ORYZA OIL & FAT CHEMICAL CO., LTD.

Kuzu Lactic Acid Bacteria
SkinBarrier Lactic Acid ™
ImmnoRise Lactic Acid ™

Effect of protecting skin

Effect of reducing risk of virus infection

■**SkinBarrier Lactic Acid™-P**
(Powder, food application)

■**ImmnoRise Lactic Acid™-P**
(Powder, food application)

ORYZA OIL & FAT CHEMICAL CO., LTD.

ver. 1.0 NS

Effect of protecting skin
Effect of reducing risk of virus infection

Kuzu Lactic Acid Bacteria

SkinBarrier Lactic Acid Bacteria™
ImmnoRise Lactic Acid Bacteria™

1. Introduction

Kudzu (*Pueraria montana var. lobata*) is a perennial vine that belongs to the genus Pueraria in the Fabaceae family (Fig. 1). In Japan, its roots are used to make kudzu powder as well as Chinese medicine, and it has been listed as one of the seven plants of autumn since the age of Manyo (Nara Period between 629 and 759).

The Japanese name "kudzu" is said to come from the fact that Kudzu in the upper reaches of the Yoshino River (Kino River) in Yamato Province (currently known as Nara Prefecture) used to be the center of kudzu powder production. There was a theory that the plant became known as "kudzu" because people from Kudzu traveled around to sell it [1].

It is distributed in warm temperate zones and can be found throughout Japan from Hokkaido to Kyushu, as well as in China, the Philippines, Indonesia, and New Guinea [2].

Kudzu has been consumed as food, and the starch extracted from the large tuberous roots has been used as "Kudzuko" (kudzu powder) since ancient times [2]. People dig up the roots during the fall and winter and crush them. Then, they add water to remove the fibers, and refine to extract only the starch. It has long been used as an ingredient for Japanese sweets such as kudzu starch gruel, kudzu starch noodles, kudzu starch cake, and kudzu sweets (dry sweets), as well as for thickening cooling materials.

Fig. 1 Flower and vine of Kudzu

Kudzu can also be used for medicinal purposes. In Japan and China, kakkon (root of kudzu) is considered to have perspiring, antipyretic, and analgesic actions, thus it is used as a remedy for the early stages of cold. The flowers are called Kakka (kudzu flower) and are known to contain isoflavones, which are believed to be good for hangovers in folk remedies. Also, the leaves are called Katsuyo (kudzu leaf) and are used as a crude drug to stop bleeding in cases such as injuries on mountain hikes [3].

Inoue Tengyokudo, a long history kudzu manufacturer that has been producing Yoshino-hon kudzu in Nara since 1890, isolated kudzu-derived lactic acid bacteria (*Leuconostoc mesenteroides*) from kudzu which is used for food and medicinal purposes as described above. With the supply of kudzu from the company, Oryza Oil & Fat Chemical has discovered new functionalities.

Oryza Oil & Fat Chemical has confirmed that Leuconostoc mesenteroides has the potential to improve the function of the cornified envelope, which is important for the suppression of water evaporation from the skin as well as the maintenance of the skin's barrier function, and has named it "SkinBarrier Lactic Acid Bacteria™" to enable its wide use in foods as a brand for beauty enhancement.

Furthermore, Oryza Oil & Fat Chemical has been keenly focusing on the research on its immunostimulatory action and viral infection defense functions conducted at Inoue Tengyokudo & Co Ltd, and has named the bacteria "ImmnoRise Lactic Acid Bacteria™" as lactic acid bacteria essential for modern society, where the importance of immunity is being emphasized. The application of the product for food products is currently expanding.

1) Supervised by Toshiaki Oshima, "Hana iro de hikeru sanyaso/kouzan syokubutsu. (Wildflowers and alpine plants by flower color.)" SEIBIDO SHUPPAN, p. 158, ISBN 4-415-01906-4, (2002)
2) Yasuhiro Asai, "Midori no shinnyusyatachi: Kikasyokubutsu no hanashi. (Green Invaders: Stories of Naturalized Plants.)", The Asahi Shimbun, ISBN 4-02-259574-4, (1993)
3) Yoshitaka Kaizu, "Nihon no yakuso. (Medicinal plants of Japan.)" Shogakukan, p. 93, ISBN 4-09-208016-6, (1995)

2. Lactic acid bacteria

Lactic acid bacteria is a general term for anaerobic microorganisms that produce lactic acid from sugars through fermentation. It is known to suppress the propagation of bad bacteria in the intestines and regulate the intestinal environment.

They produce lactic acid from sugars through fermentation, and are called "good bacteria" because they are beneficial to the human body. As a food ingredient, they are used in the manufacturing of fermented foods such as yogurt, cheese, pickles, Japanese sake, etc. Bifidobacteria, which is well known for its use in yogurt, is also a type of lactic acid bacteria.

Lactic acid bacteria suppress the propagation of bad bacteria such as E. coli in the intestines and regulate the balance of bacteria in the intestines. In addition to improving bowel movements, they are said to have various functions such as lowering cholesterol level, improving immunity, and preventing cancer. Recently, researches have been conducted on lactic acid bacteria that have unique functions such as eliminating Helicobacter pylori.

Among the microorganisms that help regulate the intestinal environment, such as lactic acid bacteria, useful microorganisms that can reach the intestines alive are particularly called probiotics. Live bacteria are sensitive to heat and stomach acid, so it is difficult for them to reach the intestines alive. However, even if they are broken down by stomach acid, they can still be a source of food for good bacteria. On the other hand, there are some effects that can only be observed in live bacteria. Oligosaccharides and other organisms that serve as a source of nutrients and help probiotics to propagate are called prebiotics. Furthermore, in recent years, dead lactic acid bacteria that have been treated through heating and other processes also called biogenics, are widely used. Although those bacteria are already dead, they become less vulnerable to the effects of heat and stomach acid. It is said that some types of bacteria can be more effective in dead condition than when alive.

3. Kudzu-derived Lactic Acid Bacteria

Kudzu-derived lactic acid bacteria is a strain discovered and isolated by Inoue Tengyokudo & Co., Ltd. and it belongs to Leuconostoc mesenteroides species.

Leuconostoc mesenteroides is a gram-positive, spherical lactic acid bacterium. It is known as a heterolactic fermentative lactic acid bacterium that breaks down sugar and

generates lactic acid and acetic acid or alcohol as well as carbon dioxide. It is also known to be separated from fermented plant-derived foods such as sauerkraut.

The human body is made of about 60 trillion pieces of various cells. For any living organism to live in a healthy condition, it must distinguish its own cells and other cells to prevent destruction of an individual body by the invasion of external enemies or continuous parasitism. The mechanism to distinguish self and non-self cells is called "immunity". Immune systems have an important role to keep our body in a normal condition by recognizing bacteria and viruses that have invaded into the body as foreign matters (non-self matters) and attacking them.

