

aurafirm S

DATA PACK



INTRODUCTION.....1-2

aurafirm S PROFILE: FT-IR ANALYSIS.....3

VIABILITY OF LACTOBACILLUS BACTERIA: FLOW CYTOMETRY STUDY.....4

SKIN MICROBIOME: BACTERIAL ADHESION AND GROWTH STUDY (IN VITRO).....5-6

EFFECT OF **aurafirm S** ON DISRUPTED SKIN MICROBIOTA (IN VIVO).....7-10

EXPERTS' PERCEPTION STUDY: HAIR APPLICATION.....11-12

HUMAN REPEAT INSULT PATCH TEST (IN VIVO).....13

REFERENCES.....14

Oat Cosmetics' **aurafirm** ingredients are a family of active ingredients created by the fermentation of Oat COM, our advanced colloidal oatmeal, using a proprietary culture of Lactobacillus.

aurafirm S is a mobile, crystal clear, slightly straw-coloured liquid, rich in highly active water-soluble molecules. This ingredient has been centrifuged and finely filtered to produce a powerful postbiotic material which gives a rapid boost to a disrupted microflora. As well as giving benefits to the microbiome, **aurafirm S** improves radiance and complexion. This ingredient is ideal for clear, colourless products such as serums and toners.

FERMENTATION

aurafirm S is made by the fermentation of 25% of Oat COM (our advanced colloidal oatmeal). Oat COM is fed to the Lactobacillus strain which undergoes a patented fermentation process where Oat COM is completely converted to biomass and organic acids. Fermentation breaks down the cell wall structures of the oat, leading to the release or synthesis of bioavailable molecules as various antioxidant compounds and amino acids. These antioxidant compounds can act as free radical terminators, metal chelators, singlet oxygen quenchers or hydrogen donors to radicals.

The fermentation results in a filtered paste, **aurafirm P** – 5 to 10% of Oat COM, and a filtrate, **aurafirm N** – 1 to 5% of Oat COM. The filtrate can be further processed to create a clear serum, **aurafirm S** – 1 to 2% of Oat COM.

PREBIOTICS

Molecules that promote the growth of beneficial microorganisms on the skin and provide a healthy and balanced diet for skin microbiota.

PHENOLIC COMPOUNDS

Polyphenols are secondary metabolites with antioxidant and anti-inflammatory activity. Phenolic acids are a key class of polyphenols. The fermentation of Oat COM increased the antioxidant capacity of **aurafirm S**.

ECTOIN

Ectoin is a natural substance which is produced by bacteria to protect against extreme conditions. It promotes hydration by maintaining the correct water balance in skin meaning the skin appears smooth and soft preventing dehydration of the epidermis.

POSTBIOTICS

Range of metabolites produced by live bacteria during the fermentation process that help to regulate the composition of the skin microbiome ecosystem.

AHAs

Strains of Lactobacilli can produce α -hydroxy acids (AHAs) to exhibit pH-adjustments and antibacterial activity against most dermal pathogenic bacteria. **aurafirm S** contains hydroxy acids, particularly Lactic acid. Lactic acid can exfoliate by breaking down and dissolving the structure of dead skin cells without causing sensitivity. Lactic acid stays on the outer layers of the skin due to its large molecular size where it works to lift and remove dead skin cells to reveal brighter, more even skin, as well as adding moisture to the outer layers of the skin.

AMINO ACIDS

Amino acids, which are important to the metabolic activity of the living epidermis, are essential in maintaining the integrity of the skin barrier, for protein synthesis and nutrient absorption. Microorganisms must synthesize amino acids in order to grow, develop and perform all routine metabolic functions¹. **aurafirm S** will supply the skin with essential amino acids - mainly glutamic acid and proline.

BIOACTIVE PEPTIDES

The fermentation process produces peptides that help the skin's cellular renewal. Peptides are involved in the modulation of cell proliferation, cell migration, inflammation and protein synthesis and regulation. Peptides have high bioavailability.

CONCLUSION

Fermentation is the enzymatic decomposition and utilisation of nutrients, particularly carbohydrates, by microbes. The process of fermentation enhances the accessibility of actives in Oat COM and this is responsible for the development and improvement of **aurafirm S**, a fermented product.

The levels of bioactive compounds can be modified during fermentation by the metabolic activity of Lactic acid bacteria and enzymes derived from oat. The fermentation process induces structural breakdown of oat cell walls leading to the liberation and synthesis of various bioactive compounds. As the skin is incapable of breaking down large molecules, fermentation allows greater bioavailability of potentially protective and reparative molecules. This bioavailability is because proteins are broken down into peptides and amino acids, many of which are small enough to penetrate past the stratum corneum^{2,3}.

