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Physiological effect of a probiotic on skin

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Synopsis

Introduction and objective: Normal human skin can produce a range of antimicrobial chemicals that play an important part in eliminating potential cutaneous pathogens. *Lactobacillus plantarum* is a gram-positive bacteria that produces antimicrobial peptides, which when applied to the skin can act like an anti-inflammatory as well as enhance the antimicrobial properties of the skin. Clinical studies were conducted to determine the effect of lactobacillus extract on improvement of skin barrier and reduction of erythema from chemical irritant, skin microflora, and acne. Results show that lactobacillus extract was effective in reducing skin erythema, repairing skin barrier, and reducing skin microflora, thereby exhibiting an effective reduction in acne lesion size and erythema at 5%, but not at 1%.Conclusion: Based on the results of these studies, lactobacillus extract (5%) can be used to treat mild acne lesions.

INTRODUCTION

The concept of probiotics likely dates back to 1908, when Nobel Prize winner Eli Metchnikoff suggested that the long life of Bulgarian peasants resulted from their consumption of fermented milk products (1). The term "probiotic" was first used in 1965 by Lilly and Stillwell for describing the substances secreted by one organism, which stimulate the growth of another (2). Marteau *et al.* in 2002 (3) defined them as "microbial preparations or components of microbial cells that have a beneficial effect on health and well being" (2,3).

Probiotics from *Lactobacillus* cultures are well recognized as possessing certain health benefits, such as antimicrobial and immune-stimulating properties. A recent study (4) showed that prenatal administration of *Lactobacillus GG* reduced the risk of children developing eczema. *Lactobacillus* cultures have also been reported to possess antimicrobial activity against pathogenic bacteria such as *Staphylococcus aureus* (5).

Normal human skin is remarkably resistant to infection from a myriad of microorganisms that routinely colonize its surface. In addition to the role of skin as a mechanical barrier,

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it has long been recognized that skin and other epithelia can produce a range of antimicrobial peptides that play an important part in eliminating potential cutaneous pathogens (6,7). Antimicrobial peptides are induced in keratinocytes of the skin via the binding of bacterial lipopolysaccharides, beta glucans, some mannose-containing carbohydrates, peptidoglycans or bacterial DNA to "pattern recognition" receptors, or the toll-like receptors. Heat-killed bacteria or fungi alone can provoke the transcription of antimicrobial peptides. *Lactobacillus* and other types of lactic acid bacteria have been reported to produce specific antimicrobial peptides known as bacterocins (8) that possess broad-spectrum antimicrobial activity against gram-negative and gram-positive bacteria (9).

Oral administration of probiotics has been shown to be effective in improving skin barrier functions (10). Impaired skin barrier function is invariably caused by decreased amounts of ceramides that may be responsible for comedone formation, since barrier dysfunction is accompanied by hyperkeratosis of the follicular epithelium (11).

Since microbial agents, impaired barrier, and inflammation play an important role in acne, we considered observing the effect of a probiotic on acne. Acne vulgaris is a complex, chronic, and common skin disorder of pilosebaceous units (12). The major pathogenic factors involved are ductal hyperkeratinization, obstruction of sebaceous follicles resulting from abnormal keratinization of the infundibular epithelium, stimulation of sebaceous gland secretion by androgens, and microbial colonization of pilosebaceous units by *Propionibacterium acnes*. Both viable and non-viable *P. acnes* have been shown to induce an immunostimulatory effect that activates an inflammatory response (13). The inflammatory stage of acne vulgaris is usually of greatest concern to the patient. A number of morphologically different inflammatory lesions may form that can be painful and unsightly and can lead to scarring. Inflammatory acne and acne scarring can have significant psychological effects on the patient, including depression, anxiety, and poor self-image. The onset of non-inflammatory lesions is understood as the consequence of follicular keratinocytes failing to differentiate, thus producing hypergranulosis, resulting in the formation of microcomedones (12).

Acne is commonly treated with antibiotics, bactericidals, retinoids, and so on, however, most of such treatments come with side effects. In this study, we addressed alleviation of three aspects of acne, namely skin microflora, barrier strength, and inflammation using a probiotic: lactobacillus extract.

