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Original Article

Bifidobacterium longum lysate, a new ingredient for reactive skin

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Abstract: Reactive skin is characterized by marked sensitivity to physical (heat, cold, wind) or chemical (topically applied products) stimuli and by the impairment of the skin barrier's ability to repair itself. Several lines of evidence suggest that beyond their capacity to positively influence the composition of intestinal microbiota, some probiotic bacteria can modulate the immune system both at local and systemic levels, thereby improving immune defense mechanisms and/or down-regulating immune disorders such as allergies and intestinal inflammation. Several recent human clinical trials clearly suggest that probiotic supplementation might be beneficial to the skin. Using a probiotic lysate, *Bifidobacterium longum* sp. extract (BL), we demonstrated first *in vitro*, and then in a clinical trial, that this non-replicating bacteria form applied to the skin was able to improve sensitive skin. The effect of BL were evaluated first on two different models. Using *ex vivo* human skin explant model we found a statistically significant improvement versus placebo in various parameters associated with inflammation such as a decrease in vasodilation, oedema, mast cell degranulation and TNF-alpha release. Moreover, using nerve cell cultures *in vitro*, we showed that after 6 h of incubation in culture medium (0.3–1%), the probiotic lysate significantly inhibited capsaicin-induced CGRP release by neurones. Then, a topical cream containing the active extract was tested in a randomized, double-blind, placebo-controlled trial. Sixty-six female volunteers with reactive skin were randomly given either the cream with the bacterial extract at 10% (n = 33) or the control cream (n = 33). The volunteers applied the cream to the face, arms and legs twice a day for two months. Skin sensitivity was assessed by stinging test (lactic acid) and skin barrier recovery was evaluated by measuring trans-epidermal water loss following barrier disruption induced by repeated tape-stripping at D1, D29 and D57. The results demonstrated that the volunteers who applied the cream with bacterial extract had a significant decrease in skin sensitivity at the end of the treatment. Moreover, the treatment led to increase skin resistance against physical and chemical aggression compared to the group of volunteers who applied the control cream. Notably, the number of strippings required to disrupt skin barrier function was significantly increased for volunteers treated with the active cream. Clinical and self-assessment scores revealed a significant decrease in skin dryness after 29 days for volunteers treated with the cream containing the 10% bacterial extract. Since *in vitro* studies demonstrated that, on one hand, isolate sensitive neurones release less CGRP under capsaicin stimulation in the presence of the bacterial extract and, on the other hand, increased skin resistance in volunteers applying the test cream, we speculate that this new ingredient may decrease skin sensitivity by reducing neurone reactivity and neurone accessibility. The results of this studies demonstrate that this specific bacterial extract has a beneficial effect on reactive skin. These findings suggest that new approaches, based on a bacteria lysate, could be developed for the treatment and/or prevention of symptoms related to reactive skin.

Key words: aging – *Bifidobacterium* – probiotics – sensitive skin – skin barrier resistance – xerosis

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Introduction

The term probiotic, popularized by Fuller (1), was recently defined by an expert committee as 'living microorganisms which, when consumed in adequate amounts, confer a health effect on the host' [joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powdered milk with live lactic acid bacteria (LAB) (2)].

Specific strains of probiotic LAB have been shown to beneficially influence the composition and/or metabolic activity of endogenous microbiota (3–6) and some of these have been shown to inhibit the growth of a wide range of enteropathogens (7,8). Competition for essential nutrients, aggregation with pathogenic micro-organisms (9), competition for receptor sites (10), and production of anti-microbial metabolites (7,8) have all been reported to play a role.

Probiotics can be consumed in various forms of fermented or non-fermented food products. As a common feature, probiotics become, after ingestion, transient constituents of the intestinal microbiota capable of exerting their biologic effects, thus giving a rationale for their use as component of functional foods. Weaning, stress, dietary changes, use of antibiotics, and intestinal infections are all conditions that affect the natural balance of intestinal microbiota for which the application of probiotics might be beneficial.

The most often used probiotic genera in humans and animals are enterococci, lactobacilli and bifidobacteria, which are natural inhabitants of the intestinal tract.

