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The positive benefit of *Lactobacillus paracasei* NCC2461 ST11 in healthy volunteers with moderate to severe dandruff

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RESEARCH ARTICLE

Abstract

Dandruff is a common persistent, relapsing inflammatory condition affecting the scalp. An imbalanced proportion of the major bacterial and fungal populations colonising the scalp, a skin barrier dysfunction, and hyperseborrhoea are three main etiological factors of dandruff. The efficacy of *Lactobacillus paracasei* NCC 2461 ST11 (ST11) to manage dandruff and to restore a balanced scalp microbiome was assessed. Sixty healthy male volunteers aged 18 to 60 years with moderate to severe dandruff consumed on a daily basis a sachet containing ST11 (1×10^9 cfu) or a placebo for 56 days. Clinical efficacy (free and adherent dandruff, erythema, scalp seborrhoea, global clinical score), subject self-assessments, safety reporting as well as scalp microbiota assessments were performed every two weeks (day 1, 15, 29, 43, 57 and 64/follow-up). Free and adherent dandruff, erythema and the global clinical score improved significantly (all $P<0.05$) over time in the ST11 group and as compared to the placebo when day 57 was compared to day 1. Self-assessments paralleled these findings. ST11 enhanced restoring the scalp microbiota after 56 days of supplementation when compared to the placebo. No adverse events were reported. Regular intake of ST11 over 56 days is safe and reduces significantly the severity of signs and symptoms of moderate to severe dandruff. Its efficacy is potentially due to its positive impact on the skin barrier and skin immune system.

Keywords: dandruff, *Lactobacillus paracasei, Malassezia restricta,* probiotic

1. Introduction

Dandruff is a common, persistent and relapsing inflammatory condition affecting skin rich in sebaceous glands (Hay and Graham-Brown, 1997). Its prevalence varies between 30 to 95% of humans (Xu *et al.*, 2007). Besides the discomfort it causes, dandruff is also socially embarrassing and affects the patient's self-esteem (Manuel and Ranganathan, 2011). *Malassezia* species have been suggested to play a major role in the pathogenesis of the condition together with stress, fatigue, weather extremes, oily skin, inadequate shampoo use, immunosuppressed status (AIDS), and neurological disorders (Rudramurthy *et al.*, 2014). Among these yeasts, *Malassezia restricta* and *Malassezia globosa* are generally considered associated with dandruff (Zisova, 2009).

Seborrheic dermatitis, like dandruff, is observed in skin rich in sebaceous glands. It is thought that an association exists between *Malassezia* yeasts and seborrheic dermatitis, which may partly be due to an abnormal or inflammatory immune response to these yeasts (Gupta *et al.*, 2004a; Gupta *et al.*, 2004b).Both dandruff and seborrheic dermatitis (D/SD) share an aetiology depending from three factors: sebum, microbial metabolism (specifically *Malassezia* yeasts), and individual susceptibility (Dawson, 2007). More recently, research work demonstrated that dandruff is associated with imbalance in the proportion of the major bacterial and fungal populations colonising the scalp (Clavaud *et al.*, 2013; Wang *et al.*, 2015).

A healthy *stratum corneum* (SC) not only forms a barrier to prevent water loss and to maintain hydration of the scalp, it also protects against the skin from external injuries.

Severe or chronic scalp barrier damage can impair proper hydration, and leads to atypical epidermal proliferation, keratinocyte differentiation and SC maturation, which may underlie some dandruff symptoms (Turner *et al.*, 2012). The depleted and disorganised structural lipids of dandruff SC are consistent with the weakened barrier indicated by elevated transepidermal water loss (DeAngelis *et al.*, 2005). Scalp with dandruff: (1) showed profound changes in the expression and maturation of structural and epidermal differentiation related proteins, that are responsible for the integrity of the skin; (2) showed altered relevant factors that regulate skin hydration; and (3) showed an imbalanced physiological protease-protease inhibitor ratio (Cavusoglu *et al.*, 2016). Further evidence of a weakened scalp skin barrier in subjects with dandruff includes subclinical inflammation and higher susceptibility to topical irritants (Dieamant Gde *et al.*, 2008; Turner *et al.*, 2012).

In recent years, nutritional approaches, including ingesting living microorganisms, such as probiotics, have gained increasing interest. The classification of a strain as a probiotic requires that its beneficial physiologic effects has been proven scientifically, that the strain has been of human origin, that it is safe for human use, stable in acid and bile, and that it adheres to the intestinal mucosa (Salminen *et al.*, 1998). The most frequently used bacteria fulfilling these criteria are *Lactobacillus* and *Bifidobacterium* (Isolauri, 2001).

Nowadays, it is acknowledged that maintaining and protecting the gastrointestinal tract contributes to the general homeostasis of the body. Although no direct link has been established between probiotics and bioavailability of nutrients, the fact remains that probiotics are involved in maintaining the digestive equilibrium: digestive homeostasis supports promoting the assimilation of dietary nutrients in the intestinal mucosa which are vital for the cell metabolism and for the synthesis of various functional and structural elements in the skin and the overall well-being.

