)
Baked goods and baking mixes	cookies	30 to 40	150	0.5 to 0.375
Beverages and beverage bases	Meal replacement beverage (not milk-based)	240	150	0.375
Cereal and cereal products	Nutritional bars (breakfast, granola, protein)	40	150	0.375
Dairy product analogs	Soy milk	240	150	0 0625
Milk and milk products	Meal replacement beverage	240	150	0.0625
	Probiotic beverage	240	150	0.0625
	Yogurt	225	150	0.0666
	Yogurt beverage	240	150	0.0625
Plant protein products	Soy protein bars	40	150	0.375
Processed fruits and fruit juices	Fruit beverages (drinks, juices, smoothies)	240	150	0.0625
Soft candy	Chocolate confections	40	150	0.375
Soup and soup mixes	soups	245	150	0.0612
Meat products	Processed meat	55	150	0.2727

[a]RACC = reference amounts customarily consumed per eating occasion [21 CFR § 101 12 (U S FDA, 2007b)] when a range of values is reported for proposed food use, particular foods within that food use may differ with respect to their RACC

Using the United States Department of Agriculture(USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSF II 1994-1996) survey (USDA, 2000), under the conditions of intended use, the total population all-user mean intake of black yeast beta-glucan(Polycan®), an estimated heavy consumer (90th percentile) all-user intake of black yeast beta-glucan(Polycan®), the greatest mean and percentile all-user intakes of black yeast beta-glucan(Polycan®) on an absolute basis, and the greatest mean and 90th percentile all-user intakes of black yeast beta-glucan(Polycan®) on a body weight basis are 338.7 mg/person/day, 678.4 mg/person/day, 476.4 and 972.9 mg/person/day, 22.9 and 44.2 mg/kg body weight/day in infants, respectively (See Table 3 and 4). Furthermore, black yeast beta-glucan(Polycan®) is not intended directly to market for infant and toddler foods, resulting in limiting infants and toddlers to consume black yeast beta-glucan(Polycan®).

Table 3. Summary of the estimated daily intake of black yeast beta-glcuan (Polycan®) from all proposed food categories in the U.S. by population group (1994-1996, 1998 USDA CSF II data)

Population group	Age group (years)	% users	Actural # of total users	All person consumption		All-users consumption	
				Mean (mg)	90th percentile (mg)	Mean (mg)	90th percentile (mg)
Infant	0 to 2	71.6	2,564	224 1	521.6	287.3	568.4
Child	3 to 11	98.4	6,205	389.8	712.6	397 7	713.9
Female teenager	12 to 19	91.5	642	330 9	702.6	361 8	726.6
Male teenager	12 to 19	91 7	638	436.2	930 3	476.4	972 9
Female adult	20 and up	85	3,887	238.7	526 9	278.2	551.9
Male adult	20 and up	81.6	3,876	290.6	678.8	355 1	743.7
Total population	All ages	86 4	17,812	292 7	644 7	338.7	678 4

Table 4. Summary of the estimated daily per kilogram body weight intake of black yeast beta-glucan(Polycan®) from all proposed food categories in the U.S. by population group (1994-1996, 1998 USDA CSF II data)

Population group	Age group (years)	% users	Actural # of total users	All person consumption		All-users consumption	
				Mean (mg)	90th percentile (mg)	Mean (mg)	90th percentile (mg)
Infant	0 to 2	71.6	2,564	17 8	41.0	22 9	44.2
Child	3 to 11	98.4	6,205	16.1	31.1	16.5	31.2
Female teenager	12 to 19	91.5	642	6 1	14.3	6.7	14.5
Male teenager	12 to 19	91 7	638	7.2	15.1	7.8	16 3
Female adult	20 and up	85	3,887	3.7	8.4	4.3	8.9
Male adult	20 and up	81.6	3,876	3.6	8.6	4.4	9.5
Total population	All ages	86.4	17,812	6.3	15.4	7.3	16 9

F. Expert Consensus

The safety studies described above for black yeast beta-glucan(Polycan®) have been published in four peer-reviewed scientific journals. The full version of those journals are presented in Appendix 7-10. A human clinical study[400mg/day/human (60kg)], which is Randomized, Double-Blind, Clinical Trials of Immunomodulating Polycan® of Black Yeast for Its Efficacy and Safety presented in detail as Appendix 11, has been completed at Daegu Oriental Hospital of Daegu Haany University, a GCP(Good Clinical Practice) certified institute. The authors who are qualified by scientific training and experience to evaluate the safety of black yeast beta-glucan (Polycan®) consistently concluded in the 4 journals and 1 clinical study that no adverse effects associated with the repeated consumption of black yeast beta-glucan(Polycan®) produced from *Aureobasidium pullulans* SM-2001 has been observed. Furthermore, the authors concluded that their findings of black yeast beta-

glucan(Polycan®)'s safety are quite similar to other types of β-glucan isolated from different origins (Chihara,1983; Feletti et al., 1992; Spicer et al., 1999; Delaney et al., 2003).

In addition, two reports in Appendix 12 and 13 from a US FDA registered laboratory, RCH Pharmaceutical Cosmetic and Food Analytical Laboratories, show that no parameter indicated any safety risk in both Organochlorine Pesticides and PCBs by GC and Heavy Metals by ICP.

As a result, based on all the safety data in the peer-reviewed journals, studies, and reports, the compositional similarity to Biothera's bakers yeast beta-glucan, and the lack of toxicity associated with black yeast beta-glucan (Polycan®), the conclusion that the proposed uses of Glucan's black yeast beta-glucan(Polycan®) are Generally Recognized as Safe(GRAS) would be highly agreeable with any expert.

In summary, the expert consensus and the general recognition elements of GRAS status are satisfied by the fact that all the conclusions of our published and peer reviewed journals, toxicity studies, toxicity reports, and human clinical trial consistently indicate black yeast beta-glucan(Polycan®)'s safety.

REFERENCES

1. Hyeung-Sik Lee, Kun-Ju Yang, Hyun-dong Shin, Bok-Ryeon Park, Chang-Woo Son, Hee-Jeong Jang, Dong-Chan Park, Young-Mi Jung and Sae-Kwang Ku (2005): Single Oral Dose Toxicity Studies of Polycan, β-Glucan Originated from Aureobasidium in Mice.J.ToxicolPub.Health,21,361-365

2. Jaehyun Lee, Kunju Yang, Hyundong Shin, Bokryeon Park, Changwoo Son, Heejeong Jang, Dongchan Park, Hyeung-Sik Lee and Saekwang Ku(2005): Single Subcutaneous Dose Toxicity of Polycan, a β-Glucan Originated from Aureobasidium in Mice.Lab.Anim.Res.21,299-305

3. Hyeung-Sik Lee, Hyun-Rea Cho, Kun-Ju Yang, Seung-Bae Moon, Bok-Ryeon Park, HyunDong Shin, Hee-Jeong Jang, Lin-Su Kim and Sae-Kwang Ku(2008):Micronucleus Test of Polycan, β-Glucan Originated from *Aureobasidium*, in Bone Marrow Cells of Male ICR Mice.Toxicol.Res.24,11-15

4. Hyun Dong Shin, PH.D.,Kun Juh Yang, B.Sc, Bok Ryeon Park,M.Sc, Chang Woo Son, M.Sc, Hee Jeong Jang, M.Sc, and Sae Kwang Ku, Ph.D.(2007): Antiosteoporotic effect of Polycan, β-Glucan from *Aureobasidium*, in ovariectomized osteoporotic mice.Nutrition.23,853-860

5. Hyeung-Sik Lee, Hyung-Rae Cho, Kun-Ju Yang, Hyun-Dong Shin, Bok-Ryeon Park, Hee-Jeong Jang, Kwang-Ho Cho, Heong-Dong Kim and Sae-Kwang Ku(2006): Local Irritation Test of 3 Types of β-Glucan after Subcutaneous Injection in Rats.Lab.Anim.Res.22,339-342

6. Song,HeBok, Dong Chan Park, Gyung Min Do, Seing-Lark Hwang, Won Kyu Lee, Heun-Soo Kang, Bok-Ryun Park, Hee-Jeong Jang, Chang Woo Son, Eui Kyun Park, Shin-Yoon Kim, and Tae-Lin Huh(2006): Effect of Exopoymers of *Aureobasidium pullulans* on Improving Osteoporosis Induced in Ovariectomized Mice. J.Microbiol.Biotechnol.16,37~45

7. Hyeung-Sik Lee, Hyung-Rea Cho, Seung-Bae Moon, Hyun-Dong Shin, Kun-Ju Yang, Bok-Ryeon Park, Hee-Jeong Jang, Kin-Su Kim, Sae-Kwang Ku(2008):Effect of β-Glucan from *Aureobasidium pullulands* on Rat Rib Fracture Healing.Lab.Anim.Res.24,39-44

8. Hyeong-Dong Kim, Hyung-Rea Cho, Seung-Bae Moon, Hyun-Dong Shin, Kun-Ju Yang, Bok-Ryeon Park, Hee-Jeong Jang, Lin-Su Kim, Hyeung-Sik Lee, and Sea-Kwang Ku(2006):Effect of Exopolymers from *Aureobasidium pullulan*s on Formalin-Induced Chronic Paw Inflammation in Mice.J.Microbiol.Biotechnol.16,1954-1960

9. Hyeong-Dong Kim, Hyung-Rae Cho, Seung-Bae Moon, Kun-Ju Yang, Bok-Ryeon Park, Hee-Jeong Jang, Lin-Su Kim, Hyeung-Sik Lee, and Sae-Kwang Ku(2007):Effect of β-Glucan from *Aureobasidium pullulands* on Acure Inflammation in Mice. Arch Pharm Res. 30,323-328

10. Joseph F. Borzelleca, Robert J. Nicolosi, Gary M. William(2007):Expert Panel Consensus Statement Concerning The Generally Recognized As Safe (GRAS) Status of Yeast Beta-Glucan For Use as A Food Ingredient. Biothera Inc.

11. FAO/WHO 1996. Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety. Available from http://www.fao.org/ag/agn/food/pdf/biotechnology.pdf

12. USDA 2000. 1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS). U.S. Department of Agriculture(USDA); Riverdale. Maryland[PB2000-500027]

13. U S. FDA 1997. Substances generally recognized as safe; Proposed rule (21 CFR Parts 170, 184, 186, and 570) Federal Register (US) (Volume 62, number 74). Page 18937-18964. Available from http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm

14. U S FDA 2009. Part 101-Food labeling. § 101.12-Reference amounts customarily consumed per eating occasion In. U.S. Code of Federal Regulations (CFR) Title 21-Food and Drugs (U.S.F ood and Drug Administration). Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=101.12

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APPENDIX 1

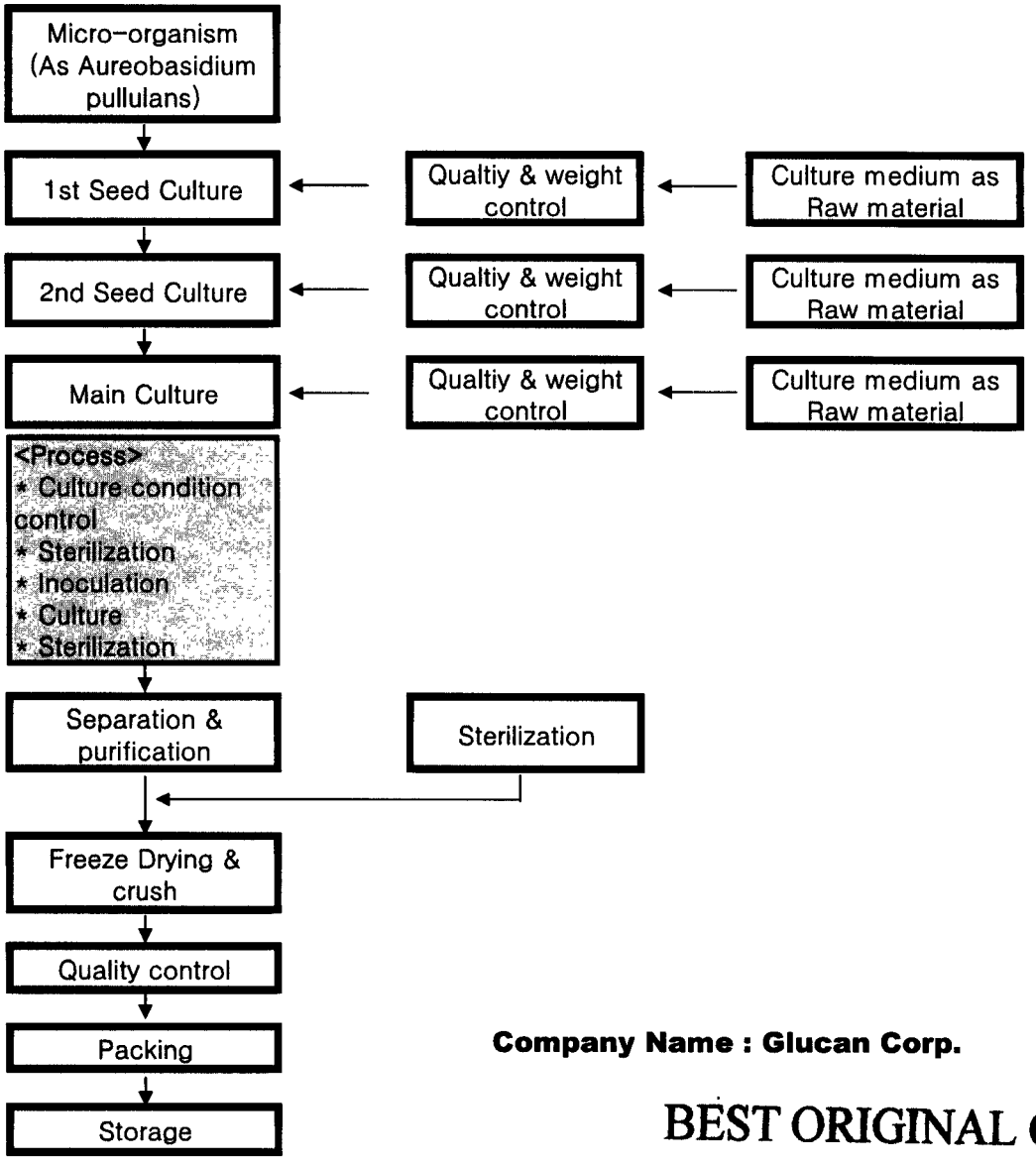
Manufacturing method for black yeast beta-glucan(Polycan®)

Issue Date: 2008.02.01 ISO9001 : QP-704(1)

Manufacturing Process

Product Name: Polycan
Using Type: Food (Korean FDA standard)
Ingredient: cuture extract powder by Aureobasidium pullulans

Flow chart



Company Name : Glucan Corp.

BEST ORIGINAL COPY

Address: Marine Bio center 221 Silla Univ. Busan, Korea
Tel: +82-51-999-5886 Fax: +82-51-999-5887 www.glucan.co.kr
Glucan Corp.