Numerous criteria have been defined for the selection of probiotic strains (reviewed in 11). Obviously, the most important criterion is that selected strains have to be safe for use in the host and for the environment. One of the most commonly reported selection criteria is the ability to survive during passage through the gastrointestinal tract (GIT) of the host for which the capacity of a strain to withstand the conditions prevailing in the stomach (high acid level) and intestinal tract (bile acids, pancreatic and other digestive enzymes) is crucial. Adhesion to intestinal epithelial cells is considered important for immune modulation, pathogen exclusion and prolonged residence time in the GIT. Viability of the probiotic strain is assumed to be important and metabolic activity may be pivotal for the expression of anti-pathogenic activity. However, there is increasing evidence that bacterial compounds such as DNA (some CpG motifs) or cell-wall fragments and/or dead bacteria can elicit certain immune responses (12–15).

Although species-specific origin is thought to be important for host specific interactions with the probiotic, there are cases of non-species specific probiotic strains showing some interesting properties. Hence, health benefits have been reported from using the yeast *Saccharomyces boulardii* in humans (16,17).

Beyond their capacity to positively influence the composition of intestinal microbiota (18–24), several lines of evidence suggest that some probiotic bacteria can modulate the immune system both at the local and systemic levels (5,25,26) thereby improving immune defense mechanisms and/or down-regulating immune disorders such as allergy or intestinal inflammation (27–29).

Several strains of LAB were shown to modulate cytokines and growth factor production *in vitro* and *in vivo* (30–32). Moreover, results from different pre-clinical and clinical trials have revealed the ability of various probiotic strains to enhance non-specific and specific immunity (33–38).

As far as skin is concerned, numerous recent human clinical trials widely suggest that probiotic supplementation might be useful in the management of atopic dermatitis and dry skin (27,29,39–41).

If live probiotic form appears to be essential for applications in which their metabolic properties are of importance, some scientific groups showed that some semi-active or non-replicating bacteria preparations retained activities comparable to the live forms (42,43). Such semi-active and non-replicating bacteria forms seemed to be of interest for topical preparations designed for skin, beauty and health purposes.

To date, only a few specialized suppliers propose primarily ultrasound-inactivated bacterial extracts from lactic bacteria or yeasts for potential use in cosmetic products. Many authors have demonstrated that certain bacterial extracts (*Bacillus coagulans*, *Lactobacillus johnsonii*, *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus acidophilus*) have anti-adhesion and anti-microbial properties when applied to cutaneous and mucous surfaces (19,44).

Using *in vitro* models, we demonstrate here that an inactivated bacterial extract from *Bifidobacterium longum* sp. modulates neurogenic inflammation sensory neurone stimulation by capsaicin. Moreover, in a pilot clinical trial we demonstrated that the bacterial extract regulates skin reactivity and dryness in healthy female volunteers.

Materials and methods

Bacterial extract

Bifidobacterium longum reuter lysate (BL) is an ultrasound inactivated suspension in aqueous medium. This strain has the following INCI code: Bifida ferment lysate. The active form is at 5% in the total suspension

Part I: neurogenic changes induced by substance P (SP) in organ culture of human skin

Organ culture of human skin

Bifidobacterium longum efficacy was assessed using a previously described alternative method on neurogenic inflammation of the skin (45). For this purpose, SP was added to an organ culture of human skin directly to culture medium in an attempt to reproduce *ex vivo*, the effects described *in vivo*.

Human skin samples ($n = 8$) were obtained from plastic surgery on patients (Caucasian women 35–45 years old). They were washed three times with antibiotics and then cut into 1 cm^2 full-thickness pieces. Subcutaneous fat and lower dermis were mechanically removed under stereomicroscope using surgical scalpel.

Skin biopsies were placed on culture inserts (filter pore size 12 μm; Costar, Poly-Labo Paul Block, Strasbourg, France) with the epithelium uppermost at an air/liquid interface. The inserts were set on 12 well plates (Costar) and culture medium was added to the wells so that the surface of the medium reached the filter level. Organ cultures were performed using Dulbecco's minimal essential medium (Gibco BRL, Invitrogen, Cergy Pontoise, France) con-

taining antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin; Gibco BRL), 200 μg/ml L-glutamine (Gibco BRL), bovine pituitary extract, growth factors and foetal calf serum (Gibco BRL). Cohesion between skin and insert was obtained with polysiloxane vinyl seal in such a way that neither skin retraction nor lateral passage of any applied topical product towards the dermis was possible.

