aura*firm* P

DATA PACK

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aura*firm*

DCI: Issue Date 23 Jul 2020

Contents

Introduction

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

aura*firm* P is a creamy liquid with a high concentration of fermented oat bran. **aura***firm* P shows a selective recovery of a disrupted microbiome, boosting the diversity of the microflora in a selective way, favouring useful species such as *S. epidermidis*, giving rise to healthier skin. It also benefits the skin by improving smoothness, radiance and complexion.

FERMENTATION

aura*firm* P 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, **aura***firm* P – 5 to 10% of Oat COM, and a filtrate, **aura***firm* N – 1 to 5% of Oat COM. The filtrate can be further processed to create a clear serum, **aura***firm* 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.

BETA GLUCAN

aura*firm* P contains beta-glucan - long-chain carbohydrates naturally present in oats - which can form a thin film on the skin and keep it moisturised. Research shows that oat beta-glucan can have an effect on skin bacteria by nourishing the bacterial strain of the skin and therefore stimulating the growth of bacteria. Prebiotics are high-fibre food for live bacteria, therefore we can conclude that oat beta-glucans are prebiotics on the skin.[3]

STARCH

Oat COM contains starches, naturally present in oats. They are highly hydrophilic and help to hold in water, which enhances the moisturising abilities of **aura***firm* P. Improvement in starch digestibility during fermentation is due to the break down of starch oligosaccharides. The enzymes bring about cleavage of amylase and amylopectin to maltose and glucose.[1]

ANTIOXIDANTS

Most phenolic compounds in oats are insoluble-bound phenolics that are covalently bonded to the structural components of the cell wall. The crude enzymes from fermented oats had the ability to hydrolyse the bound between phenolics and cell wall macromolecules, leading to the increase of the soluble phenolic content[3]. The fermentation of Oat COM increased the antioxidant capacity of **aura***firm* P.

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.

PROBIOTICS

Live microorganisms which when administered in adequate amounts confer a health benefit by strengthening the skin microbiome.

LACTOBACILLUS BACTERIA

Lactobacillus bacteria can inhibit pathogen attachment to epidermal cells of the skin, by blocking the site of attachment and attracting bacteria of the same or similar species.[4] Data shows that heat-killed bacteria, their fractions or purified components have probiotic effects with advantages over live probiotics.

DIACETYL

Strains of Lactobacillus bacteria can produce diacetyl. It has potential to exhibit dermal antimicrobial activities, with greater sensitivity against Gram-negative bacteria and fungi as compared to Gram-positive bacteria.[4]

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. AHAs can exfoliate the uppermost layer of the skin. AHAs have the ability to hydrate the skin, improve the stratum corneum barrier function and enhance the production of ceramides by keratinocytes. **aura***firm* P contains hydroxy acids, particularly Lactic acid.

AMINO ACIDS

Amino acids, which are important to the metabolic activity of the living epidermis, are essential for maintaining the integrity of the skin barrier, protein synthesis and nutrient absorption. During fermentation, proteins are digested by microbial proteases and peptidases resulting in amino acids.[1] **aura***firm* P will supply the skin with essential amino acids.

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 **aura***firm* P, 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 (amylases, xylanases and proteases). 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

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Introduction (Cont.)

bioavailability is because proteins are broken down into peptides and amino acids, many of which are small enough to penetrate past the stratum corneum[1,2].

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.

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aura*firm* P Profile: FT-IR Analysis

BACKGROUND

Fourier–transform infrared spectroscopy (FT-IR) was used to profile and create a fingerprint for **aura***firm* P.

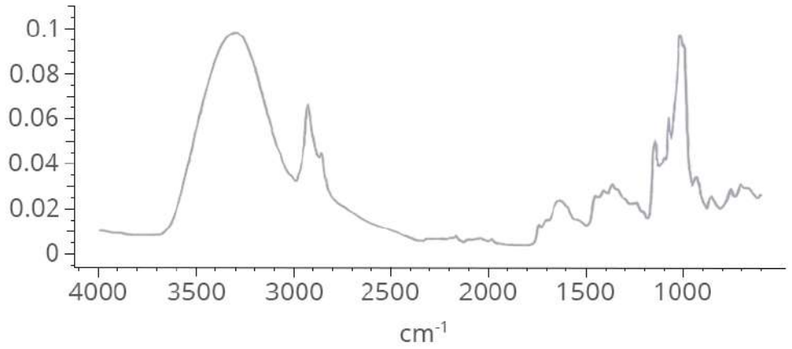
METHOD

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 **aura***firm* P 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.

aura*firm* P is a complex sample that cannot be easily resolved.