Oat COM, a colloidal oatmeal, contains proteins, polysaccharides and vitamin B. It has been widely accepted as a prebiotic for gut health and data suggests benefits for the skin too as demonstrated in our studies. In addition, Oat COM exhibits prebiotic benefits to enhance growth of healthy bacteria on the skin, its composition makes it an optimal starting material for fermentation.

BACKGROUND

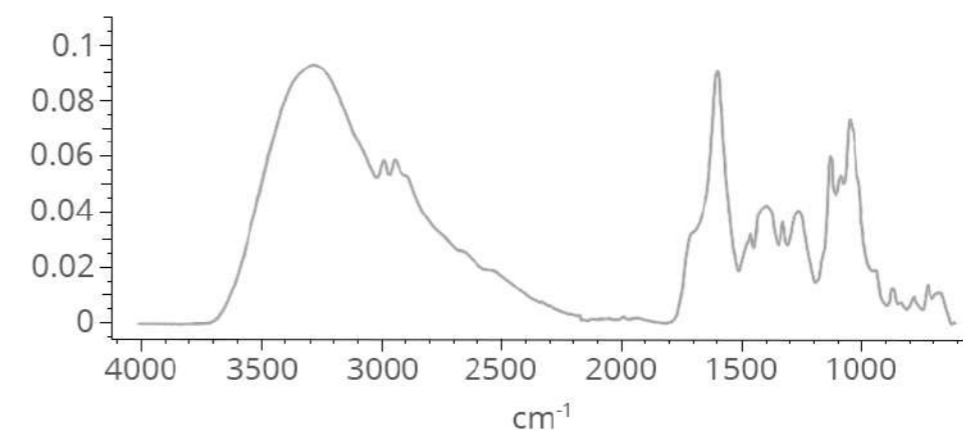
Fourier-transform infrared spectroscopy (FT-IR) was used to profile and create a fingerprint for **aurafirm S**.

METHOD

FT-IR analyses molecules that absorb light in the infrared region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in a compound. The absorption spectrum obtained from the FT-IR analysis indicates the presence of various chemical bonds and functional groups in the sample. FT-IR was used to characterise **aurafirm S** due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infrared range.

aurafirm S is a complex sample that cannot be easily resolved.

RESULTS



Peak Wavenumber (cm ⁻¹)	Associated Chemical Bond
3300-3400	O-H
2900-2950	C-C bonds close to a double bond C=O or particularly C-H bond stretching
1610-1720	C=O or C=C
1300-1400	C-H
1050	C-O
860	C=C-H
680-780	Aromatic C-H

Wavenumber Assignment of FT-IR

CONCLUSION

The peaks around 3300 cm⁻¹, 2900 cm⁻¹, 1720 cm⁻¹ and 1050 cm⁻¹ represent hydroxy carboxylic acids such as lactic acid. This type of compound is the major component of the sample.

The bonds around 1610 cm⁻¹ and 860 cm⁻¹ are likely to correspond to the preservative Sorbic Acid and the peaks around 680-780 cm⁻¹ to the preservative Benzoic Acid.

BACKGROUND

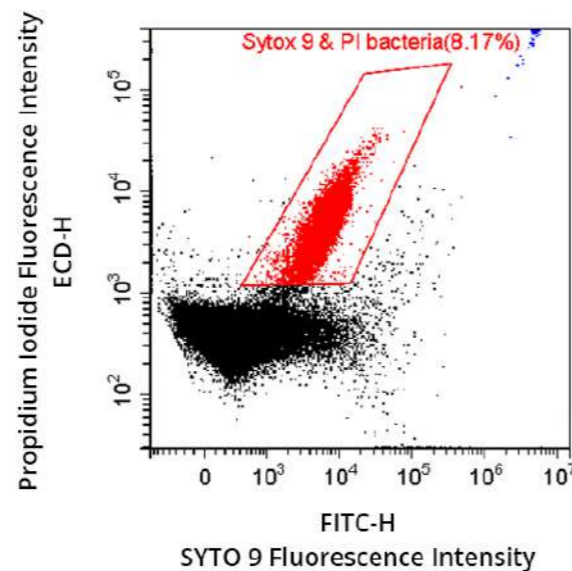
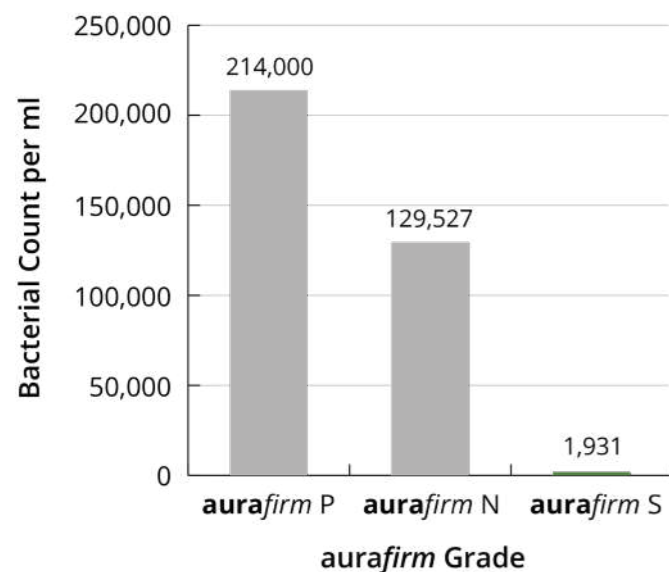
A study was performed to confirm the viability and count the Lactobacillus bacteria in **aurafirm S** using flow cytometry.