Skin samples were kept under these survival conditions for 5 h at 37°C in a humidified incubator with 5% CO_2.

Application of substance P (SP) and BL lysate

Skin samples were maintained in culture for 24 additional hours in the presence or absence of 10 μM of SP (Bachem, Weil am Rhein, Germany) incorporated in the culture medium. In the same time a preparation of 10% BL lysate (40 $\mu l/cm^2$) was topically applied to skin.

Histological evaluation

After 24 h, skin samples were removed from inserts, set in Bouin's liquid and embedded in paraffin. Thick sections of 5 μm were stained with haematoxylin and eosin to evaluate vasodilation, oedema and inflammatory infiltrate. Toluidine blue staining (0.1% in 50% ethanol; pH 3.5) was used for mast cell counts. Sections were evaluated under Olympus light-photomicroscope and photographed with Ektachrome 64 T film.

Histological evaluation of inflammatory reaction was performed on papillary dermis and on the upper part of reticular dermis, at immediate/closer by perivascular location. Double blind assessment was carried out by two experts through visual scoring with a 10 × Olympus objective. Four slides per skin sample were evaluated for each treatment.

For semi-quantitative gradation, a score scale ranging from 0 (negative) to 4 (maximum) was defined for the following parameters:

(i) Microvascular vasodilation: Evaluation was performed on luminal areas of blood capillaries (easily detected histologically by their endothelial cells). Scores were: 0 – no vasodilation, 1 – slight, 2 – moderate, 3 – marked and 4 – severe vasodilation.

(ii) Edema: evaluation was made according to the following score: 0 – no edema, 1 – slight edema in the dermis, 2 – moderate edema, 3 – marked edema 4 – severe edema.

(iii) Inflammatory cell infiltrate mainly consisted of lymphocytes (characterized by small nuclei with dark staining by hemalun and no visible cytoplasm), histiocytes (characterized by large nuclei with pale staining by hemalun and more significant cytoplasm) or mast cells (characterized by toluidine blue staining) was scored as follow: 0 – absent, 1 – occasional cells, 2 – moderate infiltrate, 3 – marked infiltrate 4 – severe infiltrate.

Intermediate values were given when inflammatory reaction was not homogeneous within sections. The results were expressed as mean and SEM for each inflammatory parameter analysed.

TNF-alpha determination

TNF-alpha level was measured into skin culture medium using commercially available enzyme immunoassay kits (Chemicon International, USA). The results were expressed in pg per ml of culture medium.

Part II: protocol of in vitro sensory neurone stimulation by capsaicin

In vitro sensory neurones were cultured by modified reported method (46).

Dissociated cells from Dorsal Root Ganglia (DRG) were suspended in DMEM-Ham F12 (Invitrogen, Cergy Pontoise, France) supplemented with N2 (Invitrogen), Nerve Growth Factor (NGF, 10 ng/ml, Invitrogen), Neurotrophin 3, L-glutamine (Invitrogen) and penicillin/streptomycin.

Viable cells were counted in a Neubauer cytometer using the trypan blue exclusion test (Sigma, St Quentin, France) and seeded at a density of 20 000 cells/well in 96-well plates (Nunc, Langenselbold, Germany) pre-coated with poly-L-lysine (Sigma P2636). Half of medium was changed every 2 days. The culture contained sensory neurones, Schwann cells and fibroblasts (the proliferation of Schwann cells and fibroblasts was further restricted). The sensory neurones were used after 10 days of culture, when they were mature and capable to synthesize and release CGRP (46).

Following a 10 days of culture, DRG neurones were exposed to three, 1 or 0.3% *B. longum* test suspension. After 6 h, the medium was replaced by fresh medium with or without capsaicin (10^{-6}M) for 25 min.