RESULTS

The spectrum obtained from **aura***firm* P has very strong peaks at 3300 and 1050 cm^{-1} corresponding to O-H and C-O bonds. There is a minor peak at around 1720 cm^{-1} which represents the double bond C=O, meaning there is a relatively low concentration of carboxylic acid in **aura***firm* P. This spectrum shows that **aura***firm* P contains a great number of polysaccharides or cellulose.



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

Wavenumber Assignment of FT-IR

CONCLUSION

Similar FT-IR spectrums were obtained from **aura***firm* N and S proving that they have a similar content of oat-based material. **aura***firm* P is unique and contains a larger amount of pre-digested oat material.

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Viability of Lactobacillus Bacteria: Flow Cytometry Study

BACKGROUND

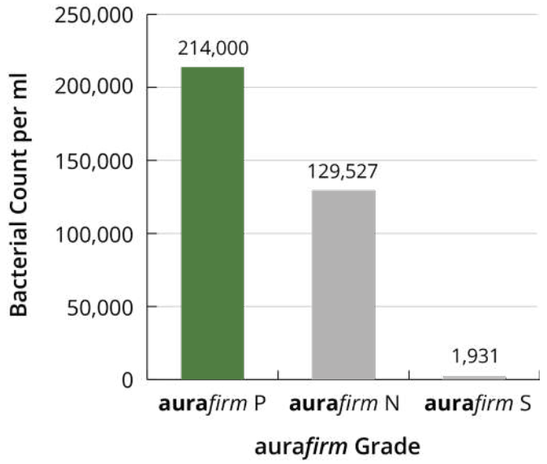
A study was performed to confirm the viability and count the Lactobacillus bacteria in **aura***firm* P using flow cytometry.

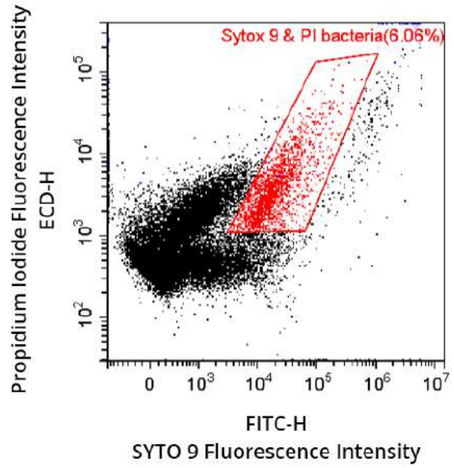
METHOD

Using a flow cytometer, **aura***firm* P 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 was average taken.

RESULTS





Red: Lactobacillus bacteria are identified as dead and account for 6% solid matter in **aura***firm* P - confirming a high concentration of pre-digested oat material.
Black: Represents pre-digested oat material.

CONCLUSION

The results show that Lactobacillus bacteria, deactivated by the pasteurisation step at the end of the fermentation process, are present in all **aura***firm* grades. However, a clear distinction is seen between **aura***firm* P and the other **aura***firm* grades.

The bacterial content in **aura***firm* P is nearly double that of **aura***firm* N, however the flow cytometry results indicate that 6.06% of all solid matter is Lactobaccilus bacteria, meaning **aura***firm* P contains a lot of other solid matter and the bacteria are not free flowing in solution. These results confirm that not only will **aura***firm* P have a probitic like effect on the skin, it also confirms that it contains a high concentration of pre-digested oat material which will act strongly as a prebiotic when applied to the skin.

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Concentration of Lactobacillus Bacteria: Confocal Microscopy

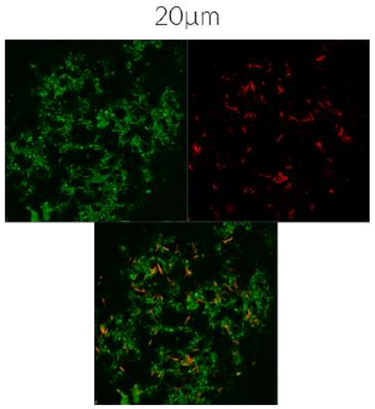
BACKGROUND

A study was performed to evaluate the concentration and viability of prebiotic Lactobacillus bacteria in **aura***firm* P under confocal microscopy.