In vitro studies showed that *Lactobacillus paracasei* ST11 (hereafter ST11) interacts on immune mechanisms and inhibits CD4+ T-cell proliferation by reducing the production of cytokines Th1 and Th2. Moreover, it increases the production of regulatory cytokines such as transforming growth factor beta (TGF-β) and interleukin (IL)-10 and induces a CD4+ T-cell population with the characteristics of regulatory T-cells (Von der Weid *et al.*, 2001). Another *in vitro* assay showed that only this strain has a beneficial impact on the skin immune system and antagonises inflammatory reactions underlying reactive skin conditions (Gueniche *et al.*, 2010).

Other, non-clinical investigations in mice demonstrated that Th1 cell-dependent immune responses such as anti-KLH immunoglobulin G(2a) [IgG(2a)] levels and delayed type hypersensitivity responses were significantly modulated after supplementation with ST11 (Vidal *et al.*, 2008). These findings were paralleled by results from several clinical studies (Isolauri, 2001; Isolauri *et al.*, 2001; Ouwehand *et al.*, 1999; Rautava and Isolauri, 2002). Moreover, increasing the number of regulatory cytokines TGF-β and IL-10 modulated the growth and the differentiation of keratinocytes: the production of such cytokines and growth factors modulates paracrine and/or autocrine the necessary metabolic responses of the cutaneous barrier repair (Ouwehand *et al.*, 1999). Thus, systemic stimulation of TGF-β synthesis may play a role in the maintainance of the cutaneous equilibrium by accelerating the epidermal repair and differentiation, activating collagen synthesis, regulating specific integrin expression by endothelial cells and by stimulating peptide synthesis involved in wound healing, such as epidermal growth factor (EGF).

Considering these elements, we aimed with the present study to assess the efficacy of a food supplement containing a probiotic, ST11, in the management of signs and symptoms of dandruff and in the restoration of a balanced microbiota on the scalp.

2. Materials and methods

Administrative features

This double-blind, randomised study comparing a food supplement containing ST11 was conducted at one single site in France (Sabouraud Healthcare Centre, Paris). The study received approval from the local ethic committee and the national health authority and was conducted in compliance with local regulatory requirements, the declaration of Helsinki and Good Clinical Practices. All volunteers provided written informed consent prior to inclusion into the study.

Patient population

The study planned for 60 healthy male, non-bald volunteers aged between 18 and 60 years. At selection visit, all subjects had to have moderate to severe dandruff, measured by the investigator on a scale from 0 = non to 4 = severe, covering at least two quadrants of the scalp. Volunteers had to wash their hair during the washout (day -42 to day 1), the treatment phase (day 1 to 57) and relapse phase (day 57 to 64) using a quantity corresponding to a soup spoon of neutral shampoo (Camomile Shampoo, DOP, France, provided by the sponsor) up to 3-times per week; subjects were not allowed to use any medication to treat their dandruff or hyperseborrhoea. Moreover, to avoid biasing of study results, volunteers were asked to consume not more than 125 g per day of fermented products containing active bacteria during the study.

Products administered

The strain ST11 was provided by Nestle (Konolfingen, Switzerland). Sachets of the product containing ST11 (1×10^9cfu) and maltodextrin were prepared by Nestle. Suitable volunteers were randomly assigned to receive either one sachet of product containing ST11 and maltodextrin or its placebo containing only maltodextrin, orally once daily for 56 days (10 g each sachet). Test products were reconstituted in a glass of water. Volunteers were asked to wash their hair 3 times per week in using not more than a quantity corresponding to a soup spoon of the neutral shampoo they were provided with.

Clinical evaluations

Study visits took place every 2 weeks up to day 57; a follow-up visit to assess the residual efficacy of the products was scheduled on day 64. Efficacy assessments included:

- Clinical assessment of scalp dandruff (free and adherent dandruff), erythema and scalp seborrhoea were assessed throughout the study and at day 64 (follow-up). Visible clinical signs were assessed by the investigator on a scale from 0 = absent to 4 = severe on each of the four head quadrants; a total head quadrant score was a score of 16. A global clinical score calculated in adding scores of free and adherent dandruff as well as of erythema, summing up to a score of 48.
- Assessment of the global clinical efficacy on day 57 (end of study), scored on a scale from 4 = total healing to -1 = deterioration.
- Self-assessment of dandruff, pruritus, greasiness, irritations, redness, stretching sensation and overall perception of the scalp. Signs of dandruff were rated by the volunteers on a scale from 0 = absent to 4 = severe. Overall perception of their scalp was rated on a scale from 0 = very good to 3 = poor.

Safety assessments included the reporting of adverse events throughout the study.

