

# Androgenetic Alopecia: Microbiota Landscape and Role of *Lindera strychnifolia* Roots Extract as a Natural Solution for Hair Loss

E. Filaire, A. Dreux, C. Boutot, F. Volat, E. Ranouille, J. Demangeon, J.Y. Berthon

## Abstract

The human scalp harbors a vast community of microbial mutualists. Androgenetic alopecia (AGA), a most common form of hair loss in males, is a multifactorial condition involving genetic predisposition and hormonal changes. The role of microflora during hair loss remains to be understood. Here, we investigated bacterial communities in 12 healthy and 12 AGA subjects at baseline and after 84 days of treatment by *Lindera strychnifolia* roots extract (LsR). Using a phototrichogram, we also compared hair density and total hair counts in 17 subjects receiving LsR treatment at baseline and after 83 days of treatment.

The analysis of bacterial distribution at the genus level showed no modifications between healthy and AGA groups. Higher *C. acnes*/*S. epidermidis* ratio in AGA subjects compared to control ones was noted. Concerning the mycobiota environment, lower abundance of Basidiomycota and higher proportion of Ascomycota, associated with lower proportion of *Malassezia* genus and increase of other fungal genus (*Wallemia*, *Eurotium*), implicated in the hair loss process, were observed in AGA scalp. Finally, lower proportion of *M. globosa* and *M. restricta* were observed. Therefore, data from sequencing profiling of the scalp microbiota strongly support a different microbial composition between normal and AGA affected the scalp. 84 days treatment with LsR extract rebalances bacteria and mycobiota for a healthy scalp. A significant increase in hair number that reach +6.9% compared to Day 0 ( $p=0.002$ ) was observed in the 17 subjects. At this time point, 71% of men had an improvement of hair density. Based on these results, we conclude that the LsR extract is a promising remedy for preventing hair loss and promoting hair growth.

## Introduction

Androgenetic alopecia (AGA) or simply baldness is the most common form of permanent hair loss in both men and women with an increasing prevalence with age. It is a hereditary pattern affecting 80% of Caucasian men and 40% of women throughout a life time [1]. Although there is racial variation in the incidence of androgenetic alopecia, it affects at least 50% of men by the age of 50 years and up to 80% of them in later life [2]. Prevalence in women has wide variation ranging from 6% in women aged under 50 years to 30-40% of women over 70 years [2]. The occurrence of AGA has been considered to have a great negative impact on the patient's psychology and the quality of life [3].

AGA is characterized by a progressive loss of hair diameter, length, and pigmentation. Shortening of the hair growth phase (anagen phase) and a slow progressing miniaturization of the hair follicle occurs over time, hair growth cycle comprising of anagen, catagen, and telogen phases. During the transition between these phases, a group of specialized fibroblasts known as dermal papillae cells (DPCs) present in the hair follicle bulb plays an essential role in the regulation of the hair growth cycle, and factors affecting the functions of DPCs are of great importance for the development of therapy for the treatment and/or prevention of hair loss [4]. These factors include, but not limited to, multiple signaling molecules, such as Wnts, Sonic hedgehog (Shh), and transforming growth

factor-beta (TGF- $\beta$ ), which contribute to the anagen initiation of multipotent epithelial stem cells [5]. Targeting these biochemical signaling pathways of hair growth regulation would be a rational approach for the treatment of alopecia.

The genetic inheritance of AGA is well known. However, its etiology is multifactorial including micronutrients, stress, alterations of hormonal secretion [6]. Infiltration of mononuclear cells and lymphocytes is also detected in about skin samples of AGA. This micro-inflammation takes place in the upper third of the hair follicle, where a great number of microorganisms are harbored. Besides these factors, strongest evidence supporting correlations with microorganisms colonizing the scalp has been found in seborrheic dermatitis and in dandruff [7]. The most abundant bacteria found in scalp swabs of healthy individuals are *Cutibacterium* spp. (with the vast majority of *C. acnes*) and *Staphylococcus* spp. (with the predominance of *S. epidermidis*), comprising approximately 90% of the total gene sequences.