METHOD

Using a flow cytometer, **aurafirm S** was passed through a laser light beam to measure the interaction of its components with the light. Fluorescent markers are bound to the Lactobacillus cells and the fluorescence intensity represents the count of bacteria. As with the confocal microscopy, SYTO 9 and propidium iodide were used; the bacteria are dead and will therefore show up red.

The concentration of bacteria was calculated using counting beads as a reference. Three replicates were analysed and an average taken.

RESULTS



Red: Lactobacillus bacteria are identified as dead and make up 8.17% of **aurafirm S**
Black: Represents other digested material

CONCLUSION

The absence of intact bacteria in **aurafirm S** confirms that the Lactobacillus bacteria were lysed during the additional refining step, with Lactobacillus membranes broken down and the inner content of the bacterial cell released into the solution. DNA that is no longer contained within an intact bacterium cannot be observed under confocal microscopy or flow cytometry.

BACKGROUND

An in-vitro study was performed to evaluate the effects of **aurafirm S** on the growth and adhesion of microorganisms. The bacterial communities used in this study represent cutaneous microflora.

METHOD

To monitor the influence of **aurafirm S** on the growth and adhesion of three different bacterial communities, 3 cultural plates were cultivated in a 48-wells plate in presence and absence of **aurafirm S**.

Three different bacterial communities, representing the most abundant phylum, were used in the study:

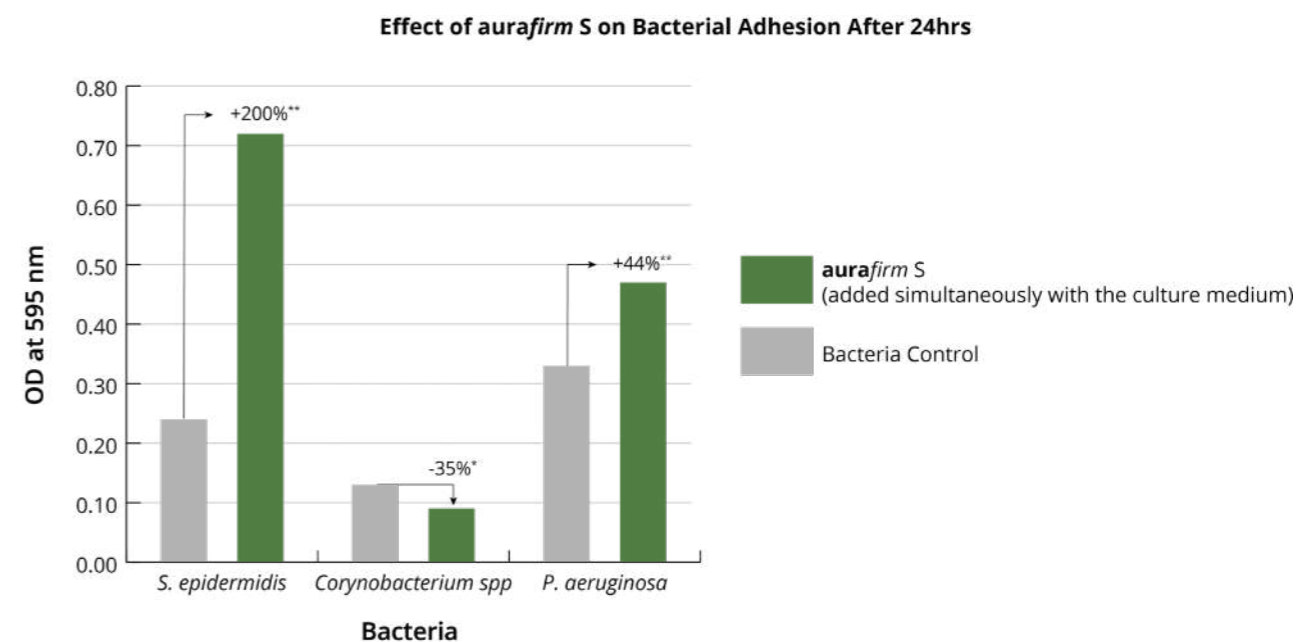
- *Staphylococcus epidermidis* (Firmicute – representing 24% of skin bacteria)
- *Corynebacterium spp.* (Actinobacteria - representing 52% of skin bacteria)
- *Pseudomonas aeruginosa* (Proteobacteria - representing 16% of skin bacteria)