MATERIALS AND METHODS

The test material was the probiotic lactobacillus extract (14). The *Lactobacillus* ferment was prepared in MRS broth (Neogen Corporation, Lansing, MI) inside a 1000-L fermenter sterilized for 20 min at 121°C to insure sterility. The vessel was cooled to 37°C and nitrogen was pumped into the vessel until the total dissolved oxygen was down to zero in order to ensure anaerobic conditions for the growth of *Lactobacillus plantarum*. The vessel was inoculated with 10 L (or 1% of total volume) of *L. plantarum* prepared in the same MRS media broth and same conditions as the final 1000-L vessel. The organism was allowed to grow in the vessel for 18–24 h after which the ferment was passed through a heat exchanger to lyse most of the cells. The ferment was then filtered first through a 0.45-μm filter, and finally through a 0.22-μm filter to produce the final broth (14).

Oil-in-water (o/w) formulations were prepared at 1% and 5% concentrations of the probiotic for the studies. Triclosan (BASF, NC), a known antibacterial, at 0.1% concentration prepared in a similar formulation base, was used as a control for some parts of the study. In addition, a similar formulation base with 1% salicylic acid (Rhodia Inc., NJ) was used as an internal control for the acne study presented in this paper.

All clinical studies were conducted following good clinical practice standard (ICH Topic E 6-R1 July 2002 CPMP/ICH/135/95). The subjects recruited in these studies were in normal health with no evidence of acute or chronic disease other than acne. Written informed consent was obtained from each volunteer before entering into the study. The subjects were not on any antibiotic, antihistamines, retinoid, anti-inflammatories or steroid therapy, benzoyl peroxide, and/or salicylic acid treatment for at least 2 weeks prior to commencement of this study. The subjects were not under the care of a dermatologist and were not on any acne treatment for at least 1 month before the study started. Pregnant or lactating females were excluded, also subjects exhibiting current sunburn, rashes, scratches, burn marks, etc., which might interfere with the evaluation of test results.

SKIN SENSITIVITY

The anti-inflammatory properties of the test materials were tested by observing the reduction of onset and intensity of erythema induced by an irritant, Balsam of Peru. The test site was on the volar forearms of subjects with a history of sensitivity to Balsam of Peru (15). "Balsam of Peru" (8% w/w in petrolatum), an irritant that contains approximately 0.9% cinnamic aldehyde (16,17), was applied at a dose of approximately 4 mg/cm^2. Erythema was measured with a Minolta Chromameter (Konica Minolta, Ramses, NJ).

Part I: Reduction of onset of skin redness. Ten subjects with a history of skin sensitivity to Balsam of Peru were chosen for the study.

The test compounds were applied on the volar forearms of the subjects. The material was allowed to absorb for 30 min and then Balsam of Peru, the irritant, was applied on the test sites. When erythema appeared, even on one site, the arms were washed with wet towels and skin redness was measured with the Chromameter. Red (a* values) subtracted by the baseline skin redness determined an "increase in redness (Δa*) due to irritation." A comparison of Δa* with the positive and negative controls exhibited the potential of the test materials for reducing the onset of skin irritation. The positive control was a* values of skin treated with Balsam of Peru alone.

Part II: Reduction of intensity of skin redness. A total of ten subjects participated in the study. Using a pen, 1.5 in.^{2} areas were marked on each volar forearm of the subjects corresponding to the test materials and the positive and negative controls. Baseline color measurements were obtained from all the sites using a Minolta Chromameter. Balsam of Peru (8% pet) was applied on all the sites at the rate of approximately 4 mg/cm^2 in a 1.5-cm diameter circle. When redness appeared approximately evenly on all the sites, the irritant was wiped off with a wet towel and then washed with warm water. The degree of redness was measured with the Chromameter on all the sites as the baseline redness.

The test materials were applied on their respective sites at the rate of 2 mg/cm^2 and color measurements were obtained after 15 min, 30 min, 1 h, 1.5 h, and 2 h.

Increase in color was determined by subtracting the baseline skin color values of all the sites and color values were plotted against time. Area under the curve was obtained for all the sites.

SKIN BACTERIAL COUNT

The test materials used in this section were an o/w base cream containing lactobacillus extract (*L. plantarum)* at 1% and a similar formulation with Triclosan (an antibacterial) at 0.1% as a control.

A total of 29 females between the age of 25 and 55 participated in the study. The panel was divided into three groups of 9–10 each. The subjects were provided with the test material to use twice a day for 2 months on the full face. They were instructed not to use any other moisturizers or treatment products; however, they could continue to use the cleansers and makeup that they normally use as long as they did not change products during the course of the study. On the day of the study, the subjects reported to the lab with a clean face and forearms, with no creams, lotions, makeup, etc. Skin microflora measurements described below were obtained at baseline, 1 month, and 2 months.