APPENDIX 2

Antiosteoporotic effect of Polycan, β -glucan from *Aureobasidium*, in ovariectomized osteoporotic mice

Pages 000024 - 000031 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

APPENDIX 3

Effect of Exopolymers of *Aureobasidium pullulans* on Improving Osteoporosis Induced in Ovariectomized Mice

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APPENDIX 4

Effect of β-Glucan from *Aureobasidium pullulans*
on Rat Rib Fracture Healing

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APPENDIX 5

Effects of β-Glucan from *Aureobasidium pullulans*
on Acute Inflammation in Mice

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APPENDIX 6

Effect of Exopolymers of *Aureobasidium pullulans*
on Formalin-Induced Chronic Paw Inflammation in Mice

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APPENDIX 7

4 weeks oral dose toxicity test of exopolymers from *Aureobasidium pullulans*(Polycan®) in SD rat

Biotoxtech

Final Report

4 weeks oral dose toxicity test of exopolymers from *Aureobasidium pullulans* (POLYCAN®) in SD rat

Toxicity test of exopolymers of Aureobasidium pullulans (polycan)
(Rats, repetitive oral dosage for 4 weeks)

Test No. : B07319

Biotoxtech Inc. GLP organ

Post 363-883 Chungbuk Chungwongun Ochangoep Yangchungli 686-2 Ochang-scienceindustry group 58-1 block

Test responsor signature and GLP property

Title: 4 weeks oral dose toxicity test of exopolymers from *Aureobasidium pullulans* (POLYCAN®) in SD rat

Test number: B07319

This test was conducted with law of Good Laboratory Practice (GLP). Biotoxtech corporation conducted with standard operation practices (SOPs)

- Non-GLP test manage standard(KFDA inform 2005-79, December 21, 2005))

This test was conducted follow by approved test plan, All test schedule was conducted under responsor, and this report was wrote with basal result of this test plans

Biotoxtech Corporation
Responsor kim sun hee (b) (6)

2008년 4 월 30 일

Biotoxtech Corporation
Operation responsor Lee byung kyu (b) (6)

2008년 4월 30일

Certification of Confidence

Title: 4 weeks oral dose toxicity test of exopolymers from Aureobasidium pullulans (polycan®) in SD rat

Test number: B07319

Biotoxtech corporation trust & warranty manager check the final report following test. Checking was Biotextech's SOP, each result was reported to test responsible person and operation responsible person.

Checking achieve process and test responsible person, operation responsible person are following

Check list	date	Reporting date
Test plan	January 7, 2008	January 7, 2008
Test plan modification	February 21, 2008	February 21, 2008
Animal receipt	December 7, 2007	December 7, 2007
Materials deposit	January 15, 2008	January 15, 2008
Materials making	January 15, 2008	January 15, 2008
Administration	January 15, 2008	January 15, 2008
Clinical sign observation	January 15, 2008	January 15, 2008
Body weight measurement	December 19, 2007	December 20, 2007
Food intake measurement	December 19, 2007	December 20, 2007
Urine collection	February 10, 2008	February 11, 2008
Urine test	February 10, 2008	February 11, 2008
Opthamological test	February 11, 2008	February 11, 2008
Blood collection	January 2, 2008	January 2, 2008
serum chemistry test	January 2, 2008	January 2, 2008
Hematological test	January 2, 2008	January 2, 2008
Autopsy	February 12, 2008	February 12, 2008
Histopathologic object manufacture (trimming)	February 1, 2008	February 1, 2008
Histopathologic object manufacture (embedding)	February 4, 2008	February 4, 2008

Histopathologic object manufacture (thin section)	February 18, 2008	February 20, 2008
Histopathologic object manufacture (stain)	February 12, 2008	February 12, 2008
Histopathological test	February 15, 2008	February 15, 2008
Recording of	April 4, 2008	April 4, 2008

- Checking of accomplish procedure: There are not check on regular procedure in every test, this test was certify by checking specific procedure
- Regular procedure was not execute on each test, specific test was certified in this applicable stage

The written methods was appropriate in the this final report and biotoxtech certify that the result writing from basement data reflect correctly

Biotoxtech Corporation
Confidence responsible person kim jung-wha (b) (6)

2008 년 04월 30 일

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1. Summation

This test's object is estimation of Aureobasidium pullulans (POLYCAN®) repeated dose toxicity. Polycan® was administrated 250, 500, 1000mg/kg PO for 4 weeks in SD rat(male, female). Control was administered DW. Clinical sign, body weight, food consumption, ophthalmologic findings, urine analysis, blood and serum biochemistry analysis, organ weight, macrography of organ, and histopathological test was conducted.

There are not observed any abnormality in clinical sign, body weight, food consumption by administration of Aureobasidium pullulans (POLYCAN®)

There are not recognized any abnormality in ophthalmologic findings, urine analysis, blood and serum biochemistry, organ weight, autopsy

In histopathological finding, there are not observed abnormality from 1000mg/kg administrated both male and female rats

Consequently, there are not found abnormality by administration of Aureobasidum pullulans (POLYCAN®). Therefore, Aureobasidium pullulans(POLYCAN®) is considered that NOAEL is over 1,000mg/kg both male and female

2. Abstract of experiment

2.1 Object

To estimate of 4-week repeated oral dose toxicity of Aureobasidum pullulasn(POLYCAN®) in male and female SD rats

2.2 Good laboratory practice

This test's procedure was followed by GLP rules
- PreClinical study manage standard (KFDA bulletin No.2005-79, Dec 21, 2005)

2.3 Standard of test

This test was conducted by follow SOP
-Toxicity test standard of drugs (KFDA bulletin No. 2005-60, Oct 21, 2005)

2.4 Ethnic of animal

This test was approved by IRB of Biotoxtech grounded animal protection law (established May 31, 1991 law No. 4372, revision Jan 26, 2007 law No. 8282)

2.5 Requestor

Name : Glucan Corporation
Address: 221 Marine bio center, Silla Univ. Busan 617-736, Korea
Tel: +82-51-999-5886 Fax: +82-51-999-5887

2.6 Test organization

Name: Biotoxtech
Address: post363-883 Chungbuk Chungwongun Ochangoep Yangchungli 686-2 Ochang scienceindustry group 58-1 block
Tel: +82-43-210-7777 Fax: +82-43-210-7778

2.7 Institution of test

Title: Biotoxtech
Address: post363-883 Chungbuk Chungwongun Ochangoep Yangchungli 686-2 Ochang-scienceindustry group 58-1 block

2.8 Responsible person

Name: Kim sun-hee
Affiliation: 1 team of toxicity test

2.9 Record and custody of data

2.9.1 Custody period: 5 years from test finished, prolonged custody was decided from council with request

2.9.2 Custody place
Title: Custody depository of Biotoxtech
Address: post363-883 Chungbuk Chungwongun Ochangoep Yangchungli 686-2 Ochang-scienceindustry group 58-1 block

2.9.3 Record and custody of data

A plan, plan alteration form, law data, final report, custody materials, recoding concerning test (GLP relevant record, communication record), test materials)

2.10 Test schedule

Initial date of test	Dec. 31, 2007
Animal receipt	Jan. 8, 2008
Initial date of experiment	Jan. 15, 2008
Observed period	Jan.15, 2008 ~ Feb 11, 2008 (male)
	Jan. 16, 2008 ~ Feb 12, 2008 (female)
Autopsy	Feb. 12 ~ 13, 2008
Finished date of experiment	Mar. 24, 2008
Finished date of test	Apr. 30, 2008

2.11 Division and test charge

Quarantine charge:	Hong Kyung-mi
Test custody charge:	Kim Eun-Ae
Pathologic charge:	Park Sun-hee
Quarantine	Lim Young-whan
Body weight & SC during acclimation	Park hee-won, Park Hyung-yul, Kim yeu-jung
Verification of test materials	Kim Kin-A
Preparation of test materials	Park eun-jung, Lim hee jung, Kim jin-A
Administration	Park hee-won, Park hyung-yul, Yang young-jae, Park chan-sung
Body weight & food consumption measure	Park hee-won, Park hyung-yul
Clinical signs	Park hee-won, Park hyung-yul, Lee sun-ho
Ophthalmological findings	Park hee-won, Kim sun hee, Lee jung-min
Clinical pathology	Kim young-min, Jung jin sung, Lee mi-ju, Mun Je-in, Bae resina, Kim Hea jin, Jung eun young
Histologic sample manufacture	Jo sun jung, Kim jin suk, Mun je in, shin sun hee, Im mi hee, Jung eun young, Bae resina

3 Materials and method

3.1 Materials

3.1.1 Material name : Aureobasidium pullulans broth powder (POLYCAN®)

3.1.2 Lot. No. : CGN-07121402

3.1.3 Appearance: mild yellow, unique flavor

3.1.4 Component: ß-glucan

3.1.5 Purity: 12.79%

3.1.6 pH: 5.72 (5% solution)

3.1.7 Date of manufacture: Dec. 14, 2007

3.1.8 Stability: 2 years with package in the dark cold place

3.1.9 Validity period: 2 years

3.1.10 Custody condition: dark cold place

3.1.11 Handling caution: prevent moisture absorption and scattering

3.1.12 Sponsor

Title: Glucan corporation

Address: 221 Marinebiocenter, Silla Univ. Busan 617-736, Korea

Tel: +82-51-999-5886, Fax: +82-51-999-5887

3.1.13 Management of residue materials : return after test finished

3.1.14 Certificate of analysis: offer by requester (Appendix 12)

3.2 Identification of materials

Biotoxtech identified property, sameness of the materials that was offered from requester

3.3 Carrier

3.3.1 Name: distilled water

3.3.2 Lot No.: GBA7001

3.3.3 Custody condition: room temperature

3.3.4 Maker: Choongwae Pharma Corp., Korea

3.3.5 Reason of selection

The materials was dissolved with DW through information of request and preliminary making

3.4 Making of administration materials and analysis

3.4.1 Making of administration materials

Materials put into the bottle with Measuring by electronic scale(CP323S, Sartorius, Germany), and administration materials were suspended with carrier(distilled water) by stirrer and then control the concentration

3.4.2 Analysis of administration materials

There was not conducted of stability, homogenisity, concentration analysis of adimistration materials

3.5 Animal

3.5.1 Species: rat, Sprague-Dawley (Crl:CD(SD)), SPF

3.5.2 Product & purchase place: ORIENTBIO INC., Korea

3.5.3 Reason of selection

Sprague-Dawley rat is common-use in safety tests of drug, and there are influence basic data

3.5.4 Sex, number, years, body weight range at receipt

40 male, 5 weeks-old, 109.2~126.6g/ 44 female 5-weeks old, 84.5~95.3g

3.5.5 Sex, number, years, body weight range at administration

40 male, 6 weeks old, 176.5~193.1g/ 40 female, 6-weeks old, 109.6~133.9g

3.5.6 Carrying and discrimination

After appearance inspection, the tail was marked with red ink by the discrimination method. And body weight was measured by electronic balance(CP3202S, Sartorius, Germany)

3.5.7 Quarantine, acclimation method and period

Rats was observed once a day for 7~8 days. In the final day, body weight was measured and clinical sign, body weight was verified without abnormality

3.5.8 Grouping

Animals was selected with closed means, group was divided with 4 groups containing 10 animals

3.5.9 Treatment of residue animal

The residue animals was euthanized by SOP

3.5.10 Discrimination after grouping and cage discrimination

After grouping, discrimination card was attached on the cage. Animals was marked with blue ink on the tail.

3.6 Environment of breeding

3.6.1 Breeding room name: B325

3.6.2 Cage: stainless steel cage, 260W*350D&210H(mm)

3.6.3 Animal No. in the cage: 3 animal (in quarantine, acclimation)/1 animal(observation period)

3.6.4 Temperature: 20.1 ~ 21.7℃

3.6.5 Relative humidity: 55.0~67.5%

3.6.6 Ventilation: 10~15/hours

3.6.7 Light/dark period: 12 hours/day (from a.m. 7 to p.m. 7)

3.6.8 Intensity of illumination: 150~300Lux

3.6.9 Exchange No. of breeding instrument, cleansing, sterilization

Cage and feeding bottle was exchange every 2 weeks

Breeding instrument was cleansing with auto-washer and sterilized with autoclave

3.7 Foods

3.7.1 Kind

Laboratory pellet feed (TEKLAD CERTIFIED GLOBAL. 18% PROTEIN RODENT DIET 2918C, Lot No.: 2918C 081607MA, 2918C 090507MA)

3.7.2 Manufacturer: Harlan TEKLAD, U.S.A

3.7.3 Feeding method: ad libitum

3.7.4 Analysis and certification of foods

The feed analysis was certified with Halan TEKLAD analytical record and analytical result of Korea testing & research institute by Biotextech's request twice every year

3.8 Waters

3.8.1 Kinds

Refined water through UV irradiation after filter sterilization

3.8.2 Water feeding method

Free accessed by auto feeding system

3.8.3 Analysis and certification of water

Water analysis was request to Chungbuk, institute health & environment research (raw of water standard for feeding and inspection. No 122. 2002. Ministry of Environment) twice every year. The result was certified within permission standard range

3.9 Administration

3.9.1 Administration route

Oral administration

3.9.2 Reason of route selection

That was followed by route of clinical study application

3.9.3 Administration method and frequency

Administration volume is 20ml/kg. the administration volume was followed by the body weight of once a measure in week. Every a.m. and p.m., rats were administered with jondae twice(4 hours interval) a day for 4 weeks

3.10 Grouping and dosage

3.10.1 Grouping

	Group	Dosage	Volume	No. of animal	
				male	female
G1	Control	0	20	10(1101~1110)	10(2101~2110)
G2	Low dose group	250	20	10(1201~1210)	10(2201~2210)
G3	Middle dose group	500	20	10(1301~1310)	10(2301~2310)
G4	High dose group	1000	20	10(1401~1410)	10(2401~2410)

3.10.2 Dosage

Clinical study application dosage is 1250mg/human/day (20mg/kg for 60kg adult). This test is used 1000mg/kg(high dose group) of fifty times of clinical study application dosage. In the rate of 2 to 1, 500mg/kg(middle) and 250mg.kg(low) was determination. Control was administered DW

3.11 Observation and inspection

It is day 0 that is clinical sign observation, measure and inspection. And counted after administration day 0

3.11.1 Clinical sign

All animals were observed general condition, motion, ANS function and excretion once a day, and death existence was observed twice a day. The observation practiced for 4 weeks

3.11.2 Body weight measurement

Body weight was measured in administration day(before administration), once a

week after administration, one previous day of autopy and measured body weight on the autopy day. However, the body weight measure of autopsy day was excepted cause by fasting until previous day

3.11.3 Food consumption measurement

Food intake amount was measure from grouping to administration day 0. means food intake amount was calculated by divide 7 days food intake period. The last weeks, the means food intake amount was calculated by divide 6 days food intake

3.11.4 Ophthalmologic inspection

In 4 week of administration, ophthalomolgical inspection was practiced by macrography and ophthalmoscopy(ALL PUPIL II, Keeler, UK) after miosis drug(Ocutropic ophthalmic drops, Samil Pharma. Co., Ltd.) treatment

3.11.5 Urine analysis

Urine analysis was practiced for 4 weeks of administration, fresh urine (within 3 hours after urination) and accumulation urine(accumulation within 24 hours after urination) was collected and analysis was practiced by followings. In the fresh urine collection, administration and food fastened and water is ad libitum. Fresh urine was analysis by same volume collection

kind	items	unit	Method/instrument
Fresh urine	pH	-	Combur10Test®M stick (Roche, Germany)
	Protein	Mg/dl	
	Glucose	Mg/dl	Urine chemistry analysis (MIDITRON junior II, Roche, Germany
	Ketone body	Mg/dl	
	Biliruin	Mg/dl	
	erythrocyte	Ery/ul	
	Color, clarity	-	macrography
	침사	-	Microscope
Accumulation urine	volume	ml	messcylinder
	Specific gravity	-	Gravity instrument (No. 503, NIPPON OPTICAL WORKS, Japan
	Sodium, Na	mmol/l	Ion-selective electrode, electrolyte analytical instrument (AVL9181, Roche, Germany)
	Potassium, P	mmol/l	