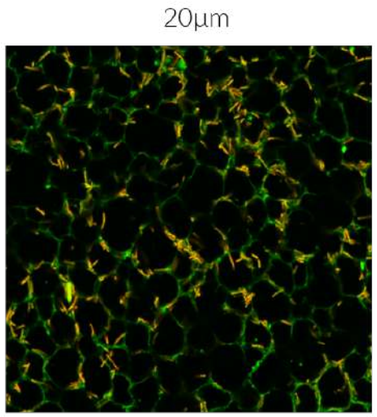
METHOD

In order to monitor the viability of Lactobacillus bacteria in **aura***firm* P, two fluorescent markers, SYTO 9 green (live bacteria) and propidium iodide red (dead bacteria), were added in solution to **aura***firm* P respectively and incubated at room temperature for 20 minutes. The image analysis was performed on a 780LSM confocal microscope.

RESULTS



Bacteria are confirmed dead but remain intact, meaning that they have not been lysed and will act similarly to live bacteria when applied to the skin



The Lactobacillus bacteria are sat on an oat-based matrix, meaning they are not as easily accessible to the skin bacteria and will be slowly released onto skin during application

CONCLUSION

Lactobacillus bacteria is present in **aura***firm* P – the dye has confirmed that these bacteria are no longer alive and have been killed during the pasteurisation stage, however the microscope clearly shows that bacteria are still withholding their shape and the cell wall and membranes are intact. This confirms that the bacteria have not been lysed and will act similarly to live bacteria when applied to the skin.

The orientation of the bacteria in **aura***firm* P is unique - the Lactobacillus bacteria are 'sat' on an oat-based matrix. This orientation will have a significant impact on the probiotic type effect that **aura***firm* P will have on the skin microbiome when topically applied as the bacteria are not as easily assessible to the skin bacteria and are therefore slowly released onto the skin during application.

Independently tested by The University of York, April 2019. Full report available upon request.

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Lignocellulosic Composition of aura*firm* P Biomass

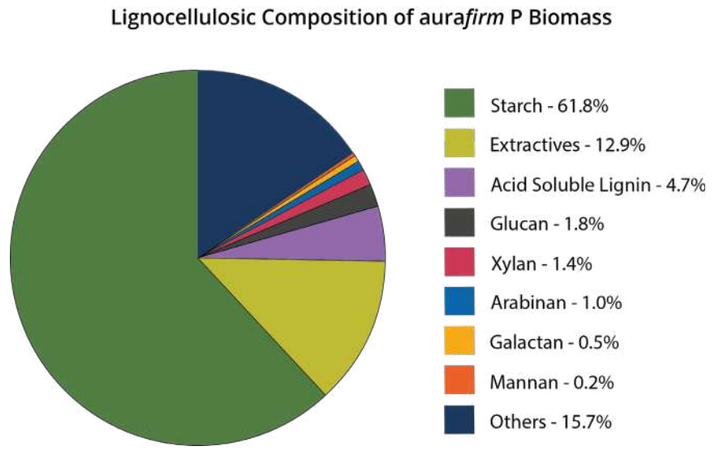
BACKGROUND

The skin microbiome is defined as all the micro-organisms living on the human skin. The microbial development of these organisms is characterised by their growth – this means the increase in cell size and cell mass during the development of a bacteria. Bacteria require basilic parameters for their energy generation and cellular biosynthesis. The growth of bacteria is affected by physical and nutritional factors.

METHOD

The analytical procedure of this study was first to determinate ash content. **aura***firm* P was hydrolysed using sulfuric acid in order to determine the lignocellulosic sugars (glucan, xylan, arabinan, galactan, mannan, rhamnan) content. The hydrolysate is diluted and analysed by ion chromatography (Dionex ICS-3000 system) and Near Infrared Spectrosocopy (FOSS XDS NIR). The chemical bonds that can be analysed, at Infrared wavelengths, are only C-H, O-H and N-H.

