

Science &
Conscience
NATURALLY®



Calybiota® Bio

A microbiota balance active ingredient

Summary

Science &
Conscience
NATURALLY®

1 *Sourcing & process*

2 *Concept*

3 *In vitro tests*

Skin protection (immune defenses & anti-inflammatory)

Protective & prebiotic effect on vagina flora

Other skin microbiome interest
(atopic dermatitis, acne, deodorant)

4 *Clinical study on the intimate flora*

Confirmation of the prebiotic effect on Lactobacillus

Soothes sensation of discomfort & moisturizes

Anti-inflammation

Sexual life improved

1



2



3

Wellness



Science &
Conscience
NATURALLY®



Sourcing & process Calybiota® Bio



Calybiota® Bio



Calyx



- ✳ Calyx extract of red kapok tree flowers (*Bombax costatum*)
- ✳ African sourcing
- ✳ Rich in polysaccharides
- ✳ INCI : Glycerin, Water, Bombax Costatum Flower Extract

The red kapok tree is a small tree (max height 15m) with red-orange flowers

The calyxes of the flowers are traditionally used to bind sauces in Africa

Sourcing

Science &
Conscience
NATURALLY®

- Red kapok trees grow in savanna zones of West Africa
- Harvesting in the classified forest and biosphere reserve of the Balla Hippopotamus Pond, in the Satiri township
- UNESCO “Man and the Biosphere” Program
- Siguibana cooperative (Balla village): in charge of harvesting and drying calyces

Calybiota® Bio



CSR actions

Science &
Conscience
NATURALLY®



Harvesting area located in the classified forest and biosphere reserve UNESCO “Man and the Biosphere” program



Good harvesting practices training dispensed in 2019 and field study for the NIES



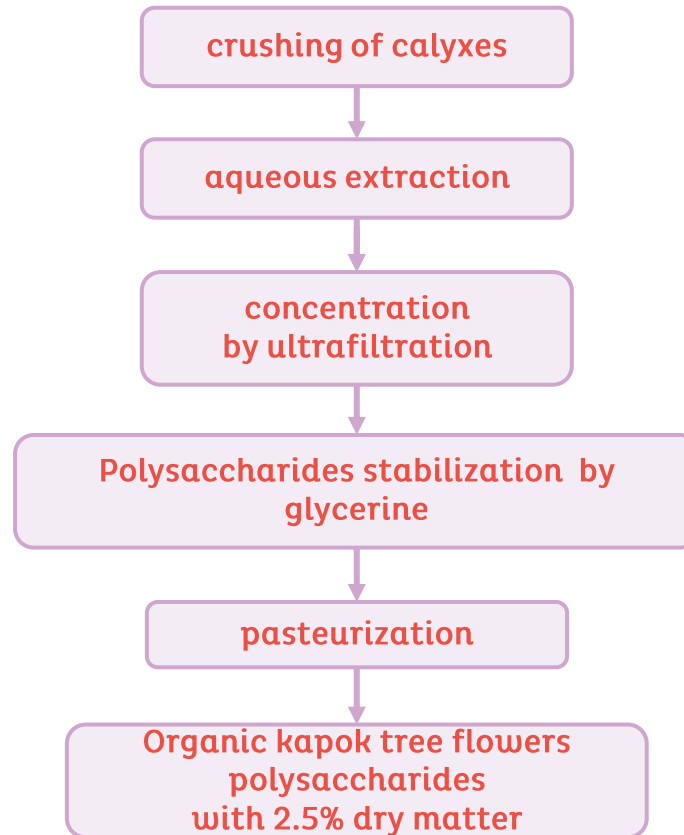
Cooperative's headquarters construction in the village of Balla (storage, office & meeting room)



Red kapok tree is an oxalogen tree (carbon sink)



Process



Calybiota® Bio



Science &
Conscience
NATURALLY®

Science &
Conscience
NATURALLY®



Calybiota® Bio Concept

What is microbiota ?

- The skin microbiota encapsulates all the microorganisms (bacteria, viruses, fungi...) residing on and in the skin
- The microbiota is present even in the dermis, adipose tissues and eccrine glands (*Nakatsuji et al 2013*)

On the skin:

- 10^{12} bacterial cells ($10^6/\text{cm}^2$) compared to 3.10^{10} human cells (**33:1 ratio**)
- 500 different germs . Resident or transient flora . 4 predominant Phyla

Vagina :

- 10^8 to $10^9/\text{ml}$ of vaginal secretion
- composed of **90% lactic acid bacteria (lactobacilli)**

Variation with multiple factors:

- **Location (sebum, pH, humidity...)** ; **Sex** ; **Age** ; **Immune system...**
- Everyone has his own bacterial identity influenced by genetics, diet, lifestyle, climate...



Calybiota® Bio restores microbiota balance especially the vaginal flora for an improved sexual life!

