LACTOPHYT

I- Background

The bacterial population present at the axillary level belongs to the axillary microbiota. Certain bacterial species present in this community have the capacity to transform non-odorous substances into volatile odorous substances by the action of enzymes. Before this transformation, sweat does not smell. Amino acids, fatty acids with long carbon chains and other compounds such as the hormones composing the sweat are transformed by various processes involving enzymes of the axillary microbiota (e.g. N-α-acylglutamine aminoacylase). Molecular tools such as metagenomics have made it possible to identify the function of some bacterial genes involved in these phenomena. Thus, we can mention a very special involvement of *Staphylococcus spp*. and *Corynebacterium spp*. Recent studies have resulted in further analysis and gave us more information concerning the impact of *Staphylococcus hominis* and *Corynebacterium tuberculostearicum* as members of the bad-odorous bacteria. Although the relationships within this bacterial community are not fully elucidated, it appears that a low abundance coupled with the presence of *corynebacteria* amplifies the formation of volatile odorous compounds.

The repeated use of deodorant to destroy the bacterial flora of the axillary microbiota does not appear to be the appropriate solution. Bacterial abundance is decreased and the presence of malodorous compounds is only masked by the use of perfumes. It has been shown that the presence of *S. epidermidis* and *P. acnes*, two commensal species, correlated with the decrease of bad odors (1).

Based on these data, it has been considered to reverse the presence of certain bacterial species using probiotic strains to implant new competitive strains without affecting the production of volatile compounds. However, the axillary area is a particular environment in which there are parameters not adapted for all bacterial species and the establishment of these strains of beneficial potential can not persist over time (Figure 1). For this reason, GREENTECH designed a new active based on the potential of probiotic strains without the need of cell viability.

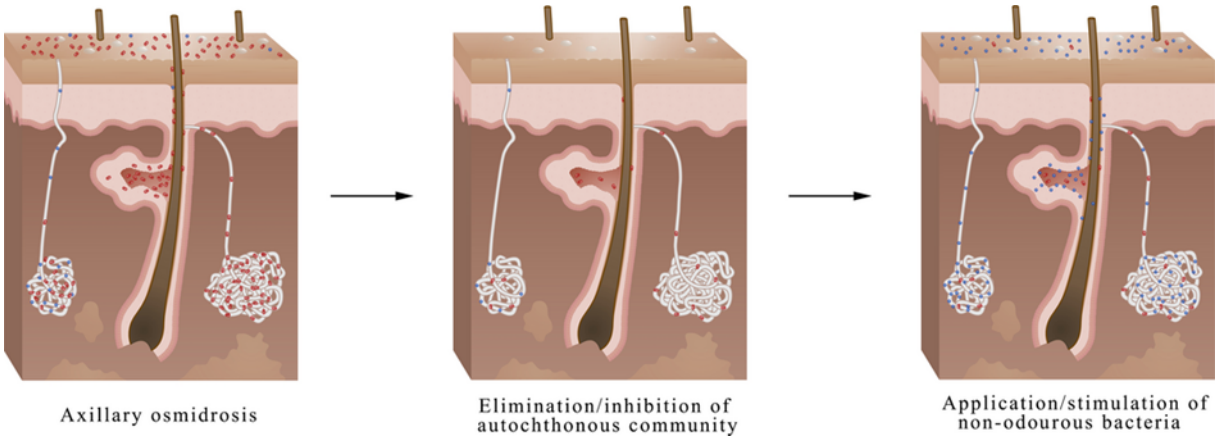


Fig 1. Strategy to implant new bacterial flora. *From Callewaert et al. Experimental Dermatology, 2017 DOI: 10.1111/exd.13259 (2).*

II- Development

GREENTECH has partnered with BIOVITIS for the production of an extract from *lactococcus lactis,* a bacterial species known for its antibacterial properties. A new production process has been specifically developed to increase the antibacterial activity of this secreted extract. To reinforce the selectivity of this ingredient, GREENTECH has demonstrated a synergy between this extract from bacterial origin and a low concentration of Glyceryl caprylate, an emollient also possessing an antibacterial activity.

