Expo’biota: identifying impacts and solutions

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ABSTRACT

A study of the characterization of bacterial populations in aerosols of polluted environments was carried out by high-throughput sequencing (16S rRN^{2}A) combined with bioinformatics analyses.

After highlighting some differences in the composition of the air and skin microbiota according to the level of pollution, we were able to show significant effects of a topical treatment versus placebo on specific skin parameters. This involved the development of a plant extract derived from buckwheat seeds (*Polygonum Fagopyrum* syn. *Polygonum fagopyrum*) applied topically *in vivo* to identify its effects on these parameters. We demonstrated the properties of this buckwheat extract applied to a panel of Parisian women naturally subjected to a polluted environment during one month of exposure. Significant evolutions were demonstrated both in terms of biometric and biological characteristics (skin tone, oxidation of stratum corneum proteins) associated with an impact on the cutaneous microbiota.

This first candidate to help the microbiota of skin to adapt to this polluted environment led us to consider a new field of research of interest, the ‘Expo’biota’: ‘expo’ for exposome, ‘biota’ for skin flora.

The mutual relations between cutaneous microorganisms, and their responses to environmental impacts may contribute to skin diseases.[1] The diversity of the skin microbiome is partly determined by intrinsic factors – i.e. gender, age, anatomical site or ethnicity - as well as extrinsic factors related to the exposome. i.e. UV, pollution, smoking, climate.

Today, the direct consequences of the exposome on skin physiology is better characterised,[2,3] but its impact on the cutaneous microbiota is not very well documented. Yet statistical studies show that demographic, lifestyle and physiological factors can collectively explain 12 to 20% of the variability in the composition of microbiomes.[4]

Pollution impacts our microbial composition and some human skin diseases such as acne, atopic dermatitis or eczema are reported to be associated with dysbiosis of skin microbiota.[5] Closely linked to the human immune system through the production of bacteriocins and the induction of antimicrobial peptides, skin flora ensures an effective protection against invading microorganisms and shapes the skin’s immune system as well as its barrier function.[6]

Like other environments such as water or soil, atmospheric air contains circulating bacteria in permanent contact with our skin and its microbiota for which very little data is available. While the water and food we ingest is monitored, the microbial quality of the air is only controlled in specific environments such as operating theatres or cleanrooms. Yet air quality directly impacts the composition of microbial aerosols.

In terms of pollution, bioremediation is a process used to treat contaminated media thanks to living organisms. Therefore, it represents an interesting source of inspiration to develop bioactive solutions adapted to urban skin.

In this context, we screened different plants capable in their natural environment of both decreasing high levels of pollution – known as phytoremediation - and adapting the bacterial composition of its environment to help their development and protect themselves.

From this perspective, we identified buckwheat, a promising plant for phytoremediation used for lead extraction in polluted soils, with an ability to tolerate and accumulate Pb^{2+} as well as other heavy metals.[7]

The rhizosphere of buckwheat can also impact the soil microbiome, with beneficial effects on soil health.[8] It was thus starting from a buckwheat extract that we carried out this clinical study to validate its interest in promoting the adaptation of urban cutaneous microbiota. Moreover, certain bacteria are also particularly studied in bioremediation.

As an example, *Paracoccus aminovorans* is known to have interesting potential for soil depollution.[9] They thus represent promising candidates for bioremediation of PAH (Polycyclic Aromatic Hydrocarbons)-contamination, such as benzopyrene also found in atmospheric pollution and harmful to the entire human organism, including the skin.[10]

Therefore, we have sought to investigate *Paracoccus aminovorans* in this study, both in the polluted air and on the scale of exposed skin. In addition, *Kocuria* sp. are also known for their ability to degrade some adverse pollutants notably contained in crude oils.[11]

The skin microbiome can be modulated via cutaneous transplant or bacteriotherapy.[12] Yet in this study, we are interested in the regulation of certain strains capable of in *situ* bioremediation through the development of plant extracts inspired by phytoremediation.

Materials and methods

Clinical study design

26 female volunteers living and/or working in Paris and its close suburbs with normal skin were included. This double-blind study was carried out versus placebo with an application of these products (placebo and 3% active formula) twice daily for 28 days in randomised hemi-face.