Science &
Conscience
NATURALLY®



Calybiota® Bio

- Balances the microbiota
- Limits inflammation
- Reduces discomfort sensations

Microbiota balance

INTIMATE CARE

- **Prebiotic** effect on the vaginal bacteria
L. gasseri, L. acidophilus, L. rhamnosus, L. crispatus, L. jensenii
- **Protective effect** through a stimulation of Bacterium/Host communication systems
- **Bacteriostatic** on pathogenic strains

ACNE

- **Inhibits** *M. furfur* & *C. acnes*
- Limit biofilm formation

ATOPIC DERMATITIS

- **Inhibits** adhesion & formation *S. aureus*
- Protect skin barrier

DEODORANT

- **Inhibits** *C. xerosis*
- Limits the odors from *S. epidermidis* & *C. stratum*

Science &
Conscience
NATURALLY®



In vitro tests
Calybiota® Bio

Calybiota® Bio restores the vaginal microbiota balance

Science &
Conscience
NATURALLY®

Microbiota balance

INTIMATE CARE

- **Prebiotic** effect on the vaginal bacteria
L. gasseri, *L. acidophilus*, *L. rhamnosus*, *L. crispatus*, *L. jensenii*
- **Protective effect** through a stimulation of Bacterium/Host communication systems
- **Bacteriostatic** on pathogenic strains

ACNE

- **Inhibits** *M. furfur* & *C. acnes*
- Limit biofilm formation

ATOPIC DERMATITIS

- **Inhibits** adhesion & formation *S. aureus*
- Protect skin barrier

DEODORANT

- **Inhibits** *C. xerosis*
- Limits the odors from *S. epidermidis* & *C. stratum*



Calybiota® Bio

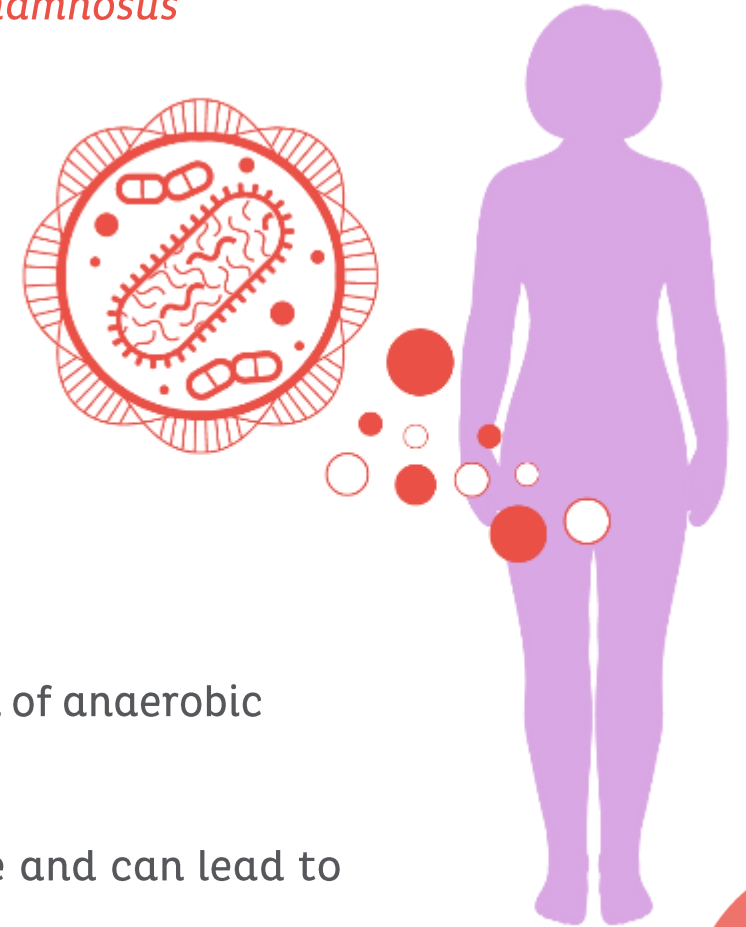
- **Balances** the microbiota
- **Limits** inflammation
- **Reduces** discomfort sensations