A culture medium with known quantity of each bacteria (colony-forming unit, CFU/ml) was added to the wells of the 3 plates as well as **aurafirm S**, at a concentration of 1% (diluted with culture media), this addition corresponded to the following conditions:

1. Simultaneously with bacteria in the culture medium (during bacterial adhesion)
2. 8h after the culture medium (during the bacterial growth)

After being incubated for 24hrs, the solutions were taken from the wells and optical density (OD) was measured, with a spectrophotometer at 595 nm, to evaluate the quantity of planktonic bacteria.

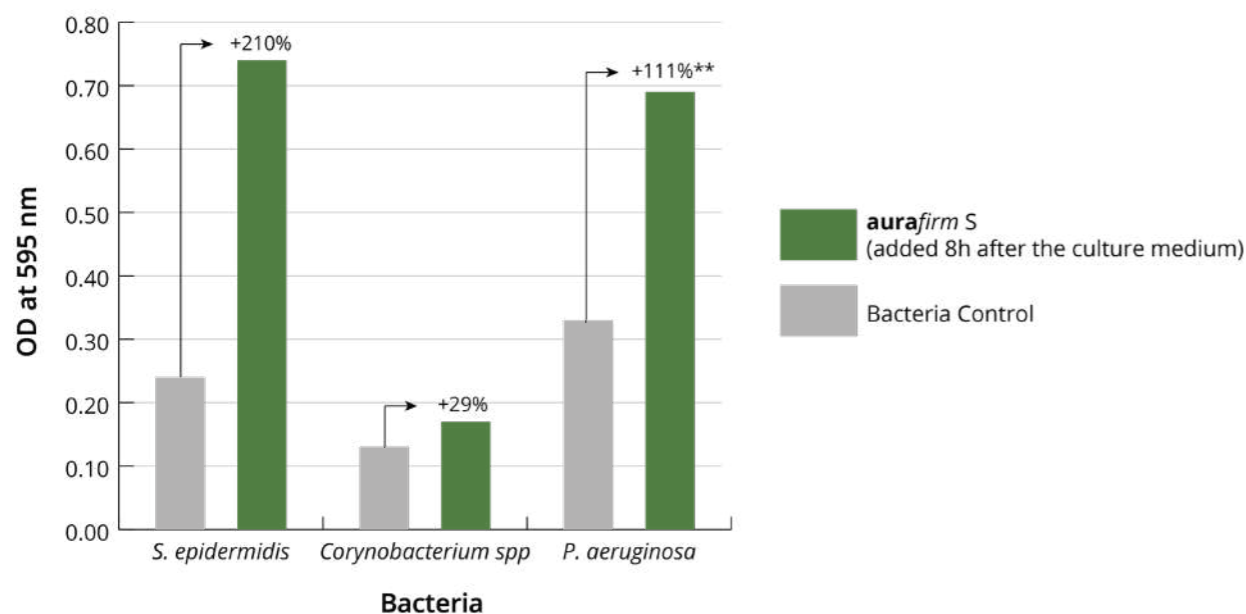
RESULTS



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Significant: * = p<0.05 (95%), **= p<0.01 (99%)

Effect of *aurafirm S* on Bacterial Growth After 24hrs



The results show that:

- Application of 1% *aurafirm S* induces a significant increase, +200%***, on *S. epidermidis* adhesion and on its growth, +210%***, after 24h.
- On *Corynebacterium spp*, *aurafirm S* induces a significant decrease, -35%*, on its adhesion.
- On *P. aeruginosa*, *aurafirm S* induces a significant increase, +44%***, on its adhesion and on its growth, +111%***.

CONCLUSION

The results show that *aurafirm S* induces an increase in growth of all bacteria found on the skin, it is not selective between 'good' and 'bad' bacteria. *aurafirm S* enhanced the adhesion effect and growth of *S. epidermidis* significantly, the most out of all the *aurafirm* grades, showing *S* would be an expectational ingredient to apply to the skin after the microbiome had been severely disrupted, for example after washing with harsh soap.

BACKGROUND

Skin bacteria actively work on maintaining an environment favourable to their survival via interactions with the skin. Constant use of harsh cleansers disrupts your skin's natural bacterial ecosystem. The use of harsh cosmetics which stress the skin can cause skin problems from acne to psoriasis and eczema issues.

Following in vitro analysis, this study was performed to evaluate the ability of *aurafirm S* to rebalance a disrupted microbiota and improve the diversity of the skin microbiota by the determination of the taxonomical composition of microbiota and the calculation of their respective alpha diversity indexes (Observed OTUs, Chao1 and Shannon indexes).