Following the 25 min incubation, supernatants were individually collected and frozen at –80°C. The amount of CGRP in each sample was measured using Enzyme-Linked Immuno Sorbant Assay (ELISA, SpiBio kit, Montigny le Bretonneux, France).

Part III: clinical trial: efficacy of BL extract on skin reactivity and skin dryness

Proceeding *in vitro* and *ex vivo* data prompted us to conduct a clinical trial aimed to study the efficacy of a topical cream containing *B. longum* lysate on reactive skin symptoms.

A randomized, double-blind, placebo-controlled trial was conducted in 66 female volunteers with reactive skin who were selected based on answers to a questionnaire for sensitive skin, skin leg dryness and face roughness. Volunteers applied either the cream containing 10% bacterial extract (test cream) ($n = 33$) or control cream/placebo ($n = 33$), twice daily to face, arm and leg skin for 2 months. Control cream and test cream were randomly assigned.

In order to prevent any bias effect from other previously applied topical cream, a washout period of about 1 week

was implemented before starting the 2 months treatment with either the test or the placebo cream.

Skin sensitivity

Sensitive skin is a condition uneasy to assess since no truly pertinent bio-instrumental method is available to measure it. For that reason, a number of standard tests have been developed to identify subjects with sensitive skin.

The best known test method to evaluate skin reactivity is the lactic acid stinging test developed by Frosch and Kligman (47). The test consists in recording stinging feeling by subject at given times following application of a lactic acid solution to the nasolabial sulci. Sulci are very reactive areas where the stratum corneum where the stratum corneum is very permeable. They contain a large number of hair follicles and sweat glands promoting product penetration. Moreover the peripheral sensory nerve network of sulci is very dense. In order to monitor skin sensitivity over time, lactic acid stinging test was performed at baseline and after one and two months treatment with the test cream or the placebo cream.

Skin barrier function

In order to follow the recovery of skin barrier function, trans-epidermal water loss (TEWL) was determined after tape stripping using an evaporimeter. TEWL was determined immediately before treatment initiation and then after one month and two months of treatment with cream (48–50).

The method of individual skin barrier impairment by tape stripping was first reported by Pinkus in 1951. The method has been widely used as a model to impair the stratum corneum barrier function (51,52).

The method consists in repeated application of dermatological tape to the skin on the medial surface of volunteer's forearm. The procedure is repeated as many times as necessary until a TEWL level of 15 g/cm^2/h is reached. The method only removes layers of the stratum corneum without inducing damage to living keratinocytes or inflammatory reaction (53). Follow-up of TEWL over 5 days generates data that reflects the speed of barrier function recovery (54).

Skin hydration

In order to follow the markers associated with a decrease in skin dryness, such as components of natural moisturizing factor (NMF) that play an important role in skin hydration, epidermal samples were harvested for further biochemical evaluation (55–58). Selected NMF components were pyrrolidone carboxylic acid, sodium lactate, serine and urea. Pyrrolidone carboxylic acid is formed from glutamic acid located in the *stratum corneum* as sodium or potassium salts. The acid is endowed with marked hygroscopic properties. Sodium lactate has the same properties. Serine plays a role in establishing osmotic pressure, while urea increases skin water penetration and retention in the *stratum corneum.*

All assessments were done at day 1, day 29 and day 57.

Part IV: statistical analysis

For histological parameters, changes in qualitative variables (edema, vasodilation, lymphocyte, and mast cell infiltrates) and quantitative variables (cytokine releases) were analysed with chi-square tests and Student's *t*-test, respectively. Analysis of CGRP data was carried out using analysis of variance (ANOVA) followed by post hoc pair-wise comparisons using Dunnett's test. Data from quantitative stinging tests were processed using a linear mixed model for longitudinal data. The number of strippings necessary to reach the TEWL level of 15g/cm2/h and the qualitative clinical and self assessment scores for skin roughness and dryness were analyzed using generalized linear mixed models. The stripping count data were specifically modelized using a Poisson distribution and a logarithmic link function.

Statistical analyses were carried out using the SAS or SPSS statistical software. The two-sided significant threshold was set at 5%.

