

USE OF LACTIC ACID BACTERIUM FERMENTED PRODUCT FOR MANUFACTURE OF COMPOSITION FOR IMPROVING SKIN DISEASE

BACKGROUND

Technical Field

[0001] The present disclosure relates to a use of a lactic acid bacterium fermented product. More particularly, the present disclosure relates to an application of a lactic acid bacterium fermented product in the improvement of a skin disease.

Description of Related Art

[0002] Acne, also known as whelk, pimple or folliculitis, includes the manifestations of the formation of acne, papules, pustules and cysts, and thus the acne has become one of the most annoying skin diseases. Further, if the acne is without proper treatments, the sequelae such as skin depression, grooves, pigmentation, scars, etc., may occur even after recovery. Furthermore, although the acne is more common in teenagers, the occurrence thereof is not limited to age, and the acne may also develop on the skin of an adult.

[0003] The causes of the acne are mainly related to the excessive sebum secretion, the follicular hyperkeratinization, and the proliferation of *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) and the accompanying inflammatory response. *Cutibacterium acnes* is a facultative anaerobic Gram-positive bacterium commonly found in the pores of the skin and lives on the sebum fatty acids secreted by the pilosebaceous glands. When the sebaceous glands secrete too much oil, *Cutibacterium acnes* may breed in large numbers,

and at this time, *Cutibacterium acnes* will induce the occurrence of the inflammatory response of the skin, leading to the formation of the acne.

[0004] In clinical practice, the common and effective treatment method for the acne is to use the antibiotics such as clindamycin and erythromycin to inhibit the proliferation of *Cutibacterium acnes*, and the application of the retinoic acid (such as isotretinoin) can also reduce the sebum secretion and promote the proliferation of the collagen so as to renew the skin, so that the effect of reducing the scar can be achieved. However, the use of the antibiotics to treat the acne is prone to the potential risk of drug resistance or side effects, and the use of the retinoic acid is also likely to increase the risk of fetal malformations in the pregnant women. Further, the long-term use of the retinoic acid is more likely to cause the lips, the eyes or the skin to be dry and chapped.

[0005] Therefore, how to develop a method that can effectively reduce the stimulation of external factors and microorganisms on the skin, and thus prevent or treat the skin diseases, has become an important topic in the related arts.

SUMMARY

[0006] According to one aspect of the present disclosure, a use of a lactic acid bacterium fermented product is provided for a manufacture of a composition for improving a skin disease, wherein the lactic acid bacterium fermented product is obtained by fermenting a substrate with a lactic acid bacterium. The lactic acid bacterium is a *Bifidobacterium lactis*, a *Lactobacillus plantarum*, a *Lactobacillus paracasei*, a *Bifidobacterium longum* or a combination thereof, and a weight ratio

of the lactic acid bacterium fermented product in the composition for improving the skin disease ranges from of 1% to 5%.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The present disclosure can be more fully understood by reading the following detailed description of the embodiment, with reference made to the accompanying drawings as follows:

[0008] Fig. 1 is a flow chart of a method for preparing a lactic acid bacterium fermented product of the present disclosure.

[0009] Fig. 2 shows the analysis results of the effect of the lactic acid bacterium fermented product of the present disclosure on the expressed amount of IL-8 in the HaCaT cells co-cultured with the *Cutibacterium acnes*.

[0010] Fig. 3 shows the analysis results of the effect of the lactic acid bacterium fermented product of the present disclosure on the expressed amount of MMP-1 in the Hs68 cells treated with UV light.

[0011] Fig. 4 shows the analysis results of the secreted amount of the hyaluronic acid of the HaCaT cells after being treated with the substrate and the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure.

[0012] Fig. 5 shows the analysis results of the secreted amount of the hyaluronic acid of the HaCaT cells after being treated with the substrate and the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure.