Analysis of the scalp microbiota

Analysis of the scalp microbiota included in addition to *Malassezia* strains, sampled on the scalp of the subjects, a quantitative assessment of the total, anaerobic and aerobic bacteria represented by Gram positive cocci. All *Malassezia* strains to be analysed were cultured in adequate mediums. *Malassezia* and bacteriological samples were taken from the scalp of subjects using dry, sterile, cotton swabs.

Swabs were placed in 3 ml of phosphate buffered saline containing 0.1% Triton X and then shaken thoroughly for at least 30 s. This suspension became the parent solution (PS) from which two successive 1:10 dilutions (d1 and d2) were prepared. For the quantitative analysis, 0.1 ml of the pure sample and its dilutions were inoculated on the surface of the following media:

- Trypticase soy agar (AES-Chemunex, Bruz, France) + 1% Tween 80 (48 hours at 37 °C) for total mesophile aerobic flora count.
- CNA (colimycine nalidixic acid) blood agar (BioMérieux, Marcy L'Etoile, France) with 5% sheep blood) (24-48 h at 37 °C) for the Gram positive flora count, essentially coagulase-negative staphylococci and streptococci for the skin flora.
- Blood medium (Columbia agar (BioMérieux) with 5% sheep blood) (8 days at 37 °C under anaerobic conditions) for the *Propionibacterium* count, essentially *Propionibacterium acnes* for the skin flora.

For the qualitative analysis: 0.1 ml of the pure samples was spread on:

- Baird Parker medium (BioMérieux) (48 h at 37 °C) to isolate *Staphylococcus aureus.*
- Drigalski medium (BioMérieux) (24 h at 37 °C) to isolate Gram negative bacteria, including enterobacteria and specifically *Escherichia coli.*

Inoculation of plates was carried out in duplicate. Aerobic bacteria were incubated at 35 to 37 °C for 48 h, while anaerobic bacteria were incubated at 35 to 37 °C under anaerobic conditions for five to six days. The number of micro-organisms/cm^2 was expressed in log10 scale in quantitative analyses. If specific colonies appeared at a significant quantity, a special identification process was implemented.

Analyses were carried out on mycological samples using a sterile swab from the scalp of subjects. Culture conditions were validated for each *Malassezia* strain originating from the Westerdijk Fungal Biodiversity Institute collection centre (Utrecht, the Netherlands, Table 1). Media were tested for each new production batch. Incubation conditions were monitored at the beginning and end of the study. Antibiotics were added to the culture media to suppress prolific bacteria; microscopic examinations checked for the efficacy of antibiotics.

To isolate and cultivate *Malassezia* species, isolation procedures and growth conditions defined by Leeming and Notman (1987) and Sandström Falk *et al.* (2005) were followed. Inoculation on a Leeming medium modified with antibiotics and a Sabouraud medium (AES-Chemunex) with chloramphenicol (8 to 10 days at 30 to35 °C) was performed to isolate *Malassezia* yeasts (*M. furfur, M. globosa, M. restricta, M. obtusa, M. pachydermatis, M. sloofiae* and *M. sympodialis*). The colonies obtained were subcultivated on the surface of LNm agar and incubated for 48 h before being identified.

Table 1. Selected *Malassezia* reference strains (Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands).

Strain	Reference number
Malassezia furfur	CBS 7019
Malassezia globosa	CBS 7986
Malassezia sympodialis	CBS 7977
Malassezia obtusa	CBS 7876
Malassezia slooffiae	CBS 7971
Malassezia restricta	CBS 7991

Statistical analyses

Clinical scores of free and adherent dandruff, scalp erythema and seborrhoea, as well as criteria for the general scalp microbiota were analysed using a repeated measurement model for quantitative data (SAS Proc Mixed). The clinical scores for self-assessment (pruritus, greasiness, irritation, redness, perception of the scalp, stretching sensation, and dandruff) were analysed using a repeated measurement model for qualitative data ('Generalised Estimating Equations' approach, SAS, Proc GENMOD). In both cases, a covariance pattern model with factors time, treatment, and the interaction treatment×time was accounting for the within-subject correlation between measurements. Overall efficacy at the end of the study (day 57) was analysed using a Wilcoxon test for independent groups. The two-sided significance threshold was set at 5%. All analyses were carried out using SAS statistical software version 9.3, SAS Institute, Cary, NC, USA.

3. Results

A total of 60 healthy male volunteers meeting all study inclusion criteria were included; two in the ST11 group discontinued from the study upon their request. Detailed demographic and clinical data at day 1 are shown in Table 2 and Figure 1.

Table 2. Summary of general characteristics of subjects at day 0.

	Placebo (n=30)	ST11 (n=28)[2]
Age (years)[1]	40±9	40±11
Phototype		
I	0	0
II	5	1
III	19	19
IV	6	10
Size (cm)[1]	175±7	173±8
Weight (kg)[1]	73±11	69±9

[1] Values are mean ± standard deviation.
[2] ST11 = *Lactobacillus paracasei* NCC2461 ST11.