3.11.6 Hematological analysis

All animal were fasting during 18 hours before autopsy. In isoflurane anesthesia, blood collected in the descending aorta. Collected blood was inserted in the EDTA treated tube (BD vacutainer™ K2 EDTA, Becton Dickinson, USA). Following items was analysis by blood analytical instrument ADVIA 120, SIEMENS, Germany)

Items	Unit	Method
Total erythrocyte count, RBC	x 10^6 cells/ul	Flow cytometry
Hemoglobin concentration, Hb	g/dl	Flow cytometry, cyanmethemoglobin
Hematocrit, HCT	fl	Flow cytometry
Mean cell hemoglobin, MCH	pg	Calculated
Mean cell hemoglobin concentration, MCHC	g/dl	
Platelet, PLT	x 10^3 cells/ul	Flow cytometry
Total leukocyte, WBC	x 10^3 cells/ul	Flow cytometry, peroxidase stain
WBC differential counting		
• Neutrophils, NEU	%	
• Lymphocytes, LYM		
• Monocytes, MONO		
• Eosinophils, EOS		
• Basophils, BASO		
Reticulocyte, Retic	%	Flow cytometry, RNA stain

For agglutination test, 2ml collected blood was inserted in the tube containing 3.2% sodium citrate (VACUTTE® 9NC Coagulation sodium citrate 3.2%, greiner bio-one, Austria) and then centrifuged 3000rpm, 10 minutes. Plasma was collected Agglutination tester (ACL 7000, Instrumentation Laboratory, USA)

Items	Unit	Method
Prothrombin time, PT	Sec	Nephelometry
Activated partial thromboplastin time, APTT	sec	

3.11.7 Serum chemistry analysis

Collected blood from descending aorta centrifuged 3000rpm for 10 minutes and collected serum. The following are analyzed with hematochemical analyzer(7080,

HITACHI, Japan) and electrolyte analyzer(AVL9181, Roche, Germany

Items	unit	Method
Alanine aminotransferase, ALT	U/L	JSCC(UV kinetic)
Aspartate aminotransferase, AST	U/L	JSCC
Alkaline phosphatase, ALP	U/L	p-Nitrophenylphosphate substrate
Blood urea nitrogen, BUN	mg/dl	Urease-GLDH
Creatinine, Crea	mg/dl	Jaffe
Total bilirubin, T-bil	Mg/dl	Vanadate oxidation
Total protein, TP	g/dl	Biuret
Albumin, Alb	g/dl	BCG
A/G ratio		Calculated
Total cholesterol, T-chol	mg/dl	Cholesterol oxidase, HDAOS
Tryglycerides, TG	mg/dl	GPO•HDAOS
Phosphours, P	mg/dl	Fiske subbarow
Glucose, Glu	mg/dl	Hexokinase-G6PDH
Calcium, Ca	mg/dl	OCPC
Chloride*, Cl	nmol/l	
Sodium*, Na	nmol/l	Ion-selective electrode
Potassium*, K	nmol/l	

*: measure with Electrolyte analyzer

3.11.8 Autopsy

All animals's oragans were inspected by macrograpy of surface of body, head, cervical region, thoracic region, abdominal region

3.11.9 Organ weight measurement

Following organ was measured and calculated the relative weight of organ. *; sum of right and left organ

- Brain
- Heart
- Kidney*
- Adrenal*
- Ovary
- Lung
- pituitary
- liver
- spleen
- testis*
- prostate
- uterus

3.11.10 Histological analysis

Following organs were removed and fixed with 10% neutral buffered formalin.

Testis was fixed with Davidson solution. The tissue was then processed and embedded in paraffin. Sample was stained with Hematoxylin & Eosin stain

- Brain
- Thyroid and parathyroid
- Thymus
- Trachea
- Liver
- Kidney
- Salivary gland
- Stomach
- Jejunum
- Cecum
- Rectum
- Testis
- Prostate
- Ovary
- Vagina
- Submandibular lymph node
- Mammary gland
- Eye & etc.
- Femur and sternum

pituitary
lung with bronchi
heart
spleen
adneral
esophagus
duodenum
ileum
colon
pancreas
epididymis
seminal vesicle
uterus
urinary bladder
mesenteric lymph node
skin
tongue
spinal cord

3.11.11 Histopahtological analysis

Histopathological analysis was compared with control group.

3.12 Statistical analysis

All data fo body weight, food consumption, urine test, hematological test, organ weight were analyzed using statistical program (version 9.1.3, SAS Institute Inc., Cary, NC, USA).

Bartlett test for homoscedasticity was conducted (significance: 5%). One-way analysis of variance(ANOVA) was conducted in the homoscedasticity. If the ANOVA indicate significance. Dunnett's test for multiple comparison was conduced to determine the specific paris of group comparison (significance: 5% and 1%). If the ANIVA indicated no significance, Kruskal-wallis test was conducted. When a significant difference is observed in the Kruskal-wallis test, Mann-whitney u-test was conducted to determine the specific pairs of group comparison.

4 RESULT

4.1 Clinical sign (Table 1 & Appendix 1)

There are not observed abnormality during experimental period in male and female

4.2 Body weight change (Figure 1,2 & Table 2 & Appendix 2)

There are not significant variance of body weight compared with control group in male and female

4.3 Food intake (Table 3, Appendix 3)

There are not significant food consumption variance compared with control group

4.4 Opthalmological inspection

There are not observed abnormality

4.5 Urine analysis

There are not observed abnormality compared with control group

4.6 Hematologic analysis

There are not observed abnormality compare with male control group in female 500mg/kg dosing group, APTT was significant increased compared with control group. There are not toxicological finds cause by no dose-dependency

4.7 Serum chemistry analysis

In male 250mg/kg dosing group, phosphorus was significantly decreased but there are not toxicological findings cause by no dose-dependency. In male 500 and 1000mg/kg dosing group, ALP was significantly increased (respectively $P<0.05$, $P<0.01$) but there are not toxicological finding cause by no histopathological change of relative organ(liver, bone marrow etc)

In female 1000mg/kg dosing group, potassium was significantly decreased compared with control group, but there are not toxicological finding cause by no dose-dependency

4.8 Organ weight

In absolute organ weight and relative organ weight ratio, there are not significant variance compared with control group.

4.9 Atopsy and histological analysis

There are not observed abnormality in all male and female autopsy. Heart enlargement was observed In female control group. But there are not toxicological findings.

In histopathological findings, there are not observed abnormality of 1000mg/kg dosing group of male and female

In control and 1000mg/kg dosing group of male and female, myocardial hypertrophy, ultimobranchial cyst of thyroid gland or ectopic thymus, and cell infiltration of other organ•tissue and so on were occurred spontaneously or by accident. So there are no toxicological findings

5 Discussion and Conclusion

In order to test toxicity in the repetitive oral dosage of Exopolymers of *Aureobasidium pollulans* (Polycan) the repetitive oral dosage of Polycan was given to male & female SD rats for 4 weeks.

The change of weight, the change of food consumption, and any kind of clinical sign caused by the testing material were not observed in the group of male & female rats.
There are no odd and specific clinical signs

The group of male & female rats did not show the change in the ophthalmological test, the urinalysis, the hematological analysis, the serum biochemistry, the internal organ weight measure, and the autopsy result.

The change was not observed in the histopathological test from the male & female group dosed 1000 mg/kg.

As a result of the entire test above, it is concluded that any change resulted from the repetitive oral dosage of Polycan was not observed in the group of male & female rats who took the dosage. Therefore, NOAEL in this test is over 1000mg/Kg for both male & female rats.

APPENDIX 8

Micronucleus test of Polycan®, β-Glucan Originated from *Aureobasidium*, in Bone Marrow Cells of Male ICR Mice

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APPENDIX 9

Single Oral Dose Toxicity Studies of Polycan, β-Glucan Originated from Aureobasidium in Mice

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APPENDIX 10

Single subcutaneous dosage toxicity of Polycan, a β-Glucan Originated from Aureobasidium in Mice

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APPENDIX 11

Randomized, Double-Blind, Clinical Trials of Immunomodulating Polycan® of Black Yeast for Its Efficacy and Safety

Randomized, Double-Blind, Clinical Trials of Immunomodulating Polycan of Black Yeast for Its Efficacy and Safety - Summary

2009. 8

[Request authority]
Glucan corporation

[Clinical Test Centers]
Daegu Oriental hospital of Daegu Haany university
Oriental medicine industry support center of Daegu technopark

1. Purpose

This study is randomized, placebo-controlled study, evaluating immuno-modulating efficacy and safety of Polycan, which is the culture powder produced by black yeast(*Aureobasidium pullulans* SM-2001), having β-1,3-1,6-glucan as the active ingredient. A total 56 subjects were enrolled into 2 groups and within each group, randomized treatment groups receiving Polycan or placebo in the regimen, oral dosing 2 tablets after 30 minute of every meal.

2. Products Tested

2-1. Product Clinically Tested

1) Characteristics of the tested product

Polycan, a registered trademark of Glucan, is a dried powder form of culture supernatant of a black yest *Aureobasidium pullulans* SM-2001 after removal of cell and its debris followed by additional manufacturing processes. This has a weak unique smell and yellowlish brown colored tabletized product which is produced from manufacturing processes including sterilization, filtration and drying of culture broth of *Aureobasidium pullulans*. β-Gucan, an active (indicator) ingredient, of Polycan is β-1,3-1,6-glucan was a branched polysaccharide having β-1,6-linked D-glucose and the ratio of glucose to gentibiose implying the degree of branching was found to be 5:1. This glucan has better biological modulating activity than unbranched linear form due to the branched form, and the degree of branching is similar with those (0.2~0.33) of schizophyllan and lentinan, known as the glucans having a most strong anti-cancer activity. This glucan has amorphous structure in aqueous phase and up to multi-million molecular weight, in addition, a lactic acid residue linked to gentibiose residue of this polymer may induce solubilizing it in water.

2) Product Information

○ Name : Polycan
○ Type : Yellowlish brown colored tablet
○ Storage : Room temperature/secret container shading
○ Expiration: 24 months after manufacturing
○ Company: Glucan LLC

2-2. Placebo product

○ Type : Yellowlish brown colored tablet

○ Storage : Room temperature/secret container shading
○ Expiration: 24 months after manufacturing
○ Company: Glucan LLC

3. Subject

3-1. Subject

56 Healthy community-dwelling adults between the ages 20 and 60, who have normal white-blood cell count(4×10^3 ~ $9\times10^3/\mu\ell$), were enrolled into the study. The subject were divided to A total 56 subjects were enrolled into 2 groups and within each group, randomized treatment groups receiving Polycan or placebo in the regimen, oral dosing 2 tablets after 30 minute of every meal. During 4 weeks, white-blood cell count(WBC) and cytokines (TNF-α, INF-γ, IL-2, IL-4) of subjects were analyzed and compared between Polycan group and placebo group.

3-2. Inclusion criteria

① WBC: $4\times10^3/\mu\ell$ ~ $9\times10^3/\mu\ell$
② Agree to all study visits and visit procedure
③ community-dwelling
④ Generally healthy without exclusion criteria

3-3. Exclusion criteria

① Subject with hyper-sensitivity against functional food
② Active liver disease with ALT & AST >100IU/L or severe, renal failure
③ Pregnant, nursing women or fertile women disagreeing birth control
④ Subject with multisystemic diseases, such as malignant tumor, active asthma requiring ongoing treatment, leukemia, reactive perforating collagenosis, multiple sclerosis, allergic dermatoses, autoimmune disorder etc.
⑤ Subject with surgery in last 3 months
⑥ Subject with nerve or mental disorder
⑦ Subject with myocardial infarction or cerebrovascular disease in last 6 months
⑧ Subject with eating other clinical drug in last 3 months or antibiotics in last 1 month
⑨ Subject with negative doctor's opinion

4. Protocol and Physical Examination

4-1. Procotol

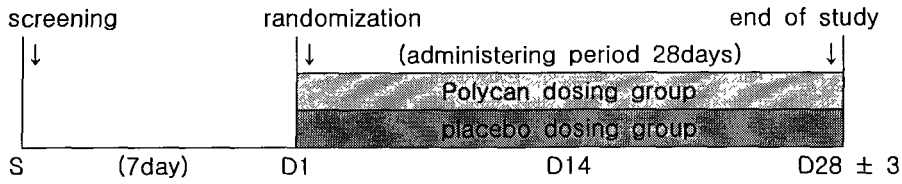
The subject were divided to A total 56 subjects were enrolled into 2 groups and within each group, randomized treatment groups receiving Polycan or placebo in the regimen, oral dosing 2 tablets after 30 minute of every meal during 4 weeks. In visit 1, white-blood cell count(total WBC, differential WBC count) and cytokines (TNF-α, INF-γ, IL-2, IL-4) of subjects were analyzed and compared between polycan group and placebo group for efficacy evaluation of products tested.

For safety test, blood test(RBC, hemoglobin, platelet, MCV, MCH, MCHC, HCT etc.), serum electrolytes(Na, K, Ca, Cl) or biochemical test(AST, ALT, ALP, γ-GTP, total protein, triglyceride, total cholesterol, total bilirubin, albumin, glucose, uric acid, creatinine, BUN) and urine test(pH, protein, glucose, ketone body, blood, bilirubin) were performed. Use of drug or other symptoms were investigated by phone call in visit 2 and the efficacies of the product and placebo were compared through the blood test in visit 3.

4-2. Physical Examination

Hematologic, biochemical, urine and pregnant test of screening items in visit 1, and hematologic and urine test in visit 3 were carried out at Clinicopathologic laboratory of Daegu Haany University Center. Electrolyte test were performed by SungYoon Reference Lab. contract with Daegu Haany University Center and immunological, electrolytes and biochemical tests of visit 1 and 3 were carried out by Eone reference laboratory.

4-3. Schedule



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visiting	screening	dosing		
	screening visit (day -7 ~ -1)	Visit 1 (day 0)	Visit 2 (tele visit) (day 14)	Visit 3 (day 28 ± 3)
Subject agrement	◉			
Demographic survey	◉			
History survey	◉	◉	◉	◉
Physical test	◉	◉		◉
Vital sign test	◉	◉		◉
Inclusion/exclusion	◉	◉		
Hematologicai test	◉	◉		◉
Serum electrolytical test	◉	◉		◉
Serum immunological test		◉		◉
Serum biochemistry test	◉	◉		◉
Urine test	◉	◉		◉
Pregnance test	◉			
Product supply		◉		
adverse monitoring			◉	◉
Product compliance test				◉

검 사	항 목
immunological test	TNF-α, INF-γ, IL-2, IL-4
electrolytical test	Na, Cl, K, Ca
hematological test	RBC, Hb, Hct, MCV, MCH, MCHC, WBC, Differential WBC, platelet
biochemical test	uric acid, creatinine, total bilirubin, albumin, AST, ALT, γ-GTP, ALP, total protein, glucose, triglyceride, total cholesterol, BUN
urine test	pH, protein, glucose, ketone body, blood, bilirubin
pregnance test	HCG

5. Statistical Analysis

All data(demographic and clinical data etc.) were summarized as basic statistic values (continuous variable mean, standard deviation, frequency of categorical variables, percentage). For identity, efficacy and safety evaluation of Sample(A) and Control(B),

1) Two sample t-test was used for continuous variable, i.e. average difference between before and after dose was analyzed, and then further analyzed between two groups.
2) For categorical variables, Chi-square test was used. Especially, if the expected frequency (< 5 in the cell) was over than 20%, Fisher's exact test was used instead of chi-square test.

In addition, the data of sample(A)/control(B) before and after dose were analyzed by repeated measured ANOVA(mixed-effects models) in the view points of immunomodulating activities, time effect of dose, interaction effect of group and dose time in both groups. The used statistical package was SAS version 9.1 and significance level was set at $p<0.05$.