Results

Part I: neurogenic inflammation induced by SP

Neurogenic skin inflammation following topical application of an irritant substance or environmental stimulation induces vascular changes and the production of inflammatory mediators. Substance P is one of the main neuropeptide that triggers skin inflammatory response. Even in the absence of systemic blood circulation, SP addition to culture medium of surviving skin induced a dose dependent edema, vasodilation and extravasation of lymphocytes through microvascular walls and mast cell degranulation. Moreover, the inflammatory response is associated with the release of pro-inflammatory mediators such as TNFα.

Histological changes after SP exposure

Edema and vasodilation

When applied directly to the culture medium, SP induced vasodilatation in the superficial dermis: the global % of capillary dilatation was 77.7 ± 17.8% compared to control with 69 ± 8.9% ($p = 0.05$). The application of the bacterial extract during SP stimulation tends to decrease the % of vessel dilatation, 69.06 ± 20.1% compared to the stimulation with SP alone ($p = 0.054$).

From measurements of mean capillary surface, we found that SP induced statistically significant vasodilation compared to control [144.4 ± 71.2 μm^2 with SP vs 107.66 ± 71.63 μm^2 without ($p = 0.02$)].

When BL bacterial extract was applied onto the skin, SP-induced vasodilation significantly decreased [97.4 ± 60.7 vs 144 ± 71.2 μm^2 ($p = 0.0003$)].

Adding SP to culture medium raises oedema score to 1.8 ± 0.7 compared with 1.1 ± 0.64 in unstimulated skin samples ($p = 0.02$). When bacterial extract is applied to stimulated skin samples, oedema score significantly decreases to 1.17 ± 0.7 ($p = 0.009$).

Mast cell extravasation

SP induces a statistically significant increase in semi-quantitative evaluation of mast cell degranulation as reflected by a significant decrease in highly granulated mast cells (12.54 ± 3.4% vs 30.94 ± 7.94% in control skin, $p = 0.016$ and an increase of degranulated mast cells (37.4 ± 6.5% vs 18.46 ± 5.72% in control skin, $p = 0.007$).

Thus, the application of the bacterial extract led to a significant decrease in mast cell degranulation (13.2 ± 1.85% vs 37.4 ± 6.5% in skin SP treated, $p = 0.0024$).

TNF-α levels after SP stimulation

When 10 μM SP was added to culture medium of *ex vivo* surviving skin, we observed also a significant increase in TNF-α level ($p = 0.00001$) reaching 49.2 ± 14.9 vs 32.3 ± 13.8 pg/ml in unstimulated control skin.

Applying bacterial extract to SP-stimulated skin resulted in a significant decrease in TNF-α release (28.8 ± 12.3 vs 49.2 ± 14.9 pg/ml, $p = 0.05$).

Part II: CGRP release by sensory neurones without or with capsaicin stimulation

After 6 h of incubation with BL bacterial extract, no significant stimulation of CGRP spontaneously released was notice (Fig. 1) (34.0 ± 5.2 compared with 38.2 ± 11.8 pg/ml in the control group).

After 6 h of pre-incubation with BL bacterial extract (0.3% and 1%) followed by neurone stimulation by capsaicin, a significant decrease in CGRP release was observed ($p < 0.01$) (37.5 ± 12.2 pg/ml after 0.3% of BL compared to 91.0 ± 16.4 pg/ml in the control group treated with capsaicine alone (decrease of 36%) and 32.6 ± 5.3 pg/ml after 1% of BL compared with 91.0 ± 16.4 pg/ml in the control group (decrease of 41%), $p < 0.01$) (Fig. 2).

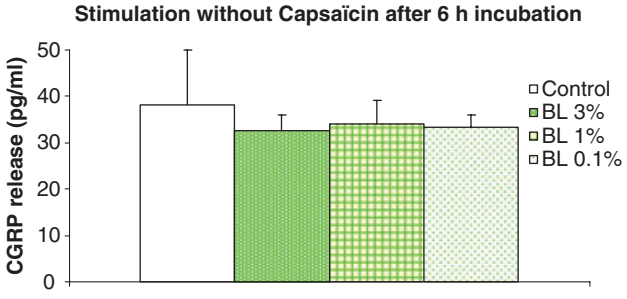

Figure 1. Effect of BL bacterial extract on spontaneous CGRP release after 6 h incubation with sensory neurones.