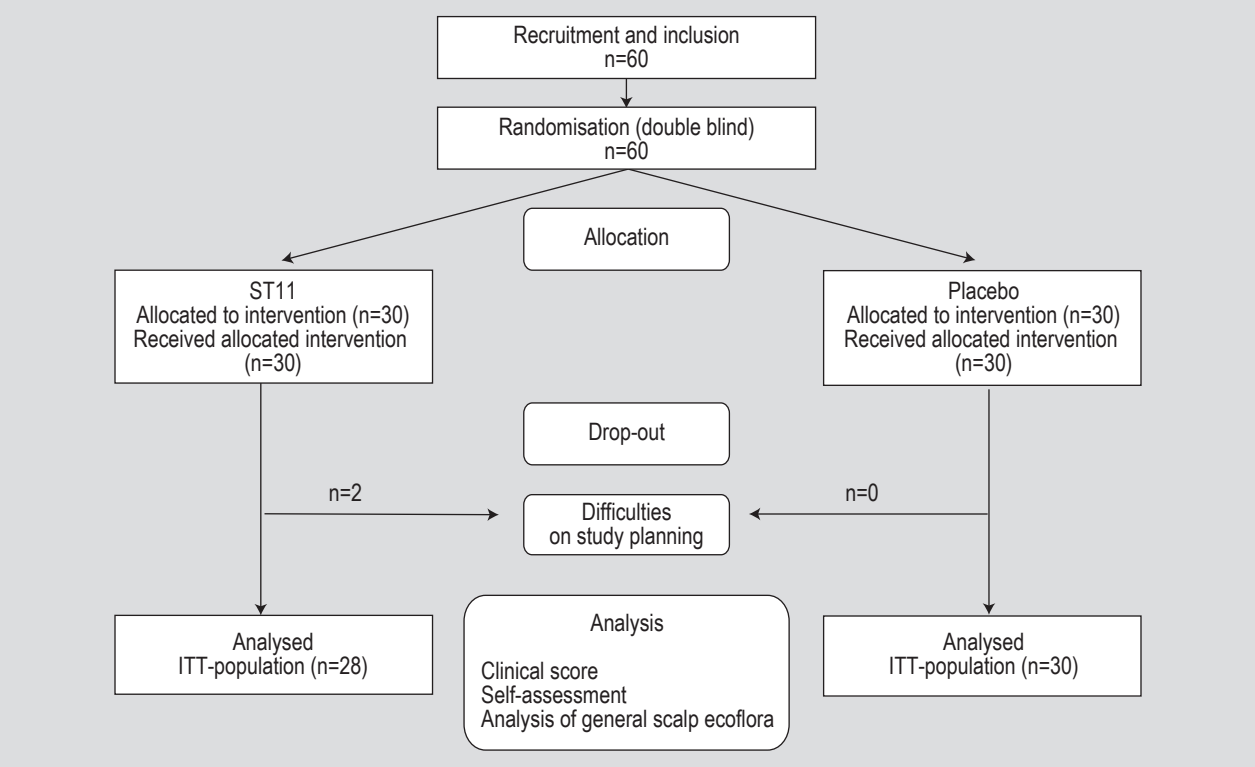

Figure 1. Flow diagram of the trial.

Efficacy

During the wash-out period (day -42 to day 1), no variation was observed for both dandruff scores (free and adherent) within the two groups of treatment in volunteers included in the study. Both groups ST11 and the placebo, were comparable on day 1. Both dandruff scores started decreasing significantly ($P<0.05$) in both groups after two weeks of supplementation sustaining until day 57 (Figure 2). When comparing day 1 versus day 57, a 70% reduction of the free dandruff score for ST11 was observed versus 23% for the placebo (day 1-57 placebo/ST11 $P=0.0005$). Reduction of the adherent dandruff score was 72% for subjects treated with ST11 and 34% for the placebo when comparing day 1 versus day 57. The between-group difference was statistically significant in favour of ST11 ($P=0.0005$). One week after discontinuation of the product (day 64), the residual effect of ST11 was still significantly superior compared to that of the placebo for both, free and adherent dandruff ($P=0.0403$ and $P=0.0002$, respectively comparing day 57 versus day 64).

The erythema score had significantly decreased over time in both groups between day 1 and 57 (Figure 3). The reduction of the erythema score between day 1 and 57 was significantly ($P=0.0469$) in favour of ST11 with a 58% reduction of the erythema score with ST11 versus a 31% reduction with the placebo. At day 1, a significant between-group difference ($P=0.0295$) for scalp seborrhoea was observed. Therefore, any comparison between the experimental groups should be considered carefully. Despite this issue, the scalp seborrhoea score decreased significantly over time in both groups after two weeks until day 57 ($P\leq0.0001$; Figure 4), and had decreased more importantly in the ST11 group than in the placebo group ($P<0.05$). At the end of the study, the scalp seborrhoea score with ST11 had reduced by 46% and by 37% with the placebo.