6. Results

6-1. Baseline characteristics before dose

1) sex, age, weight and height

(1) continuous variable statistical data

Items	N	means	standard deviation	minimum	maximum
age	56	34.77	12.00	21.00	59.00
body weight	56	65.70	10.80	44.80	92.70
height	56	166.4	10.05	143.9	186.7

(2) simple frequency analysis by categorical variables

Frequency table of Sample(A)/Control(B)

group	frequency	percentage	cumulative frequency	cumulative percentage
A	28	50.00	28	50.00
B	28	50.00	56	100.00

Frequency table of sex

sex	cumulative frequency	cumulative percentage	frequency	percentage
female	27	48.21	27	48.21
male	29	51.79	56	100.00

2) Distribution of variables, Cross-analysis and Homogeneity test

(1) Distribution and homogeneity test of sex in treats

Male and female in treat A (28 adults) were 14, respectively (50%), and this is similar with that of treat B(13 females(46.4%), 15 males(53.6%)), which p-value of chi-square is 0.7891.

(2) Basic stastics and homogeneity test of age in treats

Treat A age: means ± SD = 34.86 ± 12.122, 95% Confidence interval of average age = (30.157, 39.558)

Treat B age: means ± SD = 34.68 ± 12.095, 95% Confidence interval of average age = (29.988, 39.369)

Therefore, both treats have similar average age and homogeneity (p- = 0.9562 in t-test), similar with p-=0.9215 of Wilcoxon statistic test.

<Statistical conclusion of Baseline variable analysis before treatment>

Before treatment, Baseline variables(demographic variables: age, sex) of sample(A) and control(B) were very similar and no significant difference in statistical analysis.

6-2. Efficacy evaluation analysis

6-2-1. 1st efficacy evaluation (TNF-α)

Almost of subjects have negative in difference of TNF-α before and after treatment, except for subject R18 ,R41, R42, R51, R52 ,R56), indicating enhancing immune activity although significant variation between subjects.

1) TNF-α level before and after treatment

TNF-α levels of both treats were significantly increased after treatment compared to before treatment, and SDs were also increased. This means that immune activities of both treats were enhanced, but variation between subjects was also observed.

2) Intra-Group comparison

Average difference of TNF-α level between before and after treatment was -14.5(< 0), so that TNF-α values of after treatment were higher than those of before treatment. Therefore, sample(A) has a significant immuno-modulating activity, however, control (B) also has a similar activity with sample(A) according to statistical analysis including t-test.

3) Inter-Group comparison

The equal variation of before and after treatment was p=0.5197>0.05, indicating non-equal variation. Both groups were quite similar because significance level of the parametric statistical t-tests was p=0.3116>0.05. Additionally, difference of TNF-α of both groups at each time showed a good significance before treatment (p-=0.0287), but it did not show significance after treatment.

5) Analysis of repeated measures

In this test, repeated measured ANOVA, mixed-effect model was used because all data was longitudinal data obtained from repeated analysis of identical subjects at each visit. Especially, it is well known that the estimation of the fixed effect in mixed-effect model is not sensitive in the case of normal distribution. Therefore, F-tests for treat, time, and interaction effect using this method as follows.

Treat effect(i.e. difference between two treats) and interaction effect(i.e. difference between two treats before and after treatment) have not statistical

significance, however, time effect (difference between before and after treatment) showed good significance (p-<0.0001) so, TNF-α levels were significantly increased after treatment.

<Conclusion of 1st efficacy evaluation>

TNF-α levels were significantly increased after treatment in sample (A) as well as control(B), so both treats have immuno-modulating activity, however, no difference between both groups. Therefore, it can be concluded that sample(A) has better immuno- modulating activity than control(B).

6-2-2. 2nd efficacy evaluation analysis (INF-γ, IL2, IL4)

1) Basic statistics and graphical analysis before and after treatment

(1) INF-γ

Both groups have an increase of INF-γ level after treatment, but no difference between two groups.

(2) IL2

Both groups have an decrease of IL2 level after treatment, and it was found a difference between two groups.

(3) IL4

IL4 level of Both group were conflicted before and after treatment, i.e. sample(A) has decreased level, but control(B) has a slightly increased after treatment.

2) Intra-group comparison

(1) INF-γ

INF-γ level of sample(A) was not significant difference after treatment, however, control(B) was some changed.

(2) IL2

IL2 level of sample(A) was some significance after treatment as well as control (B).

(3) IL4

IL4 level of sample(A) was some significance after treatment, but control (B) has not meaningful significance of IL4.

3) Inter-group comparison

IL4 level in regular tests were not followed to normal distribution, however, non-parametric statistical test showed significant difference of IL4 level between two groups (p-=0.0478)

4) Analysis of repeated measures

(1) INF-γ

(2) IL2

(3) IL4

INF-γ and IL2 have time effect (difference before and after treatment) with good significances, i.e. INF-γ level was significantly increased and IL2 was decrease after treatment, however, IL4 did not show any significance.

<Conclusion of 2nd efficacy evaluation>

In 2nd evaluation variables for efficacy test, these variables (INF-γ, IL2, IL4) showed some various significances of their average differences before and after treatment. In tra-group, INF-γ was significant in control(B), IL2 in sample(A) and control(B), and IL4 in sample(A). However, only IL4 showed some significance inter-group.

6-3. Safety analysis

6-4-1. Adverse Symptom

<Adverse Symptom 1: after 2 week treatment>

11 of 28 subjects in sample(A) had adverse symptoms (39.29%), 5 among 28 in control(B) (17.86%). however, it was not significance between both groups (chi-square p-=0.0759).

<Adverse Symptom 21: after complete treatment>

3 subjects of A group (R34, R38, R52) were found adverse symptoms, however, it was not significance between both groups. Especially, 16 subjects had "adverse symptom 1", but it was decreased to 3 subjects in "adverse symptom 2".

6-4-2. Adverse symptom related items

1) Adverse symptom 1

- Degree: light symptom in all 16 - 11 subjects in group A (68.75%), 5 subjects in group B(31.25%)
- Severe symptom: No
- Healing : complete healing
- Relationship of product: No-relationship in 10 of group and 5 of group B
  Uncertain in 1 of group A(R52)

- Treatment related to product: No

2) Adverse symptom 1

3 subject having adverse symptom 2, found in group A were not related to above 5 items.

- Degree: light symptom in all 3 cases
- Severe symptom: No
- Healing : complete healing
- Relationship of product: No-relationship
- Treatment related to product: No

<Conclusion of safety evaluation>

Two groups, sample(A) and control (B) were not significant difference in statistical analysis of above items.

6-5. Conclusion

In this study, Polycan (dried powder of black yeast culture broth) showed an increased TNF-α level, 1st evaluation variable in intra-group after 4-week treatment, and average levels of INF-γ, IL2 and IL4 (2nd evaluation variables) were found in different significances. In intra-group, INF-γ in placebo group, IL2 in Polycan and placebo group, and IL4 in Polycan group were showed a good significances, respectively. However, IL4 showed slight significance in inter-group. Therefore, it was found that both of Polycan and placebo groups have immuno-modulating activity. In addition, Safety test did not show any significant statistical difference in clinical investigation items and adverse symptoms in 4-week treatments. This can be concluded that Polycan does not have adverse symptoms or toxicity. Therefore, the tested product, Polycan (dried powder of

black yeast culture broth) strongly induced the increase of TNF-α, immunological parameter in intra-group study as well as other cytokine analysis although no-difference in inter-group study with placebo group.

In future works, other evaluation variables for immuno-modulating activity and different treatment conditions is need to be applied to support the immuno-modulating activity of Polycan in various situations.

APPENDIX 12

Organochlorine Pesticides and PCBs by GC, Certificate of Analysis by RCH Pharmaceutical Cosmetic and Food Analytical Laboratories

RCH Pharmaceutical Cosmetic and Food Analytical Laboratories

113 W. Victoria Street
Long Beach, CA 90805
Phone (310) 763-5400
Fax (310) 763-4000
www.rchlabs.com

U.S. FDA Registered Laboratory
- Pharmaceuticals
- Cosmetics
- Foods

CERTIFICATE OF ANALYSIS

CLIENT: **GLUCAN CORP.**
MARINE BIO CENTER 221,
SILLA UNIVERSITY, BUSAN, 617-736
PROJECT CONT. PERSON: CHO HYUNG RAE
PROJECT. NAME/NO.: CHO HYUNG RAE
SAMPLE I.D.: POLYCAN

REPORTING DATE: 6/15/06
SAMPLE RECEIVED: 6/7/06
LABORATORY NO.: 06-1791
DATE SAMPLED : 6/7/06
FDA REGISTRATION NO.: 2030950
INVESTIGATION : SEE BELOW

Organochlorine Pesticides and PCBs by GC

Parameter	Result	Detection Limit	Method	Date Analyzed
a-BHC	ND	0.50 ug/kg	EPA8080	6/13/06
r-BHC	ND	0.50 ug/kg	EPA8080	6/13/06
b-BHC	ND	0.50 ug/kg	EPA8080	6/13/06
Heptachlor	ND	0.50 ug/kg	EPA8080	6/13/06
d-BHC	ND	0.50 ug/kg	EPA8080	6/13/06
Aldrin	ND	0.50 ug/kg	EPA8080	6/13/06
Heptaclor Epoxide	ND	0.50 ug/kg	EPA8080	6/13/06
Endosulfan I	ND	1.00 ug/kg	EPA8080	6/13/06
DDE	ND	1.00 ug/kg	EPA8080	6/13/06
Dieldrin	ND	1.00 ug/kg	EPA8080	6/13/06
Endrin	ND	1.00 ug/kg	EPA8080	6/13/06
DDD	ND	3.00 ug/kg	EPA8080	6/13/06
Endosulfan II	ND	1.00 ug/kg	EPA8080	6/13/06
DDT	ND	3.00 ug/kg	EPA8080	6/13/06
Endrin Aldehyde	ND	3.00 ug/kg	EPA8080	6/13/06
Endosulfan Sulfate	ND	0.50 ug/kg	EPA8080	6/13/06
Methoxychlor	ND	0.50 ug/kg	EPA8080	6/13/06
Chlordane	ND	45.2 ug/kg	EPA8080	6/13/06
Toxaphene	ND	45.2 ug/kg	EPA8080	6/13/06
Aroclor 1016	ND	45.2 ug/kg	EPA8080	6/13/06
Aroclor 1221	ND	45.2 ug/kg	EPA8080	6/13/06
Aroclor 1232	ND	45.2 ug/kg	EPA8080	6/13/06
Aroclor 1242	ND	45.2 ug/kg	EPA8080	6/13/06
Aroclor 1248	ND	45.2 ug/kg	EPA8080	6/13/06
Aroclor 1254	ND	45.2 ug/kg	EPA8080	6/13/06
Aroclor 1260	ND	45.2 ug/kg	EPA8080	6/13/06

ND = The concentration is below the detection limit or is non-detected.
Note: 1. The above analyses were performed by FDA and California Department of Health Services (DOHS) certified laboratories.

Respectfully Submitted
(b) (6)

Filipina A. Joven
Chief Chemist

BEST ORIGINAL COPY

APPENDIX 13

Heavy Metals by ICP, Certificate of Analysis
by RCH Pharmaceutical Cosmetic and Food Analytical Laboratories

RCH Pharmaceutical Cosmetic and Food Analytical Laboratories

113 W. Victoria Street
Long Beach, CA 90805
Phone (310) 763-5400
Fax (310) 763-4000
www.rchlabs.com

U.S. FDA Registered Laboratory
- Pharmaceuticals
- Cosmetics
- Foods

CERTIFICATE OF ANALYSIS

CLIENT: **GLUCAN CORP.**
MARINE BIO CENTER 221,
SILLA UNIVERSITY, BUSAN, 617-736
PROJECT CONT. PERSON: CHO HYUNG RAE
PROJECT. NAME/NO.: CHO HYUNG RAE
SAMPLE I.D.: POLYCAN

REPORTING DATE: 6/15/06
SAMPLE RECEIVED: 6/7/06
LABORATORY NO.: 06-1791
DATE SAMPLED : 6/7/06
FDA REGISTRATION NO.: 2030950
INVESTIGATION : SEE BELOW

Heavy Metals by ICP

Parameter	Result	Detection Limit	Method	Date Analyzed
Arsenic	ND	1.00 mg/kg	EPA6010B	6/8/06
Barium	3.00 mg/kg	1.00 mg/kg	EPA6010B	6/8/06
Cadmium	ND	1.00 mg/kg	EPA6010B	6/8/06
Chromium	3.50 mg/kg	1.00 mg/kg	EPA6010B	6/8/06
Lead	ND	1.00 mg/kg	EPA6010B	6/8/06
Mercury	ND	1.00 mg/kg	EPA6010B	6/8/06
Selenium	ND	1.00 mg/kg	EPA6010B	6/8/06
Silver	ND	1.00 mg/kg	EPA6010B	6/8/06

ND = The concentration is below the detection limit or is non-detected.
Note: 1. The above analyses were performed by FDA and California Department of Health Services (DOHS) certified laboratories.

Respectfully Submitted,

(b) (6)

Filipina A. Joven
Chief Chemist

BEST ORIGINAL COPY

Ramos-Valle, Moraima

From: Ramos-Valle, Moraima
Sent: Thursday, December 10, 2009 11:14 AM
To: 'chowonho@gmail.com'
Cc: Martin, Robert L
Subject: GRAS submission on black yeast betaglucan

Dear Mr. Cho,

We have received your GRAS determination dated December 3, 2009 for "black yeast beta-glucan". At this moment we have not file your submission as a GRAS notice because we noted something that needs to be address before we can file it. I have not been able to reach you at the telephone number provided in the cover letter header (832-494-1180).

Please give me a call at 301-436-1248 at your soonest convenience or provide me with a phone number that I can reach you at.

Thanks,

Moraima J. Ramos Valle
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Food and Drug Administration
Phone: 301-436-1248
Email: Moraima.Ramos-Valle@fda.hhs.gov

PEOPLE AND TECHNOLOGIES, LLC

1424 W. Sam Houston Parkway, Suite 160. Houston, TX. 77043 / T 832.494.1180 F 832.494.1182 / www.glucan.co.kr

RECEIVED
DEC 15 2009
Division of
Biotechnology and
GRAS Notice Review

SENT VIA FEDEX

December 10, 2009

Robert L. Martin, Ph.D
U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Div. of Biotechnology and GRAS Notice Review
HFS-255, 5100 Paint Branch Pkwy.
College Park, MD 20740-3835.

Re: GRAS Notice for black yeast beta-glucan(Polycan®)

Dear Dr. Robert L. Martin

Pursuant to proposed 21 CFR§170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the Federal Register [62 FR18938 (1 7 April 1997)] and on behalf of Glucan Corp. headquartered in Busan, S. Korea, , on Dec. 3rd, 2009, I have submitted in triplicate, as the notifier [PeopleAndTechnologies, LLC, 1424 W. Sam Houston Parkway, Suite 160, Houston, TX 77041], a Notice of the determination, on the basis of scientific procedures, that black yeast beta-glucan(Polycan®) produced from Aureobasidium pullulans SM-2001, produced by Glucan Corporation, Limited, as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient.

However, since our intended food uses include processed meat products, I am submitting the fourth copy of GRAS Notice for a USDA review.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me (chowonho@gmail.com, 832-814-3506) at any point during the review process. All of the information that serves as the basis for this GRAS determination can be sent - upon request - to FDA and is available for review and copying at reasonable times.