DETAILED DESCRIPTION

[0013] The present disclosure provides a novel use of the lactic acid bacterium fermented product, especially the use of the lactic acid bacterium fermented product for a manufacture of a composition for improving a skin disease, wherein a weight ratio of the lactic acid bacterium fermented product in the composition for improving the skin disease ranges from 1% to 5%, and the composition for improving the skin disease is used to inhibit the inflammatory response of the skin induced by a *Cutibacterium acnes*. Accordingly, the expressed amount of the collagenase in the skin cells can be reduced, and the secretion of the hyaluronic acid in the skin cells can be enhanced. Therefore, the lactic acid bacterium fermented product of the present disclosure can be used to manufacture a composition for improving the skin disease so as to effectively resist the stimulation of external factors and the microorganisms on the skin, thus the skin disease can be prevented or treated, and it has the application potential in related markets.

[0014] Reference is made to Fig. 1, which is a flow chart of a method 100 for preparing a lactic acid bacterium fermented product of the present disclosure. The method 100 for preparing the lactic acid bacterium fermented product includes Step 110, Step 120, Step 130 and Step 140. In detail, the lactic acid bacterium fermented product of the present disclosure is prepared by the method 100 for preparing the lactic acid bacterium fermented product, and the preparation details of the lactic acid bacterium fermented product of the present disclosure are illustrated based on Fig. 1.

[0015] In Step 110, a lactic acid bacterium and a substrate are provided. In detail, the lactic acid bacterium of the present disclosure is a *Bifidobacterium lactis*, a *Lactobacillus plantarum*, a *Lactobacillus paracasei*, a *Bifidobacterium longum* or a combination thereof. Further, the *Bifidobacterium lactis*, the *Lactobacillus plantarum*, the *Lactobacillus paracasei* and the *Bifidobacterium longum* are respectively collected and separated by the inventors, wherein the *Bifidobacterium lactis* is *Bifidobacterium lactis* GKK2, the *Bifidobacterium lactis* GKK2 of present disclosure has been deposited at the China General Microbiological Culture Collection Center (CGMCC) in China (address of depositary institution: #100101, No.1 West Beichen Road Chaoyang District Beijing, China) on January 12, 2018, and the deposit number thereof is CGMCC 15205; the *Lactobacillus plantarum* is *Lactobacillus plantarum* GKM3, the *Lactobacillus plantarum* GKM3 of present disclosure has been deposited at CGMCC in China on August 25, 2017, and the deposit number thereof is CGMCC 14565; the *Lactobacillus paracasei* is *Lactobacillus paracasei* GKS6, the *Lactobacillus paracasei* GKS6 of present disclosure has been deposited at CGMCC in China on August 25, 2017, and the deposit number thereof is CGMCC 14566; and the *Bifidobacterium longum* is *Bifidobacterium longum* GKL7, the *Bifidobacterium longum* GKL7 of present disclosure has been deposited at the NITE (National Institute of Technology and Evaluation) Patent Microorganisms Depositary in Japan (address of depositary institution: #122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan) on November 22, 2024, and the deposit number thereof is NITE BP-04220. Further, the substrate can include a carbohydrate, a yeast extract, a peptone, an inorganic salt, an amino

acid and other essential nutrients for the growth of the lactic acid bacterium, but the present disclosure is not limited thereto.

[0016] In Step 120, the lactic acid bacterium is inoculated in the substrate so as to obtain a mixture, wherein an inoculating amount of the lactic acid bacterium in the substrate ranges from a weight ratio of 0.01% to 10%.

[0017] In Step 130, the mixture is cultured and fermented at 30°C to 37°C for 16 hours to 48 hours so as to obtain a fermentation raw liquid.

[0018] In detail, after the lactic acid bacterium is inoculated in the substrate with the inoculating amount of the weight ratio of 0.01% to 10%, the mixture is cultured and fermented at 30°C to 37°C under an aerobic environment or an anaerobic environment for 16 hours to 48 hours so as to obtain the fermentation raw liquid. Within the aforementioned temperature range, the lactic acid bacterium has the best fermentation efficiency. If the fermentation time is too short, the lactic acid bacterium may not be able to fully utilize the substrate and then convert it into effective ingredients. On the contrary, if the fermentation time is too long, other bacteria may grow and then affect the fermentation quality.