*Corynebacterium* spp., *Streptococcus* spp., *Acinetobacter* spp., and *Prevotella* spp. are listed among other significantly less numerous species [8]. Among fungi, *Malassezia* spp. largely predominate on the scalp, *Malassezia globosa* (*M. globosa*) and *M. restricta* being the most abundant species. Ascomycota (*Acremonium* spp., *Didymella bryoniae*), other Basidiomycota (*Cryptococcus liquifaciens* and *C. diffluentis*),

*Coniochaeta spp.*, *Rhodotorula spp.*, were also identified on healthy scalp. It appears that fungal invasion and prevalence of *Cutibacterium acnes* result in an increased hair shedding [9]. Nevertheless, the data are scarce.

Management of alopecia is an essential aspect of clinical dermatology given the prevalence of hair loss and its significant impact on patients' quality of life. Over the centuries, a wide range of remedies has been suggested for androgenetic alopecia and current treatments include surgery, hormone action modifiers and non-hormonal therapy. Pharmacological therapies are based on the understanding of androgen action mechanisms in hair follicle. Use of natural products has been quite common in hair care industry and the search for natural products is being continuously promoted [10]. It seems that polyphenols and terpenes have positive effects on hair growth cellular pathways. Indeed, polyphenols have been shown to enhance proliferation of human dermal papilla cells, to increase growth factors concentrations such as IGF-1 and VEGF and to reduce oxidative stress, resulting in an improved hair growth [11]. Lignans were also shown to exert hair growth-promoting effects by increasing Wnt/ $\beta$ -catenin signaling pathway in human dermal papilla cells [12]. Terpenes such as linderane were also able to inhibit the cAMP/PKA/CREB pathway [13] whereas agents increasing cAMP levels were identified as potent inhibitors of human hair follicle growth. These molecules can be found in some plants such

as *Lindera strychnifolia* roots (LsR) [14]. The plant, is distributed in several Asian countries and is considered as a drug promoting longevity and as an elixir of life. Extracts of roots are used as traditional medicine and recent studies reported antioxidant and anti-inflammatory effects [15].

The aim of this investigation was to evaluate the effect of LsR extract versus placebo on the bacterial and fungal scalp microflora using a panel of 12 volunteers presenting a hair loss / chronic alopecia. The strategy used was based on high throughput DNA sequencing targeting the encoding 16S ribosomal RNA for bacteria and ITS1 (Internal Transcribed Spacer 1) ribosomal DNA for fungi. Finally, the efficiency of LsR extract in preventing hair loss was assessed using phototri-chogram analysis during 84 days in 17 subjects.

## Material and Methods

### Preparation of Extract

Dried roots of *Lindera strychnifolia* were firstly washed by alkaline water, and then extracted by 70% alcohol at 65°C during 12 hours. After 10  $\mu$ m clarification, the extracted solution was concentrated, under vacuum at 55°C, up to 6% of dry matter in propan-1,3-diol (less than 10% of residual water). The decontamination is realized by filtration under 2  $\mu$ m.

The advertisement features a central illustration of a smiling male scientist with grey hair and glasses, wearing a white lab coat and holding a tablet and a bottle. To his right is a banner celebrating 25 years of proDERM. To his left, a blue banner reads 'Your partner for trustworthy clinical studies.' Below the scientist, three statistics are listed with icons: a magnifying glass for '+10,000 STUDIES CONDUCTED', a checkmark for '+50,000 PRODUCTS TESTED', and a group of people for '+225,000 SUBJECTS ENROLLED'. The slogan 'Research to rely on.' is written in a cursive font at the bottom right.

*Lindera strychnifolia* roots extract (LsR) contains polyphenols (29.4% per dry matter), linderane (1.2% per dry matter) and linderalactone (1.8% per dry matter). The polyphenols content is mainly constituted by tannins (24% per dry matter) and catechin derivatives (5% per dry matter).