Skin microflora. The subjects (n = 29) reported to the laboratory and washed their face with a (non-antibacterial) mild liquid soap. Normally, after washing with soap and water, the bacterial count of skin drops to almost zero with gradual increase in microflora over time. The normal microflora was allowed to appear on the skin for the next 3 h. During this period, the subjects were advised to keep their hair away from the forehead and refrain from touching the face or wash it or apply anything on it. At the end of this 3-h time point, saline washings were obtained from the forehead of the face for microbiological analysis.

Dulbeccos phosphate-buffered saline (PBS) washings of the forehead area were obtained using a sterile glass cylinder and a sterile rubber policeman. One milliliter of the saline was poured in the cup and then the skin was scrubbed with rubber policeman (10 strokes) and washed and then the saline was aspirated and collected in 9 ml of PBS. The samples were analyzed for aerobic and anaerobic bacterial count. The data were averaged to determine the total microflora.

The samples were analyzed for microflora as per the U.S. Pharmacopeia Chapter 61 (18) where 1:10 dilutions of the samples were prepared in PBS and plated on tryptic soy agar. After 48 h of incubation at 37°C, the plates were examined and the recovered organisms were quantified. All recovered organisms were then streaked for single colony isolation, gram stained, and examined under the microscope. The organisms were identified using the Becton–Dickinson BBL Crystal™ Identification System.

BARRIER FUNCTIONS

The test site was the jawline of the face of the subjects. Basal skin barrier was determined by measuring trans epidermal water loss (TEWL), using a Servomed Evaporimeter (ServoMed AB, Stockholm, Sweden). To determine barrier integrity, a tape (Tuck tape) was used to cover the test area and after a firm stroke in both directions the tape was peeled off (19). A total of three strippings were obtained. TEWL was recorded again. Strippings followed

by TEWL measurements were continued in groups of three. Damage to the barrier was described as TEWL of 18 g/m^2/h. The number of strippings to disrupt skin barrier was calculated using a linear equation. Barrier repair was observed by measuring TEWL at the stripped skin site, 3 h and 24 h after stripping. For each subject, a linear graph was plotted for TEWL versus time (hours) and using a linear equation, the number of hours to reduce disrupted barrier TEWL to 50% was calculated for every time point (15, 19–21).

The subjects used an o/w base cream containing lactobacillus extract (*L. plantarum*) at 1% for 2 months and the same measurements were observed after 1 and 2 months of use.

ACNE LESION REDUCTION

Oil-in-water base formulations containing lactobacillus extract at 1% and 5% were tested against a formulation containing 1% salicylic acid as a control.

Ten volunteers between the age of 18 and 50 were recruited from a local population. The subjects exhibited acne with several similar acne lesions on the upper back. Two inflamed acne lesions were selected for each treatment and for the untreated. Each lesion was marked, photographed, and graded. A skin surface microscope (Scopeman, Moritex U.S.A., Inc., San Jose, CA) was used to visualize, size, and grade the lesion by two MDs at the testing laboratory. The lesions were treated with the respective treatment once a day for 4 days. Acne lesion size and erythema were plotted against time and area under the curve was calculated (22), and statistical significance was calculated using students *t*-test.

RESULTS

SKIN SENSITIVITY: EFFECT OF LACTOBACILLUS EXTRACT ON THE REDUCTION OF THE ONSET OF SKIN IRRITATION

As observed in Figure 1, there was 57% and 40% reduction in the onset of erythema with 5% and 1% lactobacillus extract, respectively, showing a dose response.

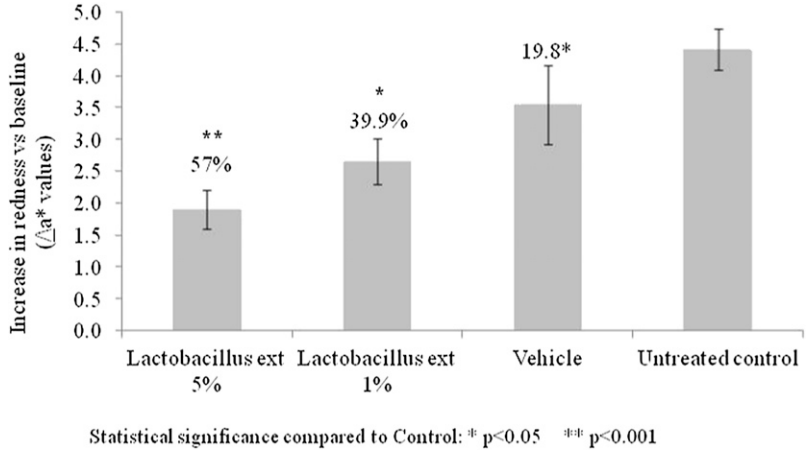

Figure 1. Effect of lactobacillus extract (in o/w emulsion) on the reduction of the onset of skin irritation.