Executive Summary

aurafirm S has been shown to be beneficial for the skin's natural microbiome

- Defends microbiota from external aggression
- Faster recovery of a disrupted microbiome
- Rebalances skin's natural bacterial species' ratios
- Rebalances skin's natural pH

METHOD

For the identification of skin bacteria, a 16S rRNA gene sequence analysis (the Amplicon method)¹ was used. The generated data was compared to a DNA database for taxonomic classification and more than 300 different species of bacteria were identified. This allows an indication to be given of the impact of *aurafirm S* on the whole diversity of skin microorganisms, identify major bacterial phylum and evaluate the ratio between major bacterial phylum (skin microbiota balance).

3 Caucasian women aged between 32 and 37 applied 2% *aurafirm S* on their forearms twice-daily (morning/evening), for 10 days, after the application and rinsing of an ordinary soap: solid savon de Marseille to disrupt skin microflora. The soap was selected to represent an every day soap commonly used by a consumer and is known for being gentle on the skin. *aurafirm S* was diluted with distilled water in the laboratory of the test facility: fresh test solutions were prepared every day to avoid microbial contamination. Samples were prepared by swabbing the skin surface (6 hours after the application of products) and then rinsed with phosphate-buffered saline to collect bacteria from the surface. A standardised protocol was used for extraction of RNA from swabs.

The bioinformatic analysis data was carried out by detection and elimination of chimeras and the clustering of sequences in Operational Taxonomic unit (OTU) at 97% homology.

Additionally, pH of the skin was measured for each sampling (Day 0, Day 1 and Day 10), indicating the effect of the test product on the skin microbiota recovery.

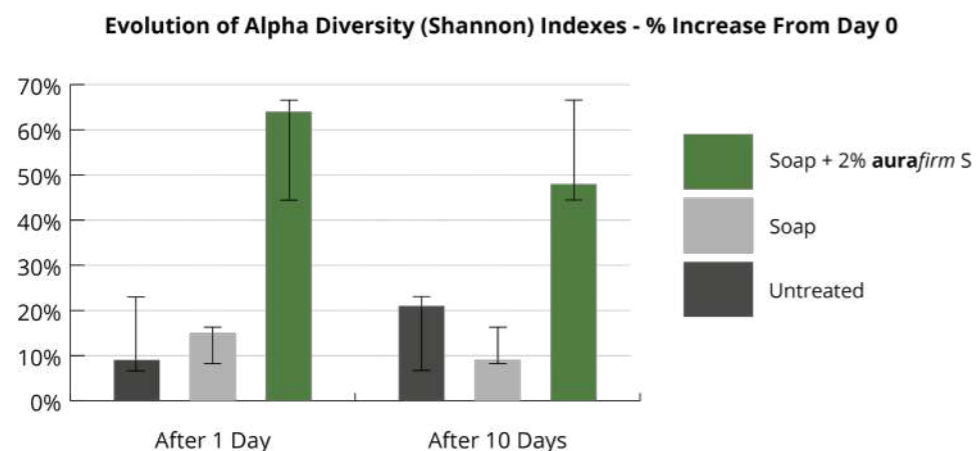
RESULTS

The effect of *aurafirm S* on the skin microbiome was analysed using three parameters: alpha diversity, genus profiling and change in skin pH.

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Significant: * = p<0.05 (95%), **= p<0.01 (99%)