### Subjects recruitment

Twelve males AGA subjects (40–65 years old) were recruited. They had chronic alopecia of androgenetic origin with a stage of III to IV according to the Norwood Hamilton classification. All enrolled subjects had to meet the following criteria: 1) no antibiotics in the 30 days leading up to the sampling 2) no probiotics in the last 15 days 3) the last shampoo was performed 48h before sampling 4) not suffering from other dermatological diseases 5) no anti-tumor, immunosuppressant or radiation therapy in the last 3 months 6) no topical or hormonal therapy on the scalp in the last 3 months 7) Any local or general treatment that may affect hair growth or hair loss in the last few months prior to the investigation 8) no subject with hair loss treatment or who has used this type of treatment (application of Minoxidil or taking of Finasteride within six months before the start of the study or taking another product for oral or topical hair loss treatment during the 3 months prior to the selection visit 9) No inflammatory skin disease or progressive skin lesion on the scalp (psoriasis, seborrheic dermatitis, severe erythema, severe excoriation, severe sunburn, etc.) The control group included the following criteria: (1) the age and sex of healthy volunteers were basically matched with those who participated in the hair loss group; (2) perms and hair dyes were not used 2 months prior the treatment. Anti-hair loss shampoo was also not used; (3) oral or topical antifungal preparations were not given within 1 month prior the treatment; (4) no scalp-related diseases such as scalp folliculitis, head lice, and alopecia areata were observed in the individuals.

### Treatment

Applications of a lotion with LsR extract at 1% and a placebo lotion were performed twice daily during 84 days on a randomised half-head (one product per half-head).

#### Swab sample collection

The scalp surface has been sampled by means of swab procedure.

#### DNA extraction

Genomic DNA was extracted with the “DNeasy PowerSoil kit” following supplier’s recommendations. Genomic DNA samples were stored at -80°C and were then sent to an external firm for next generation sequencing.

#### Amplification and sequencing of 16S RNA gene

Microbiota composition analysis of samples was performed by amplifying the hypervariable regions V1-V3 of the 16S

RNA gene. Sequences were processed using Mothur (version 1.36.1) according to MiSeq SOP pipeline (Schloss, 2009).

#### Amplification and sequencing of ITS1 RNA gene

Fungal composition analysis of samples was performed by amplifying the ITS1 regions of the RNA gene. This amplification was done using universal primers ITS5 (GGAAGTAAAGTCGTAACAAGG) and 5.8S-1R (GTTCAAAGAYTCGATGATTCAC) which target the conserved regions of this gene common to all fungi. The products of this amplification were sequenced by MiSeq Illumina technology. Sequences were processed using Mothur (version 1.36.1) according to pipeline developed by our provider.

#### Phototrichogram test

For this evaluation at D0 and D84, we focused on type III alopecia (n= 17 men, mean age: 51 years old). Type III androgenetic alopecia (according to Norwood Hamilton scale) represents the minimal extent of hair loss sufficient to be considered as baldness. The treatment was the same as the first study.

The phototrichogram is a non-invasive technic that allows studying the hair growth cycle by the determination of the proportion of growing and resting hair. Analyses of the photographs allow the determination of hair number of in growing (Anagen) and resting/shedding (Telogen) stages.

#### Self-assessment questionnaire

At the end of the study, all subjects completed a self-evaluation questionnaire to evaluate their overall opinion and their attitude towards the effectiveness of the lotion being tested.

#### Statistical analysis

Results are presented as mean  $\pm$  SEM.

Statistical analysis of variations over time and analysis comparing LsR extract and Placebo were performed using paired t-test (if the normality of the distributions was confirmed using the Shapiro-Wilk) or with the Wilcoxon test (if the normality of the distributions was rejected). The level of significance was set at 5%.