RESULTS



The results show that **aura***firm* P is full of polysaccharides (long chain sugars) which attract water, helping to lubricate the skin and correct imbalances. Polysaccharides can form a protective film on the surface of the stratum corneum, avoiding the penetration of external molecules[1].

CONCLUSION

This study shows the benefits of **aura***firm* P composition for skin microbiota, which will provide a healthy and balanced diet for skin microbiota. Glucan or other carbon sources encourage the growth of bacteria as they are an available source of energy for the cutaneous flora.

Consequently, the skin barrier reinforcing activity of **aura***firm* P is due to the polysaccharides: pre-digested oat molecules that are known to have a prebiotic action. These molecules are able to form a skin-protecting film (by protecting bacteria) and maintain stable environmental conditions.

Independently tested by Celignis, September 2019. Full report available upon request.

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BACKGROUND

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

METHOD

To monitor the influence of **aura***firm* P 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 **aura***firm* P.

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

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

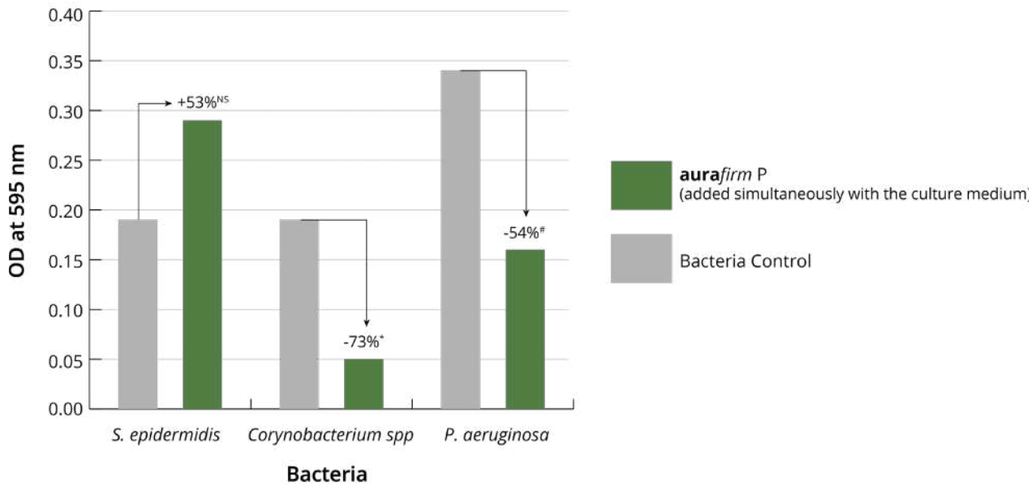
A culture medium with known quantity of each bacteria (colony-forming unit, CFU/ml) was added to the wells of the 3 plates as well as **aura***firm* P, at a concentration of 1% (diluted with culture media), this addition corresponded to the following conditions:

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

Effect of aura*firm* P on Bacterial Adhesion After 24hrs



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Significant: *=p<0.05 (95%), **=p<0.01 (99%), #=p<0.10 (90%) | Non-significant: NS

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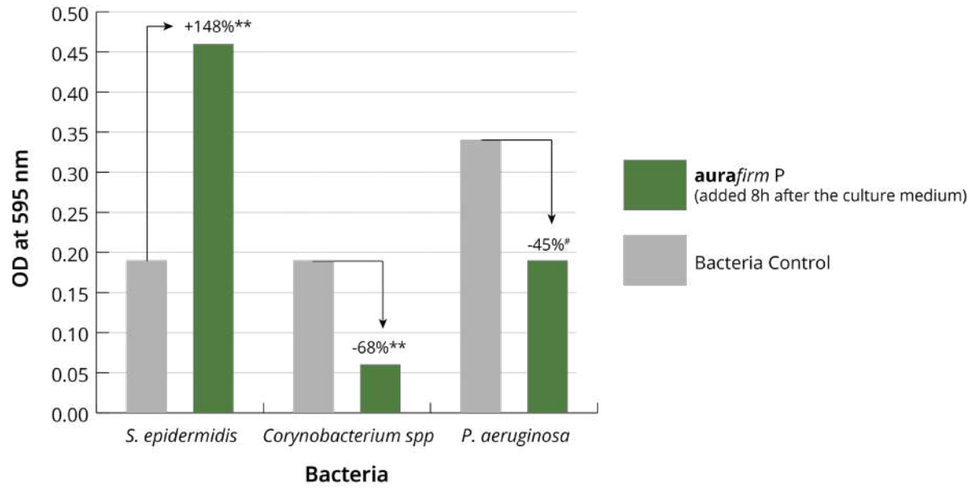
Skin Microbiome: Bacterial Adhesion and Growth Study (In Vitro)