Best regrads,

(b) (6)

Wonho Cho
PeopleAndTechnologies, LLC
US Sales Division of Glucan Co., Ltd
chowonho@gmail.com
M. 832.814.3506
O. 832.494.1180

SUBMISSION END

Reference List for Industry Submission, GRN 000309

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB Info*
000024 - 000031	Shin, Hyun Dong; Yang, Kun Juh; Park, Bok Ryeon; Son, Chang Woo; Jang, Hee Jeong; Ku, Sae Kwang	Antiosteoporotic effect of Polycan, Beta-glucan from Aureobasidium, in ovariectomized osteoporotic mice	2007	Nutrition	Volume 23, pgs 853-860
000033 - 000041	Hebok, Song; Park, Dong Chan; Do, Gyung Min; Hwang, Seung-Lark; Lee, Won Kyu; Kang, Heun-Soo; Park, Bok-Ryun; Jang, Hee-Jeong; Son, Chang-Woo; Park, Eui Kyun; Kim, Shin-Yoon; Huh, Tae-Lin	Effect of Exopolymers of Aureobasidium pullulans on Improving Osteoporosis Induced in Ovariectomized Mice	2006	Journal of Microbiology and Biotechnology	Volume 16, Number 1, pgs 37-45
000043 - 000048	Lee, Hyeung-Sik; Cho, Hyung-Rae; Moon, Seung-Bae; Shin, Hyun-Dong; Yang, Kun-Ju; Park, Bok-Ryeon; Jang, Hee-Jeong; Kim, Lin-Su; Ku, Sae-Kwang	Effect of Beta-Glucan from Aureobasidium pullulans on Rat Rib Fracture Healing	2008	Laboratory Animal Research	Volume 24, Number 1, pgs 39-44
000050 - 000055	Kim, Hyeong-Dong; Cho, Hyung-Rae; Moon, Seung-bae; Shin, Hyun-Dong; Yang, Kun-Ju; Park, Bok-ryeon; Jang, Hee-Jeong; Kim, Lin-Su; Lee, Hyeung-Sik; Ku, Sae-Kwang	Effects of Beta-Glucan from Aureobasidium pullulans on Acute Inflammation in Mice	2007	Archives of Pharmacal Research	Volume 30, Number 3, pgs 323-328
000057 - 000063	Kim, Hyeong-Dong; Cho, Hyung-Rae; Moon, Seung-Bae; Shin, Hyun-Dong; Yang, Kun-Ju; Park, Bok-Ryeon; Jang, Hee-Jeong; Kim, Lin-Su, Lee, Hyeung-Sik; Ku, Sae-Kwang	Effect of Exopolymers from Aureobasidium pullulans on Formalin-Induced Chronic Paw Inflammation in Mice	2006	Journal of Microbiology and Biotechnology	Volume 16, Number 12, pgs 1954-1960

NA- Not applicable

Pages	Author	Title	Publish Date	Publisher	BIB Info
000089 - 000093	Lee, Hyeung-Sik; Cho, Hyung-Rae; Yang, Kun-Ju; Moon, Seung-Bae; Park, Bok-Ryeon; Shin, Hyun-Dong; Jang, Hee-Jeong; Kim, Lin-Su; Ku, Sae-Kwang	Micronucleus test of Polycan, Beta-Glucan Originated from Aureobasidium, in Bone Marrow Cells of Male ICR Mice	2008	Toxicol Res.	Volume 24, Number 1, pgs 11-15
000095 - 000099	Lee, Hyeung-Sik; Yang, Kun-Ju; Shin, Hyun-Dong; Park, Bok-Ryeon; Son, Chang-Woo; Jang, Hee-Jeong; Park, Dong-Chan; Jung, Young-Mi; Ku, Sae-Kwang	Single Oral Dose Toxicity Studies of Polycan, Beta-Glucan Originated from Aureobasidium in Mice	2005	J. Toxicol. Pub. Health	Volume 21, Number 4, pgs 361-365
000101 - 000107	Lee, Jaehyun; Yang, Kunju; Shin, Hyundong; Park, Bokryeon; Son, Changwoo, Jang, Heejeong; Park, Dongchan; Lee, Hyeung-Sik; Ku, Saekwang	Single Subcutaneous Dose Toxicity of Polycan, a Beta-Glucan Originated from Aureobasidium in Mice	2005	Laboratory Animal Research	Volume 21, Number 4, pgs 299-305

NA- Not applicable

AM

Weinsetel, Natalia

From: Wonho Cho [chowonho@gmail.com]
Sent: Monday, March 15, 2010 4:32 PM
To: Weinsetel, Natalia
Subject: Re: GRN000309 - questions

Dear Dr. Weinstetel

I have one question.

Under Chemistry, Number 2, what do you mean by "the specifications for items"?

Thanks

On Tue, Mar 9, 2010 at 12:05 PM, Weinsetel, Natalia <Natalia.Weinsetel@fda.hhs.gov> wrote:

Dear Mr. Wonho Cho,
Below are FDA's questions from the review of GRN000309. Please reply to this email acknowledging receipt of these questions.

Regulatory/Administrative

1. The review team has considered the name for GRN0000309, black yeast beta-glucan. To prevent misleading or incorrect assumptions in our correspondence, this substance is going to be termed: beta-glucan derived from *Aureobasidium pullulans*.

2) According to the notice, beta-glucan derived from *Aureobasidium pullulans* is a reddish yellow powder. We request that you present your view

on whether any of the intended uses of beta-glucan derived from *Aureobasidium pullulans* would be exempt from the definition of a color

additive. Please refer to section 201(t)(1) of the Food, Drug, and Cosmetic Act (FDCA) and FDA's implementing regulations in 21 Code of

Federal Regulations (CFR) section 70.3(f) for the definition of a color additive.

Microbiology

1. Please provide general information about *A. pullulans* (ex. where is the organism normally found).

2) Please provide a statement about the nonpathogenic/nontoxigenic nature of *A. pullulans.*

3) Please include and explain published case reports in which *A. pullulans* is described as an opportunistic organism.

4) Please provide information about the physiological effects of the UV treatment of *A. pullulans* SM-2001. How is the mutant strain different from the wild type strain?

Chemistry

1. On pg 4, Section D please provide: (1) a statement about the safety and toxicity of the sources of the genes used, (2) statement of how strain identity is confirmed, and how contamination is controlled, (3) statement of how fermentation conditions are controlled (e.g. time, pH, temp, etc.), (4) the identity of the materials used in the processing and purification steps (where appropriate) and whether the materials are in compliance with FDA's regulations in 21 CFR, (5) description of the processing controls and potential residues, and measures to ensure removal of the residues (where appropriate).

2) Provide the specifications for items listed in Table 1 (pg 11) and Appendix 13.

3) In Table 1 (pg 11), what is "na" cited on the bottom of the table referring to?

Toxicology

1. The last paragraph on pg 12 states that black yeast beta-glucan is not intended directly to be marketed for infant and toddler foods. Table 2 on the same page describes the proposed food uses of beta-glucan and some of those food categories include milk and milk products (ex. Yogurt and soymilk) and fruit juices which are two types of foods often consumed by infants and toddlers. Please explain the rationale for these limitations and any safety concerns.

2) Given that the data reported was for Polycan, which includes one peer-reviewed tox article (Lee et al., 2005), a micronucleus test bone marrow, a sc study, and 2 GLP studies, please elaborate further in how these articles and GRN 239 support the safety of black yeast beta-glucan. In addition, clarify why GRN 239 supports safety, for example, where you state that: ....."black yeast beta-glucan is soluble and Biothera's bakers yeast is insoluble" (page 10). Is black yeast beta-glucan completely soluble or just more soluble than bakers yeast?

3) Please discuss if the ingestion of Polycan would have deleterious effects in humans or subgroups (ex. infants) for example, in terms of allergenicity or if the organism *A. pullulans* has been reported to be pathogenic.

4) The GLP study using randomized double-blind clinical trial did not report the concentration used in tablets, please provide or explain why the data is not available.

5) Please clarify the discrepancy in these two data points. In the Lee et al., 2005 article, the authors concluded that "the LD50 and approximate LD in mice after a single oral dose of Polycan were considered over 1000 mg/kg, respectively in both male and female" whereas in the GLP study on rats, the report states the NOAEL is over 1000 mg/kg for both male and female.

Please feel free to call or email if you need clarification and/or additional information.
Thank you,
Natalia
Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

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--
Wonho Cho
1424 W. Sam Houston Parkway N.
Suite 160
Houston, TX 77043
O.832.494.1180
M.832.814.3506

AM

From: Wonho Cho [mailto:chowonho@gmail.com]
Sent: Monday, March 22, 2010 10:55 AM
To: Weinsetel, Natalia
Subject: Re: GRN309

Dr. Natalia Wensetel,

I am fine with 11:00-12:00 tomorrow. Please let me know the call-in information.

Thanks

On Mon, Mar 22, 2010 at 9:30 AM, Weinsetel, Natalia <Natalia.Weinsetel@fda.hhs.gov> wrote:

Mr. Cho,
Unfortunately, FSIS is only able to meet from 11-12 tomorrow 03/23. Is that still alright with you? Please let me know as soon as you get a chance so that I can get you call-in information. If you are not available from 11-12 tomorrow, please let me know your availability for the rest of the week.

Thank you!
Natalia

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

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--
Wonho Cho
1424 W. Sam Houston Parkway N.
Suite 160
Houston, TX 77043
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--
Wonho Cho
1424 W. Sam Houston Parkway N.
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Houston, TX 77043
O.832.494.1180
M.832.814.3506

AM

Weinsetel, Natalia

From: Wonho Cho [chowonho@gmail.com]
Sent: Friday, April 02, 2010 11:29 AM
To: Weinsetel, Natalia
Subject: Re: GRN309

Dr. Weinsetel

I will try to finish up the translation by Wednesday, April 7, 2010. If I am not able to complete by the date, I will let you know but I will finish it by Friday 9, 2010 at latest.

Thanks
Wonho Cho
On Fri, Apr 2, 2010 at 10:19 AM, Weinsetel, Natalia <Natalia.Weinsetel@fda.hhs.gov> wrote:

> Mr. Cho,
> I was wondering if you had additional questions for the review team regarding GRN 309. It has been three weeks since our meeting and we had discussed you needing approximately two weeks to complete your responses to our questions. In order for us to complete our review and respond to your notification in a timely manner, we would appreciate receiving your responses to the reviewers' comments by Wednesday, April 7, 2010. If you will be unable to respond by this date, please let me know.
>
> Best regards,
> Natalia
>
> Natalia Weinsetel, Ph.D.
> Consumer Safety Officer
> Division of Biotechnology and GRAS Notice Review
> Office of Food Additive Safety
> Center for Food Safety and Applied Nutrition
> U.S. Food and Drug Administration
> Tel: (301) 436-2907/Fax: (301) 436-2965
> E-mail: Natalia.Weinsetel@fda.hhs.gov
>
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--
Wonho Cho
1424 W. Sam Houston Parkway N.

Suite 160
Houston, TX 77043
O.832.494.1180
M.832.814.3506

AM

Weinsetel, Natalia

From: Wonho Cho [chowonho@gmail.com]
Sent: Tuesday, April 06, 2010 10:53 AM
To: Weinsetel, Natalia
Subject: Re: GRN309

Dear Dr. Weinsetel

I won't be able to finish the translation by tomorrow, Wed.(April 07, 2010). I will finish it up by Fri. (April 9, 2010).

Is it OK to simply email you the responses in MS Word format?

In addition, do I need to send you a hard copy of the amended GRAS Notice? Or is it fine to email you a soft copy (PDF) of it?

Thanks
Have a nice day.
Wonho Cho

On Tue, Mar 23, 2010 at 3:59 PM, Weinsetel, Natalia <Natalia.Weinsetel@fda.hhs.gov> wrote:

Dear Wongho Cho,
Thank you for participating in the teleconference with USDA's Food Safety and Inspection Service (FSIS) and FDA this morning.

Based on the outcome of that meeting, we need you to amend the notice in order to remove the use of beta-glucan in meat and poultry products. When you submit the answers to our review questions, please include a paragraph where you address this issue.

On page 3 of the notice (first paragraph), please remove the use of beta-glucan in processed meat products. In addition, please state that the soup bases and sauces would be non-meat and non-poultry based (as those are also affected). Please check to see if your exposure estimates will change with the removal of this category and submit a revised Table 2 (pg. 12). The table should reflect the updated estimates (if necessary), not contain the meat products food category, and include a footnote that specifies that the soups and soup mixes category would be non-meat and non-poultry based.

Please let me know if you need any additional information or clarification on this request.
Thank you!
Natalia

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

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From: Wonho Cho [mailto:chowonho@gmail.com]
Sent: Monday, March 22, 2010 10:55 AM
To: Weinsetel, Natalia
Subject: Re: GRN309

Dr. Natalia Wensetel,

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Thanks

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> Unfortunately, FSIS is only able to meet from 11-12 tomorrow 03/23. Is that still alright with you? Please let me know as soon as you get a chance so that I can get you call-in information. If you are not available from 11-12 tomorrow, please let me know your availability for the rest of the week.
>
> Thank you!
> Natalia
>
> Natalia Weinsetel, Ph.D.
> Consumer Safety Officer
> Division of Biotechnology and GRAS Notice Review
> Office of Food Additive Safety
> Center for Food Safety and Applied Nutrition
> U.S. Food and Drug Administration
> Tel: (301) 436-2907/Fax: (301) 436-2965
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1424 W. Sam Houston Parkway N.
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O.832.494.1180
M.832.814.3506

--

Wonho Cho
1424 W. Sam Houston Parkway N.
Suite 160
Houston, TX 77043
O.832.494.1180
M.832.814.3506

AM

Weinsetel, Natalia

From: Wonho Cho [chowonho@gmail.com]
Sent: Tuesday, April 06, 2010 4:47 PM
To: Weinsetel, Natalia
Subject: Re: GRN309

Dear Dr. Weinsetel

I have one question to clarify what a reviewer in toxicology wanted to ask.

In Number 3 under Toxicology, does the reviewer think those articles does not support the safety of beta-glucan derived from A. pullulans? The reason why I ask is that I found out that responses from Korea were repeating the same things in those articles because they believed those articles showed the safety of beta-glucan. Does "please elaborate further in how these articles and GRN239 support the safety of black yeast beta-glucan" mean that I need to address an rationale why we think these articles support the safety of it?

Thanks.

Wonho

On Tue, Apr 6, 2010 at 10:23 AM, Weinsetel, Natalia <Natalia.Weinsetel@fda.hhs.gov> wrote:

Dear Wonho Cho,
Friday is fine, thank you for letting me know. You may send the answers in word, but please only send the corrected pages to the original notice (in pdf), not the whole notice. Please let me know if you have any other questions/concerns.
Thank you!
Natalia

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

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Sent: Tuesday, April 06, 2010 10:53 AM

To: Weinsetel, Natalia
Subject: Re: GRN309

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Wonho Cho

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Dear Wongho Cho,
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Please let me know if you need any additional information or clarification on this request.
Thank you!
Natalia

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
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Tel: (301) 436-2907/Fax: (301) 436-2965
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AM

From: Wonho Cho
To: Weinsetel, Natalia;
Subject: Responses on additional questions
Date: Friday, April 09, 2010 11:56:25 AM
Attachments: Additional Q&A.zip

April 09, 2010

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
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Re: GRAS Notice for beta-glucan derived from Aurebasidim pullulans

Dear Dr. Natalia Weinsetel

Pursuant to additional questions raised by FDA and USDA regarding GRAS Notice for beta-glucan derived from Aureobasidium pullulans submitted on Dec. 9, 2010 and on behalf of Glucan Corp. headquartered in Busan, S. Korea, , I am submitting the responses on those questions. In addition, I also notify that Glucan Corporation decided to exclude processed meat products and soup bases and sauces from the list of the intended uses. The responses and the pages amended accordingly are included in a zipped file attached.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process. All of the information that serves as the basis for this GRAS determination can be sent - upon request - to FDA and is available for review and copying at reasonable times.