The immunity is known to decline as the differentiation ability of T-cells, B-cells, and lymphocyte that are associated in immunity declines, the number of cells working normally reduces, or the function of immune cells declines due to various factors such as aging or stress. It is known that this lowers protection against infection and increases the risk of viral or bacterial infection. Declined immunity that suppresses excessive inflammation and increases the risk of allergies, auto immune diseases, and rheumatoid arthritis. Stimulating immunity (immunostimulation) is important to maintain our body at its normal condition.

4. Immunostimulatory Action [ImmnoRise Lactic Acid™]

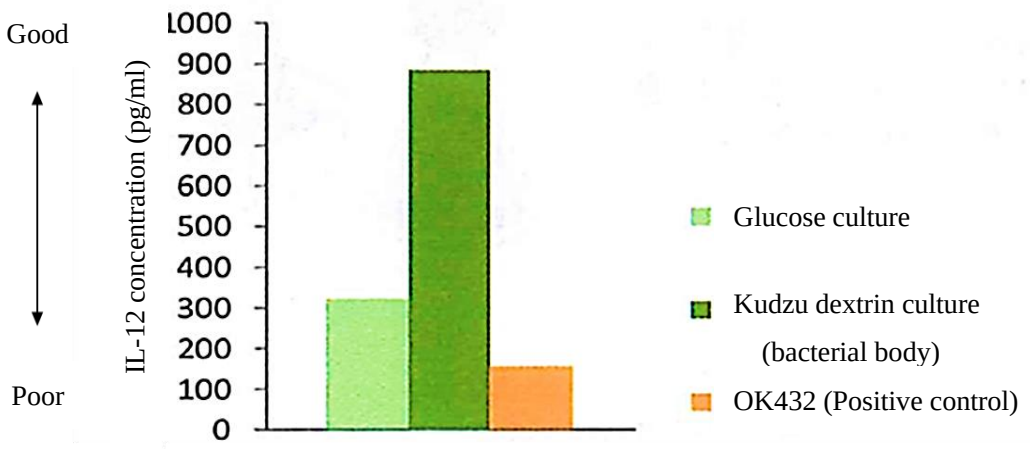
(1) Effects on the immunostimulatory action (IL-12) production capacity

Interleukin 12 (IL-12) is a cytokine that was originally reported as the "NK cells stimulating factor" and is characterized by its action to activate NK cells. IL-12 is also known to be generated from B-cells and monocyte lineage cells, and to demonstrate actions to accelerate the growth of T-cells and NK cells, induce cytotoxic activation IFN-γ production, and LAK cells etc. For these cell-mediated immunity functions, IL-12 is expected to be used in clinical applications for the prevention of infections, anticancer therapies, and the improvement of immunodeficiency diseases. For example, IL-12 production in peripheral blood lymphocytes, IFN-γ production, or NK cells activation are all significantly lowered in HIV-infected patients. Administration of IL-12 is believed to improve these to normal levels equivalent to healthy people. So, we used it as the immunostimulatory marker and evaluated the results in own study.

Method: ImmnoRise Lactic Acid™-P and the control OK432 (Streptococcus pyogenes formulation that deactivates cell-mediated immunity) were added to spleen cells of mice and cultivated. Then, the concentration of IL-12 was measured via the ELISA technique.

Results: As that is kudzu-derived lactic acid bacteria. It was confirmed that the concentration of IL-12 was increased when cultured with kudzu dextrin compared to group which cultured with glucose (Fig. 2).

Fig. 2 Effect of kudzu dextrin on IL-12 production capacity



(2) Effect on influenza virus and neutralizing antibodies

In order to check the effects of ImmnoRise Lactic Acid™ which has been confirmed to have a strong immunostimulatory action, we conducted a joint test with Inoue Tengyokudo & Co., Ltd. and Kyoko HAYASHI's laboratory office at Chubu University on its effects to prevent infection from the influenza virus using mice infected with the influenza virus.

<Effect of prevention of virus infection after administration of lactic acid (*in vivo*)>

Method: Female, 6 weeks-old BALB/c mice were infected with type A influenza virus (A/NWS/33,H1N1 subtype) and ImmnoRise Lactic Acid™-P (1 mg/0.4 ml/day) as well as the positive control anti-influenza drug Tamiflu (0.2 mg/0.4 ml/day) were orally administrated after three days and two weeks. Then, the amount of virus-specific antibodies were measured after three days and two weeks infection.

Results: After infected with influence virus for three days, ImmnoRise Lactic Acid^{TM}-P was confirmed to significantly suppress the increase of the amount of virus in the lung and bronchoalveolar lavage fluid as compared to the control. When infected with influence virus for 14 days, ImmnoRise Lactic Acid^{TM}-P showed a significantly higher value of the neutralizing antibody in serum compared to the control (Fig. 3).

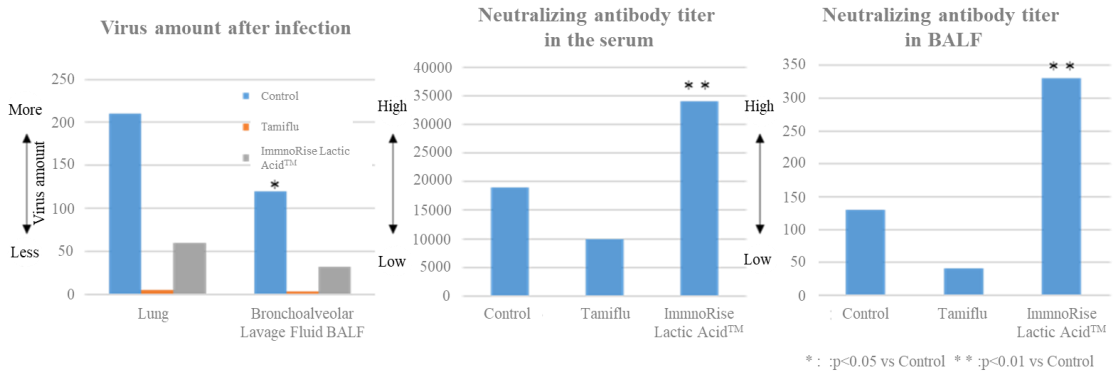


Fig. 3 Effect on the influenza virus and neutralizing antibodies

As the administration of ImmnoRise Lactic Acid^{TM}-P is assumed to increase the neutralizing antibody titer by activating natural immunity which is important in the early stage of infections as well as working on acquired immunity, it is also expected to reduce the risk of infection.