Effect of aura*firm* P on Bacterial Growth After 24hrs



The results show that:

- Application of 1% **aura***firm* P induces a significant increase, +148%**, on *S. epidermidis* growth after 24h
- On *Corynobacterium ssp*, **aura***firm* P induces a significant decrease on its adhesion, -73%*, and on its growth, -68%**
- On *P. aeruginosa* **aura***firm* P induces a significant decrease on its adhesion, -54%#, and on its growth, -45%#

CONCLUSION

The results show that **aura***firm* P has a selective effect on the growth of 'good' bacteria and potentially 'pathogenic' bacteria. **aura***firm* P enhanced the adhesion effect and growth of *S. epidermidis* significantly yet hindered the bacterial adhesion and growth of *Corynobacterium* and *P. aeruginosa*, both considered as pathogenic when growth is not controlled. These two potentially pathogenic bacteria will increase sebaceous gland activity which stimulates sebum secretion. An abundance of these bacteria can lead to Atopic dermatitis or acne. It is reported that *S. epidermidis* can protect the skin by producing lactic acid which lowers the pH of the skin and controls the growth of pathogens. It also regulates the skin by helping to constrain inflammation and repairs it by speeding up the barrier function recovery and enhancing tight junction[1].

Interestingly, **aura***firm* P had a stronger antimicrobial effect on 'bad' bacteria compared to **aura***firm* N, this is due to the 'probiotic' type effect that the deactivated Lactobacillus is having on this bacteria and difference in orientation of this bacteria between P and N.

The skin microbiome is a matter of balance: the higher the diversity is, the healthier the skin will be. The above results have proven that **aura***firm* P will help to rebalance and significantly improve the diversity of common skin bacteria.

Significant: * = p<0.05 (95%), **= p<0.01 (99%), #= p<0.10 (90%) | Non-significant: NS

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Effect of aura*firm* P on Skin Microbiota (In Vivo)

BACKGROUND

The skin is naturally enveloped in its own microbiome and these are unique to each individual, with no two persons microbiome being identical. The skin's microbial biodiversity is heavily influenced by many external and internal factors such as host physiology, environment, lifestyle habits, immune system and ageing which impact its evolution.

Following in vitro analysis, this study was performed to evaluate the ability of **aura***firm* P to maintain diversity and balance 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

aura*firm* P has been shown to be beneficial for the skin's natural microbiome.

- Defends microbiota from external aggression
- Maintains a healthy microbiota
- Maintains skin's natural bacterial species' ratios
- Microbiome friendly
- 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 us to register the impact of **aura***firm* P 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 22 to 31, applied 2% **aura***firm* P on their forearms twice-daily (morning/evening) for 10 days. **aura***firm* P was diluted with distilled water in the laboratory of the test facility: fresh test solution was 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 11) to represent the effect of **aura***firm* P on the skin microbiota recovery.

RESULTS

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

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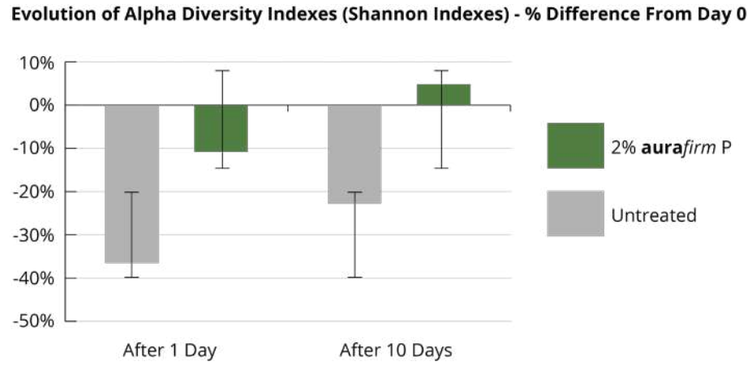
Effect of aura*firm* P on Skin Microbiota (In Vivo)