Best regrads,

Wonho Cho
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Regulatory/Administrative

1)The review team has considered the name for GRN0000309, black yeast beta-glucan. To prevent misleading or incorrect assumptions in our correspondence, this substance is going to be termed: beta-glucan derived from Aureobasidium pullulans.

Glucan corporation agrees on changing the name of the substance from black yeast beta-glucan to beta-glucan derived from Aureobasidium pullulans.

2) According to the notice, beta-glucan derived from Aureobasidium pullulans is a reddish yellow powder. We request that you present your view on whether any of the intended uses of beta-glucan derived from Aureobasidium pullulans would be exempt from the definition of a color additive. Please refer to section 201(t)(1) of the Food, Drug, and Cosmetic Act (FDCA) and FDA's implementing regulations in 21 Code of Federal Regulations (CFR) section 70.3(f) for the definition of a color additive.

In fact, "reddish yellow powder" was a mis-translation of a hard-to-translation color description in Korean. A correct color description of the substance should be light brown or beige.

Due to the nature of light color and the limited use-levels ranging 1% to the maximum 10% (120mg-150mg)/serving, when added or applied to a food, drug, or cosmetic, or to the human body or any part thereof, beta-glucan derived from Aureobasidium pullulans is not capable (alone or through reaction with other substance) of imparting color thereto. In other words, beta-glucan derived from Aureobasidium pullulans should be exempt from the definition of a color additive in section 201(t)(1) of the Food, Drug, and Cosmetic Act (FDCA) and FDA's implementing regulations in 21 Code of Federal Regulations (CFR) section 70.3(f).

Below is pictures of the test showing that beta-gluan derived from Aureobasidium pullulans is not capable of imparting color to a food (milk in this test).

1.

2.

Picture 1. Cup A contains whole milk and cup B contains 240ml whole milk plus 100mg beta-gluan derived from Aureobasium pullulans.
Picture 2. Cup A contains whole milk and Cup B contains 240ml whole milk plus 150mg beta-glucan derived from Aureobasidium pullulans.

Microbiology

1)Please provide general information about A. pullulans (ex. where is the organism normally found).

Aureobasidium pullulans, the "black yeast," is a ubiquitous fungus that has been found in soil, on the leaves, in lake water, on weather wood, as well as in used cosmetics and on foods such as fruits, ceretal, tomatoes and cheese. The fungus contains multiple life forms (polymorphic) including blastospores, hyphae, chlamydospores, and swollen cells. The chlamydospores and swollen cells are considered resting forms. The fungus produces a green melanin which turns black over time. The fungus also produces the polysaccharide pullulan, which is currently used in industrial applications such as a coating for pill tablets, cosmetics, foods, and dissolvable breath fresheners.

Figure shows seven-day-old *Aureobasidium pullulans* colonies grown on Ueda medium at 28°C in a humid environment. The culture is exhibiting dark green to black coloration due to melanin production. The sheen on the green area represents pullulan production, and white vegetative hyphae can be seen extending into the culture medium at the edge of the culture.

Reference

1. Anna Oller, http://www.microbelibrary.org/asmonly/details_print.asp?id=1444&lang=
2. Cooke, WB. (1961) A taxonomy study in the 'black yeasts'. Mycopathol Mycol Appl, 17: 1-43.
3. Durrell, LW. (1967) Studies of Aureobasidium pullulans (De Bary) Arnaud. Mycopathol Mycol Appl, 18: 113-120.
4. Zabel, RA & Terracina F.(1980) The role of Aureobasidium pullulans in the disfigurement of latex paint films. Dev Ind Microbiol, 21: 179-190.
5. Domash, KH, Gams, W & Anderson, TH. (1993) Aureobasidium. IN: Complendium of soil Fungi. Eching, Germany, IHT-Verg, Vol 1, pp. 130-134.
6. Mislivec, PB, Yoneyama, M & Sasaki, S. (1990) Growth promoting agent for bacteria containing pullulan with or without dextran. European Patent Specification EP 0382355B1.
7. Weidenborner, M, Berleth, M, Kramer, J & Kunz, B. (1997) Mold spectrum of four cereal brands of the German crop 1995. Nahrung, 41:139-141.
8. Webb, JS, van der Mei, HC, Nixon,M, Eastwood, IM, Greenhalgh, M, Read, SJ, Robson, GD & Hanley, PS.(1999) Plasticizers increased adhesion of the deteriogenic fungus Aureobasidium pullulans to polyvinyl chloride. Appl Environ Microbiol, 65:3575-3581.
9. Cronin, LA, Tiffney, WN & Eveleigh, DE. (2000) The greying of cedar shingles ina maritime climate – a fungal basis?. J Ind Microbiol Biotechnol, 24: 319-322.

2) Please provide a statement about the nonpathogenic/nontoxigenic nature of A. pullulans.

Below is a statement excerpted from a memorandum included in GRAS Notice No. 000099, Pullulan GRAS Notification. Pullulan is an extracellular polysaccharide, excreted by the polymorphic fungus *Aureobasidium pullulans* which is the same organism as Glucan corporation uses for manufacturing beta-glucan.

" *Aureobaridium pullulans* is a common black saprobic mould with a world-wide distribution in both indoor and outdoor environments. As a member of the phyllosphere community and a common biofilm member on painted surfaces and shower curtains, it is inhaled and ingested with fresh fruits and vegetables on a daily basis; Prior to the mid- 1980's, the species was associated occasionally with superficial infections in humans, but many of these reports have been considered questionable (McGinnis, M.1980, Laboratory handbook of medical mycology. Academic Press Inc., *N.Y.).* Early clinical studies either failed to establish a pathogenic association or the taxonomic procedures failed to distinguish their isolates from *Exophialia* spp. In the past several decades

there have been a few additional reports (e.g. see Salkin et al. 1986. J. Clin Microbiol. 23:826) on the pathogenicity of *A. pullulans* for seriously immunocompromised patients, a phenomenon that is considered possible for most fungi including the baker's yeast *Saccharomyces cerevisiae.* Indeed there are far more reports associating this beneficial and safe industrial yeast with various disease syndromes than the rare associations indicated for *A. pullulans*

Host debilitation is by far the primary factor in the opportunistic or adventitious involvement of saprobic fungi with humans. Nevertheless, in our over 30 years of experience with yeasts and moulds in environmental , industrial and clinical settings, the involvement of *A.* pullulans with any adverse human health related problems is extremely rare."

3) Please include and explain published case reports in which A. pullulans is described as an opportunistic organism.

Below are two case reports in which A. pullulans is described as an opportunistic organism. However, both of reports concluded that severe host debilitation is by far the primary factor in the opportunistic or adventitious involvement of A. pullulans with humans. These two articles are attached in a PDF form.

a. Salikin, IF, Martines, JA & Kemna, ME.(1986) Opportunistic infection of the spleen caused by Aureobasidium pullulans, J Clin Microbiol. 23:828-831.

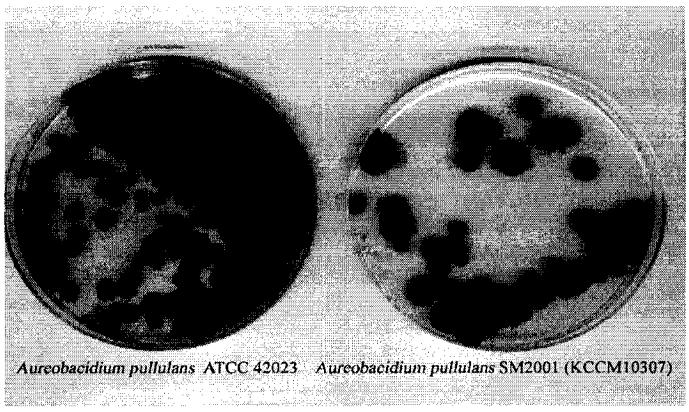
The mold Aureobasidium pullulans was isolated on several nutrient media from a splenic abscess in a patient with disseminated lymphoma. Examination of stained smears and paraffin sections revealed fungal structures characteristic of this organism. This is the first reported association of A. pullulans with an opportunistic visceral infection.

b. Michael H, Rennie R, Crystal S, Wendy V. 2005. Aureobasidium pullulans infection: fungemia in an infant and a review of human case, Diagnostic microbiology and infection disease. 51: 209-213

This article reports the first case of A. pullulans fungemia with invasive infection in an infant and reviews the previously reported 23 cases of human infection from the English literature (1966–2003). This infant case is also, the first case of documented invasive pulmonary infection and the first patient with a recently repaired cardiac lesion as the identified risk factor.

4) Please provide information about the physiological effects of the UV treatment of A. pullulans SM-2001. How is the mutant strain different from the wild type strain?

A. pullulans SM-2001 produced by the UV treatment of A. pullulans ATCC 42023 possesses the same mycological as well as taxonomical properties. However, the colony of A. pullulans SM-2001 is larger, less glossy, and lighter in color as shown in a picture below.



Aureobacidium pullulans ATCC 42023 *Aureobacidium pullulans* SM2001 (KCCM10307)

Chemistry

1) On pg 4, Section D please provide:

(1) a statement about the safety and toxicity of the sources of the genes used

A. pullulans is the same source that Hayashibara International Inc. uses to produce Hayashibara pullulans, GRAS NOTICE No.000099. Therefore, the safety and toxicity of the source of the gene had been evaluated in GRAS NOTICE No. 000099.

Any particular gene was not added to A. pullulans to produce A. pullulans SM-2001, causing no change in the nature of the safety and toxicity. A. pullulans SM-2001 is an improved strain, producing more beta-glucan rather than simple pullulans, by the UV induction of A. pullulans.

(2) a statement of how strain identity is confirmed, and how contamination is controlled

The strain identity is confirmed by the size of its colony and color at first. Then, , the amount of beta-1,3/1,6-glucan secreted during a pre-fermentation is another indicator of its strain identity. Glucan corporation's original strain of A. pullulans SM-2001 is kept in Korean Culture Center of Microorganisms(KCCM) as KCCM 10307.

Contamination during the fermentation is controlled through measuring heterotrophic plate count out of fermented medium sample by the clean bench.

(3) a statement of how fermentation conditions are controlled (e.g. time, pH, temp, etc.)

Culture medium composition: Vitamin C Ascorbic acid, yeast extract, sterilized sucrose solution

Fermentation Process: Temperature, time, viscosity, and contamination are periodically measured and checked in a stainless steel fermentation vessel whose temperature and time are automatically controlled. pH is not manually controlled. In a normal condition, pH is 5.0-6.0 at the beginning and 4.0-4.5 at the end..

1. Agar plate culture: Preparing an agar plate culture medium. After sterilization, inoculate the agar plate with cells or spores and culture with agitation for 48 hrs at 25°C
2. Seed culture: After sterilizing the flask of culture medium, inoculate spores from the agar plate and agitate the flask for another 72 hrs at 25C
3. Main culture in 100 L fermentation vessel and 5 ton fermentation vessel : After sterilizing (15 min at 121°C) and cool down the seed culture, inoculate and culture in 100 L fermentation vessel for another 24 hrs at 25°C and then culture in 5 ton fermentation vessel for 48 hrs at 25°C until ph level become 4.0-4.5.

(4) the identity of the materials used in the processing and purification steps (where appropriate) and whether the materials are in compliance with FDA's regulations in 21 CFR

Culture medium constituents
a. Vitamin C Ascorbic acid: 21CFR 182.3013
b. Bakers yeast extract: 21CFR 184.1983
c. Sucrose: 21CFR184.1854

Purification Steps
After the sterilization by 15 min at 121°C, Fungal cells are removed by the multi filtration/purification system (MF :microfiltration to remove residues and fungal cells and UF: ultrafilteration to obtain beta-glucan with the certain molecular weight). These multi filtration/purification steps use only the physical force to filter/purify. No chemical or additional materials are used in the steps

Therefore, all the materials in the processing and purification steps are in compliance with FDA's regulations in 21CFR.

(5) a description of the processing controls and potential residues, and measures to ensure removal of the

residues (where appropriate).

The processing controls:

1. During fermentation, temperature, time, viscosity, and contamination are periodically measured and checked in a fermentation vessel whose temperature and time are automatically controlled.
2. After fermentation, using a viscometer the quality of the fermented culture is checked.
3. Beta-glucan content (40%) in a pre-dried sample is checked.
4. Sterilize the whole batch for 15 min at 125°C
5. Fungal cells and residues are removed by the multi filtration/purification system (MF/UF)
6. The final product is freeze- spray-dried.

The residues: Turbidity is measured by UV spectrometer to ensure removal of the potential residues.

2) Provide the specifications for items listed in Table 1 (pg 11) and Appendix 13.

COA (Certificate of Analysis)			
Product name	**POLYCAN (PN-101)**		
Package	**1kg**	**Product No.**	**CGN-091117**
Delivery quantity	**3kg**	**Product date**	**2009.11.04**
Delivery company	**Masterasia marketing co.Ltd**	**Test date**	**2009.11.04 ~ 11**
Result			
Test	**Specification**	**Test result**	**Reference**
1. Appearance	**Mild unique smell, pale beige**	**pass**	
2. Principle component	**Beta-glucan 40%**	**40.8%**	**loss on dry weight**
3. pH	**5.5 ~ 8.5**	**6.5**	**5% solution**
4. Moisture	**< 8.0 %**	**3.4%**	
5. Tar pigment	**Negative**	**pass**	**Korea Food Code**
6. Arsenic	**< 1.5 ppm**	**pass**	**Korea Food Code**
7. Hevy metal	**< 3 ppm**	**pass**	**Korea Food Code**
8. Microbe	**< 3,000 CFU/g**	**pass**	**Korea Food Code**
9. *E. coli*	**Negative**	**pass**	**Korea Food Code**

3) In Table 1 (pg 11), what is "na" cited on the bottom of the table referring to?

"na" was mistakenly inserted. In other words, no data in Table 1 falls under "na". "na" is removed in page 11 amended.

Toxicology

1)The last paragraph on pg 12 states that black yeast beta-glucan is not intended directly to be marketed for infant and toddler foods. Table 2 on the same page describes the proposed food uses of beta-glucan and some of those food categories include milk and milk products (ex. Yogurt and soymilk) and fruit juices which are two types of foods often consumed by infants and toddlers. Please explain the rationale for these limitations and any safety concerns.

Infants and toddlers foods in pg 12 meant foods manufactured and marketed specifically for infants and toddlers; for example, formulas and baby purees. Therefore, the rationale behind the statement is that beta-glucan derived from A. pullulans will not be consumed by infants or toddlers through foods manufactured specifically for them, limiting their consumption of beta-glucan derived from A. pullulans to the extent that they consume it from other foods, such as milk and yogurt, which are not being manufactured specifically for them.

Any adverse health effect or allergenic effect to infants and toddlers have not been reported in S. Korea in which milk and yogurt containing beta-glucan derived from A. pullulans have been being sold more than for 5 years by the biggest diary product manufacturer.

2)-1 Given that the data reported was for Polycan, which includes one peer-reviewed tox article (Lee et al., 2005), a micronucleus test bone marrow, a sc study, and 2 GLP studies, please elaborate further in how these articles and GRN 239 support the safety of black yeast beta-glucan.

To elaborate further why Glucan corporation included these articles to support the safety of beta-glucan derived from A. pullulans, a part of the conclusion or discussion of each article is excerpted below.