<Effect of administration of lactic acid to the prevention of virus infection (human trial on volunteer subjects)>

The children in nursery school in Nara were orally taken 10 billion bacteria (50 mg/day) of ImmnoRise Lactic Acid^{TM} from January to February which is influenza season and compared the infection rate with adjacent nursery schools who did not take.

Method: Test period: 2 months

Test subjects: 496 nursery school children (3 to 5 years old)

Breakdown: ImmnoRise Lactic Acid^{TM}-P administration/taken group: 209 children,

Non-taken group: 287 children

Evaluation: Prevalence of viral infection diseases

Results: It was confirmed that the prevalence of viral infection diseases was significantly low in nursery schools where children ingested ImmnoRise Lactic Acid™ as compared to other three nursery schools where children did not ingest it (Fig. 4).

Prevalence of viral infection diseases				
Disease	Nursery schools with ingestion of lactic acid bacteria	No ingestion nursery school 1	No ingestion nursery school 2	No ingestion nursery school 3
Type A influenza	10	35	27	16
Adenovirus	0	9	1	0
rotavirus	0	1	1	0
Hand, food, and,mouth disease	0	3	1	0
RS virus	0	4	0	0
Human metapneumovirus	0	2	0	0
Norovirus	0	0	1	0
Total no. of infected children	10	54	31	16
No. of children	209	112	89	86
Infection rate	4.8%	48.2%	34.9%	18.6%

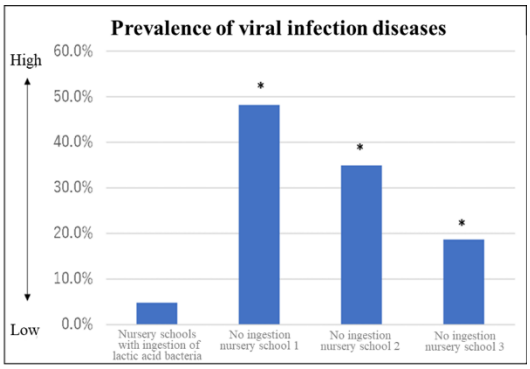


Fig. 4 Effect on ingestion of lactic acid to the prevention of virus infection (human trial on volunteer subjects)

According to the above results, ImmnoRise Lactic Acid™ is expected to improve immunostimulatory action and prevent infections.

5. Skin Moisturizing/Barrier Function [SkinBarrier Lactic Acid™]

(1) Damage to skin because of wearing a mask for long time during the COVID-19

In Japan, people sometimes wear a mask during the influenza season in winter or some people with pollen allergy in specific seasons wear. Meanwhile, due to the pandemic outbreak of COVID-19 (novel coronavirus) started in 2020, now we need to wear a mask for a long time regardless of the season in order to prevent infection from this novel coronavirus. While wearing a mask reduces the risk of infection, it is said that many people are suffering from skin damage due to increased friction on their face where the skin tends to be sensitive as well as due to changes in temperature and humidity.

One of the reason, induced skin problems due to wearing a mask is believed to be the high humidity inside the mask. In general living environment, a comfortable humidity is considered to be 50 to 60%. When the skin is continuously exposed to excessive humidity, the stratum corneum that protects the outer most layer of the skin becomes excessively humid and reaches a so-called pruney condition. The stratum corneum in this condition is susceptible to the influence of the external environment. As a result, its moisture content rapidly changes when putting on or taking off a mask, which affects the barrier function of the skin and allows moisture to evaporate easier. Skin problems caused by wearing a mask are also related to the action of putting on and taking off a mask. As we repeatedly put on and take off masks to eat, drink, or relieve the feeling of smothering, the skin and mask brush each other, damaging the stratum corneum. Furthermore, when removing a mask after wearing it, the skin is suddenly exposed to the outer air from a high temperature/humidity condition, being exposed to changes in the temperature and humidity, which is also believed to be a problem.

As described above, it is believed that the barrier function is weakened by sudden and rapid changes in the temperature and humidity when the stratum corneum has become pruney and weak due to wearing a mask.

(2) Cornified envelope

In the outermost layer of the skin, the stratum corneum forms a thin barrier of just 20 microns from the outside world. In addition, the barrier function to prevent the infiltration of foreign matters from the outside world, the stratum corneum plays a biologically important role of maintaining moisture within the body. As this structure is thought of in terms of blocks and mortar, it is constructed of corneocytes that are equivalent to the blocks and the intercellular lipids that act like the mortar. The corneocytes are filled with keratin fibers and they include natural moisturizing factors (NMF) mainly consisting of amino acids that play an important role in the previously described moisture retention function (Fig. 5).

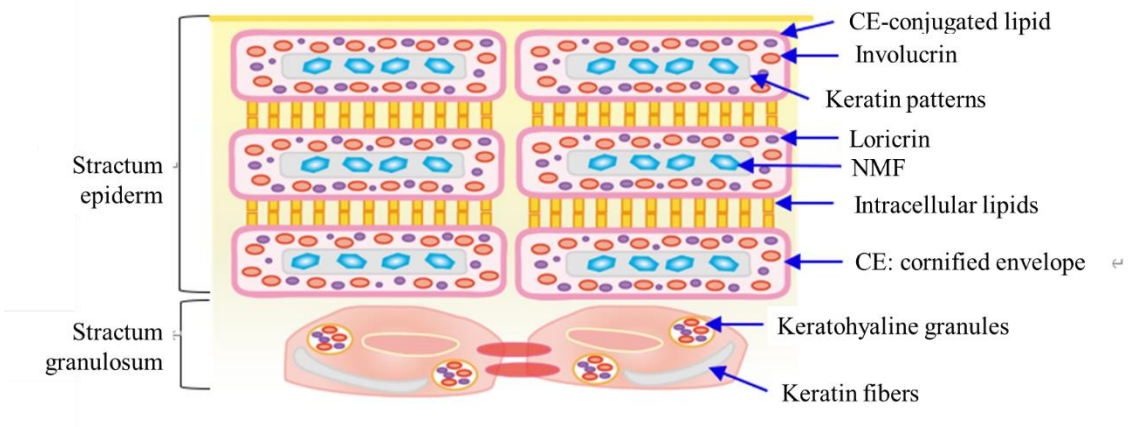


Fig. 5 Structure of Epidermis

On the other hand, the intercellular lipids of the mortar section consist of ceramide, cholesterol, and free fatty acids, and these are organized into a repeated structure (lamellar structure) of oil layers and water layers. As the barrier function changes greatly based on quantitative and qualitative changes to these fats and disturbances in their orientation, these intracellular lipids are considered to play a vital role in the barrier function of the stratum corneum.