Vaginal flora and lactobacilla



Science &
Conscience
NATURALLY®

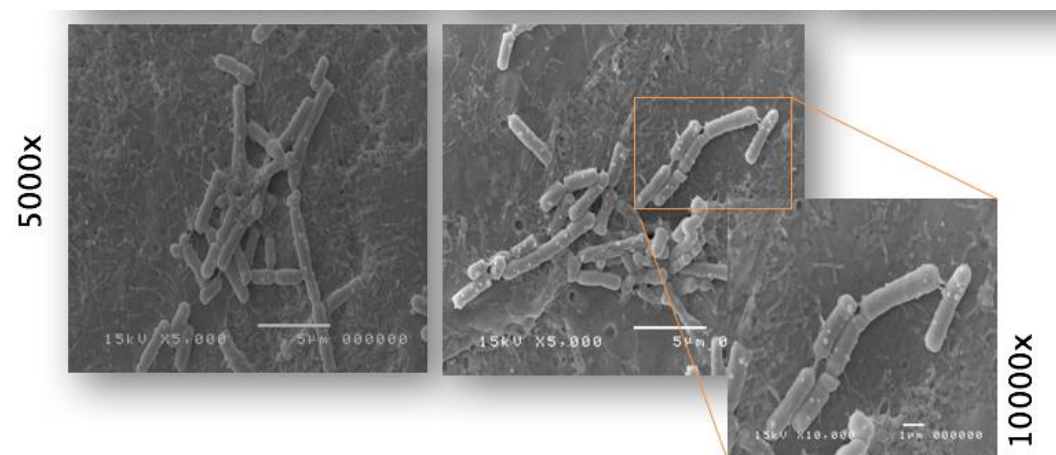
- It is composed of **90% lactic acid bacteria (lactobacilli)** ; Predominant species in healthy vaginal microbiota: *L. crispatus*, *L. gasseri*, *L. acidophilus*, *L. jensenii*, and *L. rhamnosus*
- **Lactobacilli** ensure a **low pH**. They are essential:
 - to stimulate the local immune system and form biofilms that create protective shields
 - To transform the glycogen secreted by the cells into lactic acid, creating an acidic environment necessary to maintain a **healthy vaginal pH ($3.5 < \text{pH} < 4.5$)**
- **A pH > 4.5** causes **a vaginal flora imbalance (dysbiosis)**
Symptoms: unusual discharge, discomfort, vulvar pain or even infections
- An imbalance in the vaginal ecosystem can induce an increase in the growth of anaerobic bacteria, leading to bacterial vaginosis.
- Vaginal dysbiosis syndrome is most common in women of childbearing age and can lead to **feelings of discomfort** (itching, burning).



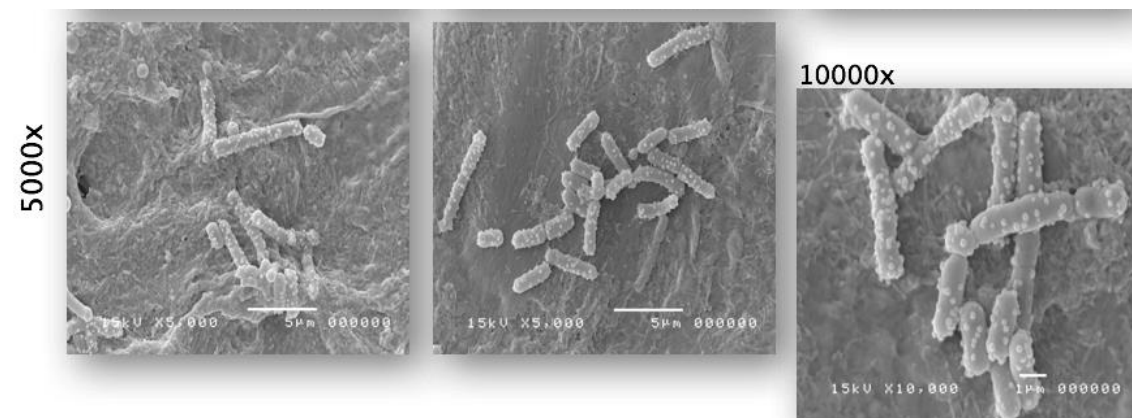
Calybiota® Bio protective effect on reconstructed vaginal epithelium

Observation of 2 Lactobacillus strains on reconstituted vaginal epithelium (HVE) in the presence of Calybiota® Bio (incubation 6h) vs control: *L. crispatus* & *L. gasseri*

Control



Calybiota® 0,5%



Calybiota® Bio 0.5% was applied to the surface of (HVE), at the same time the epithelia was colonized by a mixture of *L. crispatus* and *L. gasseri*, mimicking the physiological resident microflora.

After 6 hours of incubation, the surface of colonized tissues was observed with SEM.

- In the presence of Calybiota® Bio, Bacteria seem **strongly attached** to the surface of the tissue (no interference with the establishment of the vaginal microflora)
- Observation of **membrane vesicles** formation