[0019] In Step 140, a solid content of the fermentation raw liquid is removed so as to obtain the lactic acid bacterium fermented product. The solid content can be removed by the centrifugation method, the filtration method or a combination thereof, and the remained liquid component is the lactic acid bacterium fermented product of the present disclosure. In detail, after the processing of Step 110 to Step 130, the nutrients of the substrate are fully utilized by the lactic acid bacterium and then converted into the effective ingredients, and all of the effective ingredients have remained in the fermentation raw liquid. Thus, after removing

the solid content of the fermentation raw liquid, the remained liquid component is the lactic acid bacterium fermented product of the present disclosure.

[0020] Further, the lactic acid bacterium fermented product of the present disclosure can further include the fermented products of other lactic acid bacteria, such as *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Streptococcus salivarius*, *Streptococcus thermophilus*, *Lactobacillus pentosus*, *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus brevis*, *Lactobacillus johnsonii*, *Lactobacillus gasseri*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactococcus lactis*, *Bifidobacterium breve*, *Bifidobacterium animalis* subsp. *animalis*, and *Bifidobacterium bifidum* (*Lactobacillus rhamnosus*), but the present disclosure is not limited thereto.

[0021] Further, the lactic acid bacterium fermented product of the present disclosure can be prepared into the composition for improving the skin disease according to the conventional pharmaceutical methods, and except for the lactic acid bacterium fermented product, the composition for improving the skin disease can further include pharmaceutically acceptable carriers, excipients, diluents, adjuvants, solvents, dispersants, coatings, antibacterial agents or antifungal agents so as to carry the lactic acid bacterium fermented product, but the present disclosure is not limited thereto.

[0022] [Example]

[0023] In order to illustrate the effects of the lactic acid bacterium fermented product of the present disclosure used to reduce the impact of the external factors on the skin condition and inhibit the inflammatory response of the skin induced by the *Cutibacterium acnes*, the following specific embodiments are provided so

as to facilitate utilizing and practicing the present disclosure completely by the people skilled in the art without over-interpreting and over-experimenting. However, the readers should understand that the present disclosure should not be limited to these practical details thereof, that is, in some embodiments, these practical details are used to describe how to implement the materials and methods of the present disclosure and are not necessary.

[0024] 1. Preparing the lactic acid bacterium fermented product of the present disclosure

[0025] When preparing the lactic acid bacterium fermented product of the present disclosure, the *Bifidobacterium lactis* GKK2, the *Lactobacillus plantarum* GKM3, the *Lactobacillus paracasei* GKS6 and the *Bifidobacterium longum* GKL7 are respectively inoculated in the substrates with a weight ratio of 0.01% to 10% and then cultured and fermented at 30°C to 37°C for 16 hours to 48 hours so as to obtain a fermentation raw liquid. The substrate includes the carbohydrate with a weight ratio of 1% to 20%, the yeast extract with a weight ratio of 0.5% to 10%, the peptone with a weight ratio of 0.5% to 10%, the inorganic salt with a weight ratio of 0.01% to 5% and the amino acid with a weight ratio of 0.01% to 0.1%. Next, the solid content of the fermentation raw liquid is removed by the centrifugation method, the filtration method, etc., and the post-processing can be performed by the heat treatment, the concentration treatment, the freezing treatment, the drying treatment, etc. so as to obtain the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure, the fermented product

of *Lactobacillus paracasei* GKS6 of the present disclosure and the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure.

[0026] 2. Analysis of the effects of the lactic acid bacterium fermented product of the present disclosure used to inhibit the inflammatory response induced by the *Cutibacterium acnes*

[0027] In the present test, the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure, the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure and the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure are respectively applied to the human keratinocyte cell line HaCaT ("HaCaT cells" hereafter), and then the HaCaT cells are stimulated with the *Cutibacterium acnes* and then analyzed for the expressed amount of interleukin-8 ("IL-8" hereafter) thereof so as to illustrate the effects of the lactic acid bacterium fermented product of the present disclosure used to inhibit the inflammatory response induced by the *Cutibacterium acnes*.