LACTOPHYT has two objectives: 1) Maintain the presence of commensal population not involved in the formation of unpleasant odors and 2) Inhibit the populations of *corynebacteria* and *staphylococcus* responsible for these discomforts.

1) *Lactococcus lactis extract*

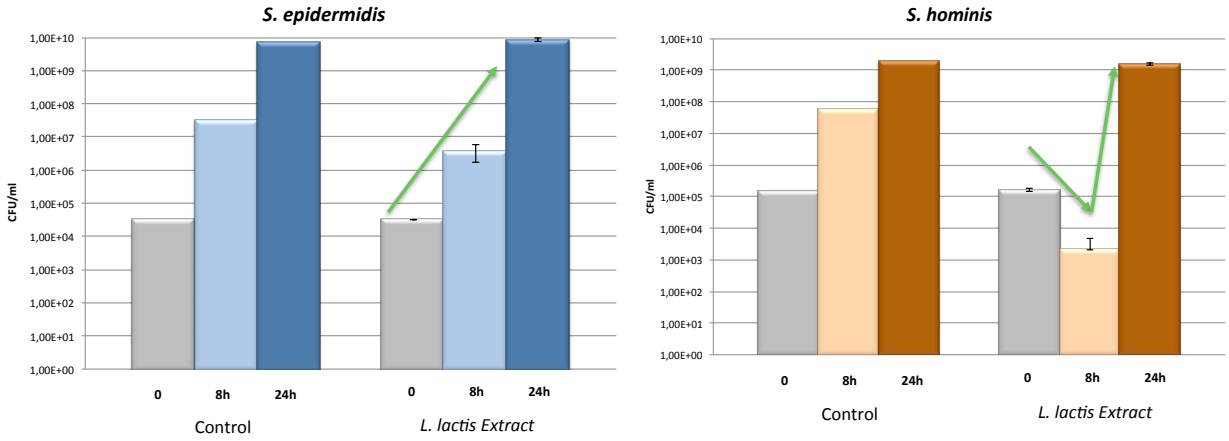
The action of the extract from *L. lactis* alone was evaluated *in vitro* against 4 axillary strains selected according to their presence in the axillary microbiota: *Staphylococcus epidermidis*, as a positive control for the commensal flora non involved in the formation of bad odours *; Staphylococcus hominis, Corynebacterium* xerosis and *Corynebacterium tuberculostearicum*, for their contributions to the production of axillary odours (Table 1).

STRAIN	MIC at 72h (%)
S. epidermidis	>5%
S. hominis	>5%
C. tubercuostearicum	5%
C. xerosis	0,8%

Table 1: Results of MIC assays - L. lactis extract tested alone *from 5% to 0.2% (v/v) against the 4 axillary strains. The results were obtained after 72 hours of incubation at 37 ° C. * MIC = Minimum Inhibitory Concentration*. Initial concentration of the strains at 10^5 CFU/mL.

After 72 hours, corynebacteria species were affected by the use of the extract alone. Both Staphylococcus species were apparently less sensitive to the extract of *L. lactis.* Based on the time of deodorants classical use, effect on staphylococcus was tested for 24h.

A more detailed estimatation of the S*. epidermidis* and *S. hominis* populations was performed at 0, 8 and 24h to evaluate the impact of this extract at 2% (v/v) on the growth of these strains :



These results demonstrate a difference between these two species. *S. hominis*, a species involved in molecular transformation processes, appears to be more affected by the presence of *L. lactis extract*. During the first hours of exposure, a bactericidal effect is observed. After 8 h, the growth of *S. hominis* reaches a stationary level. In the case of *S. epidermidis*, the presence of the extract does not appear to affect the strain which shows growth similar to the control.