To form corneocytes, keratinocytes first divide in the basal membrane, they produce keratin, and move toward to the upper layer while differentiating and maturing. At this time, keratin 5 and keratin 14 in the vicinity of the stratum basal and keratin 1 and keratin 10 in the prickle cell layer and granular layer are formed respectively. The keratin fibers in the granular layer, at the time of keratinization, are aggregated with filaggrin protein, caused dramatic shape changes in the keratin patterns. The keratohyaline granules in the granular cells contain large quantities of profilaggrin, which is the precursor to filaggrin,

then continue to decompose through the action of dephosphorylation at the time of keratinization. The isolated filaggrin aggregates keratin fibers within the cytoplasms of the corneocytes and then it is decomposed into amino acids and other substances in the upper epidermis.

Concerning the structure of corneocytes, there is a growing awareness about cornified envelope (CE) (also called cornified thick membrane, limbic body, and keratinocyte outer membrane). CE is formed when a variety of proteins such as involucrin and loricrin form bridges with each other and become insoluble. It forms a bag-shape structure wrapping corneocytes (Fig. 6).

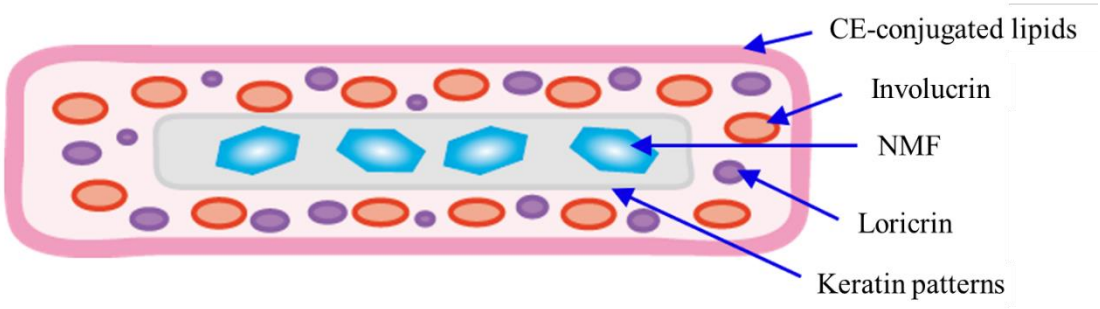


Fig. 6 Structure of CE

The precursor protein CE is manifested from the prickle cell layer to the granular layer following the differentiation of the epidermal keratin sites. Involucrin from prickle cells and loricrin from granular cells are the main components. Bag-shaped CE is formed when these proteins are sufficiently created and bridged by enzymes such as transglutaminase. Further, the wrapping of the CE by the keratin patterns and amino acids etc. existing in the CE can be considered as the maturing of the CE (Fig. 7). When the CE matures, it forms a firm barrier in combination with the surrounding intracellular lipids. CE can also become immature and the barrier function declines in the case of sleep deficiency or skin received with large volumes of UV-rays during the daytime, caused insufficient cell dispersion, differentiation, and synthesis of proteins such as keratin, involucrin and loricrin.

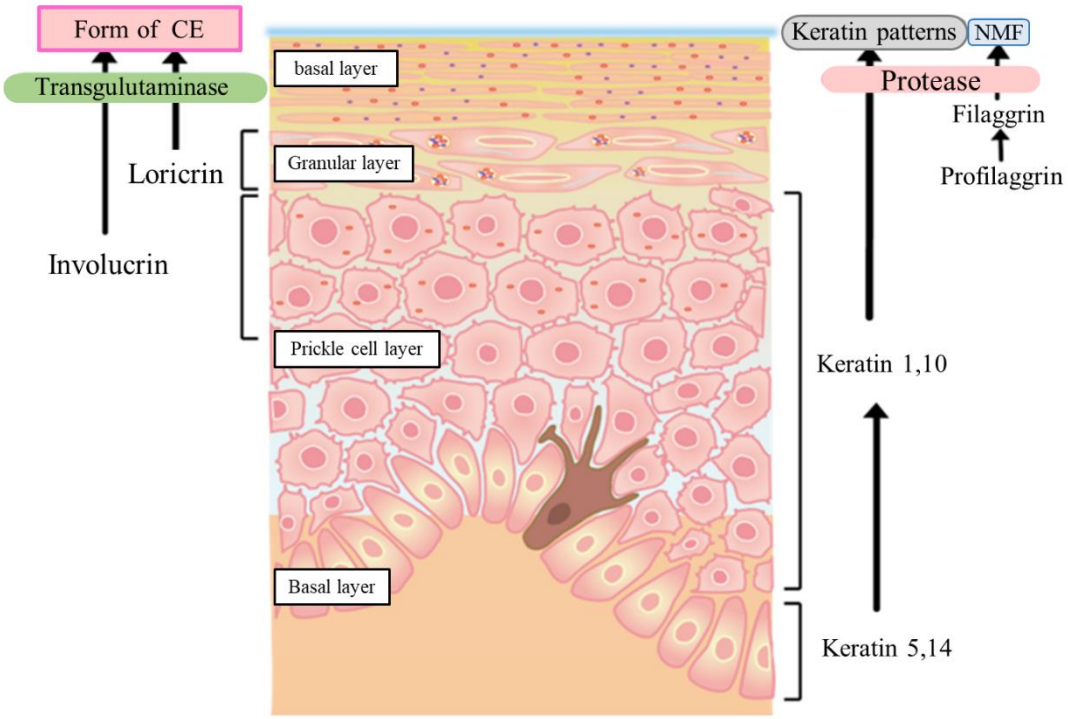


Fig. 7 Formation process of CE

(3) Effect of SkinBarrier Lactic Acid™ on skin is barrier function

<Effect of administration of lactic acid on transepidermal water loss (*in vivo*)>

Method: SkinBarrier Lactic Acid™-P (50 and 100 mg/kg) was orally administrated to hairless mice for 28 days, an SDS solution* was repeatedly applied (5 minutes) on the right side of their back for three days, and then the transepidermal water loss (TEWL) was measured (Fig. 8).