[0028] In the steps of the present test, 1×10^5 of the HaCaT cells are seeded in each well of the 24-well cell culture plate including the DMEM (Dulbecco's modified eagle medium) and then cultured at 37°C for 24 hours, wherein the DMEM includes 3.7 g/L of sodium bicarbonate, 1% of 100 U/mL of penicillin/streptomycin, 1% of 100 mM sodium pyruvate and 10% of fetal bovine serum. Next, the DMEM is removed and the HaCaT cells are washed by the PBS, and then 500 μ L of the DMEM (2% fetal bovine serum) including the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure,

the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure or the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure (with the weight ratio of 1% or 5%) is added to each well and reacted for 24 hours. Then, after removing the DMEM in the 24-well cell culture plate, the serum-free DMEM including 10^4 CFU/mL to 10^8 CFU/mL (preferably 10^6 CFU/mL) of the *Cutibacterium acnes* is added to each well, and then the HaCaT cells and the *Cutibacterium acnes* are co-cultured for 18 hours so as to induce the inflammatory response in the HaCaT cells. After co-culturing for 18 hours, the supernatant is obtained by centrifuging at 3000 rpm for 5 minutes, and the supernatant is analyzed by an ELISA kit (Invitrogen IL-8 Human Uncoated ELISA Kit, 88-8086-88) to detect the absorbance at a wavelength of 450 nm so as to measure the concentration of IL-8 in the supernatant.

[0029] Further, the *Cutibacterium acnes* used in the present test can be any of the *Cutibacterium acnes* on the market, and the *Cutibacterium acnes* is activated under an anaerobic environment by the tryptone soy broth (TSB). After culturing for 24 hours, the tryptone soy broth including the *Cutibacterium acnes* is centrifuged, and the *Cutibacterium acnes* are suspended in the serum-free DMEM for later use.

[0030] Further, the present test further includes Comparative group 1 and Comparative group 2 so as to further illustrate the effects of the lactic acid bacterium fermented product of the present disclosure used to inhibit the inflammatory response induced by the *Cutibacterium acnes*, wherein Comparative group 1 includes the HaCaT cells without any treatment, and Comparative group 2 includes the HaCaT cells that are co-cultured with the

Cutibacterium acnes but not treated with the lactic acid bacterium fermented product of the present disclosure.

[0031] Reference is made to Fig. 2, which shows the analysis results of the effect of the lactic acid bacterium fermented product of the present disclosure on the expressed amount of IL-8 in the HaCaT cells co-cultured with the *Cutibacterium acnes*. In Fig. 2, “C1” represents Comparative group 1, “C2” represents Comparative group 2, “GKK2” represents the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, “GKM3” represents the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure, “GKS6” represents the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure, and “GKL7” represents the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure.

[0032] As shown in Fig. 2, the concentration of IL-8 of Comparative group 2 is significantly higher than the concentration of IL-8 of Comparative group 1, and it shows that the *Cutibacterium acnes* does induce an inflammatory response in the HaCaT cells. Further, in the groups that the HaCaT cells are treated with the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure, the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure or the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure at a concentration of a weight ratio of 1% or 5% and then cultured with the *Cutibacterium acnes*, the concentration of IL-8 of the HaCaT cells is significantly lower than that of Comparative group 2, and it is shown that the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the

fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure, the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure and the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure can effectively inhibit the inflammatory response induced by the *Cutibacterium acnes* and have the effects to improve the skin disease. Therefore, the lactic acid bacterium fermented product of the present disclosure can be applied for the manufacture of the composition for improving a skin disease caused by the inflammatory response induced by the *Cutibacterium acnes*, and there is potential for application in relevant markets.