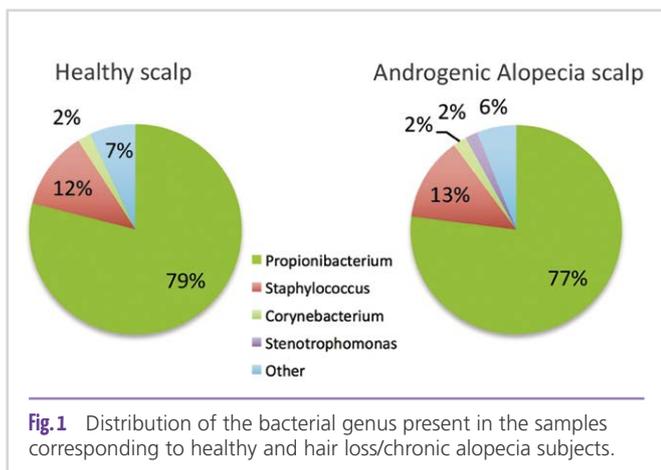
Concerning the scalp microbiome, alpha diversity (Shannon diversity index) was evaluated. It is a measure of the biodiversity of samples and is characterized by the observation of the taxonomic richness and distribution of OTUs.

## Results

### Identification of bacteria communities

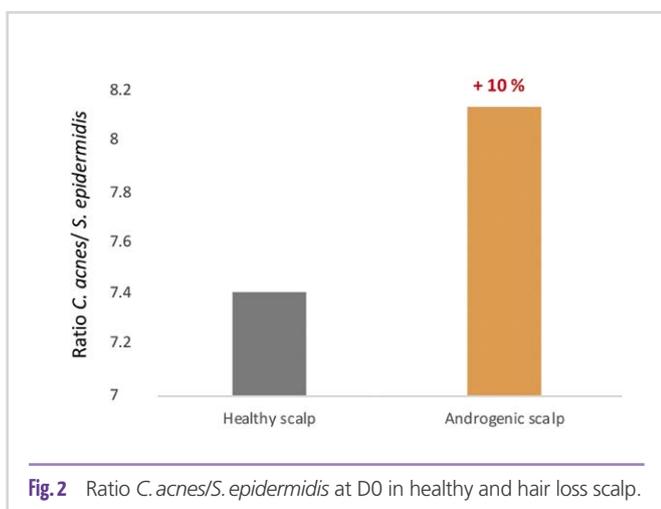
Alpha-diversity did not differ between the groups studied, whatever the period of investigation.

At D0, samples corresponding to healthy scalps were mostly composed of 3 major phyla: *Actinobacteria*, *Firmicutes* and *Proteobacteria* that account for 98% of total sequencing



reads in all samples. The bacterial landscape corresponding to AGA scalps showed no modifications as compared to healthy group.

At the genus level, *Propionibacterium* (79%) and *Staphylococcus* (12%) account for about 90% of the total scalp microflora for healthy volunteers, data in agreement with the literature (Polak-Witka, 2019) (Fig. 1). For the AGA volunteers, *Propionibacterium* (76.5%) and *Staphylococcus* (14%) account also for about 90% of the total scalp microflora.



At the species level, mean reads of *Stenotrophomonas geniculata* and *Staphylococcus epidermidis* were different between healthy and AGA group, with higher mean reads of *Stenotrophomonas geniculata* noted in the AGA population. At the same time, the ratio *C. acnes*/*S. epidermidis* was higher in AGA subjects (mean ratio = 8.1) compared to control subjects (mean ratio = 7.45) (Fig. 2).

### Identification of fungal communities

It was observed that the alpha-diversity (Shannon diversity index) for the fungal population was not significantly different in the healthy scalp compared to the AGA scalp, whatever the period of investigation.

Taxonomic fungal composition of healthy and hair loss scalp at the beginning of the study (D0) are presented in Fig. 3.