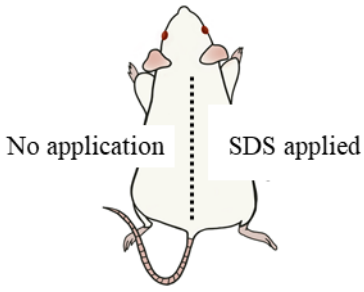
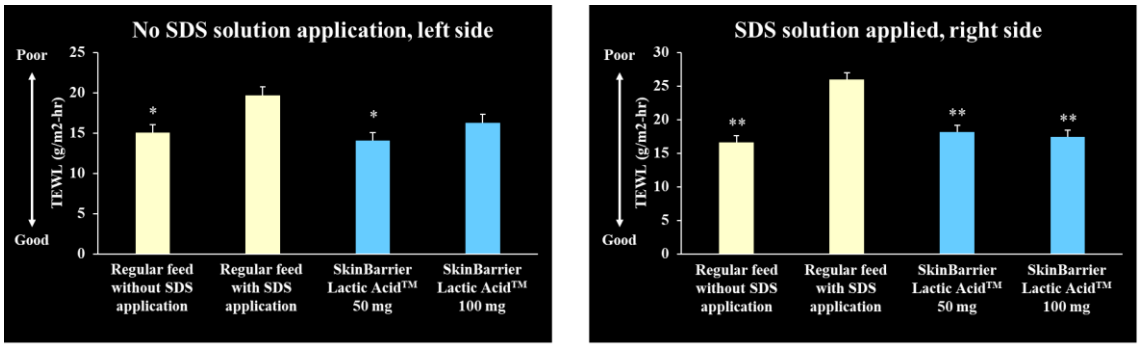


Fig. 8 : Graph of SDS applied

*Sodium dodecyl sulfate (SDS) solution, a type of surfactant:
Used to intentionally weaken skin's barrier function to create a dry skin model.

Results: SkinBarrier Lactic Acid™-P (50 mg/kg) suppressed the increase of TEWL regardless of the application of SDS. Suppression of TEWL was observed in the SDS application group (Fig. 9).



Each column indicates the average value and standard error (n=6). *: p<0.05, **: p<0.01.

Fig. 9 Effect of administration of SkinBarrier Lactic Acid™-P on TEWL of hairless mice

<Effect of cornified envelope to moisturization-related proteins (mRNA)>

We studied the changes of moisturization-related proteins in cornified envelope, which has a membrane-like structure of proteins wrapping corneocytes that make up the outermost layer of the skin. Cornified envelope is known to be responsible for suppressing moisture evaporation and perform the barrier function (function to prevent the infiltration of foreign matters from the outside world) in order to maintain the skin in a healthy condition. It is thought that the function of cornified envelope has declined on sensitive skin or skin with atopic dermatitis. As the moisturizing proteins (Fig. 10) which make up cornified envelope, involucrin and loricrin are famous to known. Transglutaminase 1 is known to be involved with a combined formation of involucrin, also loricrin and others.

Method: Skin of the hairless mice, which was measured in the TEWL test described in the previous section was used, and the expression of mRNA of moisturizing proteins was studied.

Results: Increase in the expression of the moisturizing proteins involucrin, filaggrin, and transglutaminase 1 which make up cornified envelope was observed (Fig. 11). Therefore, it was confirmed to promote the mRNA gene expression of involucrin and other proteins which have important roles for the skin's barrier function, and thus enhance the skin's barrier function.

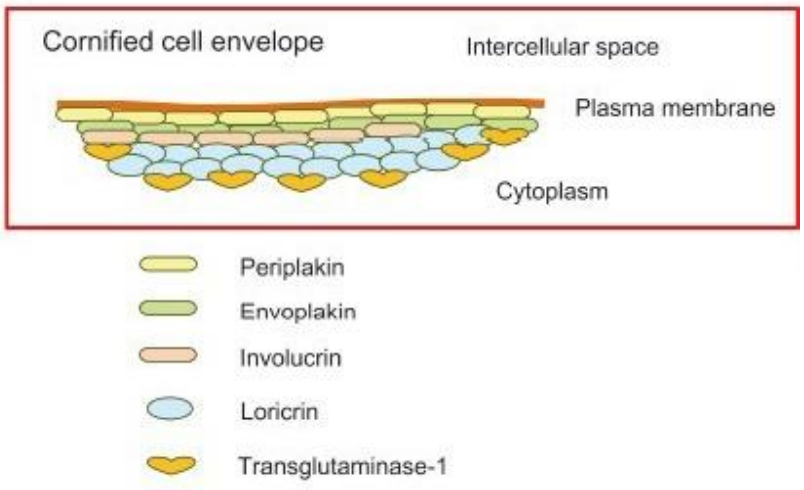


Fig. 10 Schematic Diagram of Moisturizing Proteins of Cornified Envelope

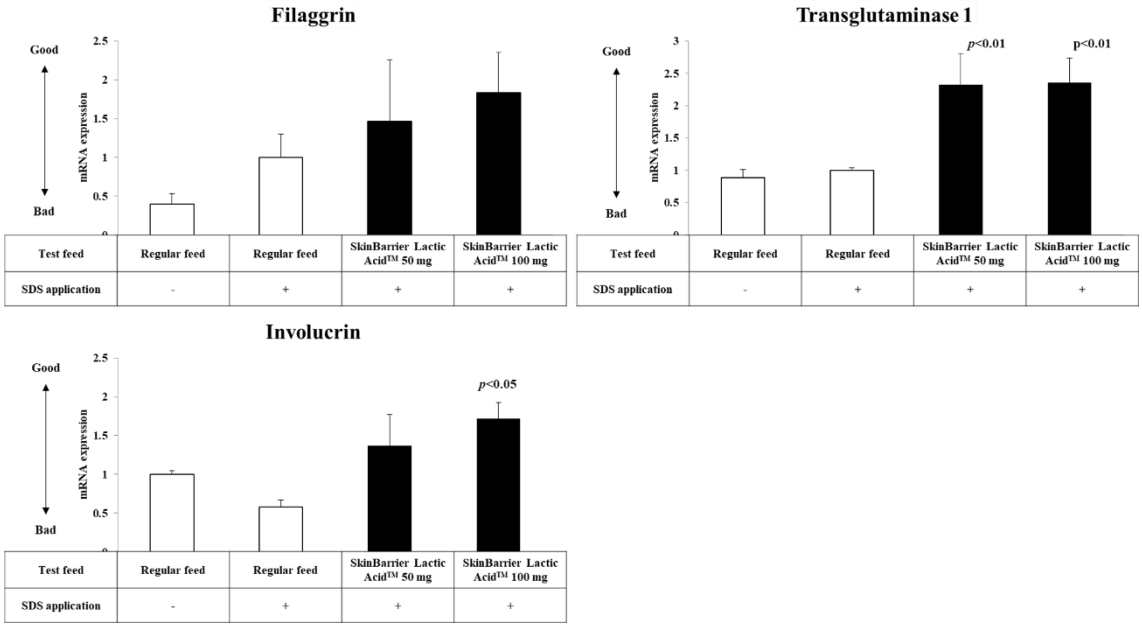


Fig. 11 Effect of moisturizing protein mRNA on hairless mice administrated by SkinBarrier Lactic Acid™-P

<Effect of moisturization-related proteins of CE (immunostaining)>

Method: Skin of the hairless mice, which was measured in the TEWL test described in the previous section was used, and the expression of moisturizing proteins on the skin was checked via immunostaining.