Appendix 9: Single Oral Dose Toxicity Studies of Polycan, β-Glucan Originated from Aureobasidium in Mice (Lee et al., 2005)

As the results, we could not find any mortalities, clinical signs, changes in the body weight and gross findings. The results obtained in this study suggest that the Polycan is non-toxic in mice and is therefore likely to be safe for clinical use.

Appendix 8: Micronucleus test of Polycan®, β-Glucan Originated from *Aureobasidium*, in Bone Marrow Cells of Male ICR Mice

Micronucleus assays were first introduced in the early 1970's for the examination of genotoxic activity of chemical agents (Matter and Schmid, 1971; Heddle,1973). The procedure is based on the observation that mitotic cells with chromatid breaks or incomplete exchanges or with malfunction of the spindle apparatus suffer from disturbances in anaphase distribution of their chromatin. After telophase, a sizable portion of this displaced chromatin is not included in the nuclei of the daughter cells but forms single or multiple micronuclei in the cell cytoplasm. The frequency of the appearance of micronuclei depends both upon the rate of chromosome breakage or loss and the rate of cell division (Von Ledebur and Schmid, 1973; Heddle et al., 1984). Although micronuclei can occur in almost all dividing cells, mouse bone marrow is usually the tissue used for the micronucleus test, and any agent which induces chromosomal aberrations can also produce micronuclei (Heddle et al., 1983, 1984).....As the results obtained in the present study, PolycanTM shows no genotoxicity effect upto 1000 mg/kg dosing levels. The highest dosage used in the present study was selected as total 1000 mg/kg, based on the results of single dose toxicity test in mice (Lee et al., 2005), and vehicle and positive control were added according to the recommendation of KFDA (2005) and OECD (1997) guidelines.

Appendix 10: Single subcutaneous dosage toxicity of Polycan, a β-Glucan Originated from Aureobasidium in Mice

In the present study, we investigated the acute toxicity of single subcutaneous dose with Polycan, a newly purified beta-glucan from Aureobasidium pullulans SM-20001 to mice as a part of the development. Test articles were once subcutaneously administered to mice at dose levels of 250, 125, and 62.5mg/kg, and the mortality and changes on body weight, clinical signs and gross observation were monitored during 14days after

dosing according to KFDA Guidelines (1999-61). As the results, we could not find moralities, clinical signs and changes in the bodyweight except for residuals of test articles and related skin ulcer at injection sites.....From these results, subcutaneous dose of Polycan caused no serious systemic toxicity effect to the male and female mice up to 250mg/kg-the highest dosage tested in this study. Therefore, Plycan was considered that it has relatively favorable toxicological profiles similar to other types of beta-glucan having different origins (Chilara, 1983: William et al., 1988: Feletti et al., 1992; Spicer et al., 1999; Delaney et al., 2003)

Appendix 7: 4 weeks oral dose toxicity test of exopolymers from Aureobasidium pullulans(Polycan®) in SD rat

In order to test toxicity in the repetitive oral dosage of Exopolymers of Aureobasidium pollulans (Polycan) the repetitive oral dosage of Polycan was given to male & female SD rats for 4 weeks. The change of weight, the change of food consumption, and any kind of clinical sign caused by the testing material were not observed in the group of male & female rats. There are no odd and specific clinical signs. The group of male & female rats did not show the change in the ophthalmological test, the urinalysis, the hematological analysis, the serum biochemistry, the internal organ weight measure, and the autopsy result. The change was not observed in the histopathological test from the male & female group dosed 1000 mg/kg. As a result of the entire test above, it is concluded that any change resulted from the repetitive oral dosage of Polycan was not observed in the group of male & female rats who took the dosage.

Appendix 11: Randomized, Double-Blind, Clinical Trials of Immunomodulating Polycan® of Black Yeast for Its Efficacy and Safety

In addition, Safety test did not show any significant statistical difference in clinical investigation items and adverse symptoms in 4-week treatments. This can be concluded that Polycan does not have adverse symptoms or toxicity.

As shown above, all these 5 articles support the safety of beta-glucan derived from A. pullulans in their conclusion.

2)-2. In addition, clarify why GRN 239 supports safety, for example, where you state that: ....."black yeast beta-glucan is soluble and Biothera's bakers yeast is insoluble" (page 10). Is black yeast beta-glucan completely soluble or just more soluble than bakers yeast?

First of all, beta-glucan from A. pullulans is not completely soluble. The correct statement should have been "black yeast beta-glucan is more soluble than Biothera's bakers yeast beta-glucan." The difference in solubility results from the difference of the process of how to obtain beta-glucan 1,3/1,6. While beta-glucan derived from A. pullulans is obtained through the fermentation of A. pullulans and the series of physical filtrations (microfiltration to remove residues and fungal cells and ultrafilteration to obtain beta-glucan with the certain molecular weight) of the culture medium, Biothera's bakers yeast beta-glucan is obtained through the lysis of the yeast's cells, the centrifugal separation of the yeast extract, the isolation of the cell wall, the caustic treatment to strip the cell wall protein, the acid treatment to form free glucosamine hydrochloride, the precipitation by the ph adjustment, and finally the spay-dry. In other words, beta-glucan derived from A. pullulans produced by the physical filtrations is naturally more soluble than Biothera's bakers yeast beta-glucan produced by the precipitation by the ph adjustment.

Although the difference in solubility, both beta-glucans have the almost identical structure of beta-glucan 1,3/1,6 as well as the similarity in the constituents composition. That's why Glucan corporation claims that GRN239 supports the safety of its beta-glucan derived from A. pullulans. That is, the rationale behind the claim was that the substantial equivalence in the composition shown in Table 1 of page 11 between beta-glucan derived from A. pullulans and GRN239 do outweigh the difference in the extent of solubility. This claim was based on a statement of US FDA as follows: In establishing the equivalence of a product to an existing food product it is important to determine that any differences in the new food product do not outweigh the similarities between the two products. Thus, the FDA has stated that a critical factor that must be determined when applying the concept of equivalence is any difference in composition between the substances being compared (U.S. FDA, 1997)

3) Please discuss if the ingestion of Polycan would have deleterious effects in humans or subgroups (ex. infants) for example, in terms of allergenicity or if the organism A. pullulans has been reported to be pathogenic.

Any pathogenic or allergenic effect from the ingestion of Polycan has not been reported in S. Korea since 2004 in which Polycan was started being used as a food additive. In addition, neither pathogenic nor allergenic effect was reported in any animal or clinical test in which dosage was under control.

However, given that beta-glucan 1,3/1,6 has been known to have efficacy to enhance the human immune system, a rare chance of hypersensitive allergy might not be completely excluded.

4) The GLP study using randomized double-blind clinical trial did not report the concentration used in tablets, please provide or explain why the data is not available.

Dosage: 2 tablets twice a day (1 tablet: 200mg), 30 min after a meal, during 4 weeks

Dosage was included in the final report of Korean version. But the dosage was left out in English version. It is translator's and proof reader's mistake.

5)Please clarify the discrepancy in these two data points. In the Lee et al., 2005 article, the authors concluded that "the LD50 and approximate LD in mice after a single oral dose of Polycan were considered over 1000 mg/kg, respectively in both male and female" whereas in the GLP study on rats, the report states the NOAEL is over 1000 mg/kg for both male and female.

The discrepancy in the two data points in the Lee et al., 2005 article and the GLP study on rats resulted from two different purposes each test was designed for. In other words, each test had two different items to target to measure: LD50 and LD of the Lee et al., 2005 and NOAEL of the GLP study on rats. In the Lee et al., 2005, the test was designed to measure LD50 and LD and 1000mg/kg was set as its maximum level of an administration. The maximum lever was administered and no adverse effect was found, concluding that the LD 50 and approximate LD in mice is over 1000mg/kg. That is, if 1500mg/kg were set as its maximum and there was no adverse effect, its LD50 and LD would have been over 1500mg/kg. That is why it was stated as over 1000mg/kg not as 1000mg/kg. In the GLP study on rats, 1000mg/kg was also set as its maximum level of an administration and no adverse effect was found. The test concluded that NOAEL of Polycan is over 1000mg/kg. That is, any administration more than 1000mg/kg was not tested and was not in the range of the particular test. In conclusion, the discrepancy came from a fact that two different tests to measure two different items happened to set their maximum level of an administration same as 1000mg/kg.

Meat processed products and Soup bases and sauces

Based on the outcome of the meeting with USDA's Food Safety and Inspection Service, Glucan Corporation decided that soup bases and sauces and processed meat products would be excluded from the intended food uses of beta-glucan derived from Aureobasidium pullulans. Therefore, GRAS Notice for black-yeast beta-glucan submitted on Dec 3, 2009 was amended accordingly and I electrically submit the pages (pg 3, 11-13) amended in a PDF form.

Pages 000150-000158 have been removed in accordance with copyright laws. The removed references are:

Salkin "Opportunistic infection of the spleen caused by Aureobasidium pullulans." J Clin Microbiol. 1986 May; 23(5): 828–831.

Hawkes "Aureobasidium pullulans infection: fungemia in an infant and a review of human cases" Diagn Microbiol Infect Dis. 2005 Mar;51(3):209-13.

The intended food uses include dairy products, baked goods and baking mixes, beverages and beverage bases, breakfast cereals, jams and jellies, processed fruits and fruit juices, and gum and soft candy. Black yeast beta-glucan(Polycan®) will be added to food products at use-levels ranging from 1 % to the maximum 10% (120mg~150mg)/serving, depending on the proposed food use.

E. Basis for the GRAS determination

Glucan's GRAS determination for black yeast beta-glucan(Polycan®) is based primarily on scientific procedures in accordance with 21 CFR§170.30. The GRAS of black yeast beta-glucan(Polycan®) has been determined on the basis of the view of experts qualified by scientific training and experience to evaluate the safety of black yeast beta-glucan (Polycan®) and the common knowledge about throughout the scientific community knowledgeable about the safety of black yeast beta-glucan(Polycan®). For a detailed discussion of the studies, peer-reviewed journals, data, materials and information that form this basis, please see Section V below and Appendices.

In addition, from the late 1990, in Japan and S. Korea, black yeast beta-glucan produced from *Aureobasidium pullulans* SM-2001, has been commonly called beta-glucan. It has been used as a natural source for the immunomodulation. Moreover, in S. Korea, black yeast beta-glucan(Polycan®) has been used as a food ingredient in dairy products, such as milk and yogurt, and processed meat products, such as ham and sausage, in which it serves as a source to improve bone health since 2004.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notice will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

PeopleAndTechnologies, LLC
5822 Santa Fe Springs Dr.
Houston, TX 77041

and

Glucan Corporation, Limited
Marine Bio Center 218, Silla University
Gwaebop-Dong, Sasang-Gu
Busan, S. Korea 617-736

Should the FDA have any questions or additional information requests regarding this notification, PNT will supply these data and information.

Table 1 Comparison of black Yeast Beta-Glucan with bakers yeast Beta-Glucan

Specification	black yeast beta-glucan	bakers yeast beta-glucan
Description	The exopolymer of *Aureobasidium pullulans SM-2001, an UV induced mutant of A. Pullulans ATCC 42023 (black yeast)*	Fine Beige/tan powder
Organism	*A. pullulans*	*S. cerevisiae*
Total Carbohydrate (%)	At least 82	At least 80
Beta-glucan (%)	At least 40	At least 70
Protein (%)	<10	<10
Fat (%)	<5	<20
Ash (%)	<10	<5
Moisture (%)	<6	<8
Lead (ppm)	ND	<0.5
Mercury (ppm)	ND	<0.05
Arsenic (ppm)	ND	<0.1[a]
Selenium (ppm)	ND	N/A
Cadmium	ND	<1[a]
Zinc (ppm)	N/A	N/A
Aerobic Plate Count (CFU/g)	<3,000	<20,000
Yeast and Mold (CFU/g)	≤20	≤25
Coliforms (CFU/g)	<10	<10
Escherichia coli	Negative	Negative
Salmonella sp	Negative	Negative
Coagulase positive *Staphylococci*, *Clostriduim perfringens*, *Clostridium botulnum* or any other recognized pathogen or hamful microbial toxin	N/A	N/A

CFU = colony forming units, ND = the concentration is below the detection limit or is non-detected.
N/A = not analyzed for by Glucan,
[a] Derived from certificates of analysis for various batches of Biothera's yeast beta-glucan

Furthermore, because the maximum use level, 150mg/serving of black yeast beta-glucan is 3/4 of the use level, 200mg/serving of Biothera's bakers yeast beta-glucan (GRAS Notice No. GRN 000239), all the major parameters of black yeast beta-glucan(Polycan®) including the total population all-user mean intake, an estimated heavy consumer (90th percentile) all-user intake, the greatest mean and percentile all-user intakes on an absolute basis, and the greatest mean and 90th percentile all-user intakes on a body weight basis are lower than those of Biothera's bakers yeast beta-glucan. Those major parameters of black yeast beta-glucan(Polycan®) are in the section of Intake Estimate below. Moreover, none of the components of black yeast beta-glucan is new to human metabolic processes.

In addition to all these scientific procedures of supporting the safety of black yeast beta-glucan(Polycan®), 5 metric ton/ year of black yeast beta-glucan(Polycan®) has been widely used in dairy products, processed meat products, and dietary supplements in S. Korea.
In conclusion, the intended use level of the 120-150 mg/serving should not pose any safety risk to any such consumer.

A. Intake Estimate

The various food products including baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, diary product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, soft candy, and soup and soup mixes, meat products are intended to market by Glucan Corporation for its Black yeast beta-glucan(Polycan®). Its intended use is at a level of 150 mg/serving shown in Table 2. The use of black yeast beta-glucan(Polycan®) for dairy products and beverages, such as milk, yogurt, cheese, and fruit juices, is self-limiting because the solubility of Black yeast beta-glucan(Polycan®) is decreased and the aggregation starts with concentrations above 5%. This aggregation in products causes a slimy or tacky mouth feel, which limits the use of black yeast beta-glucan(Polycan®) as an ingredient to level below 5% of the food weight.

Table 2. Proposed food uses and use levels for black yeast beta-glucan(Polycan®)

Food category	Proposed food-use	RACC[a] (g or ml)	Black Yeast beta-glucan (Polycan®) levels	
			(mg/serving)	(%)
Baked goods and baking mixes	cookies	30 to 40	150	0.5 to 0.375
Beverages and beverage bases	Meal replacement beverage (not milk-based)	240	150	0.375
Cereal and cereal products	Nutritional bars (breakfast, granola, protein)	40	150	0.375
Dairy product analogs	Soy milk	240	150	0.0625
Milk and milk products	Meal replacement beverage	240	150	0.0625
	Probiotic beverage	240	150	0.0625
	Yogurt	225	150	0.0666
	Yogurt beverage	240	150	0.0625
Plant protein products	Soy protein bars	40	150	0.375
Processed fruits and fruit juices	Fruit beverages (drinks, juices, smoothies)	240	150	0.0625
Soft candy	Chocolate confections	40	150	0.375

[a]RACC = reference amounts customarily consumed per eating occasion [21 CFR § 101 12 (U S FDA, 2007b)] when a range of values is reported for proposed food use, particular foods within that food use may differ with respect to their RACC

Using the United States Department of Agriculture(USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSF II 1994-1996) survey (USDA, 2000), under the conditions of intended use, the total population all-user mean intake of black yeast beta-glucan(Polycan®), an estimated heavy consumer (90th percentile) all-user intake of black yeast beta-glucan(Polycan®), the greatest mean and percentile all-user intakes of black yeast beta-glucan(Polycan®) on an absolute basis, and the greatest mean and 90th percentile all-user intakes of black yeast beta-glucan(Polycan®) on a body weight basis are 291.3 mg/person/day, 583.4 mg/person/day, 409.7 and 836.7mg/person/day, 19.7 and 38.0 mg/kg body weight/day in infants, respectively (See Table 3 and 4). Furthermore, black yeast beta-glucan(Polycan®) is not intended directly to market for infant and toddler foods, resulting in limiting infants and toddlers to consume black yeast beta-glucan(Polycan®).