Calybiota® Bio protective effect on reconstructed vaginal epithelium

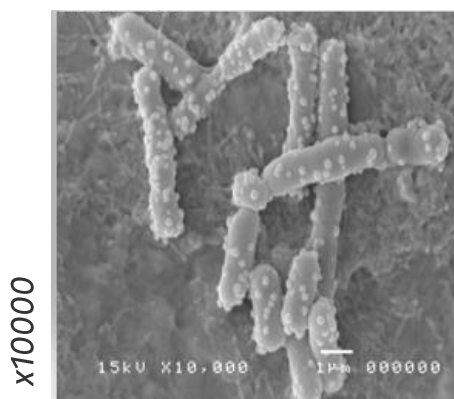
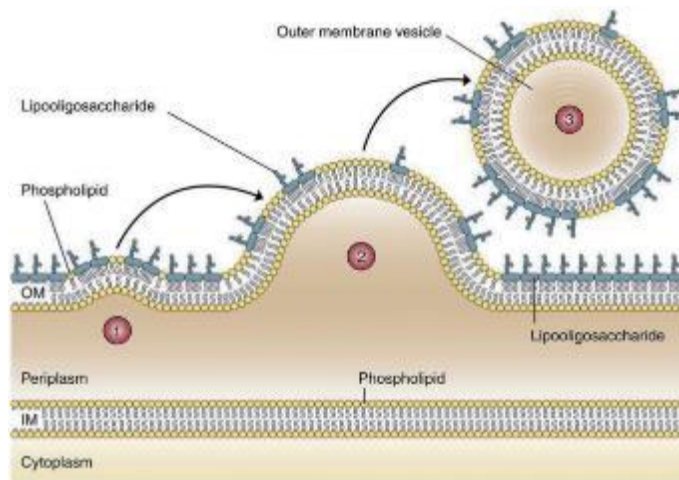
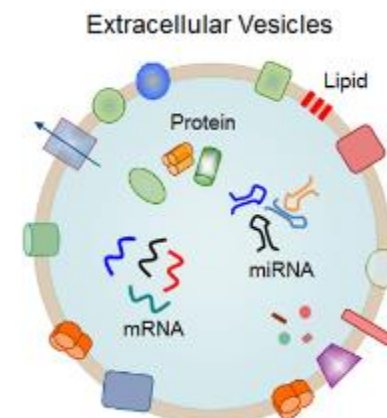
Membrane microvesicles

Recently, these microvesicles in commensal bacteria, such as Lactobacilli have been described as:

→ Associated with the probiotic properties of the bacteria :

- Immune system activation and host defense
- Regulation of the bacterial community / Maintenance of microbiotal balance

Calybiota® 0,5%



Rubio *et al.* 2017; Grande *et al.* 2017; Dean *et al.* 2018

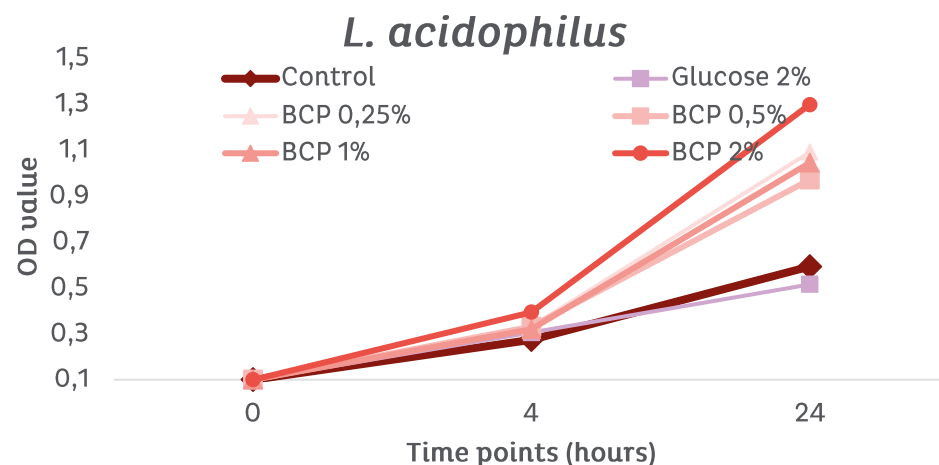
By inducing the formation of membrane vesicles, Calybiota® Bio promotes communication with the host in favor of a prebiotic and protective effect

Prebiotic effect on Lactobacilli bacterial by Calybiota® Bio

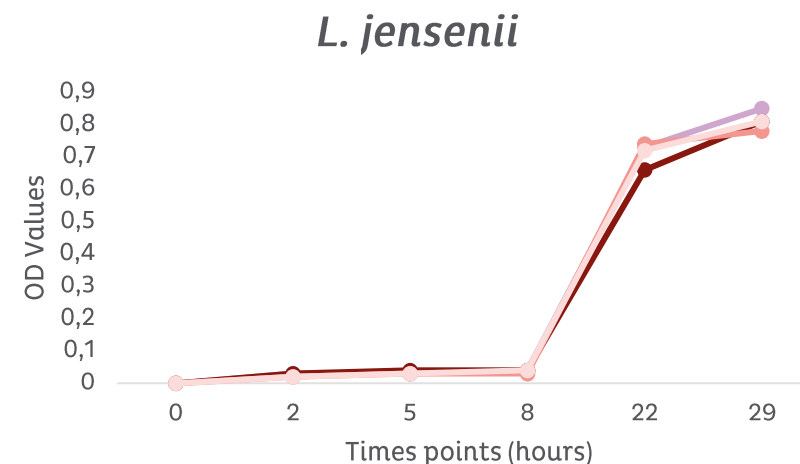
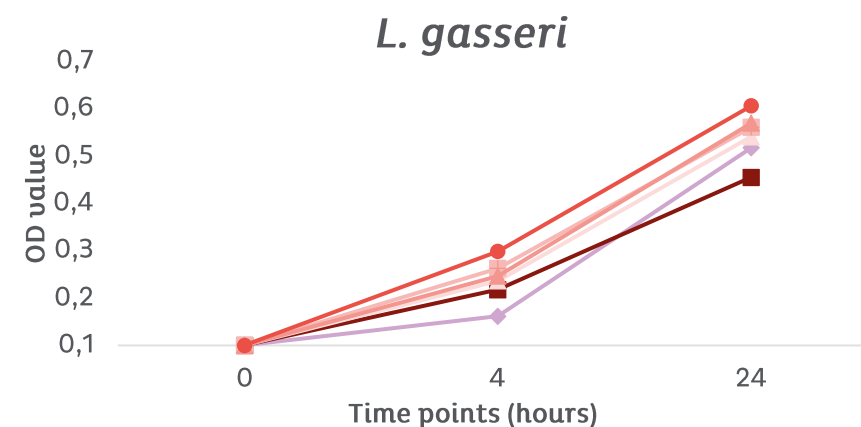
Science &
Conscience
NATURALLY®