Results: In hematoxylin and eosin (HE) staining, the stratum corneum became thicker in the group which taken lactic acid bacteria as compared to regular feed/SDS application group. Also, moisturizing proteins involucrin, loricrin, and envoplakin which make up cornified envelope were visually confirmed to be stained darkly (Fig. 12).

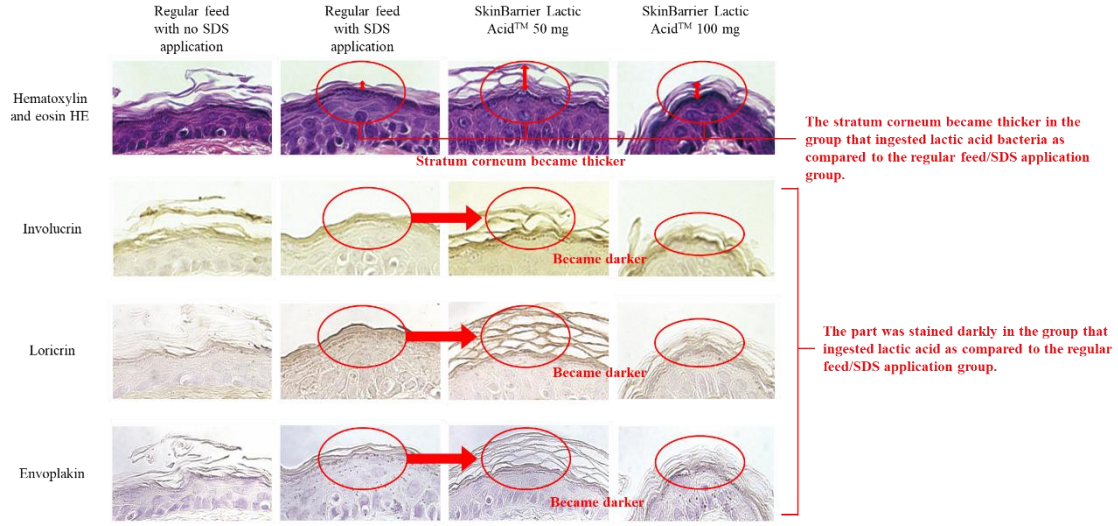


Fig. 12 Effect of moisturizing proteins of CE on hairless mice administrated by the SkinBarrier Lactic Acid™ (Immunostaining)

<Effect of cornified envelope to involucrin protein expression>

(Method)

Skin of the hairless mice, which was measured in the TEWL test described in the previous section was used and the expression of proteins was checked for involucrin, for which significant differences were observed in mRNA expression and immunostaining, so here. We used western blotting method to cornified the results again.

(Results)

Ingestion of SkinBarrier Lactic Acid™-P 50 mg was confirmed to increase the expression of involucrin protein which makes up cornified envelope (Fig. 13).

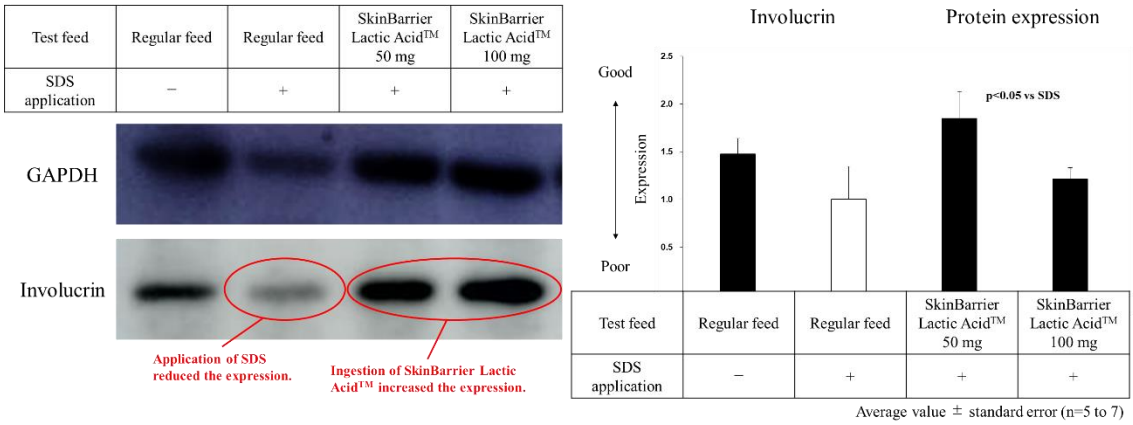


Fig. 13 Effect of SkinBarrier Lactic Acid™-P on the expression of Involucrin protein on hairless mice

Thus, we discovered that SkinBarrier Lactic Acid™-P promotes the expression of mRNA of moisturization-related proteins of cornified envelope, involucrin, filaggrin, and transglutaminase 1 that are important for the skin's barrier function. According to these the results. SkinBarrier Lactic Acid™ has been confirmed to enhance the moisturizing and barrier functions of the stratum corneum and suppress the TEWL.

<Clinical testing: Effect of SkinBarrier Lactic Acid™-P to the skin's barrier function on human>

Method: ・Test period: 6 weeks

・Test subjects: 27 people (Male: 20 people, Female: 7 people)

・ Test design: Double blind comparative study
(Placebo group: 13 people,
Lactic acid bacteria group: 14 people)

・Dose: Placebo group: Starch decomposition product 50 mg, Lactic acid bacteria group: 50 mg

・ Evaluation method: Skin flora (Staphylococcus epidermidis, P. acnes), salivary IgA, collagen score and thickness of the dermis using dermalab, transepidermal water loss (TEWL), moisture level, suppleness level, questionnaire (*questionnaire about bowel movement and skin type)

Results: SkinBarrier Lactic Acid™-P (50 mg/day) showed an improvement tendency in water loss (TEWL) on cheeks and arms as well as collagen score among evaluation items related to skin's barrier. Also, salivary IgA, which is a representative antibody for mucosal immunity, showed a tendency to increase after taken of SkinBarrier Lactic Acid™-P (Fig. 14).