Alpha Diversity

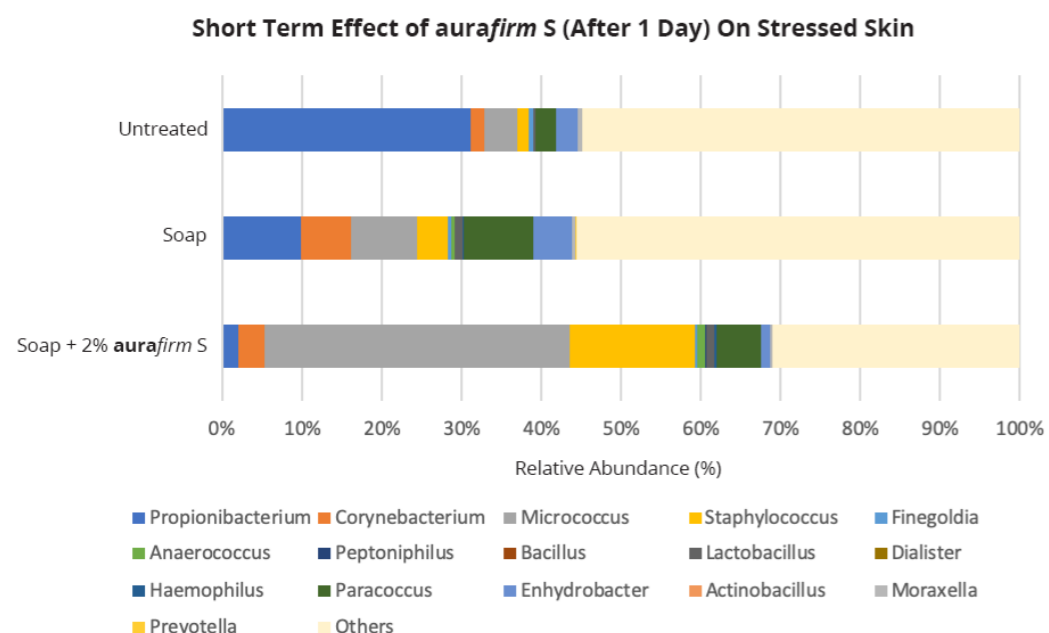


The Shannon index increases as both the richness (the number of species present) and the evenness (their relative abundances) of the community increase. It is well known that an increased biodiversity results in a healthy ecosystem, therefore an increase in Shannon index indicates a healthier skin microbiome².

Results show that washing with soap had a detrimental impact on the skin microflora after 10 days of repeated use. This was chosen to represent to washing habits of an average consumer. An imbalance in the microbial community composition and decrease in diversity is representative of 'unhealthy skin'. The increase in Shannon index shows that the skin treated with 2% of *aurafirm S* was able to recover faster from the use of soap and increase the diversity of the skin microbiome.

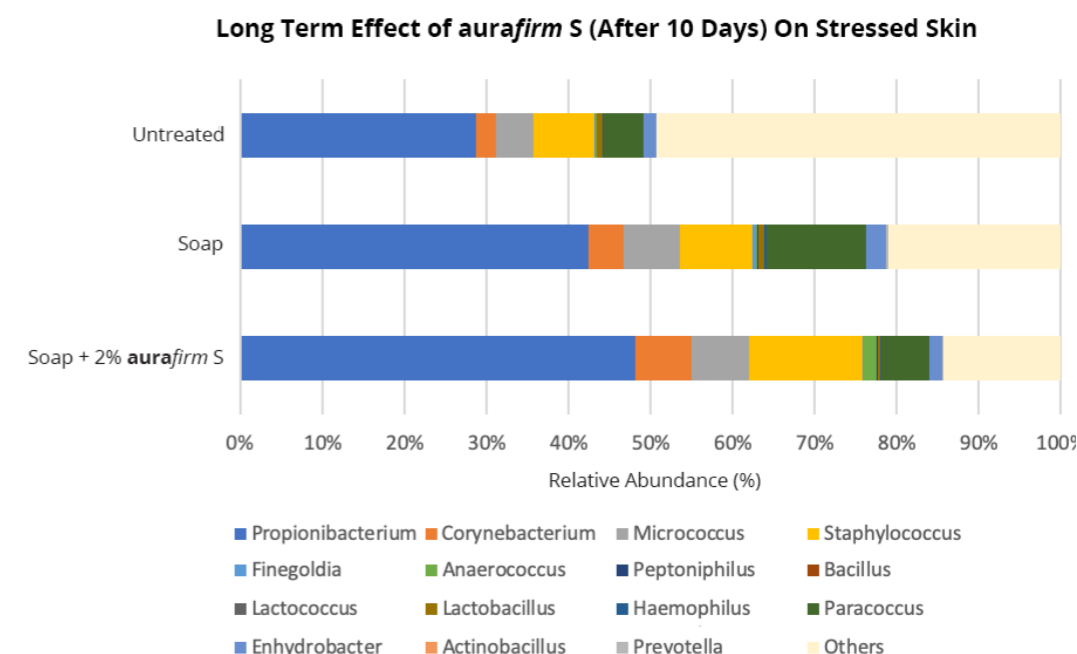
After 1 day of application of 2% *aurafirm S* the skin microbial community composition increased compared to the untreated zone. After 10 days, the soap decreased the microbial diversity of the skin, however application of *aurafirm S* counteracted this effect and increased the diversity compared to soap and untreated.

Genus Profiling



The results show that the use of even a gentle soap disrupts the skin microbiota. The application of 2% *aurafirm S*, after the use of soap, had a positive impact and helped to restore the microbiome after disruption; anaerobic bacteria, potentially pathogenic for the skin, such as *Fingoldia* and *Peptoniphilus* decreased as well as acidophilic bacteria such as *Lactobacillus*. *Corynebacterium* also decreased (resulting in an increase in sebaceous gland activity which stimulate sebum secretion) and so too did *Propionibacterium* (associated with pathogenic skin disorders). 2% of *aurafirm S* increased *Micrococcus* and *Staphylococcus*, which can contribute to regulation of the skin barrier.

After 1 day of application, *aurafirm S* helps to rebalance the skin microbiota after disruption with soap.