[0033] 3. Analysis of the effects of the lactic acid bacterium fermented product of the present disclosure used to reduce the stimulation on the skin caused by the external factors

[0034] In the present test, the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure, the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure and the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure are respectively applied to the human fibroblast cell line Hs68 ("Hs68 cells" hereafter) treated with the UV light, and then the expressed amount of the matrix metalloproteinase-1 ("MMP-1" hereafter) of the Hs68 cells is analyzed. MMP-1 is a kind of collagenase, and it can be used to illustrate the effects of the lactic acid bacterium fermented product of the present disclosure used to reduce the stimulation on the skin caused by the external factors.

[0035] In the steps of the present test, 5×10^4 of the Hs68 cells are seeded in each well of the 24-well cell culture plate including the DMEM and then cultured at 37°C for 24 hours, wherein the DMEM includes 3.7 g/L of sodium bicarbonate, 1% of 100 U/mL of penicillin/streptomycin, 1% of 100 mM sodium pyruvate and 10% of fetal bovine serum. Next, the DMEM is removed and the Hs68 cells are washed by the PBS, and then 500 μ L of the DMEM (2% fetal bovine serum) including the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure, the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure or the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure (with the weight ratio of 1% or 5%) is added to each well and reacted for 24 hours. Then, after removing the DMEM in the 24-well cell culture plate, 50 μ L of the PBS is added to each well, and then the 24-well cell culture plate is placed in the UV irradiation box (CL-3000M CROSSLINKER (AnalytikJena (UVP), 302nm, 100~115 VAC~50/60Hz H/C)) and exposed to the UV light, wherein the radiation dose of the UV light is 50 mJ/cm² so as to simulate the expression of the collagenase of the Hs68 cells induced by the external factors. After being radiated by the UV light, the supernatant is removed, and 500 μ L of serum-free DMEM is added to each well. Then, the Hs68 cells are cultured for 24 hours, and then the supernatant is obtained by centrifuging at 3000 rpm for 5 minutes. Finally, the supernatant is analyzed by an ELISA kit (R&D Human Total MMP-1 DuoSet ELISA, DY901B) to detect the absorbance at a wavelength range of 420 nm to 620 nm so as to measure the concentration of MMP-1 in the supernatant.

[0036] Further, the present test further includes Comparative group 3 and Comparative group 4 so as to further illustrate the effects of the lactic acid

bacterium fermented product of the present disclosure used to reduce the stimulation on the skin caused by the external factors, wherein Comparative group 3 includes the Hs68 cells without any treatment, and Comparative group 4 includes the Hs68 cells irradiated with UV light but not treated with the lactic acid bacterium fermented product of the present disclosure.

[0037] Reference is made to Fig. 3, which shows the analysis results of the effect of the lactic acid bacterium fermented product of the present disclosure on the expressed amount of MMP-1 in the Hs68 cells treated with UV light. In Fig. 3, “C3” represents Comparative group 3, “C4” represents Comparative group 4, “GKK2” represents the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, “GKM3” represents the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure, “GKS6” represents the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure, and “GKL7” represents the fermented product of *Bifidobacterium longum* GKL7 of the present disclosure.

[0038] As shown in Fig. 3, the concentration of MMP-1 of Comparative group 4 is significantly higher than the concentration of MMP-1 of Comparative group 3, and it shows that the UV light will accelerate the decomposition of the collagen in the Hs68 cells, leading to the loss of the collagen and affecting the skin repair. Further, in the groups treated with the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure and the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure at a concentration of a weight ratio of 1% or 5%, the concentrations of MMP-1 in the Hs68 cells are respectively lower

than that of Comparative group 4, and it shows that the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure and the fermented product of *Lactobacillus paracasei* GKS6 of the present disclosure can effectively reduce the loss of the collagen caused by the UV light or other external factors, so that the content of the collagen in the skin can be maintained, the skin repair can be enhanced, and the formation of scars on the skin after acne can be reduced. Therefore, the lactic acid bacterium fermented product of the present disclosure can be applied for the manufacture of the composition for improving a skin disease, and there is potential for application in relevant markets.