SKIN SENSITIVITY: EFFECT OF LACTOBACILLUS EXTRACT ON THE REDUCTION OF SKIN REDNESS

Lactobacillus extract at 1% and 5% mildly reduced skin redness in a dose-dependent manner displaying 3.75% and 7.5% reduction, respectively (Figures 2a and b).

EFFECT OF LACTOBACILLUS EXTRACT ON THE REDUCTION OF SKIN MICROFLORA

The formulation containing Triclosan showed an initial reduction of 66% (statistically non-significant) in bacterial count after 1 month of use; however, after 2 months of use, there was a marked increase in the microflora (Figure 3). At the 2-month time point, seven out of nine subjects exhibited a higher microbial count as compared to baseline. There is a possibility of emergence of resistance against this material. The average difference in microflora after use was not statistically significant as compared to baseline. The subjects treated with the formulation containing lactobacillus extract appeared to show a consistent reduction in the total microflora over 2-month treatment.

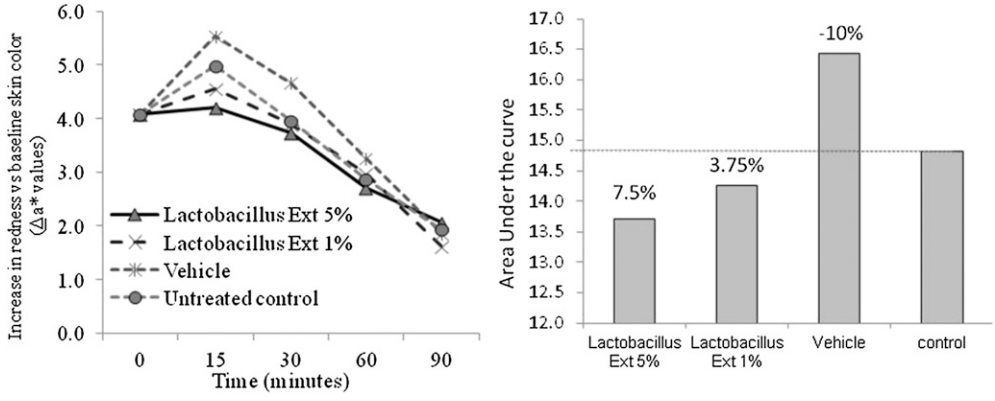

Figure 2. (a) Effect of lactobacillus extract on the reduction of skin redness. This figure exhibits the reduction of skin redness with time (normalized for baseline redness). (b) Shows the area under the curves from (a).

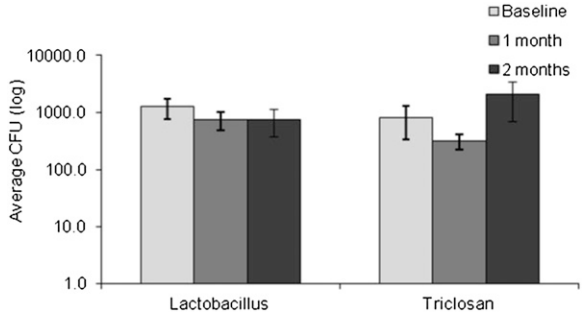

Figure 3. Effect of o/w emulsions with 1% lactobacillus extract and o/w emulsion with 1% Triclosan on aerobic and anaerobic.

EFFECT OF LACTOBACILLUS EXTRACT ON SKIN BARRIER INTEGRITY AND REPAIR

Barrier integrity in terms of baseline TEWL changed only slightly after 1-month treatment with the formulation containing 1% lactobacillus extract and persisted at this level for 2 months. There was no change in TEWL with Triclosan (Figure 4). Barrier strength in terms of increase in the number of strippings required to disrupt barrier described in Figure 5 shows 16% (p = 0.41) and 52% (p = 0.18) increase in the number of strippings required to disrupt the barrier after 1 month and 2 months of using lactobacillus extract, respectively. Triclosan exhibited 3% (p = 0.7) and 12% (p = 0.4) reduction after 1 and 2 months, respectively.