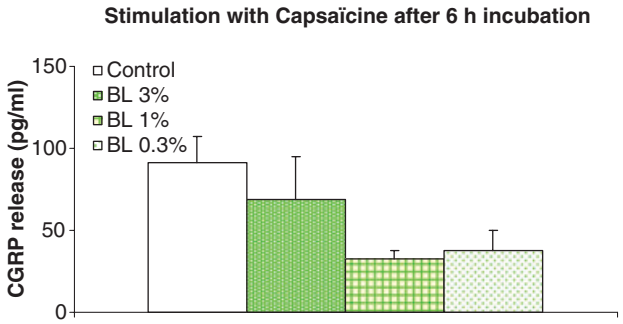

Figure 2. Effect of 6 h preincubation with BL bacterial extract on capsaicin-induced CGRP release by sensory neurones.

Part III: clinical trial

Skin integrity/homeostasis

Skin sensitivity

The results showed a significant decrease in skin sensitivity in volunteers who applied the cream containing BL bacterial extract for 2 months (day 57, $p = 0.0024$) (Fig. 3).

Skin barrier function

BL Cream application increased skin resistance to physical aggression. The number of tape strippings necessary to produce a given barrier function disruption (TEWL ≥

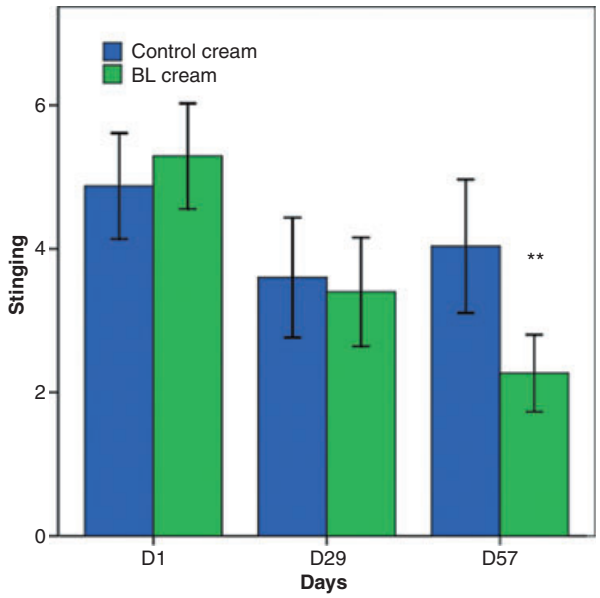

Figure 3. Influence of test cream on skin sensitivity to lactic acid: The graphic display mean values with their 95% confidence interval, ** statistically significant different at $p < 0.01$.

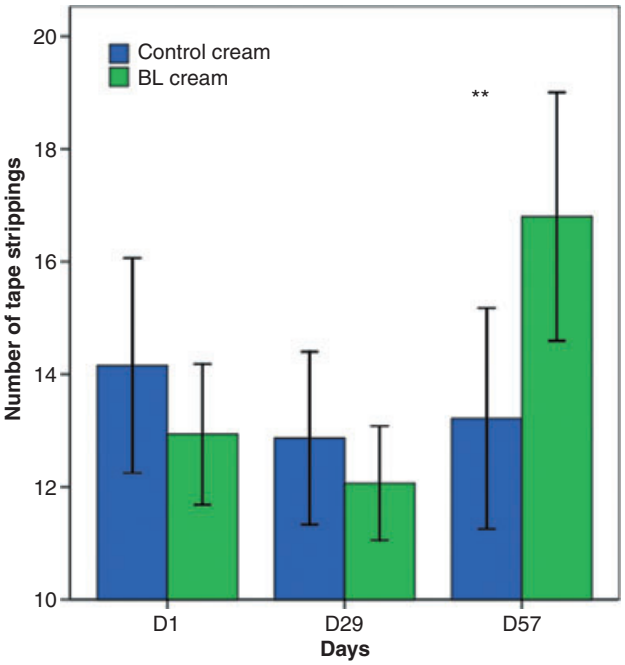

Figure 4. Influence of test cream containing *B. longum* lysate on skin barrier: The graphic display mean values with their 95% confidence interval, ** statistically significant different at $p < 0.01$.