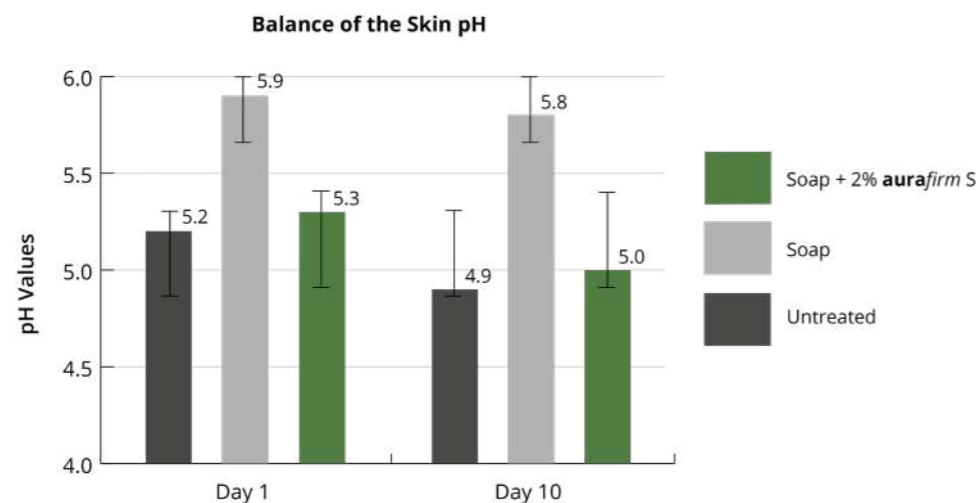
After 10 days, the application of soap disrupts the microbiome less than after 1 day of use. However, 2% *aurafirm S* still has a positive effect on the microbiome compared to the soap, showing an increase in most of the genus. Application of 2% *aurafirm S* increases abundance of *Micrococcus* and *Staphylococcus*. It is observed that *Propionibacterium* and *Corynebacterium* abundance is increased but this is not out of control, as in order to maintain a balanced microbiome both pathogenic and non-pathogenic bacteria need to be enhanced.

After 10 days of application, *aurafirm S* helps to rebalance the skin microbiota quicker than without, after disruption with soap.

Skin pH

The soap used in the study – savon de Marseille (Marseille soap) – is a common cleanser used for the body and face. A healthy skin has a slightly acidic environment: on average between 5.0 and 5.5. This environment is created by the hydrolipidic film, which provides a natural defence for the skin.

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Ordinary soap has a relatively high pH, which can reach 9. As this value increases, it will disturb the natural protective acidity of the skin. The hydrolipidic film is therefore put under strain and is unable to play its hydrating and protective role³.

The results show that the use of 2% of *aurafirm S* rebalances the skin pH and leads to a healthy skin microbiome.

CONCLUSION

Skin cleansing is an important part of the daily routine that allows oil, dirt and sebum to be washed from the skin. However, cleansing using soap can diffuse into the stratum corneum layer of the skin and disturb lipid and cellular structures.

This study shows that regularly washing the skin, even with gentle cleansers, alters bacterial diversity over time. The application of 2% *aurafirm S* can counteract this imbalance and improve skin bacterial quantity and diversity by selectively promoting the growth of certain bacteria on the skin. *aurafirm S* enables quicker recovery of skin microbiota altered by a soap cleanser. Due to its postbiotic nature, *aurafirm S* "feeds" the skin microbiota and facilitates its recovery, which is very useful to preserve skin and microbiome quality. The term 'postbiotic' refers to a range of metabolites produced by live bacteria during the fermentation process. This includes compounds such as short-chain fatty acids, antimicrobial peptides, nutrients and hydroxy acids. *aurafirm S* will act as a postbiotic when applied to the skin due to its high concentration of organic acids, particularly Lactic acid, and will be rich in other metabolites released when it is lysed.

BACKGROUND

Industry hair experts were asked to provide expert analysis on the performance of *aurafirm S* when used as part of a shampoo and conditioner.

METHOD

Shampoo and conditioner products were created using 1% *aurafirm S* and a basic shampoo and conditioner chassis, as well as placebos for both. A set of hair swatches was washed with the shampoo only and a second set of swatches was washed with shampoo and then conditioned.

For dry comb and dry feel assessment, the swatches were dried at 40°C overnight. To analyse wet comb and wet feel, the swatches were gently dried to a level equivalent to towel drying long hair (wet but not dripping).