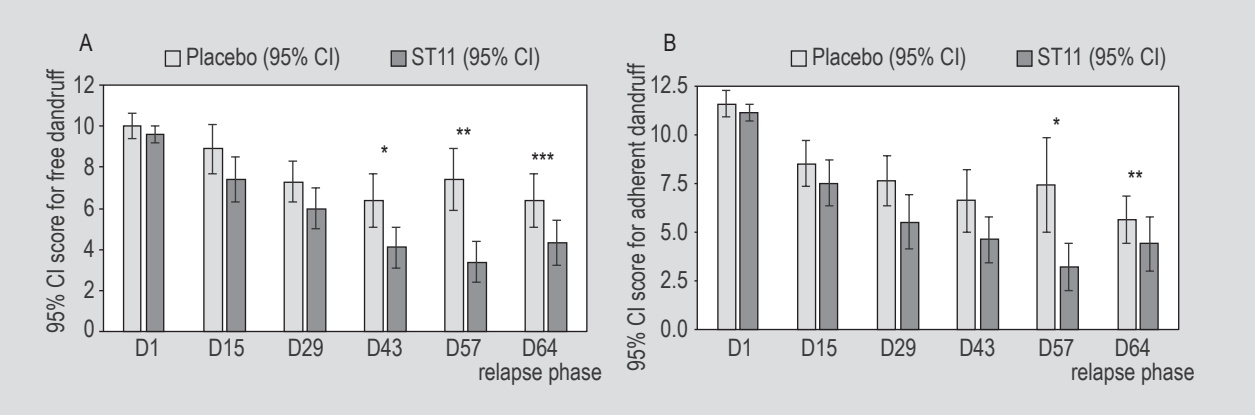

Figure 2. Evolution over time of free and adherent dandruff. (A) Evolution of scores for free dandruff over time (in days). * Day 1-43 placebo/ST11, *P*=0.0417; ** Day 1-57 placebo/ST11 *P*=0.0005; * Day 57/day 64 placebo/ST11, *P*=0.0403; Results were expressed as mean and mean confidence interval. (B) Evolution of scores for adherent dandruff over time (in days). * Day 1-57 placebo/ST11, *P*=0.0005; ** Day 57/day 64 placebo/ST11, *P*=0.0002; Results were expressed as mean and mean confidence interval.**

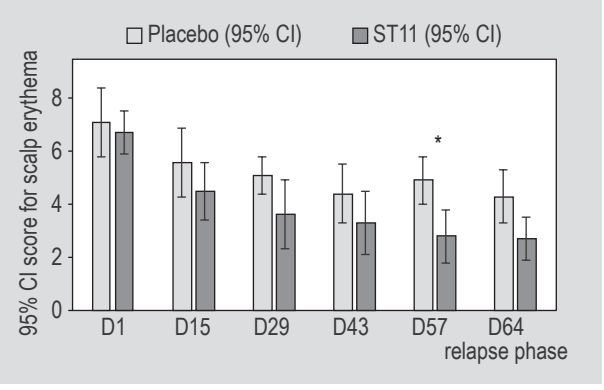

Figure 3. Evolution of scores of scalp erythema over time (in days). * Day 1-57 placebo/ST11, *P*=0.0469. Results were expressed as mean and mean confidence interval.

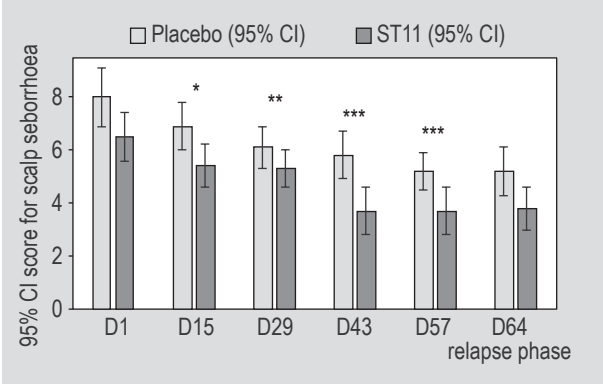

Figure 4. Evolution of scores of seborrhoea of the scalp over time (in days). * Day1-15 placebo/ST11, *P*=0.0389; ** Day 1/day 29 placebo/ST11, *P*=0.00235; * Day 1/day 43 placebo /ST11, *P*<0.001; *** Day 1/day 57 placebo/ST11, *P*<0.001; Results were expressed as mean and mean confidence interval.**

The global clinical score combining free and adherent dandruff as well as erythema significantly decreased over time in both groups when comparing day 1 versus day 15 and sustained until day 57 compared to day1 (Figure 5). At that visit, the difference was significantly in favour of ST11 compared to the placebo (P=0.0002; day 1/57). The global score was reduced by 45, 56 and 66% on day 29, 43 and 57, respectively with ST11. Conversely, reductions with the placebo reached 30, 39 and 31.3 on day 29, 43 and 57, respectively. At day 64, the residual effect of ST11 remained significantly superior over that of the placebo (P=0.0002; day 57/64).