Alpha Diversity



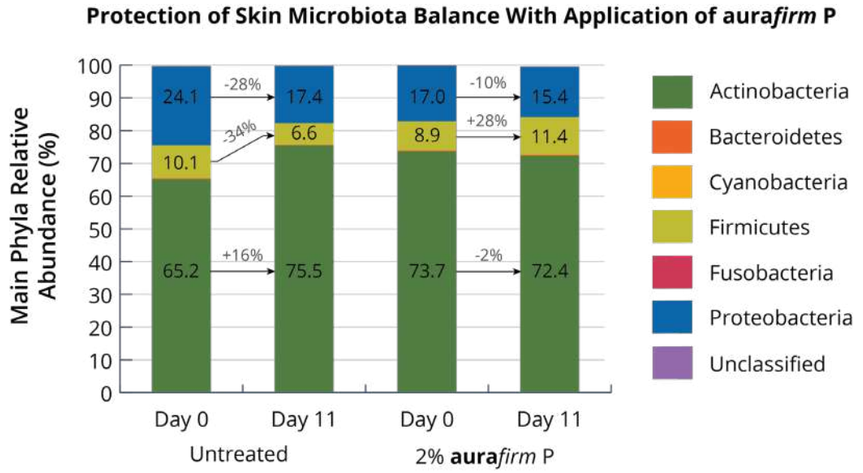
The Shannon index increases as both the richness (the number of species present) and the evenness (their relative abundances) of the community increase. It is well known that an increased biodiversity results in a healthy ecosystem, therefore an increase in Shannon index indicates a healthier skin microbiome[2].

Results show that, after 1 day, skin microbiota diversity is reduced due to external aggression. However, with the application of 2% **aura***firm* P the reduction is less. After 10 days of application of 2% **aura***firm* P we observe an increase in the skin microbiota diversity. Untreated, the skin microbiome composition is still altered after 10 days.

2% aura*firm* P increases biodiversity of the skin microbiota.

Genus Profiling

A closer look at the relative abundance of the main phyla present on the skin shows that application of 2% **aura***firm* P helps to maintain the natural ratio of bacteria on the skin after 10 days application, therefore helping to maintain a healthy microbiome compared to untreated.



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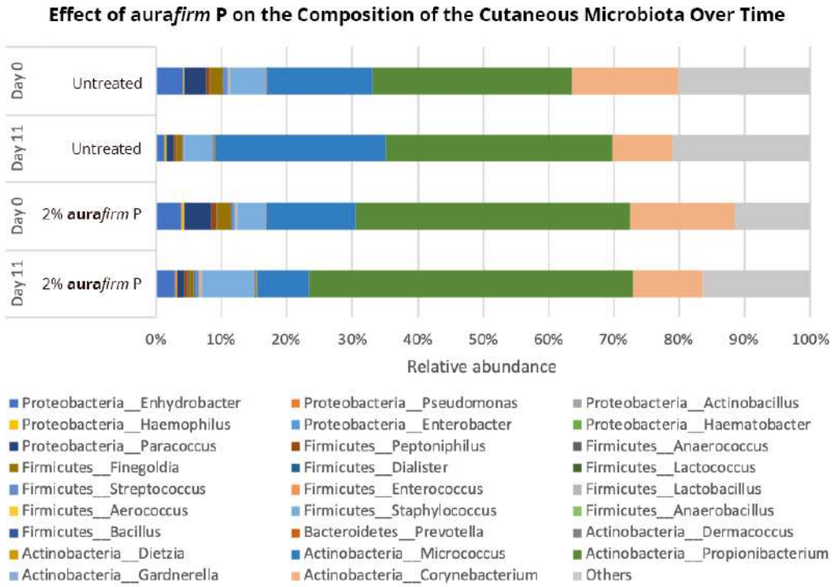
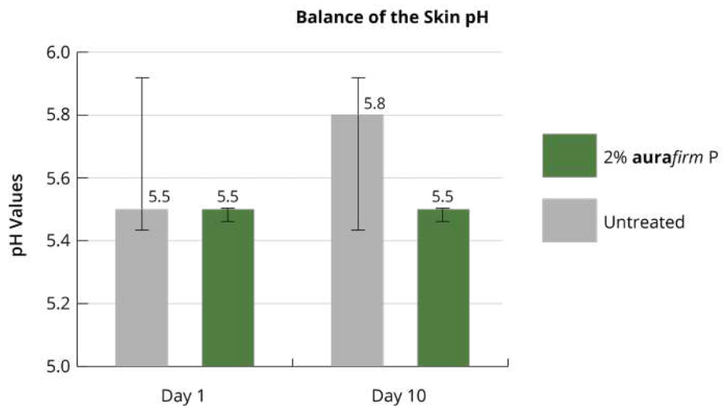