Trade Name	INCI Name	% w/w
Euxyl PE9010	Phenoxyethanol, Ethylhexylglycerin	1.00
Disodium EDTA	Disodium EDTA	0.02
Steol CS230K	Sodium Laureth-2 Ether Sulfate	45.00
Surfac B4	Cocamidopropyl Betaine	6.00
Salt Pure Vacuum Dried	Sodium Chloride	1.83
<i>aurafirm S</i>	Aqua, Avena sativa Kernel Extract, Lactobacillus Ferment, Sodium Benzoate, Potassium Sorbate	1.00
Deionised Water	Aqua	Up to 100.00

Shampoo Formula

Trade Name	INCI Name	% w/w
Euxyl PE9010	Phenoxyethanol, Ethylhexylglycerin	1.000
Dehyquart A-CA	Cetrimonium Chloride	0.500
Natrosol 250 HHR	Hydroxyethylcellulose	0.400
Citric acid 50% soln	Citric Acid	0.043
Cutina GMS	Glyceryl Stearate	0.500
Lanette O	Cetearyl Alcohol	2.000
Mineral oil 350cst	Paraffinum Liquidum	0.500
Microcare quat BHG	Behentrimonium Chloride, Glyceryl Stearate, Cetearyl Alcohol, Lauryl Alcohol, Myristyl Alcohol	1.000
<i>aurafirm S</i>	Aqua, Avena sativa Kernel Extract, Lactobacillus Ferment, Sodium Benzoate, Potassium Sorbate	1.000
Deionised Water	Aqua	Up to 100.000

Conditioner Formula

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RESULTS

Shampoo

The shampoos all foamed and rinsed out identically. The wet comb conditioning impact of the shampoo containing **aurafirm S** was very noticeable. The shampoo containing **aurafirm S** offered the best effects with good dry comb conditioning and a soft feel.

Conditioner

The swatches treated with both shampoo and conditioner containing **aurafirm S** were much better for wet feel when compared to shampoo alone. Dry comb conditioning and feel improved when using the conditioner containing **aurafirm S**. The conditioner containing **aurafirm S** did not adversely affect the shine of the hair.

CONCLUSION

aurafirm S is moisturising because it contains B vitamins, which act as humectants and penetrate to the deep layers of the hair shaft. The fermentation process breaks down proteins and turns them into amino acids and peptides - smaller molecules which penetrate the cortex of the hair more easily. They have the ability to form a protective film which imparts protection and moisturisation, improving shine and silky feel.

BACKGROUND

A Human Repeat Insult Patch Test (HRIPT) was carried out to determine the cutaneous irritation (contact dermatitis) and sensitisation (contact allergy) potential of 6 oat-derived ingredients (Oat COM USP; Oat Lipid e; AvenaPLex; and **aurafirm P, N, and S**) when applied to the skin of healthy participants.

METHOD

The study consisted of 52 volunteers (male and female aged 20-78) and 3 phases: Induction, in which 10 patches were repetitively applied over the course of 3 weeks; Incubation, a rest period; and Revealing, a challenge phase. Repeated contact with a potential allergen in the formula, if present, generates a series of immunological reactions in the body of the test subject (the volunteer) and induces a visible reaction on the application site. Any reactions were observed, recorded and evaluated by a dermatologist to confirm the allergenicity of the product and hence the product's safety.

Repeated Skin Contact Test (Induction Phase): Prior to applying the patches, the test area - upper back, between the two shoulder blades - was carefully examined. A patch containing the test products and the control was applied to the test area and left in contact with the skin for 48 hours. When this first patch was removed at the laboratory 48 hours after application, the observation area was rinsed with water, dried, and examined for any skin changes. Following the examination, a new patch with fresh test product was applied.

The test products were applied on the selected zones every second day, 3 times per week, over 3 consecutive weeks.

Rest Period (or Incubation Phase): After the completion of the Induction Phase, a Rest Period of 10 to 14 days took place.

Challenge Phase (or Revealing Phase): The application site used during the Challenge Phase was different to the one used in the Induction Phase. For this phase, the patch was removed at the laboratory 48 hours after application. The test site was cleaned and examined for any signs of intolerance or irritation.

Throughout the study, the test products (Oat COM USP; Oat Lipid e; AvenaPLex and **aurafirm P, N, and S**) were applied at 100% except for Oat COM USP which was diluted with Vaseline.

RESULTS

None of the products tested (Oat COM USP, Oat Lipid e, AvenaPLex; or **aurafirm P, N or S**) produced any signs of cutaneous irritation or skin sensitisation. That is, no volunteers showed presence of oedema, vesicles, blisters or ulcerations or reported immediate or delayed reactions such as redness, irritation, itching or other sensations.

CONCLUSION

Oat COM USP, Oat Lipid e, AvenaPLex, **aurafirm P, aurafirm N and aurafirm S** can be considered both hypo-allergenic and non-irritant. Furthermore, given the control provided by a dermatologist during the study, the test products may also bear the claim "tested under the control of a dermatologist" or "dermatologically-tested".

Introduction Pg 1-2

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Effect of aurafirm S on Disrupted Skin Microbiota (In Vivo) Pg 7-10

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