[0039] 4. Analysis of the effects of the lactic acid bacterium fermented product of the present disclosure used to promote the skin cells to secrete the hyaluronic acid

[0040] In the present test, the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure and the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure are respectively applied to the HaCaT cells to analyze the secreted amount of the hyaluronic acid in the HaCaT cells so as to illustrate the effects of the lactic acid bacterium fermented product of the present disclosure used to promote the skin cells to secrete the hyaluronic acid.

[0041] In the steps of the present test, 1×10^5 of the HaCaT cells are seeded in each well of the 24-well cell culture plate including the DMEM and then cultured at 37°C for 24 hours, wherein the DMEM includes 3.7 g/L of sodium bicarbonate, 1% of 100 U/mL of penicillin/streptomycin, 1% of 100 mM sodium pyruvate and 10% of fetal bovine serum. Next, the DMEM is removed and the HaCaT cells

are washed by the PBS, and then 500 μ L of serum-free DMEM is added to each well and reacted for 24 hours. After culturing for 24 hours, the DMEM is removed, and 500 μ L of serum-free DMEM including the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure or the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure at concentrations of a weight ratio of 1%, 2% or 5% is added to each well and then reacted for 24 hours. Then, the DMEM in the 24-well cell culture plate is removed, and then 500 μ L of serum-free DMEM is added to each well and the HaCaT cells are cultured for 24 hours, and then the supernatant is obtained by centrifuging at 3000 rpm for 5 minutes. Then, the supernatant is analyzed by an ELISA kit (R&D Hyaluronan DuoSet ELISA, DY3614-05) to detect the absorbance at a wavelength of 450 nm so as to measure the concentration of hyaluronic acid in the supernatant.

[0042] Further, the present test further includes Comparative group 5 and Comparative group 6 so as to further illustrate the effects of the lactic acid bacterium fermented product of the present disclosure used to promote the skin cells to secrete the hyaluronic acid, wherein Comparative group 5 and Comparative group 6 respectively include the HaCaT cells without any treatment, and Comparative group 5 and Comparative group 6 are respectively used to compare with the group treated with the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure and the group treated with the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure. Further, the present test further includes a group that the HaCaT cells are treated with the substrate that has not been fermented by the lactic acid bacterium of the present

disclosure, and the supernatant thereof is obtained to analyze the concentration of the hyaluronic acid.

[0043] Reference is made to Fig. 4, which shows the analysis results of the secreted amount of the hyaluronic acid of the HaCaT cells after being treated with the substrate and the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure. In Fig. 4, "C5" represents Comparative group 5, "B1" represents the substrate, and "GKM3" represents the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure.

[0044] As shown in Fig. 4, when the HaCaT cells are treated with the substrate that has not been fermented by the lactic acid bacterium of the present disclosure, the concentration of the hyaluronic acid secreted by the HaCaT cells does not have a significant difference compared with that of Comparative group 5. However, when the HaCaT cells are treated with the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure at the concentration of the weight ratio of 1%, 2% or 5%, the concentration of the hyaluronic acid secreted by the HaCaT cells is significantly higher than that of Comparative group 5. Further, as the concentration of the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure increases, the concentration of the hyaluronic acid increases, correspondingly. Therefore, the fermented product of *Lactobacillus plantarum* GKM3 of the present disclosure can facilitate the formation of the hyaluronic acid in the HaCaT cells, so that the skin moisture retention can be enhanced, the oil-water balance of oily skin can be improved, and the incidence of the acne can be reduced.

[0045] Reference is made to Fig. 5, which shows the analysis results of the secreted amount of the hyaluronic acid of the HaCaT cells after being treated with the substrate and the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure. In Fig. 5, “C6” represents Comparative group 6, “B2” represents the substrate, and “GKK2” represents the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure.