Barrier repair as a function of reduction in the number of hours for 50% barrier repair with the lactobacillus extract and Triclosan is described in Figure 6. There was 22% (p = 0.25) and 30% (p = 0.19) improvement in skin barrier repair after 1 and 2 months of using lactobacillus extract, respectively. Triclosan was not effective in improving barrier repair with a 4% (p = 0.6) improvement after 1 month and a 7% (p = 0.8) worsening after 2 months of use.

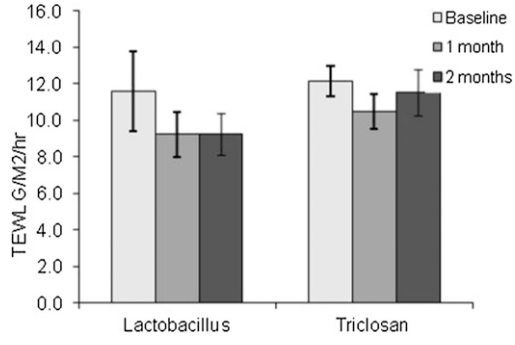

Figure 4. Effect of o/w emulsions with 1% lactobacillus extract and o/w emulsion with 1% Triclosan on barrier integrity in terms of baseline TEWL.

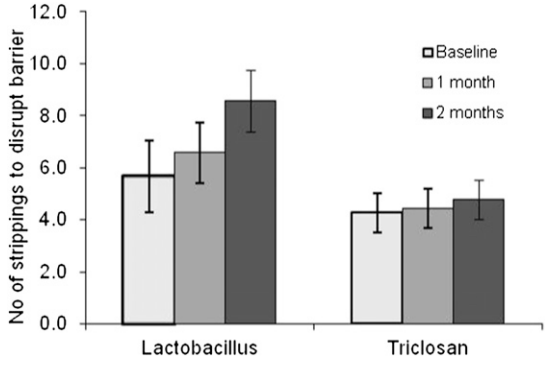

Figure 5. Barrier integrity in terms of increase in the number of strippings required to disrupt barrier. Treatments were o/w formulations containing lactobacillus extract and Triclosan.

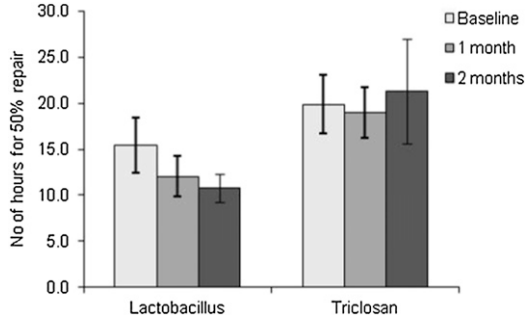

Figure 6. Barrier repair as a function of reduction in the number of hours for 50% barrier repair with o/w formulations of lactobacillus extract and Triclosan at each time point.

EFFECT OF LACTOBACILLUS EXTRACT ON ACNE LESION REDUCTION

As observed in Figures 7a and b, lactobacillus extract at 5% was the most effective in reducing acne lesion size and erythema. In this study, 1% concentration does not appear to be effective in reducing acne lesion size; however, it appeared effective in reducing erythema as compared to the untreated control. The natural immune system of the body improves untreated lesions over time, thus all data are compared to the untreated. The data from Figures 7a and b are summarized in Figure 8 as the area under the curve. It is clear from Figure 8 that a topical formulation containing 5% lactobacillus extract exhibited a substantial reduction in the size and erythema of acne lesions ($p < 0.05$). The 1% lactobacillus extract was also effective in reducing acne-induced erythema, however, the effect was not statistically significant.

DISCUSSION

In this study, we observed the activity of a probiotic: lactobacillus extract on acne and its causative components, namely inflammation, microbial activity, and poor skin barrier. Lactobacillus extract was significantly effective in reducing erythema from a topical irritant, thus showing its potential for down-regulating the expression of molecules implicated in cutaneous inflammation presumably similar to that induced by *P. acnes* (23,24).