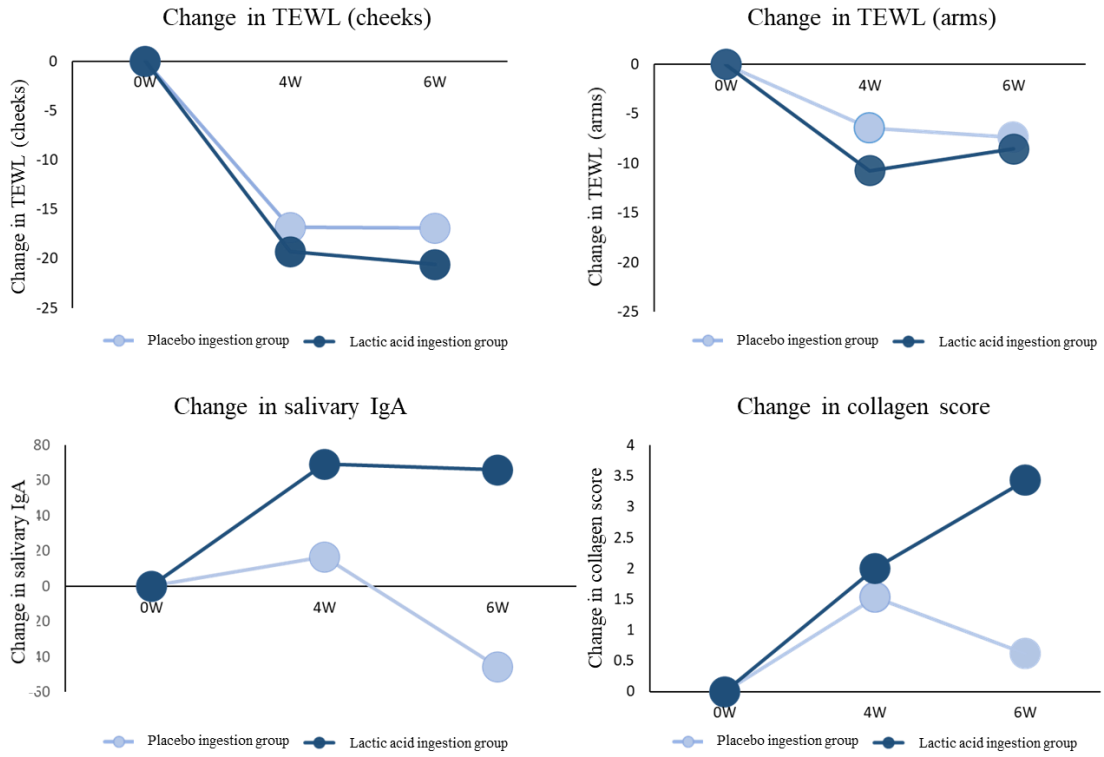


Fig. 14 Influence of the ingestion of SkinBarrier Lactic Acid™-P to Human Skin's Barrier Function

SkinBarrier Lactic Acid™ is a new type of lactic acid which improves skin's moisturizing and barrier functions and works on the improvement of mucosal immunity.

6. Stability

(1) Heat stability

Kudzu-derived lactic acid bacteria were suspended in distilled water and then heated at 100 ℃ or 120 ℃ for 20 minutes using an autoclave. Then, an IL-12 production inducing capacity test was conducted using mouse spleen cells for evaluation. OK-432 (product name: Picibanil) was used as the positive control and its specific activity was indicated.

Control	Added of lactic acid bacterial with no heating treatment
Heating at 100 ℃	Added of lactic acid bacteria and heated at 100 ℃ for 20 minutes
Heating at 120 ℃	Added of lactic acid bacteria and heated at 120 ℃ for 20 minutes
OK-432	Added OK-432
No addition	No operation

As a result, it was confirmed that the activity equivalent to that of the positive control OK-432 (no significant difference statistically) was maintained even when heated at 120 ℃ for 20 minutes. Therefore, immune activity is expected to be maintained even when kudzu-derived lactic acid bacteria are suspended in water and heated at 120 ℃ for 20 minutes.

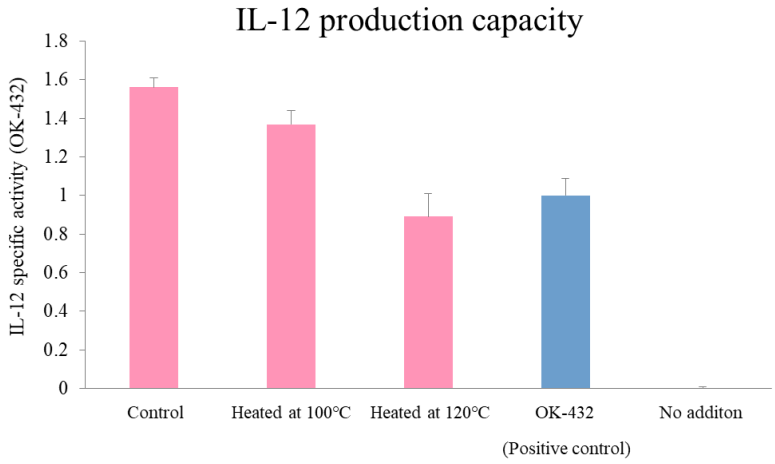


Fig. 15 Thermal Stability of Kudzu-derived Lactic Acid Bacteria (heated at 120 ℃ for 20 minutes)

(2) pH stability

Kudzu-derived lactic acid bacteria were shaken at pH 1.5 and 37 ℃ for two hours and the IL-12 production capacity was compared against the control (distilled water) treated in the same conditions.

As a result, there was no significant difference between the two groups, confirming that processing kudzu-derived lactic acid bacteria at pH 1.5 for two hours does not affect the IL-12 production amount. According to the result, kudzu-derived lactic acid bacteria are expected to be resistant to the effects of gastric acid.