Bacterial growth evaluation

- Positive effect on the growth of *L gasseri* from 4h
→ early prebiotic effect
- Positive effect on the growth of *L acidophilus* & *L jensenii*
→ prebiotic effect after 24h
- No effect on *L rhamnosus*, *L crispatus*, *E coli*, *G vaginalis*



BCP = Calybiota® Bio



Calybiota® bio has a prebiotic effect on Lactobacilli

Bacterial viability evaluation

Confirmation of the prebiotic effect on lactobacillus strains with an effect similar or even superior to the positive control (glucose)

Bacteriostatic or even anti-microbial effect on pathogenic strains

	<i>L. gasseri</i> (Log10 CFU/ml)		<i>L. acidophilus</i> (Log10 CFU/ml)		<i>L. rhamnosus</i> (Log10 CFU/ml)	
	4h	24h	4h	24h	4h	24h
Control	7,98 ± 0,01	3,10 ± 0,28	7,77 ± 0,13	5,84 ± 0,09	7,82 ± 0,21	8,23 ± 0,00
Glucose 2%	7,81 ± 0,27	3,42 ± 0,14	7,73 ± 0,23	7,22 ± 0,15	7,83 ± 0,06	9,09 ± 0,07
Calybiota® Bio 0,125%	7,89 ± 0,14	2,49 ± 0,12*	7,72 ± 0,13	8,99 ± 0,55*	7,77 ± 0,10	8,89 ± 0,27
Calybiota® Bio 0,25%	8,01 ± 0,19	0,00 ± 0,00**	7,74 ± 0,08	6,10 ± 0,57	7,83 ± 0,06	8,51 ± 0,56
Calybiota® Bio 0.5%	8,02 ± 0,10	3,27 ± 0,38	8,10 ± 0,58	6,29 ± 0,02	7,74 ± 0,06	7,82 ± 0,01*
Calybiota® Bio 1%	10,40 ± 0,24**	3,73 ± 0,07	8,83 ± 0,18	6,63 ± 0,25	8,75 ± 0,11**	10,35 ± 0,04*

↑ *L. gasseri, L. acidophilus, L. rhamnosus, L. crispatus & L. jensenii*

↓ *G. vaginalis & E. coli*



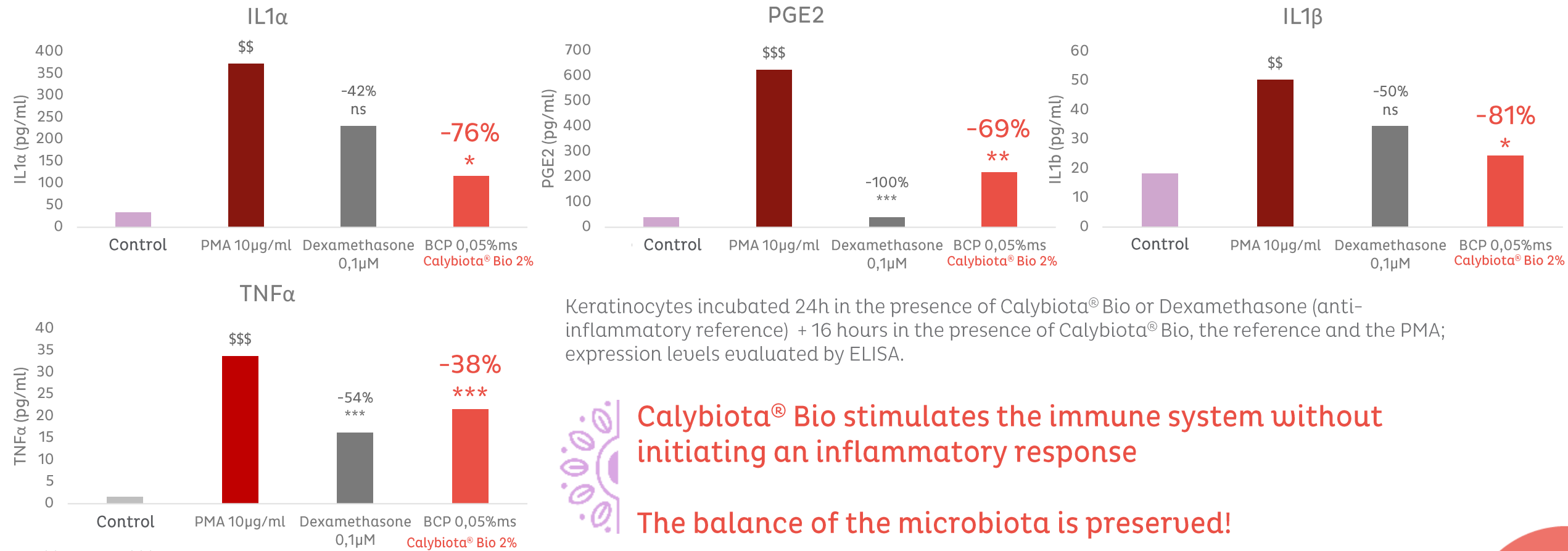
Bacteriostatic or even anti-microbial effect of Calybiota® Bio on pathogenic strains