Genus profiling shows us that, after 10 days of application, compared to untreated, aura*firm* P helps to maintain the natural ratio of the skin bacteria and maintain a healthy skin microbiome.

Skin pH

Healthy skin has a slightly acidic environment: this is on average between 5.0 and 5.5. The acidic environment is created by the hydrolipidic film, which provides a natural defence of the skin. A high pH leads to impaired barrier dysfunction and a favourable environment for the growth of *S. aureus* and *S. pyogenes*, contributing to dysbiosis of the skin's microbiome[3].



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Effect of aura*firm* P on
Skin Microbiota (In Vivo)

The results show that the use of 2% of **aura***firm* P maintains the skin pH. This is in part due to lactic acid - a product of lactobaccilus fermentation - present in **aura***firm* P which helps rebalance the skin pH.

The use of 2% of aura*firm* P rebalances the skin pH and leads to a healthy skin microbiome.

CONCLUSION

The equilibrium between protective and pathogenic species of microorganisms in the skin microbiota is important. This balance can be easily disrupted by osmotic stresses, changing of pH, low or high temperature or the use of strong cleansers. The alteration of skin microbiota may lead to dysbiosis which has been associated with skin disorders as inflammation, atopic dermatitis, psoriasis and acne[3].

Results show that the application of **aura***firm* P will maintain the natural ratio of the cutaneous microbial population and protect the balance of a healthy skin microbiome. **aura***firm* P can be considered a 'microbiome friendly' ingredient.

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Skin Radiance Study (In Vivo)

BACKGROUND

An independent study was created and undertaken, with the aim of evaluating the rapid and short-term effects of **aura***firm* P versus a placebo on skin appearance and radiance.

METHOD

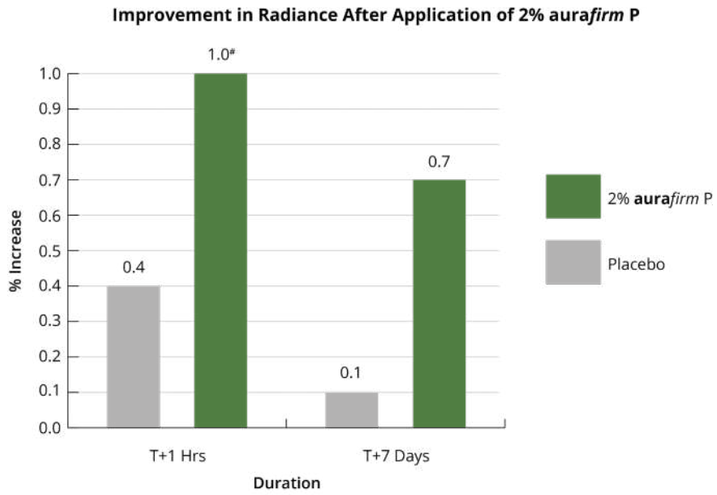
15 volunteers aged between 45 and 60 years old with dry skin were provided with a product containing 2% **aura***firm* P and a placebo and applied them twice daily on each half of the face respectively. The formulations were deliberately simple and contained no other active ingredients.

A measurement of skin colour intensity was performed by colorimetry through chromameter (Spectrocolorimeter CM2600, MINOLTA). Measurements were taken at the following periods:

- On the first visit – T0
- At T+24h
- On the last day of the study, at T+7 days

RESULTS

The Chromameter measured a significant increase after 1 hour of 1.0% when using the product containing 2% **aura***firm* P, compared to placebo. Radiance was also improved after 7 days of use.