Healthy scalp is mostly composed of one major phyla: Basidiomycota, predominant fungus being *Malassezia*. The bacterial landscape corresponding to pathogenic scalps differed from that of healthy scalps by a lower abundance of *Basidiomycota* (91%-89% vs 99% for AGA scalp and healthy scalp, respectively) and a higher proportion of *Ascomycota* (8%-10% vs 1% for AGA scalp and healthy scalp, respectively).

A lower proportion of *Malassezia* genus in samples corresponding to AGA scalps and an increase of other bacterial genera (*Wallemia*, *Eurotium*) were noted (Fig. 3).

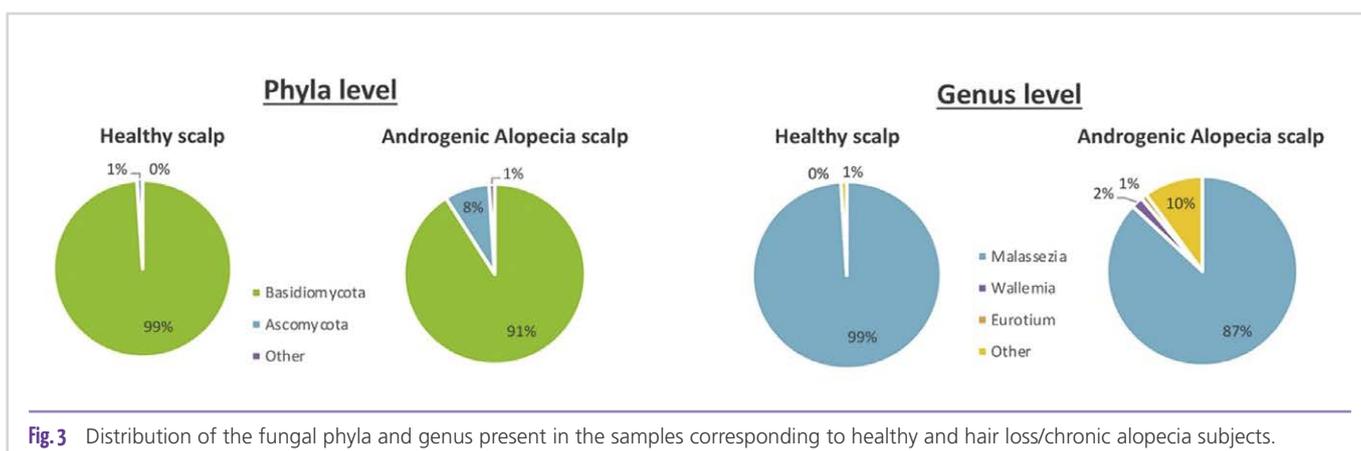
At the species level, results showed that the samples were mainly composed of fungi belonging to the *Malassezia* genus. The major component in the healthy scalp of fungal microbiome was represented by *M. restricta* and *M. globosa*.

A lower proportion of *M. globosa* and *M. restricta* were observed for the AGA group (-56% and -52%) as compared to the control group.

### Effect of LsR extract treatment on microbiome

At the phylum and genus level, LsR extract maintains the biodiversity of bacteria.

Results showed a significant decrease in the abundance of *Cutibacterium acnes* (-15%) following the treatment with



LsR extract ( $p < 0.05$ ). *Staphylococcus epidermidis* increased significantly ( $p < 0.05$ ) by 33% between D0 and D83 with LsR treatment. Thus, the ratio *C. acnes*/*S. epidermidis* decreased by 37.8% as compared to the ratio noted at D0 (Fig. 4).

At the phylum level, LsR extract tended to restore the “normal” fungal landscape for the Basidiomycota phylum and the three fungal genera studied. In fact, LsR extract allowed an increase in the abundance of *Malassezia* (+3%). A decrease of *Eurotium* and *Wallemia* were also observed. After 84 days of LsR treatment, the proportion of *M. restricta* has returned to the same value as healthy group.

### Phototrichogram test

A significant increase in hair number that reach 7% compared to Day 0 ( $p=0.002$ ) was observed (Fig. 5).