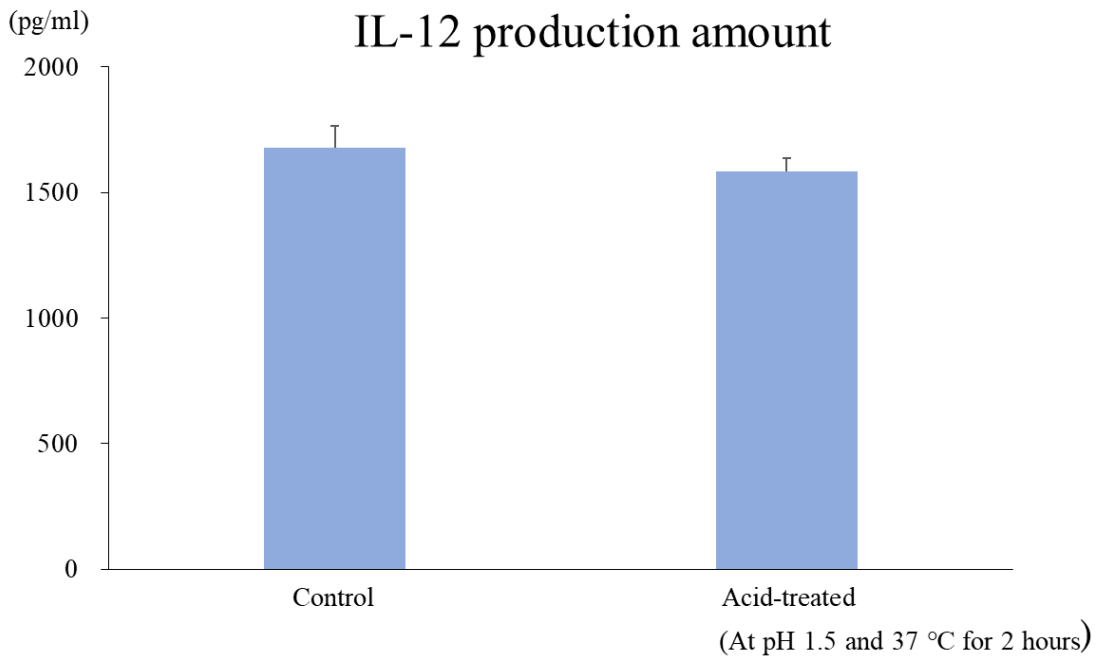


Fig. 16 pH Stability of Kudzu-derived Lactic Acid Bacteria

7. Nutrition profiles

Analyzed item (/100g)	SkinBarrier Lactic Acid Bacteria™ ImmnoRise Lactic Acid Bacteria™	Method
Water (g)	0.2	Drying method
Protein (g)	19.9	Kjeldahl method,
Fat (g)	0.5	Acid soxhlet extraction method
Ash (g)	2.4	Direct incineration method
Carbohydrate (g)	77.0	Refer note 1
Energy (kcal)	392	Modified Atwater method
Sodium (mg)	440	Atomic absorption spectrophotometory
Sodium chloride equivalent (g)	1.1	Refer note 2

Note 1: Calculation: 100-(water + protein + fat + ash)
Note 2: In terms of sodium
Test trustee: Mbic Life Co., Ltd.
Dte of analysis: October 16, 2018

8. Safety data

(1) Residual agricultural chemicals

Kudzu-derived Lactic Acid Bacteria (SkinBarrier Lactic Acid ™, ImmnoRise Lactic Acid ™) was screened and analyzed for residual Pesticides (260 items) stipulated under the Food Sanitation Act and Pesticides Control Act, presence of the test items was lower than the allowed limits.

Test trustee: Shokukanken Inc.

Date: October 29, 2018

（2） Acute toxicity（LD_{50}）

Acute Toxicity test was conducted according to the Guidelines of Single-Dose Toxicity Tests for Pharmaceutical Products. Kudzu-derived Lactic Acid Bacteria (SkinBarrier Lactic Acid ™, ImmnoRise Lactic Acid ™) 2000 mg/kg was orally given to fasted ICR mice (6 weeks old). After 14 days, no abnormalities and fatal event were observed at 2000 mg/kg. No abnormalities were observed under macroscopic examination upon autopsy. Thus, LD_{50} of Kudzu-derived Lactic Acid Bacteria (SkinBarrier Lactic Acid ™, ImmnoRise Lactic Acid ™) is deduced to be > 2000 mg/kg.

（3） Mutagenicity（Ames test）

Ames test was conducted to evaluate the mutagenicity of Kudzu-derived Lactic Acid Bacteria (SkinBarrier Lactic Acid ™, ImmnoRise Lactic Acid ™) using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coil* WP2 at concentration 19.5-5,000 μg/plate. No mutagenicity was observed.

9. Recommended dosage

We recommend 50 mg/day of SkinBarrier Lactic Acid ™-P, ImmnoRise Lactic Acid ™-P (equivalent to dead bacteria lactic acid bacteria are 100 billion) based on the result of human clinical trial.

10. Application

	Applications	Indication	Examples
Food	Nutritional supplement, Beauty food	Protect skin, reduce risk of virus infection	Beverages, Hard and soft capsules, tablets, Candies, Chewing gums, Gummies, Cookies, Chocolates, Wafers, Jellies etc.

11. Packing

SkinBarrier Lactic Acid ™-P, ImmnoRise Lactic Acid ™-P

1 kg Interior packing: Aluminium bag

Exterior packing: Cardboard box

12. Storage

Store in a dry, ventilated location. Keep away from high temperature and sun light.

13. Expression

<Food>

SkinBarrier Lactic Acid ™-P, ImmnoRise Lactic Acid ™-P

Maltodextrin, Sterilized lactic acid bacteria powder

PRODUCT STANDARD

PRODUCT NAME : **SkinBarrier Lactic Acid Bacteria-P**

(FOOD)

This product is a dried powder of heat-killed strain of lactic acid bacteria（*Leuconostoc me senteroides*）isolated from Kuzu (*Pueraria lobata*). It contains at least 2×10^{12} cfu/g of la ctic acid bacteria.

Appearance	Yellowish white to pale yellow powder with slightly characteristic odor.	
Viable cell count	Min. 2×10^{12} cfu/g	(Calculated by DAPI staining method)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 ℃、2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 3×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 3×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content (Values are just a guide)
Maltodextrin	75 %
Lactic acid bacteria (heat-killed)	25 %
Total	100 %

Expiry date	2 years from date of manufacturing.
Storage	Store in a dry, ventilated location. Keep away from high temperature and sun light.

PRODUCT STANDARD

PRODUCT NAME : **ImmnoRise Lactic Acid Bacteria™-P**

(FOOD)

This product is a dried powder of heat-killed strain of lactic acid bacteria（*Leuconostoc me senteroides*） isolated from Kuzu (*Pueraria lobata*). It contains at least 2×10^{12} cfu/g of la ctic acid bacteria.

Appearance	Yellowish white to pale yellow powder with slightly characteristic odor.	
Viable cell count	Min. 2×10^{12} cfu/g	(Calculated by DAPI staining method)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 ℃、2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 3×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 3×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content (Values are just a guide)
Maltodextrin	75 %
Lactic acid bacteria (heat-killed)	25 %
Total	100 %

Expiry date	2 years from date of manufacturing.
Storage	Store in a dry, ventilated location. Keep away from high temperature and sun light.

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

From product planning to OEM - For any additional information or assistance, please contact：

Headquarters:
ORYZA OIL & FAT CHEMICAL CO., LTD.
No.1, Numata Kitagata-cho, Ichinomiya-city, Aichi-pref.,
493-8001 JAPAN
TEL : +81 (0) 586 86 5141
FAX : +81 (0) 586 86 6191
URL/http : //www.oryza.co.jp/
E-mail : info@oryza.co.jp

Tokyo sales office:
5F of Diamant Building, Kanndasuda-cho 1-5
Chiyoda-ku, Tokyo, 101-0041 Japan
TEL (03)5209-9150 FAX (03)5209-9151
E-mail: tokyo@oryza.co.jp

“The catalog was created based on academic data. For expressions of consumer products containing this product, follow the Health Promotion Law, Pharmaceutical Low, and other related laws and regulations.”

Established Date : July 30, 2022

ORYZA OIL & FAT CHEMICAL CO., LTD.