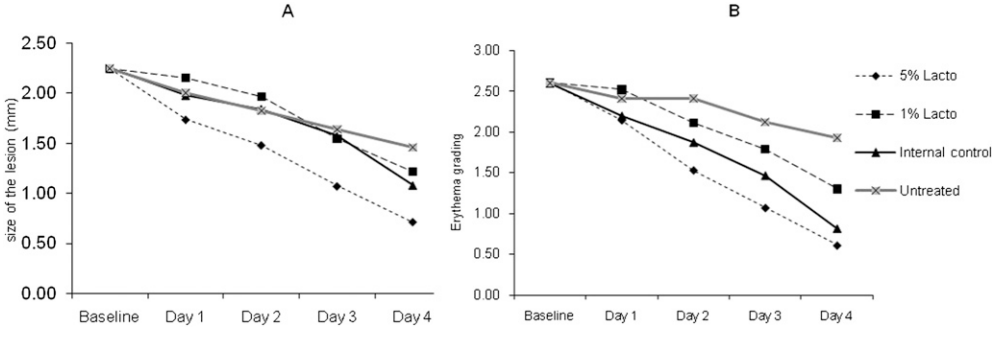

Figure 7. Acne lesion reduction. (a) Size of the lesion (mm). (b) Erythema grading of the lesion.

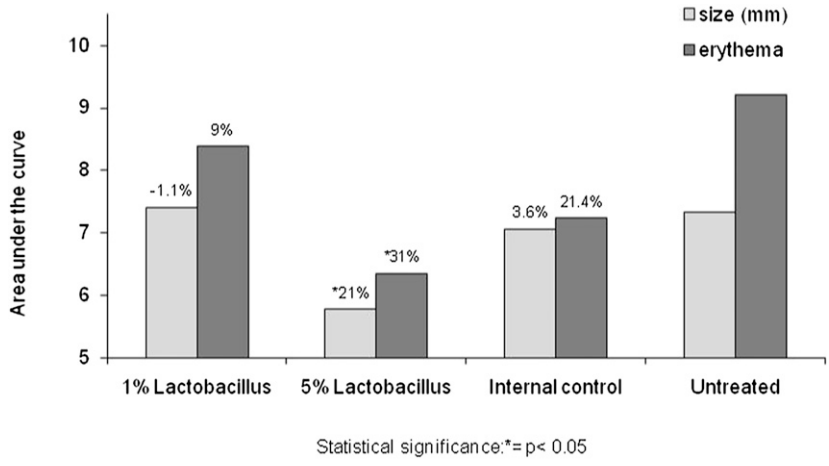

Figure 8. Area under the curve size and erythema of acne lesion. The data from Figure 7(a) and (b) are summarized in Figure 8 as the area under the curve.

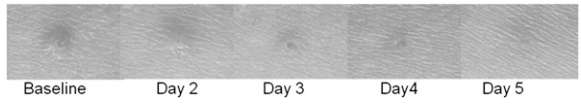

Figure 9. A sample photograph of acne lesion over time.

Lactobacillus extract also impacted a second important aspect of acne, namely infection with *P. acnes*. The human skin provides a habitat for a variety of microorganisms: the skin microflora. There is a complex network of interactions between the microbes and cells of the epidermis. Most of the resident microbes on healthy skin can be regarded as being harmless or even beneficial to the skin. In the case of diseases with some imbalance in microorganisms, such as acne or atopic dermatitis, probiotic concepts represent an effective alternative to strictly antibacterial products. Probiotic approaches predominantly consist of applying or ingesting an inactivated microbial biomass of beneficial bacteria. Ingestion of lactobacillus extract has been shown to alleviate atopic eczema/dermatitis syndrome symptoms in immunoglobulin E (IgE)-sensitized infants (25) and mice (26). *Lactobacillus acidophilus* cultures have also been advocated as a successful remedy for systemic inflammation and oxidative stress, which have important implications in acne (27). In this study, a topical approach to probiotic treatment with lactobacillus extract exhibited a successful reduction in skin microflora.

Impaired skin barrier function is also responsible for comedone formation, since barrier dysfunction is accompanied by hyperkeratosis of the follicular epithelium (11). In this study, we observed an improvement in skin barrier integrity and repair, which adds to its potential as an antiacne agent.

Lactobacillus extract exhibited a significant reduction in acne lesions when used for 4 days. Acne is a common disease, which has confounded hundreds of remedies that include strong antibiotics and retinoids. Biocides such as Triclosan are powerful antimicrobial agents commonly used in topical treatments, however, widespread use of such a material has been reported to become a potential public health risk in regard to development of concomitant resistance (28). In these studies, Triclosan treatment showed a robust reduction

in bacterial count after 1-month use; however, after 2 months of use, there was a marked increase in the microflora, suggesting the possibility of resistance. Although bacterial resistance to antibiotics is rampant, much is not known about the effect of long-term use of lactobacillus extract nevertheless, it promises to be a valuable agent in the reduction of the intensity of acne lesions.

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