The parameters are measured at T0 (corresponding to the day before the first application) and at T28 (day after the 27th and last day of application) on each hemi-face (placebo versus active formula).

Skin parameters analysis

Regarding radiance and complexion (measurement of L*/a*/b*), acquisitions are made using C-Cube (Pixience) according to the Intertek procedure at D0 (before any application) and D28 (after repeated applications for four weeks).

For skin proteins oxidation level measurements at D0 and D28, D-squame sampling of stratum corneum on each hemi-front and their analysis are carried out. The quantification of total proteins is performed by Bradford method and the samples are evenly distributed for analysis. The carbonyl-containing proteins are labelled using specific fluorescent probes and the proteins are then separated by electrophoresis (SDS-PAGE).

Regarding skin microbiota analysis, at D0 and D28 skin microbiota sampling is performed. The DNA from these samples is extracted, quantified and then sequenced using the Illumina MiSeq high throughput sequencing method.

Characterization of the air pollution

The microbiological signature of the four levels of pollution classified by AirParif (high pollution, moderate pollution, low pollution and very low pollution) was studied in the Paris region by taking microbiological samples using a biocollector (Coriolis μ from Bertin Instruments).

The analysis of the air microbiota corresponds to a sequencing of the 16S RNA for identification of its microbial population and is identical to that performed for the skin microbiota.

Results

Thanks to this clinical study, beneficial properties of this buckwheat extract versus placebo have been demonstrated when applied to skin that was naturally exposed to a polluted environment from a panel of 26 Parisian women for one month.

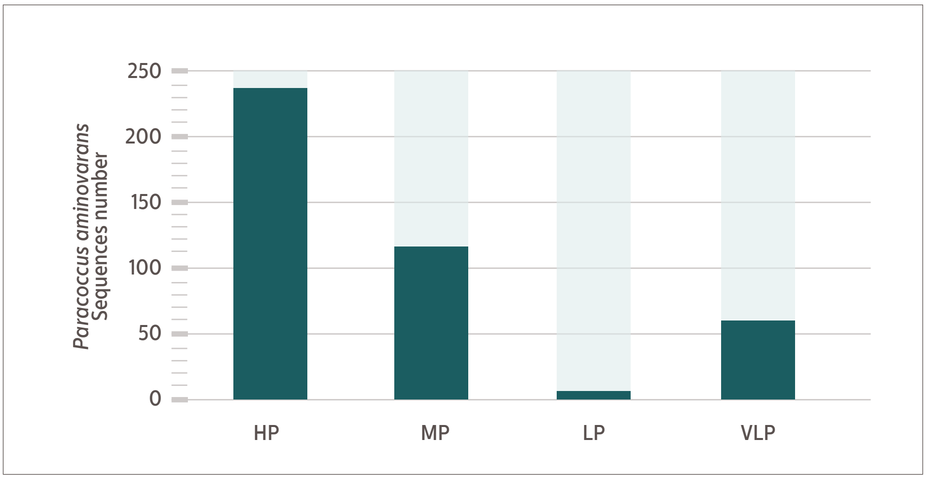

Figure 1: Variations of *Paracoccus aminovorans* sequence number in aerosol microbiome according to the level of pollution. HP: High pollution; MP: Moderate pollution; LP: Low pollution; VLP: Very low pollution