CONCLUSION

aura*firm* P significantly improves the radiance of the complexion immediately after application. Thanks to its content of AHA, it will help to stimulate the skin's cell regeneration process, to lock moisture into the skin and give radiance.

Significant: #=p<0.1

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Consumer Perception Study: Skin Application

BACKGROUND

An independent self-assessment study was created, with the aim of evaluating the rapid and short-term effects of **aura***firm* P on overall skin health.

METHOD

20 volunteers aged between 45 and 60 years old with dry skin were provided with a product containing 2% **aura***firm* P and applied it twice daily on one half of the face. The formulation was deliberately simple and contained no other active ingredients.

Assessment of the sensation felt, efficacy and cosmetic quality of the product was performed through a self-assessment questionnaire completed on Eval & Go by volunteers after 1 hour, 24 hours and after 6 days of product application during the study.

RESULTS

Results have been recorded where volunteers scored impressions of the product at 70% or higher.

Organoleptic Qualities	T+1H	T+24H	T+7
Global Appreciation of Product	✓	✓	✓
Feeling of Moisturisation	✓	✓	
Feeling of Suppleness	✓		
Feeling of Firmness	✓		✓
Feeling of Comfort	✓	✓	✓
Feeling of Radiance		✓	✓

The organoleptic qualities of the product containing 2% **aura***firm* P were a feeling of comfort throughout the study period, rapid feelings of moisturisation, suppleness, firmness and comfort within the first hour, followed by a feeling of radiance, firmness and comfort after 7 days.

Product Efficacy	T+1H	T+24H	T+7
Improved Skin Feel	✓	✓	✓
Skin is More Comfortable	✓	✓	✓
Skin is Nourished	✓	✓	✓
The Skin is Suppler and More Elastic		✓	✓
The Skin is Brighter		✓	✓
The Skin has More Radiance		✓	
The Skin is Moisturised		✓	
Unified Complexion		✓	✓
Skin Looks More Uniform		✓	
Feeling of Moisturising lasts Throughout the Day		✓	

The product efficacy results from the questionnaire highlight an improved skin feel from 1 hour to 7 days, the skin feels more comfortable and is nourished throughout. There are noticeable postive effects 24 hours after application.

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Skin Texture Analysis (In Vivo)

BACKGROUND

An independent study was created and undertaken with the aim of evaluating the short-term effects of **aura***firm* P versus a placebo on skin texture.

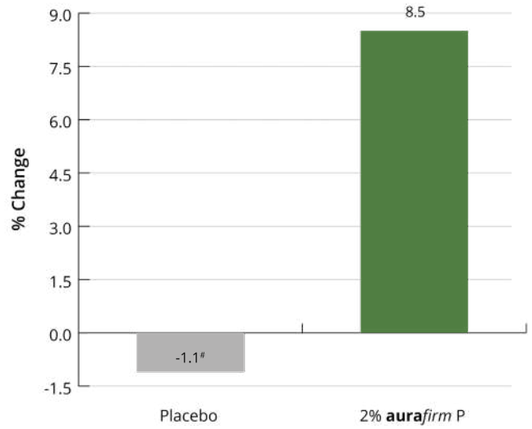
METHOD

10 volunteers aged between 45 and 60 years old with dry skin were provided with a product containing 2% **aura***firm* P and a placebo and applied them on each half of the face respectively. The formulations were deliberately simple and contained no other active ingredients.

A skin texture analysis was undertaken using Licorne for T+1h. The microrelief state of the skin was assessed in the anisotropy index, which enables the effect of a product on the topography of the skin relief to be measured. This index was calculated by analysing grey levels of an image taken using the SkinEvidence® video probe.

RESULTS

Improvement in Skin Texture 1 hour After Application of 2% aura*firm* P



aura*firm* P showed a rapid decrease of 8.5% in skin roughness within 1 hour of application when compared against the placebo.

CONCLUSION

aura*firm* P improves skin texture. Thanks to molecules like amino acids and antioxidants present in **aura***firm* P, it will hydrate and smooth the skin.

Significant: #=p<0.1

Independently tested by Bio EC, Mar 2018. Full report available on request.

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