### Self-assessment

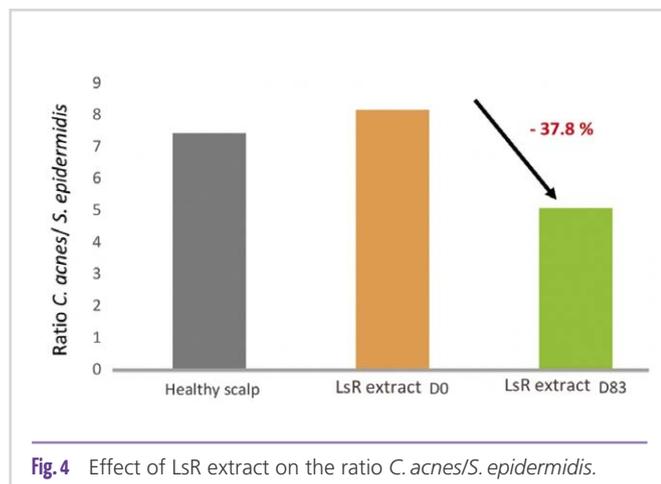
After 84 days of treatment with LsR extract, men reported their satisfaction about:

#### Hair growth and anti-hair loss efficacy

- 78% of the men reported a speed up the growth of their hair
- 72% of men observed a rapid action of the lotion with a decrease in the amount of hair in the brush, lost after the shampoo and on the clothes/pillows”.
- 75% of men reported a stimulation of hair growth and a slowdown of hair fall
- 72% of men observed that hairs are visibly denser and 69% that they seem more abundant.

#### Hair aspect and properties

72 to 88% of men observed that the hair recovers its strength (78%) and vitality (81%) and appears more vigorous (88%), more resistant (88%) and less brittle (78%).

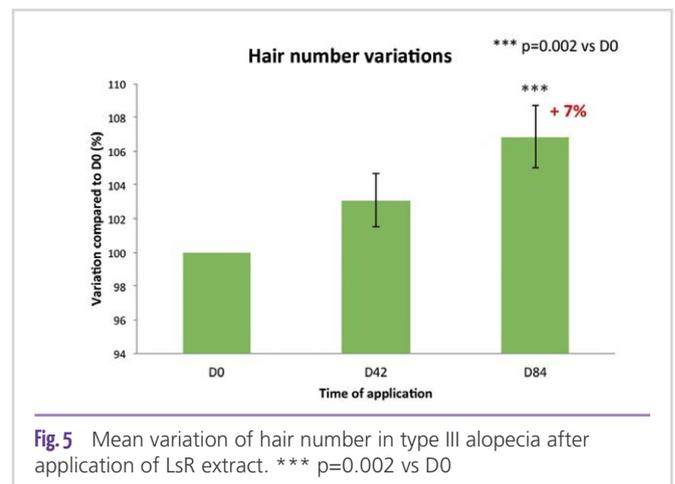


## Discussion

The skin is the largest organ in the human body. It keeps the first barrier of the immune system that not only resists the invasion of foreign pathogens, but also protects tissues and organs. A diversified and abundant microbial community host the skin and this symbiotic relationship results, most of the time, as beneficial for both the host and microbial community. Bacteria mainly belong to *Corynebacteriaceae*, *Propionibacteriae*, and *Staphylococcaceae* [16], depending to the physiochemical properties of each skin site they host. However, little is still reported with regards to the microbiome inhabiting the scalp and hair growth disorders such as andruff [17]. Characterization of scalp bacterial species involved in Alopecia androgenetica, Alopecia areata, has also been poorly investigated and, only recently, the piece bit of evidence has been reported [18]. We focused our attention on bacterial population of the scalp of healthy and AA subjects looking at main bacterial species on the scalp. Our results agree with Pinto’s work [19], showing an increase of the ratio *C. acnes*/*S. epidermidis* in AGA subjects. *C. acnes* is able to synthesize many enzymes involved in the metabolism of porphyrins that, once activated, may contribute to oxidation and follicular inflammation. Virulence in the hair follicle is noted to cause hair loss as a consequence [9]. Therefore, a speculation about the role of the hypoxic condition of the follicular region may be speculated in AGA and this may encourage *C. acnes* overgrowth [9]. *C. acnes* predominance is also identified in non-lesional scalp of patients with seborrheic dermatitis, providing support for the development in sebaceous gland in AGA, which may attract the proliferation of *C. acnes* for lipids and fatty acids [20].