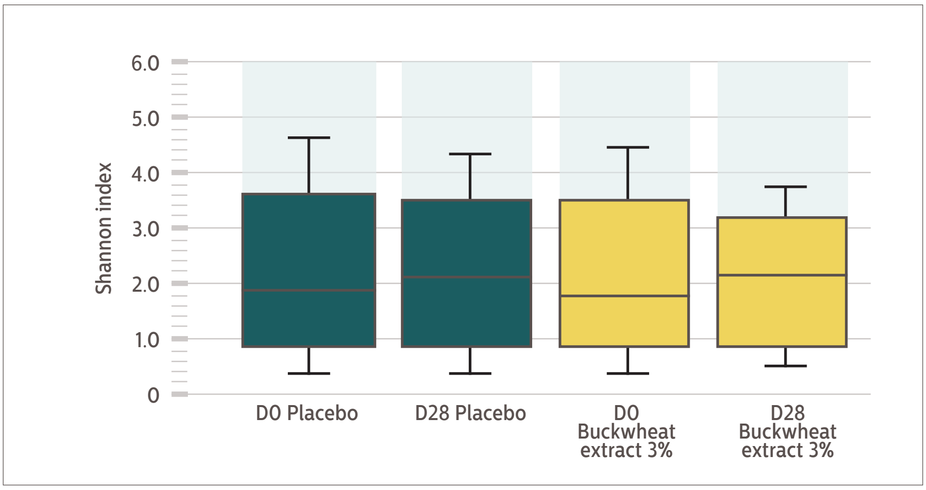

Figure 2: Shannon index of volunteers' microbiota samples before (T0) and after 28 days of treatment (T28) with a placebo or a formula containing 3% buckwheat extract

Differences were thus demonstrated both in terms of biometric characteristics (skin tone) and biological characteristics (oxidation of stratum corneum proteins) associated with an effect on the cutaneous microbiota using 16S RNA sequencing techniques.

We could first note that the more the environment was polluted, the more we observed the presence of *Paracoccus aminovorans*, which are capable of metabolising polluting compounds in the air (Figure 1).

We also proved that buckwheat extract promotes the presence of certain specific bacteria, such as *Paracoccus aminovorans*, at skin level without modifying the balance and biodiversity of the volunteers' cutaneous microbiota, measured by the Shannon diversity index (Figure 2).

At the end of the clinical study, the quantity of this bacteria increased by +139% in the presence of the active ingredient. Thus, the buckwheat extract has a real effect as a promoter of this bacterial species, firstly because of the significant increase observed between T0 and T28 and secondly because of its efficacy, which was on average three times greater than the placebo (Figure 3).

In addition, the study of the oxidation of stratum corneum proteins showed a significant decrease by 20% with buckwheat extract versus placebo (Figure 4). This extract also modifies the skin complexion in polluted conditions by reducing the yellow component and maintaining the pinkish component (Figure 5).

Discussion

This work first highlighted that the more the environment is polluted, the more we observe the presence of certain microorganisms such as *Paracoccus aminovorans*. These specific bacteria are able of metabolising polluting compounds in the air and thus decontaminate their environment.

Such natural pollutant-elimination strategies are interesting at cutaneous level if we consider urban skin. A buckwheat extract developed in this context promotes the presence of certain specific bacteria, such as *Paracoccus aminovorans*, at skin level without modifying the balance and biodiversity of the volunteers' cutaneous microbiota.

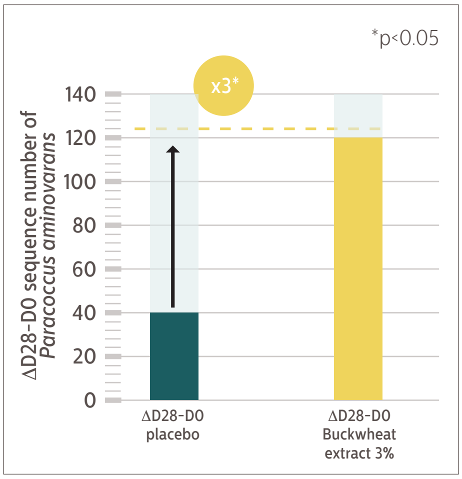

Figure 3: Variations of *Paracoccus aminovorans* sequence number before and after treatment with the active formula (buckwheat extract) versus placebo