Overall, ST11 improved significantly (P=0.001) the dandruff condition in 25/28 volunteers (89.3%) compared to 20/30 volunteers (66.7%) with the placebo (Figure 6). For all self-assessed signs, the efficacy of ST11 was rated superior compared to that of placebo. This difference was significant (all P<0.05) for dandruff, pruritus, scalp greasiness and overall perception (Figure 7). One week after the end of the study, ST11 continued to be considered superior to the placebo in managing self-perceived signs and symptoms of dandruff.

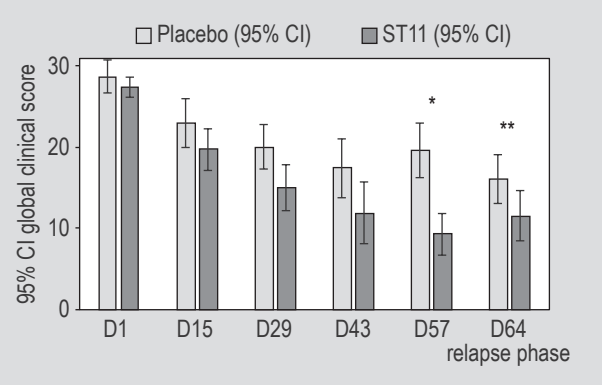

Figure 5. Evolution of scores of global clinical score over time (in days). * Day 1/day 57 placebo/ST11, *P*=0.002; ** Day 57/day 64 placebo/ST11, *P*=0.003; Results expressed as mean and mean confidence interval.

Safety

During the study, no adverse events related to the products were reported. Both ST11 and the placebo were well tolerated.

Impact on the scalp microbiota

The scalp microbiota analysis included a quantitative assessment of total, anaerobic and aerobic bacteria represented by Gram positive cocci. Moreover, a more specific identification of *Escherichia coli*, and coagulase-positive staphylococci including *S. aureus* was performed.

At day 1, the two groups were not comparable for the total mesophilic aerobic bacteria (P=0.0284) with no intragroup variation in terms of time irrespective of the treatment. However, both groups were comparable at day 1 for the Gram positive bacteria count, essentially coagulase-negative staphylococci (*Staphylococcus epidermis* and *Staphylococcus capitis*) and streptococci. The Gram positive bacteria count showed no variation over time for the placebo between day 1 and 57 and had significantly decreased between day 1 and 57 in the ST11 group (P=0.0272).

A significant decrease over time between day 1 and 57 in the placebo (P=0.0124) and in the ST11 group (P=0.0162) was observed for the anaerobic bacteria, especially for propionibacteria. Regarding the total *Malassezia* species amount, no statistical change at any time point versus day 1

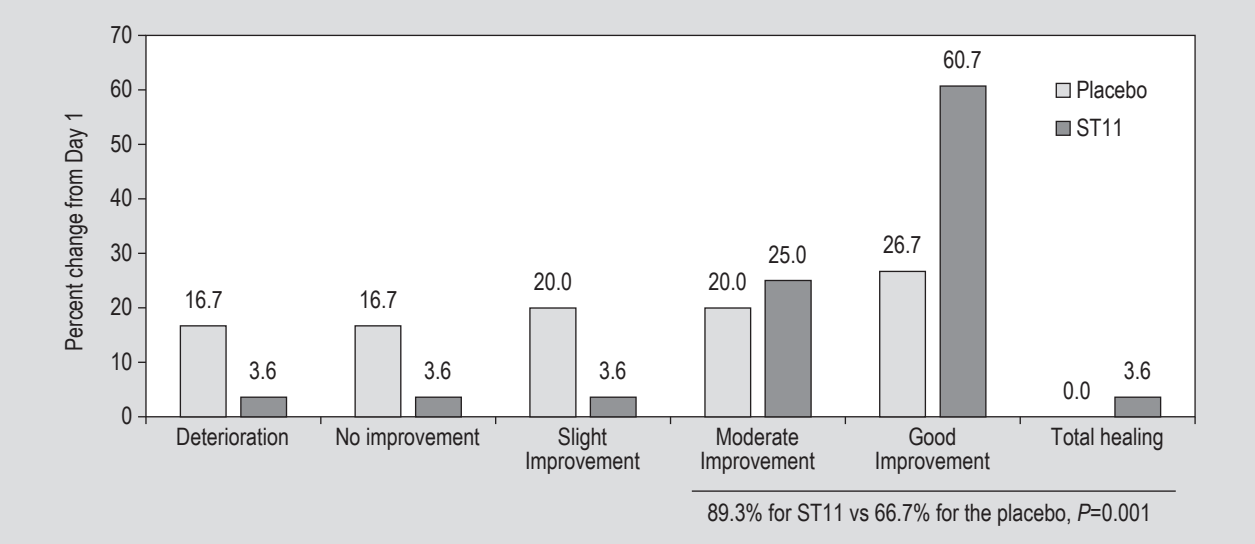

Figure 6. Global clinical improvement (%) on day 57 from day 1.