[0046] As shown in Fig. 5, when the HaCaT cells are treated with the substrate that has not been fermented by the lactic acid bacterium of the present disclosure, the concentration of the hyaluronic acid secreted by the HaCaT cells does not have a significant difference compared with that of Comparative group 6. However, when the HaCaT cells are treated with the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure at the concentration of the weight ratio of 1%, 2% or 5%, the concentration of the hyaluronic acid secreted by the HaCaT cells is significantly higher than that of Comparative group 6. Further, as the concentration of the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure increases, the concentration of the hyaluronic acid increases, correspondingly. Therefore, the fermented product of *Bifidobacterium lactis* GKK2 of the present disclosure can facilitate the formation of the hyaluronic acid in the HaCaT cells, so that the skin moisture retention can be enhanced, the oil-water balance of oily skin can be improved, and the incidence of the acne can be reduced.

[0047] Therefore, the lactic acid bacterium fermented product of the present disclosure can be used to improve the skin condition under specific concentrations, the skin moisture retention can be enhanced, and the oil-water

balance of oily skin can be maintained. Further, the lactic acid bacterium fermented product of the present disclosure can be used to reduce the stimulation on the skin caused by the external factors and inhibit the inflammatory response of the skin induced by the *Cutibacterium acnes*. Therefore, the lactic acid bacterium fermented product of the present disclosure can be applied for the manufacture of the composition for improving a skin disease, and there is potential for application in relevant markets.

[0048] Although the present disclosure has been described in considerable detail with reference to certain embodiments thereof, other embodiments are possible. Therefore, the spirit and scope of the appended claims should not be limited to the description of the embodiments contained herein.

[0049] It will be apparent to those skilled in the art that various modifications and variations can be made to the structure of the present disclosure without departing from the scope or spirit of the disclosure. In view of the foregoing, it is intended that the present disclosure cover modifications and variations of this disclosure provided they fall within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A use of a lactic acid bacterium fermented product for a manufacture of a composition for improving a skin disease, wherein the lactic acid bacterium fermented product is obtained by fermenting a substrate with a lactic acid bacterium;

wherein the lactic acid bacterium is a *Bifidobacterium lactis*, a *Lactobacillus plantarum*, a *Lactobacillus paracasei*, a *Bifidobacterium longum* or a combination thereof, and a weight ratio of the lactic acid bacterium fermented product in the composition for improving the skin disease ranges from 1% to 5%.

2. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 1, wherein the *Bifidobacterium lactis* is a *Bifidobacterium lactis* GKK2, and a deposit number thereof is CGMCC 15205.

3. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 1, wherein the *Lactobacillus plantarum* is a *Lactobacillus plantarum* GKM3, and a deposit number thereof is CGMCC 14565.

4. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 1, wherein the *Lactobacillus paracasei* is a *Lactobacillus paracasei* GKS6, and a deposit number thereof is CGMCC 14566.

5. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 1, wherein the *Bifidobacterium longum* is a *Bifidobacterium longum* GKL7, and a deposit number thereof is NITE BP-04220.

6. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 1, wherein the skin disease is an inflammatory response induced by a *Cutibacterium acnes*.

7. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 6, wherein the composition for improving the skin disease is for reducing an expressed amount of an interleukin-8 in a skin cell.

8. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 1, wherein the composition for improving the skin disease is for reducing an expressed amount of a collagenase in a skin cell.

9. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 1, wherein the composition for improving the skin disease is for promoting a skin cell to secrete a hyaluronic acid.

10. The use of the lactic acid bacterium fermented product for the manufacture of the composition for improving the skin disease of claim 1, wherein the lactic acid bacterium fermented product is prepared by following steps:

providing the lactic acid bacterium and the substrate, wherein the substrate comprises a carbohydrate, a yeast extract, a peptone, an inorganic salt and an amino acid;

inoculating the lactic acid bacterium in the substrate so as to obtain a mixture, wherein an inoculating amount of the lactic acid bacterium in the substrate ranges from a weight ratio of 0.01% to 10%;

culturing and fermenting the mixture at 30°C to 37°C for 16 hours to 48 hours so as to obtain a fermentation raw liquid; and

removing a solid content of the fermentation raw liquid so as to obtain the lactic acid bacterium fermented product.