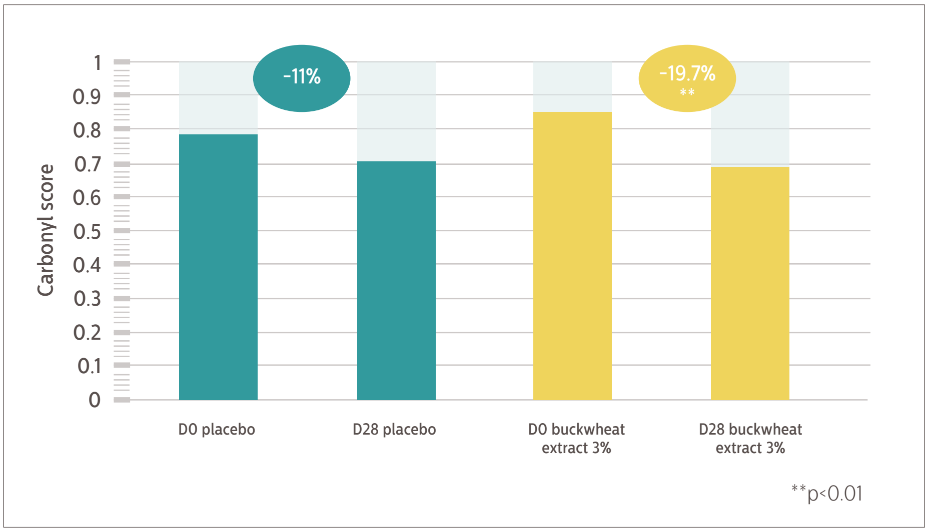

Figure 4: Variations of the Carbonyl Score (rate of oxidised proteins) between T0 and T28 with the active formula (buckwheat extract) versus placebo

Respecting this microbiota balance is essential when addressing the skin flora through the application of cosmetic ingredients.

Conclusion

The microbiota of urban skin is specific, with particularities in terms of composition and some cosmetic actives are able to target these specific characteristics. Thanks to this clinical study, the beneficial properties of this buckwheat extract versus placebo have been demonstrated when applied to skin naturally exposed to a polluted environment.

Significant evolutions were demonstrated both in terms of biometric and biological characteristics (skin tone, oxidation of stratum corneum proteins) associated with an impact on the cutaneous microbiota (*Paracoccus aminovorans*).

This natural active ingredient thus represents a first candidate of interest to help the microbiota of skin exposed to pollution, helping it adapt and limit the consequences of pollution, and paving the way for new developments within the 'Expo'biota' concept. **PC**

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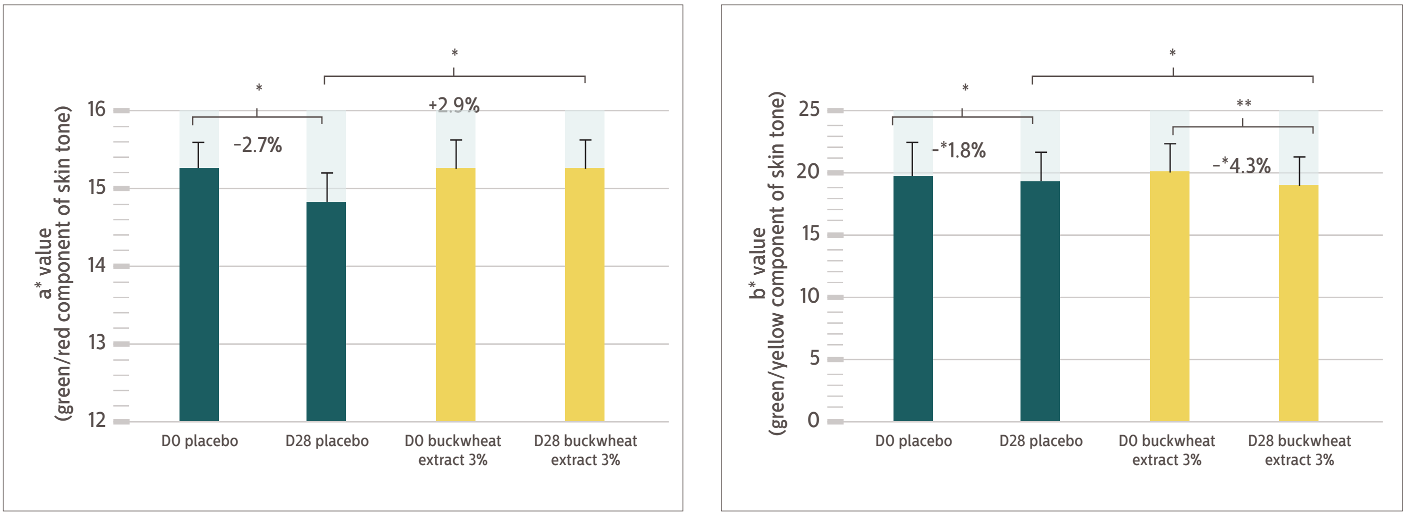

Figure 5: Skin tone. Evolution of a* value of L*/a*/b* (figure on the left) and b* value (figure on the right) before (T0) and after treatment for 28 days (T28) with placebo and buckwheat extract formulated at 3%