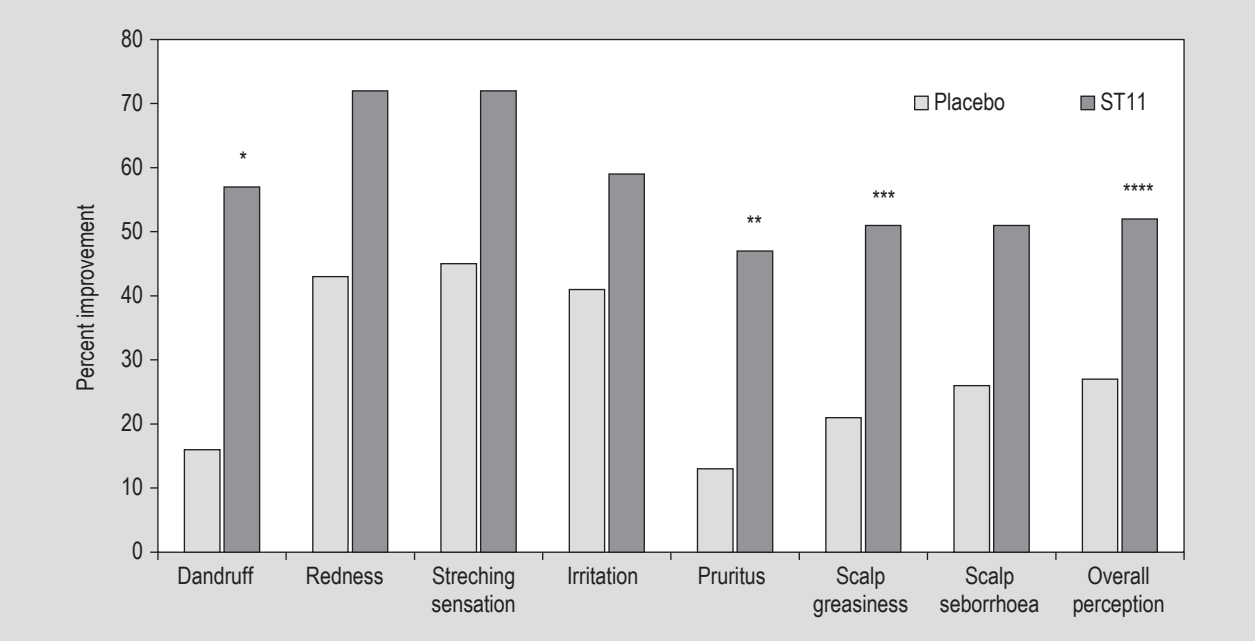

Figure 7. Percent improvement of severity of self-assessed dandruff signs and symptoms at day 57 from day 1. * *P*=0.0014; ** *P*=0.0427; * *P*=0.030; **** *P*=0.0018.**

was observed in the placebo group. Between day 29 and 1 a tendency (P=0.08) to decrease was observed with ST11; the difference was significant between day 43 and 1 (P=0.0002) and day 57 and 1 (P=0.0002). *M. obtusa, M. sympodialis, M. sloffiae* and *M. furfur* were present in very small amounts and no differences were found along the treatment when the two groups were compared. In subjects having received the placebo, a statistically significant increase of *M. restricta* and *M. globosa* between day 15, 29, 43, 57 compared to day 1 (P=0.0713; P=0.0014; P=0.0004; P=0.0003) was observed. Over the same time, the number of *M. restricta* and *M. globosa* remained stable in the ST11 group. The difference between the two groups in terms of evolution was statistically significant in favour of ST11 between day 1 and 29 (P=0.0446), day 1 and 43 (P=0.0143) and from day 1 and 57 (P=0.0111).

4. Discussion and conclusions

Results from this study showed that a 56-day supplementation with ST11 at 10^9 cfu/day has a significant and safe global anti-dandruff action. Changes in hygiene practices required by the protocol and especially the use of a mild shampoo (3 times a week from the start of the wash-out phase on day -42) reducing the dandruff score led to the discontinuation of 14 volunteers from the study prior to inclusion. The included volunteers showed no variations in their dandruff score during these 42 days of using a mild shampoo. Therefore, the placebo effect, observed during the first weeks of the study on all clinical or self-assessment parameters was not considered to be caused by any changes in hygiene habits. However, the change might be due to the natural course of the pathology as dandruff follows cyclic stages of progression. Moreover, the observed placebo effect is well-known and frequently observed with oral administration of pharmaceutical or nutritional products in clinical studies and may be related to the benefits expectations of the subjects, as explained by Howland in 2008 (Howland, 2008a,b), especially if the study is conducted at an investigational site known for its expertise in the target domain by participants in clinical studies.