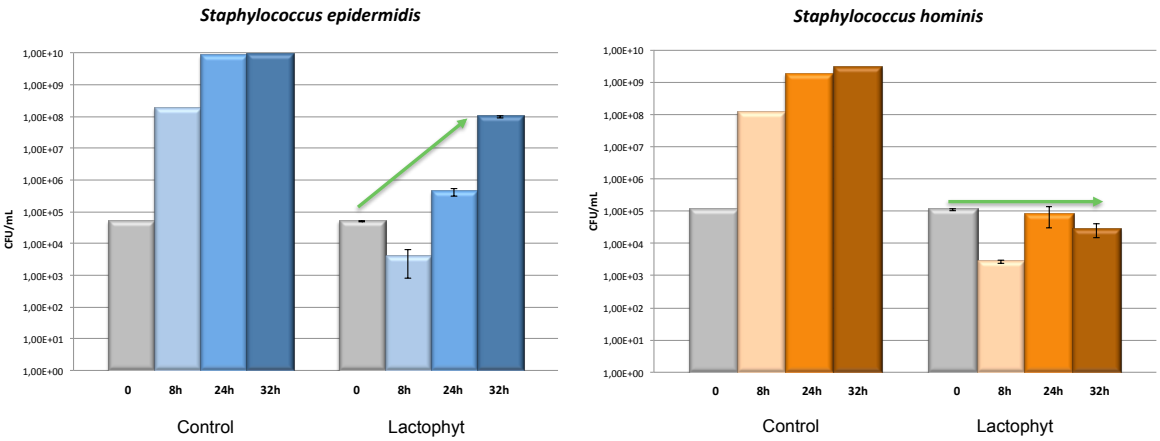
2) Lactophyt

In order to enhance the effect of this *L. lactis extract*, supplementation with a low Glyceryl caprylate content was achieved. This work made it possible to obtain a mixture presenting the following growth data for use of LACTOPHYT at 2% (v/v):

LACTOPHYT at 2% (v/v)			
STRAIN	24h	48h	72h
S. epidermidis	+	+	+
S. hominis	-	-	+
C. xerosis	-	-	-
C. tuberculostearicum	-	-	-

Table 2: In vitro growth test results - 2% (v : v) Lactophyt extract *on the 4 axillary strains. The results were obtained after 24, 48 and 72 hours of incubation at 37 ° C. The initial bacterial concentration is defined at about 105 CFU / mL. Growth is considered as positive when the* OD_{600} *is greater than 0.1.*

A more detailed estimation on populations of *S. epidermidis* and *S. hominis* was performed at 0, 8, 24 and 32 hours to evaluate the impact of the active Lactophyt use at 2% (v/v) on the growth of these strains:



During the first hours of exposure to Lactophyt at 2% (v/v), the two species appear to be sensitive to its presence. In the case of *S. epidermidis*, growth starts after 8 h of incubation. On the other hand, after an 8-hour latency period, the growth of *S. hominis* was maintained at the initial concentration (10^5 CFU/mL) and showed no growth (inhibitory effect). The use of Probiophyt Fresh P+ showed the growth of a commensal strain such as *S. epidermidis* and the inhibition of a strain associated with the formation of odors at the axillary level (*S. hominis*). Although these species are similar because they belong to the same bacterial genus, these results demonstrate the selectivity of Probiophyt fresh P+ .

Conclusion

In order to preserve the axillary microbiota while selectively reducing the bacteria involved in the production of unpleasant odors, GREENTECH has developed a product combining the defense potential of a lactic bacterial strain and an emollient. Lactophyt has for mission to respect the axillary microbiota by guiding the bacterial populations towards a new equilibrium.

References

1. Troccaz M, Gaïa N, Beccucci S, *et al.* Mapping axillary microbiota responsible for body odours using a culture-independent approach. Microbiome 2015: 3: 1-15.

2. Callewaert C, Lambert J, and Van de Wiele T. Towards a bacterial treatment for armpit malodour. Exp Dermatol. 2016; 00:1–4. doi:10.1111/exd.13259.