	<i>L. crispatus</i> (Log ₁₀ CFU/ml)		<i>L. jensenii</i> (Log ₁₀ CFU/ml)		<i>G. vaginalis</i> (Log ₁₀ CFU/ml)		<i>E. coli</i> (Log ₁₀ CFU/ml)	
	4h	24h	4h	24h	4h	24h	4h	24h
Control	7.84	7.29	7.23	8.28	8.70	8.87	8.98	8.64
Glucose 2%	9.24	8.29	9.23	8.84	9.40	9.32	8.46	8.78
Ciprofloxacin	5.89	5.25	6.10	6.13	5.47	4.71	5.39	2.56
Calybiota® Bio 0,25%	8.64	8.26*	8.55*	8.47	8.99	7.67**	8.02**	8.33
Calybiota® Bio 1%	8.79	7.78	9.01	8.97**	7.19*	7.04**	7.88*	7.25**

Calybiota® Bio protects the skin from inflammatory stress

Science &
Conscience
NATURALLY®

Calybiota® Bio shows **anti-inflammatory efficacy** against a wide range of inflammatory mediators



\$\$ $p < 0,01$; \$\$\$ $p < 0,001$ vs Contrôle

* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$ vs PMA

Calybiota® Bio restores also the microbiota for others applications such as Acne, Atopic dermatitis, Deodorant

Science &
Conscience
NATURALLY®

Microbiota balance

INTIMATE CARE

- **Prebiotic** effect on the vaginal bacteria
L. gasseri, L. acidophilus, L. rhamnosus, L. crispatus, L. jensenii
- **Protective effect** through a stimulation of Bacterium/Host communication systems
- **Bacteriostatic** on pathogenic strains

ACNE

- **Inhibits** *M. furfur* & *C. acnes*
- Limit biofilm formation

ATOPIC DERMATITIS

- **Inhibits** adhesion & formation *S. aureus*
- Protect skin barrier

DEODORANT

- **Inhibits** *C. xerosis*
- Limits the odors from *S. epidermidis* & *C. stratum*



Calybiota® Bio

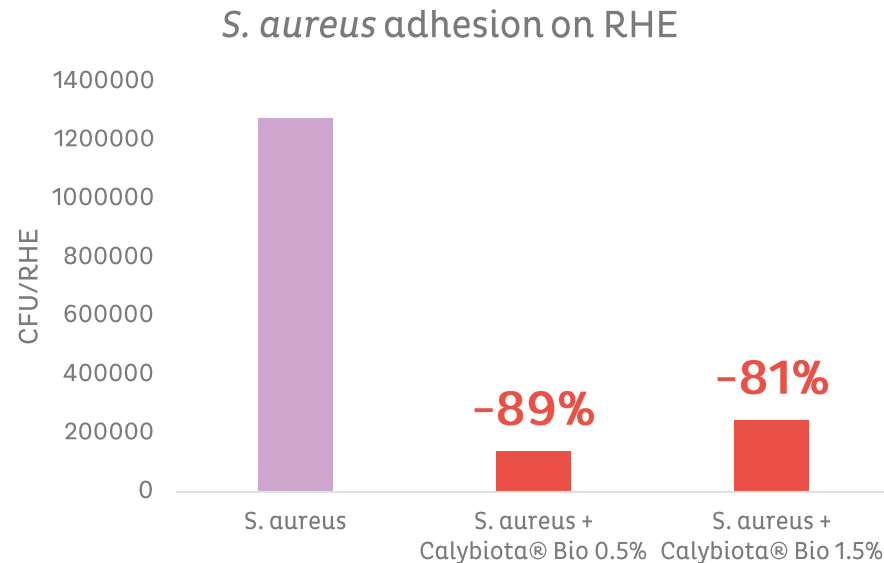
- Balances the microbiota
- Limits inflammation
- Reduces discomfort sensations