However, the observed placebo effect did not last long enough to improve the conditions as shown throughout this study: ST11 stabilised the colonisation by *M. globosa and M. restricta* on the scalp after 56 days of supplementation, while at the same time point it had significantly increased in subjects who received the placebo. These findings confirm that in individuals with dandruff, the increased number of *M. restricta* and *M. globosa* yeasts leads to disequilibrium between the proportion of the major bacterial and fungal populations (Clavaud *et al.*, 2013; Wang *et al.*, 2015). The probiotic also significantly decreased the severity of dandruff, pruritus and erythema after 56 days of continued daily supplementation. These results were paralleled by the volunteers' self-assessments.

Last, this clinical study supports recent clinical research work demonstrating that ST11, known for more than 2 decades for its beneficial effect on the intestinal immune system and in allergic patients throughout numerous studies, also exerts its effects beyond the gut (Bunout *et al.*, 2004; Chouraqui *et al.*, 2008; Ibnou-Zekri *et al.*, 2003;

Sarker *et al.*, 2005; Von der Weid *et al.*, 2001; Wassenberg *et al.*, 2011).

Undeniably, it seems as if ST11 confers a certain number of benefits at the skin level in contributing in the reinforcement of the skin barrier function, and in the modulation of the skin immune system leading to the preservation of the skin homeostasis especially in dry and sensitive skin and the rebalancing of the microbiota of the scalp (Benyacoub *et al.*, 2014; Gueniche *et al.*, 2010, 2014; Philippe *et al.*, 2011).

ST11 restores the skin barrier function altered by repeated tape stripping. This effect has been successfully and consistently demonstrated in women with reactive skin (18-35 years old) (Gueniche *et al.*, 2010) as well as in men over the 60's (Gueniche, unpublished data) following a 2-month daily consumption of 10^{10} cfu/day of ST11. Additional data obtained in an *in vitro* model using reconstructed skin (Gueniche *et al.*, 2010) and in preclinical studies using Skh/hr1 mice (Philippe *et al.*, 2011) confirm this effect. The efficiency of ST11 in modulation of skin inflammation is supported by data from *in vitro* and animal studies. In an *in vitro* model using a human abdominal plastic surgery explant, ST11-conditioned medium down-regulates skin inflammation induced by a neuropeptide (substance P) (Gueniche *et al.*, 2010), whereas in mice, ST11 antagonises, in a dose dependent manner (10^6 to 10^9 cfu), skin inflammation induced by two challenges of dinitrochlorobenzene (Philippe *et al.*, 2011).

It has been postulated that, subsequently to the interaction between probiotics and the intestinal epithelium, associated immune cells are activated resulting in a release of immune mediators, such as cytokines, into the blood stream. This release may contribute to reinforcing or restoring the skin homeostasis as cytokines are involved in modulating the growth and differentiation of keratinocytes via paracrine and/or autocrine pathways, thus orchestrating the metabolic responses necessary for skin barrier regeneration (Marionnet *et al.*, 2003; Nickoloff and Naidu, 1994).

Probiotics have been demonstrated to target immune functions and gastrointestinal disease, including paediatric rotavirus diarrhoea, antibiotic-associated diarrhoea, *Clostridium difficile* associated diarrhoea, ulcerative colitis, pouchitis, irritable bowel syndrome, or coeliac disease. Moreover, supplementation with probiotic in diseases other than those related to the gastrointestinal tract are increasingly recognised such as microbiota-influenced conditions of diabetes, metabolic syndrome, obesity, atherosclerosis, type 1 diabetes, autism, allergy, asthma, atopic disease and depression (Sanders *et al.*, 2013). The present study confirms the clinical benefit on the skin after oral supplementation with a probiotic. Results supports hypothesis of a gut-skin axis allowing the gastrointestinal microbiota to impact on the skin homoeostasis. This concept has been supported recently by Jeong *et al.* (2016) reporting that alteration of the intestinal microbiota may lead to skin diseases, such as atopic eczema. Hence, it may be hypothesised that probiotics may allow for reducing inflammation throughout induction of Treg cells, which are decisive in limiting inflammation and which may induce mediators that influence the skin barrier homeostasis.

ST11 is safe, as already shown in different clinical trials in infants, children, adults and the elderly (Bunout *et al.*, 2004; Chouraqui *et al.*, 2008; Sarker *et al.*, 2005). Currently used pharmacological active medications, such as corticosteroids, and anti-fungal preparations, are known to be rapidly efficient. However, they may also cause irritation and therefore require shampoos which are not adapted for hair conditioning (Borda and Wikramanayake, 2015). The efficacy of ST11 in dandruff was visible within 2 weeks, while it also decreased irritation and pruritus without interacting directly with the microbiota but in enhancing the rebalancing of the skin homeostasis. In conclusion, regular intake of ST11 over 56 days is safe and significantly reduces the severity of signs and symptoms of moderate to severe dandruff.

Conflict of interest

The authors have no conflict of interest to declare. However, A. Gueniche, P. Bastien and I. Castiel-Higounenc are employees of L'Oreal and D. Philippe and M Renouf are employees of Nestlé.

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