Table 3. Summary of the estimated daily intake of black yeast beta-glcuan (Polycan®) from all proposed food categories in the U.S. by population group (1994-1996, 1998 USDA CSF II data)

Population group	Age group (years)	% users	Actural # of total users	All person consumption		All-users consumption	
				Mean (mg)	90th percentile (mg)	Mean (mg)	90th percentile (mg)
Infant	0 to 2	71.6	2,564	192.7	448.6	247.1	488.8
Child	3 to 11	98.4	6,205	335.2	612.8	342.0	614.0
Female teenager	12 to 19	91.5	642	284.6	604.2	311.1	624.9
Male teenager	12 to 19	91.7	638	375.1	800.1	409.7	836.7
Female adult	20 and up	85	3,887	205.3	453.1	239.3	474.6
Male adult	20 and up	81.6	3,876	249.9	583.8	305.4	639.6
Total population	All ages	86.4	17,812	251.7	554.4	291.3	583.4

Table 4. Summary of the estimated daily per kilogram body weight intake of black yeast beta-glucan(Polycan®) from all proposed food categories in the U.S. by population group (1994-1996, 1998 USDA CSF II data)

Population group	Age group (years)	% users	Actural # of total users	All person consumption		All-users consumption	
				Mean (mg)	90th percentile (mg)	Mean (mg)	90th percentile (mg)
Infant	0 to 2	71.6	2,564	15.3	35.3	19.7	38.0
Child	3 to 11	98.4	6,205	13.8	26.7	14.2	26.8
Female teenager	12 to 19	91.5	642	5.2	12.3	5.8	12.5
Male teenager	12 to 19	91.7	638	6.2	13.0	6.7	14.0
Female adult	20 and up	85	3,887	3.2	7.2	3.7	7.7
Male adult	20 and up	81.6	3,876	3.1	7.4	3.8	8.2
Total population	All ages	86.4	17,812	5.4	13.2	6.3	14.5

B. Expert Consensus

The safety studies described above for black yeast beta-glucan(Polycan®) have been published in four peer-reviewed scientific journals. The full version of those journals are presented in Appendix 7-10. A human clinical study[400mg/day/human (60kg)], which is Randomized, Double-Blind, Clinical Trials of Immunomodulating Polycan® of Black Yeast for Its Efficacy and Safety presented in detail as Appendix 11, has been completed at Daegu Oriental Hospital of Daegu Haany University, a GCP(Good Clinical Practice) certified institute. The authors who are qualified by scientific training and experience to evaluate the safety of black yeast beta-glucan (Polycan®) consistently concluded in the 4 journals and 1 clinical study that no adverse effects associated with the repeated consumption of black yeast beta-glucan(Polycan®) produced from *Aureobasidium pullulans* SM-2001 has been observed. Furthermore, the authors concluded that their findings of black yeast beta-

AM

Weinsetel, Natalia

From: Wonho Cho [chowonho@gmail.com]
Sent: Thursday, April 15, 2010 2:49 PM
To: Weinsetel, Natalia
Subject: Re: Responses on additional questions

Dear Dr. Weinsetel,

I forwarded the revised question to engineers in Korea. I will reply as soon as I receive a reponse.

In the meanwhile, I think that the response of the question 1)-(1) in Chemistry might be an answer for the revised question.

Any particular gene was not added to A. pullulans to produce A. pullulans SM-2001, causing no change in the
nature of the safety and toxicity. A. pullulans SM-2001 is an improved strain, **producing more beta-glucan**
rather than simple pullulans, by the UV induction of A. pullulans.

Thanks

On Thu, Apr 15, 2010 at 11:30 AM, Weinsetel, Natalia <Natalia.Weinsetel@fda.hhs.gov> wrote:

Wonho Cho,
I forwarded your responses to our reviewers. In terms of microbiology question #4, the reviewer does not believe you have fully answer the question. Please see the question below from the reviewer. I will forward your response as soon as you send it.
Please let me know if you have any questions.
Thank you,
Natalia

Original question:
Please provide information about the physiological effects of the UV treatment of *A. pullulans* SM-2001. How is the mutant strain different from the wild type strain?
Answer you provided:
A. pullulans SM-2001 produced by the UV treatment of A. pullulans ATCC 42023 possesses the same mycological as well as taxonomical properties. However, the colony of A. pullulans SM-2001 is larger, less glossy, and lighter in color as shown in a picture below.

The above answer does not provide an explanation as to the reason for using the mutant over wild type.

Revised question:
Is there any relevance of the phenotype of the mutated strain to glucan production? If so, what is it?

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
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From: Wonho Cho [mailto:chowonho@gmail.com]
Sent: Friday, April 09, 2010 11:56 AM
To: Weinsetel, Natalia

Subject: Responses on additional questions

April 09, 2010

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

Re: GRAS Notice for beta-glucan derived from Aurebasidim pullulans

Dear Dr. Natalia Weinsetel

Pursuant to additional questions raised by FDA and USDA regarding GRAS Notice for beta-glucan derived from Aureobasidium pullulans submitted on Dec. 9, 2010 and on behalf of Glucan Corp. headquartered in Busan, S. Korea, , I am submitting the responses on those questions. In addition, I also notify that Glucan Corporation decided to exclude processed meat products and soup bases and sauces from the list of the intended uses. The responses and the pages amended accordingly are included in a zipped file attached.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process. All of the information that serves as the basis for this GRAS determination can be sent - upon request - to FDA and is available for review and copying at reasonable times.

Best regrads,

Wonho Cho
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US Sales Division of Glucan Co., Ltd
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Weinsetel, Natalia

Subject: FW: Responses on additional questions

From: Wonho Cho [mailto:chowonho@gmail.com]
Sent: Tuesday, April 20, 2010 1:11 PM
To: Weinsetel, Natalia
Subject: Re: Responses on additional questions

Dear Dr. Weinsetel,

In addition to the response in the question 1)-(1) of Chemistry, below is more elaborated response for the revised question. However, the main reason to use A. pullulans SM-2001 remains same: **More beta-glucan 1,3/1,6 is produced by A. pullulans SM-2001 than by normal A. pullulans.**

A. pullulans are known for excreting pullulans, extracellular polysaccharides, and the pullulans include alpha-glucan. Amid the macromolecular polymers excreted , beside alpha-glucan, the excretion of macromolecular polymers having beta-1,3- linkage as a basic structure and beta-1,6-linkage as a branch to beta-1,3 was reported. Knowing that the excretion of beta-glucan 1,3/1,6, Glucan corporation had been researched in many ways to produce more beta-1,3/1,6 and found out that pullulans excreted by the UV induced mutant of A. pulllulans, A. pullulans SM-2001, include more beta-glucan 1,3/1,6. To confirm the more content of beta-glucan 1,3/1,6 in the macromolecular polymers excreted, Glucan Corp. separated the macromolecular polymers excreted and analyzed through the quantitative analysis after the chemical treatment, the nuclear magnetic resonance(NMR) spectrum for chemical structure confirmation and Gel permeation chromatography for molecule wight measurement. The analysis support that A. pullulans SM-2001 produce more beta-glucan 1,3/1,6 than normal A. pullulans. That is why Glucan corp. uses A. pullulans SM-2001 rather than normal A. pullulans.

If the reviewer have any question, please let me know.

Thanks
Wonho Cho

On Thu, Apr 15, 2010 at 1:49 PM, Wonho Cho <chowonho@gmail.com> wrote:

Dear Dr. Weinsetel,

I forwarded the revised question to engineers in Korea. I will reply as soon as I receive a reponse.

In the meanwhile, I think that the response of the question 1)-(1) in Chemistry might be an answer for the revised question.

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nature of the safety and toxicity. A. pullulans SM-2001 is an improved strain, **producing more beta-glucan**

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Natalia

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The above answer does not provide an explanation as to the reason for using the mutant over wild type.

Revised question:
Is there any relevance of the phenotype of the mutated strain to glucan production? If so, what is it?

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
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From: Wonho Cho [mailto:chowonho@gmail.com]
Sent: Friday, April 09, 2010 11:56 AM
To: Weinsetel, Natalia

Subject: Responses on additional questions

April 09, 2010

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

Re: GRAS Notice for beta-glucan derived from Aurebasidim pullulans

Dear Dr. Natalia Weinsetel

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Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process. All of the information that serves as the basis for this GRAS determination can be sent - upon request - to FDA and is available for review and copying at reasonable times.

Best regrads,

Wonho Cho
PeopleAndTechnologies, LLC
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AM

Weinsetel, Natalia

Subject: FW: Responses on additional questions

From: Wonho Cho [mailto:chowonho@gmail.com]
Sent: Wednesday, May 19, 2010 5:38 PM
To: Weinsetel, Natalia
Subject: Re: Responses on additional questions

Dear Dr. Weinsetel

Attached is the same statement to which reference number has been added. The statement that the microbiology reviewer questioned was referenced from 14 references listed below the answer. Each part of the statement were referenced from the references numbered next to it. Furthermore, 5 additional references (1, 10-14) are added.

Thanks
Wonho Cho

On Mon, May 17, 2010 at 10:55 AM, Weinsetel, Natalia <Natalia.Weinsetel@fda.hhs.gov> wrote:

Dear Wonho Cho,
I received another question from one of the reviewers regarding beta-glucan derived from A. pullulans. Could you please respond as soon as you get a chance? Thank you!
Natalia

Microbiology:
In your response dated April 9 to our questions, you sent microbiology information regarding the organism. Can you please clarify which references state that *A. pullulans* is found on lake water, cosmetics, and foods including fruits, vegetables, tomatoes, and cheese?

Natalia Weinsetel, Ph.D.
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--
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Microbiology

1) Please provide general information about *A. pullulans*
(ex. where is the organism normally found).

Aureobasidium pullulans, the "black yeast," is a ubiquitous fungus (1,3,4) that has been found in soil(1, 6), on the leaves(12), in lake water (1,11), on weather wood(5,9,10), as well as on foods such as fruits(1), cereal(8), tomatoes(13,14) and cheese(3,4,7). The fungus contains multiple life forms (polymorphic) including blastospores, hyphae, chlamydospores, and swollen cells. The chlamydospores and swollen cells are considered resting forms. The fungus produces a green melanin which turns black over time. The fungus also produces the polysaccharide pullulan, which is currently used in industrial applications such as a coating for pill tablets, cosmetics, foods, and dissolvable breath fresheners.

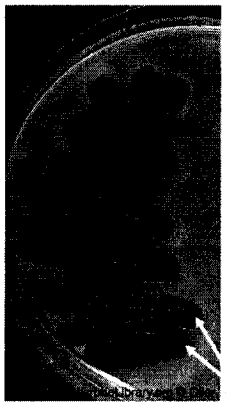

Figure shows seven-day-old *Aureobasidium pullulans* colonies grown on Ueda medium at 28℃ in a humid environment. The culture is exhibiting dark green to black coloration due to melanin production. The sheen on the green area represents pullulan production, and white vegetative hyphae can be seen extending into the culture medium at the edge of the culture.

References:

1. http://en.wikipedia.org/wiki/Aureobasidium_pullulans

2. Anna Oller, http://www.microbelibrary.org/asmonly/details_print.asp?id=1444&lang=

3. Cooke, WB. (1961) A taxonomy study in the 'black yeasts'. Mycopathol Mycol Appl, 17: 1-43.

4. Durrell, LW. (1967) Studies of *Aureobasidium pullulans* (De Bary) Arnaud. Mycopathol Mycol Appl, 18: 113-120.

5. Zabel, RA & Terracina F.(1980) The role of *Aureobasidium pullulans* in the disfigurement of latex paint films. Dev Ind Microbiol, 21: 179-190.

6. Domash, KH, Gams, W & Anderson, TH. (1993) Aureobasidium. IN: Complendium of soil Fungi. Eching, Germany, IHT-Verg, Vol 1, pp. 130-134.

7. Mislivec, PB, Yoneyama, M & Sasaki, S. (1990) Growth promoting agent for bacteria containing pullulan with or without dextran. European Patent Specification EP 0382355B1.

8. Weidenborner, M, Berleth, M, Kramer, J & Kunz, B. (1997) Mold spectrum of four cereal brands of the German crop 1995. Nahrung, 41:139-141.

9. Webb, JS, van der Mei, HC, Nixon,M, Eastwood, IM, Greenhalgh, M, Read, SJ, Robson, GD & Hanley, PS.(1999) Plasticizers increased adhesion of the deteriogenic fungus *Aureobasidium pullulans* to polyvinyl chloride. Appl Environ Microbiol, 65:3575-3581.

10. Cronin, LA, Tiffney, WN & Eveleigh, DE. (2000) The greying of cedar shingles in a maritime climate – a fungal basis?. J Ind Microbiol Biotechnol, 24: 319-322.

11. Vadkertiova, R & Slavikova, E. (1994) Yeasts from sediments and soil along the lake Jakubov. Biol. Bratislava, 49, 841-847.

12. Milind S. Deshpande, Vinay B. Rale and James M. Lynch (1992) *Aureobasidium pullulans* in applied microbiology: A status report, Enzyme and Microbial Technology 14(7): 514-527.

13. Schena L.,Ippolito A, Jahavi T, Cohen L, Nigro F and Doroby S.(1999) Genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolate against postharvest rots. Postharvest Biology and Technology 17:189-199.

14. Agnieszka Jamiołkowska, Anna Wagner. Fungal communities from the rhizosphere of tomato cultivated conventionally and with rye as cover crop. Electronic J Polish Agric Univ. 8(4). (http://www.ejpau.media.pl/volume8/issue4/art-23.html)

AM

Weinsetel, Natalia

Subject: FW: Responses on additional questions

From: Wonho Cho [mailto:chowonho@gmail.com]
Sent: Friday, June 04, 2010 10:40 AM
To: Weinsetel, Natalia
Subject: Re: Responses on additional questions

Dear Dr. Weinsetel

By Korea FDA (KFDA), heavy metal is regulated by the content of lead. The COA submitted in the amendment is our specifications being used for food grade in Korea. Accordingly, the specifications of heavy metal in the amendment is the specifications of lead. In other words, the specifications of lead is less than 3 ppm. "Less than 3 ppm" is the food grade requirement of lead by KFDA. Attached is COA revised from Heavy metal to Lead.

In addition, a test result of heavy metals including lead can be also found in Appendix 13 of GRN0000309 we submitted. In the analysis, lead was specified as ND (=the concentration is below the detection limit which is below 1 ppm or non-detected).

Please let know if the reviewer need more info regarding the specifications of lead.

Thanks
Wonho Cho

On Wed, Jun 2, 2010 at 2:24 PM, Weinsetel, Natalia <Natalia.Weinsetel@fda.hhs.gov> wrote:

Dear Wonho Cho,
I am sorry to bother you again, but I have an additional question for you regarding the specifications for beta-glucan. You submitted specifications for arsenic in the notice and for heavy metals in the amendment. Lead was not specified and we need it. Please send us the specifications for lead.
Thank you in advance for your help.
Best regards,
Natalia

From: Wonho Cho [mailto:chowonho@gmail.com]
Sent: Monday, May 17, 2010 1:08 PM

To: Weinsetel, Natalia
Subject: Re: Responses on additional questions

Dear Dr. Weinsetel

I will reply you as soon as I get an answer from Korea.

Thanks
wonho cho

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