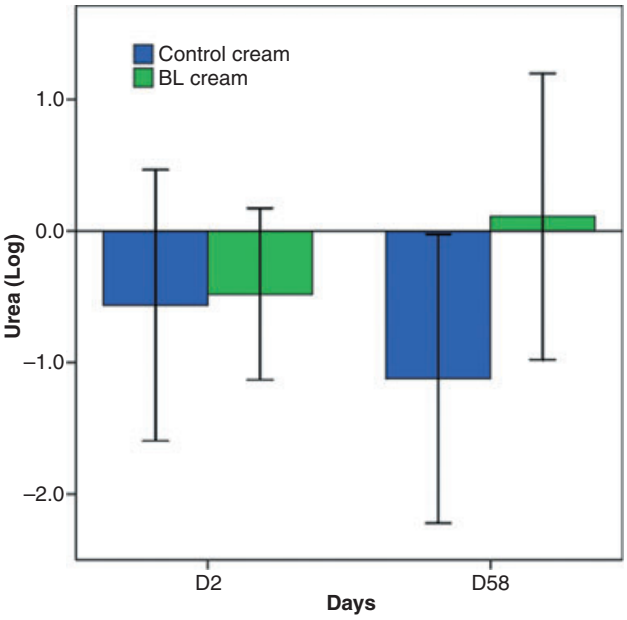

Figure 5. Effect of test cream on urea content in the *stratum corneum*. The graphic display mean values with their 95% confidence interval.

15 g/cm^2/h) was significantly increased in the group treated with BL bacterial extract/test cream for 2 months compared with the placebo group ($p = 0.0044$) (Fig. 4).

However, when the speed recovery of the skin barrier function was analysed no significant difference was noted between placebo and BL bacterial extract treatments.

Skin hydration

At day 29, clinical and self-assessment indicated a significant reduction of facial skin roughness and leg skin dryness after 29 days in volunteers applying the cream containing 10% BL bacterial extract ($p = 0.03$).

As far as skin moisturizing factors are concerned, data only show some changes in urea concentration in the stratum corneum. Whereas urea concentration of the stratum corneum decreased significantly in the placebo group over the treatment period ($p = 0.014$), no reduction could be noticed in the test cream group (Fig. 5).

Discussion

The different evaluation studies performed on *B. longum* lysate lead to the conclusion that topical application of such a bacterial extract could provide interesting skin benefits. Data show a positive effect on various skin markers and parameters assessed *in vitro*, *ex vivo* and in a clinical trial.

The *ex-vivo* human skin model stimulated by BL extract reveals an anti-inflammatory effect of the tested compound as reflected by statistically significant decrease in vasodilation, oedema, mast cell degranulation and TNF-α release compared to control.

Using nerve cell cultures *in vitro*, incubation for 6 h with 0.3% or 1% BL lysate in culture medium significantly inhibits capsaicin induced CGRP release by neurones. This activity may account for the soothing properties of BL extract observed *in vivo*.

Clinical data show that BL lysate could be appropriate to prevent problems related to skin sensitivity. Indeed, the results of lactic acid stinging test demonstrated that test cream containing BL lysate significantly decreased baseline value of skin sensitive score over the treatment period (D57 vs D1), confirming above *in vitro* data and skin culture data on neurogenic inflammation.

In the same time period, volunteers who used the control cream exhibited random variations in sensitivity limits depending on the time point (decrease at D29, then increase at D57) clearly reflecting a random placebo-like pattern (cf. Fig. 3). In contrast, during treatment with the BL *lysate*, skin sensitivity regularly and significantly decreased at all time points in the group applying the test cream. These clinical findings suggest that BL bacterial extract may prevent negative environmental effects (cold in winter, air dryness). Indeed, skin sensitivity measurements at the end of treatment time showed values close to normal as commonly found in subjects with non-sensitive skin. It seems that topical application of BL preparation contributes to reinforce skin homeostasis and to improve skin resistance to external abuse.