Atopic dermatitis and pathogenesis of *S. aureus*

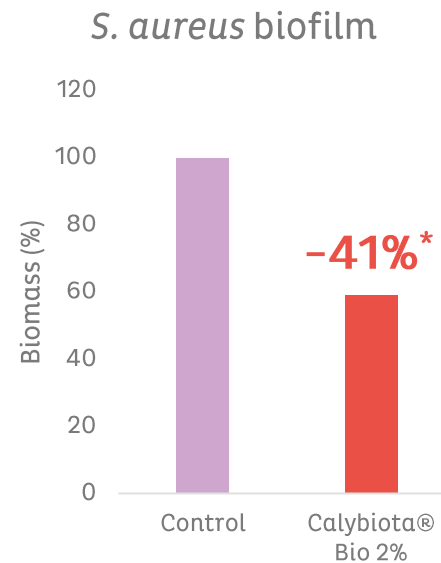
Calybiota® Bio effect on *S aureus*

S aureus (*Staphylococcus aureus*) is involved in many skin infections and its role is particularly described in the pathogenesis of **Atopic Dermatitis (AD)**

By forming a **biofilm** on the skin surface, *S aureus* participate in the pathophysiology of AD and aggravates it, notably by altering the barrier



S. aureus adhesion study on RHE surface incubated (15min + 4h) with Calybiota® Bio (0,5 and 1,5%) and the strain



Incubation 24h, 48h and 72h in microplate
Biofilm study by crystal violet technique

Biofilm: microbial cells assembly associated with a living organism or tissue and "embedded" in a polysaccharide matrix. In the skin, biofilms have been described in different pathologies such as acne, rosacea, AD.

* $p < 0,05$; ** $p < 0,01$;
*** $p < 0,001$ vs Control

Calybiota® Bio inhibits the adhesion and formation of *S aureus* biofilm on the skin surface

Atopic dermatitis

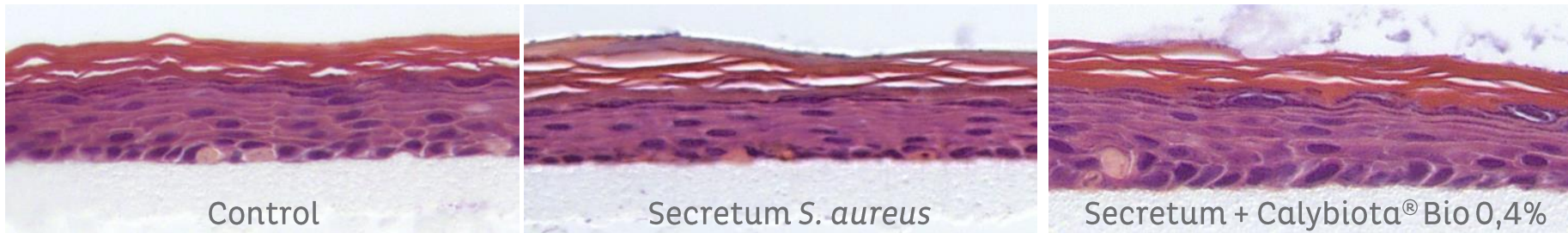
Defense against *S aureus*-induced damage

Science &
Conscience
NATURALLY®

S. aureus secretum applied to the surface of keratinocytes (RHE) induces a moderate alteration of the RHE morphology: epidermis structure disorganization (basal layer) and fewer keratohyalin grains in the granular layer

Calybiota® Bio preserves the epidermis morphology: better organized, thicker and more keratohyalin grains production

*Morphology analysis of RHE after staining
Hematoxylin/Eosin*



Pre-treatment with Calybiota® Bio 0.4% for 24h then addition of *S. aureus* secretum for 24h



Calybiota® Bio protects the skin barrier from alteration by *S aureus*, preserves the skin suffering from Atopic dermatitis