Data also suggested a higher diversity of bacterial species inhabiting the scalp of AGA subjects, such as *Stenotrophomonas sp.*, which is an opportunistic human pathogen, characterized by high keratinase activities [21].

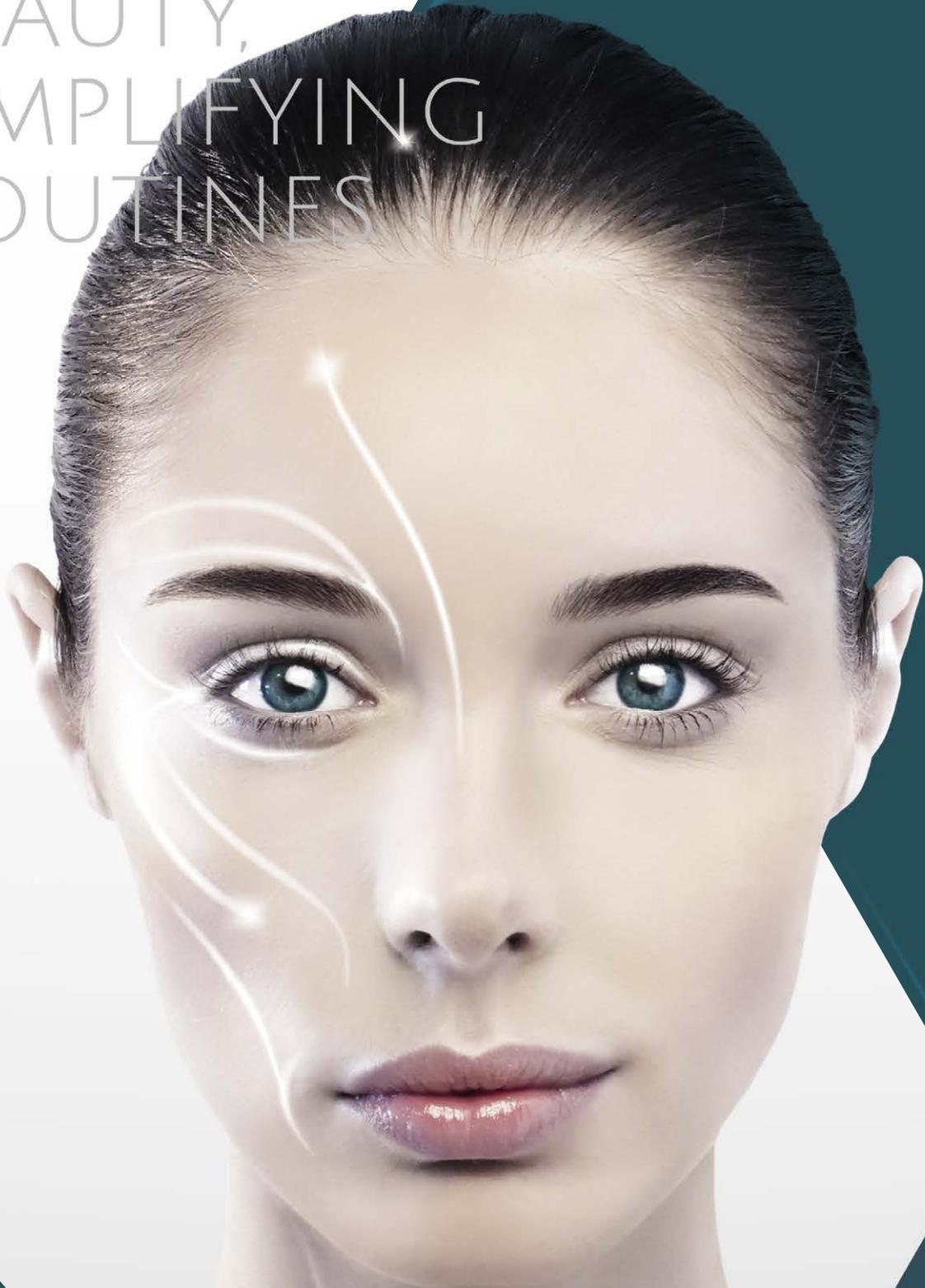
On the basis of the present and previous results, a link with a higher susceptibility of an unhealthy scalp to be colonized by microorganisms could be postulated but further analysis are needed to understand the reason behind this high variety.