Three potential mechanisms may account for the effect of *Bifidobacterium* lysate on skin sensitivity: (i) a direct action by inhibiting the release of neuro-mediators involved in sensitivity phenomena as shown by capsaicin test on neurone culture; (ii) a direct action by decreasing neurogenic inflammation frequently associated with sensitive skin symptoms as suggested by data from human skin organ culture following SP stimulation, and (iii) an indirect action by improving skin barrier function and protecting neurones from external stimuli, as suggested by clinical assessment of barrier function using the tape stripping method.

Skin is the body's interface with the environment. One of the skin's major roles is to protect the body against water loss and penetration of exogenous factors including irritants.

Water diffusion rate depends on the number of layers of compactum *stratum corneum*. The *stratum corneum* thus constitutes a uniform, poorly permeable structure which is the main barrier to water loss by diffusion.

In this study, the test cream with BL lysate did not show any positive effect on the skin barrier recovery rate but it appeared to strengthen the skin's natural barrier. The number of tape strippings necessary to reach TEWL ≥ 15 g/cm^2/h was statistically higher in the group treated for 2 months with the test cream than in the placebo group.

As previously mentioned, this property of *BL* extract on skin barrier function is of particular interest with regard to the improvement of skin sensitivity: increasing the protective role of stratum corneum against exogenous agents may limitate their penetration through the skin and indirectly protect nervous fibres from excessive external stimuli. Moreover, enhancing skin barrier may be of importance particularly in certain inflammatory diseases where barrier function is impaired such as atopic dermatitis, dry skin or in aging. Barrier function experiences a variety of tests on a daily basis including environmental, chemical or physical factors (UV radiation, pollution, hot and cold temperatures, air-conditioning, low humidity level, etc.), psychological stress and/or dietary deficiencies. The accumulation of these aggressions results in the appearance of barrier defects which in term may lead to the appearance of skin disorders as xerosis (47,59). This phenomenon is exacerbated with age when repair processes become slower and less effective.

As far as skin xerosis is concerned, results from clinical study give some evidence of the beneficial effects of BL extract through significant difference between the test cream and the control cream with respect to alleviation of facial skin roughness or leg skin dryness after 29 days of application.

Furthermore, among biochemical markers involved in skin hydration, it emerges that urea level in the *stratum corneum* increases as a result of test cream application whereas it significantly decreases in volunteers' group that used the control cream for the same period. Urea increases moisturizing by cleaving hydrogen bonds and promoting molecular unfolding, thus creating numerous water binding sites. Our data suggest that BL extract may contribute to maintain skin hydration and thus have potential interest in the prevention of dry skin in situation where skin homeostasis is impaired by environmental stresses or cold season.

Other authors reported using other specific extracts of lactic bacteria, such as those from *Streptococcus salivarium* sp. *thermophilus* commonly used in milk fermentation for yoghourt and cheese production, that these compounds even inactivated by ultrasound were able to increase the rate of ceramides in the skin. This result brings an other evidence of the beneficial role of these bacterial lysates in the maintenance of barrier function and flexibility of the *stratum corneum*) (60). This may account for positive results reported using these extracts on xerotic skin symptoms associated with atopic dermatitis (erythema, prurit) (60). In this case, the increase in ceramide levels could be related to the hydrolysis of sphingomyelin by sphingomyelinases contained in the bacterial extracts.

The results of the various efficacy studies conducted on BL lysate demonstrate that after topical application, a specific bacterial lysate or extract may cause a beneficial effect on skin [i.e. decreasing skin sensitivity, increasing resistance of skin barrier, maintaining moisturizing factor concentrations in the skin (urea)]. Since various studies show that in the presence of bacterial extract, isolate sensitive neurones release less CGRP under capsaicin stimulation, a reduced inflammatory response is observed under SP stimulation and skin resistance to tape stripping is improved, we speculated that this new ingredient may decrease clinical sensitive skin reaction by reducing neurones reactivity and neurones accessibility to external stimulti.

This leads to the conclusion that daily topical treatment with BL lysate may be an interesting solution for various skin disorders related to xerosis, skin sensitivity and aging.

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