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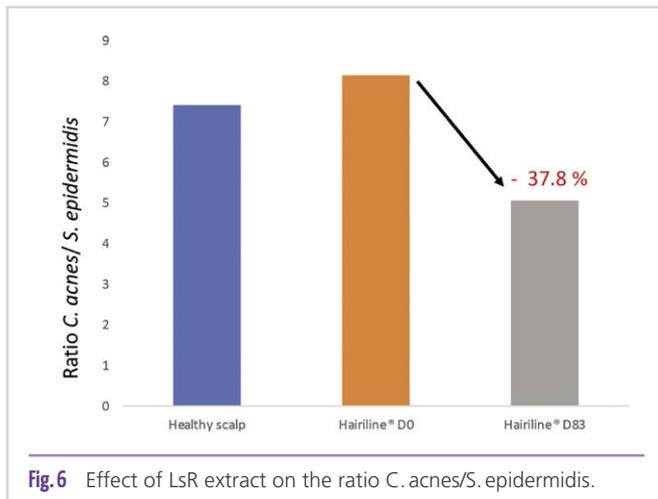
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**Fig. 6** Effect of LsR extract on the ratio *C. acnes*/*S. epidermidis*.

The prominent fungal species in healthy scalp belonged to the genera *Malassezia* (Fig. 6). At the species level, a high abundance of *M. restricta* and *M. globosa*, was observed as reported by *Grimshaw et al.* [22]. Contrary to *Huang et al.* [17], the abundance of these two strains were lower in AGA scalps. In healthy people, *Malassezia* maintains a harmonious and balanced symbiotic relationship with the host.

Treatment during 84 days with LsR extract tends to restore the “normal” fungal landscape and scalp microbiota, limiting specific species, inducing keratin alterations. The efficacy of the treatment with LsR extract on hair density was validated. Therefore, all investigated subjects after LsR extract treatment reported decrease in the amount of hair in the brush, lost after the shampoo and on the clothes/pillows, and a stimulation of hair growth and a slowdown of hair fall.

## Conclusion

Our results confirm the presence of a significant bacterial and fungal disequilibrium on the scalp of AGA subjects compared to healthy population. 84 days of LsR treatment induced a reversible microbiome environment. These data are in line with the clinical efficacy of the treatment. This active ingredient offers a new natural solution for formulated products aiming to manage hair loss by acting on the scalp microbiome.

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## contact

E. Filaire<sup>1,2</sup>, A. Dreux<sup>1</sup>, C. Boutot<sup>1</sup>, F. Volat<sup>1</sup>, E. Ranouille<sup>1</sup>, J. Demangeon<sup>1</sup>, J.Y. Berthon<sup>1</sup>

<sup>1</sup>Greentech. Biopôle Clermont-Limagne  
63360 Saint-Beauzire | France

<sup>2</sup>University Clermont Auvergne, UMR  
1019 INRA-UcA, UNH (Human Nutrition Unity), ECREIN Team  
63000 Clermont-Ferrand | France

Contact: E-mail: edithfilaire@greentech.fr, Tel.: +33 (0)473339900