Atopic dermatitis

Defense against *S aureus*-induced damage

Calybiota® Bio protects barrier markers altered by *S aureus* secretum

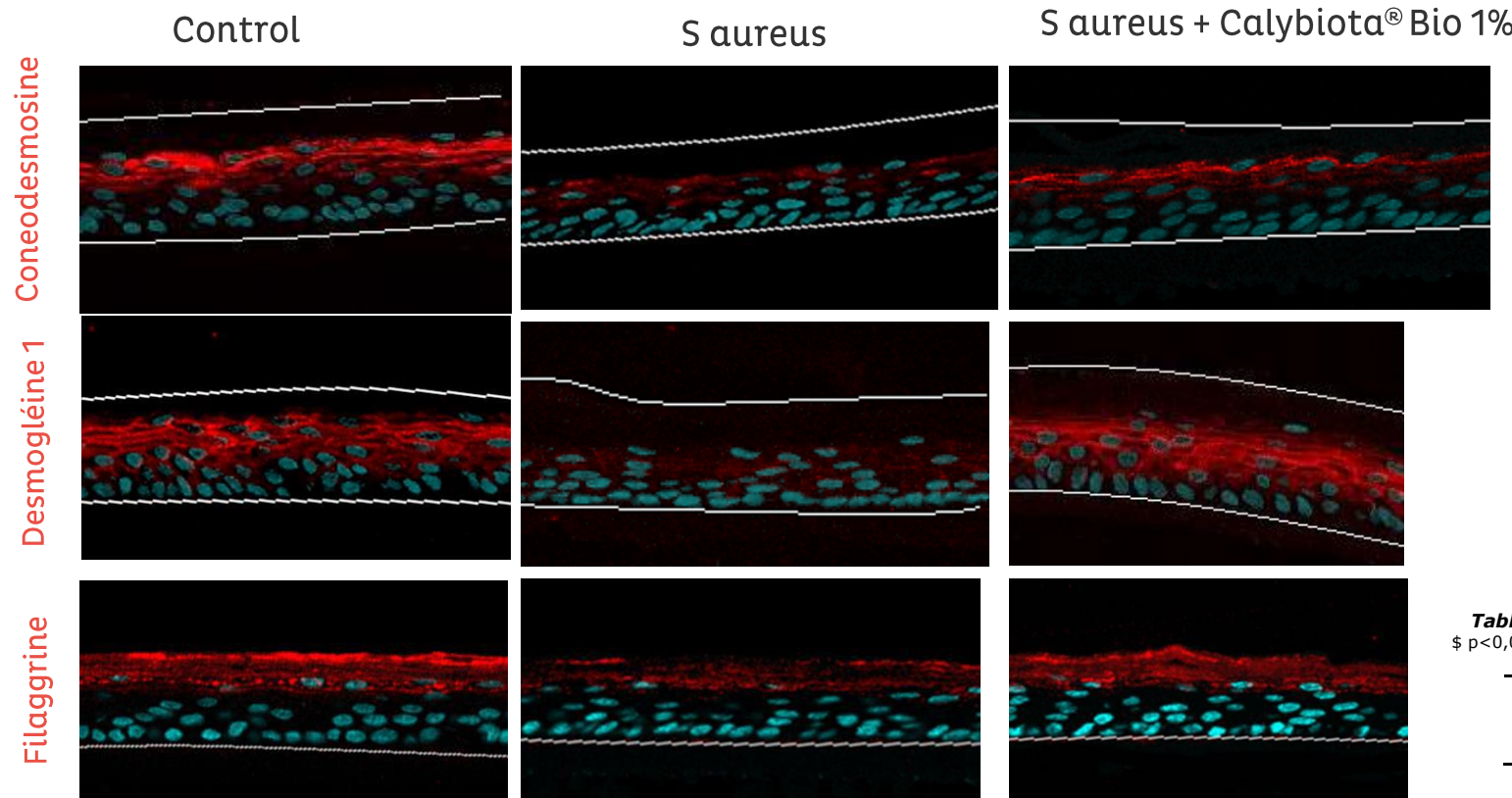


Tableau 14 : Expression des marqueurs de la barrière dans les RHE
\$ p<0,05 ; \$\$ p<0,01 vs Contrôle ; *p<0,05 ; **p<0,01 vs secretum – Unpaired t test

	Cornéodesmosine	Desmoglène 1	Filaggrine
Contrôle	100	100	100
Secretum <i>S. aureus</i>	49,6 \$	35,4 \$\$	42,5 \$\$
Secretum + BCP 1%	84,2 *	120,1 **	79,4 *

Calybiota® Bio protects the skin barrier from alteration by *S aureus*, preserves the skin suffering from Atopic dermatitis



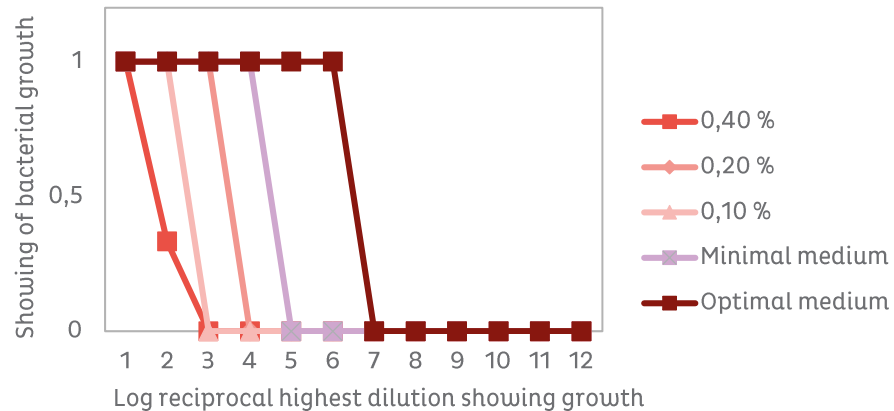
Acne and seborrheic dermatitis

Calybiota® Bio effects on *M. furfur* & *C. acnes* growth of *C. acnes* biofilm

Cutibacterium acnes: strain involved in skin acne

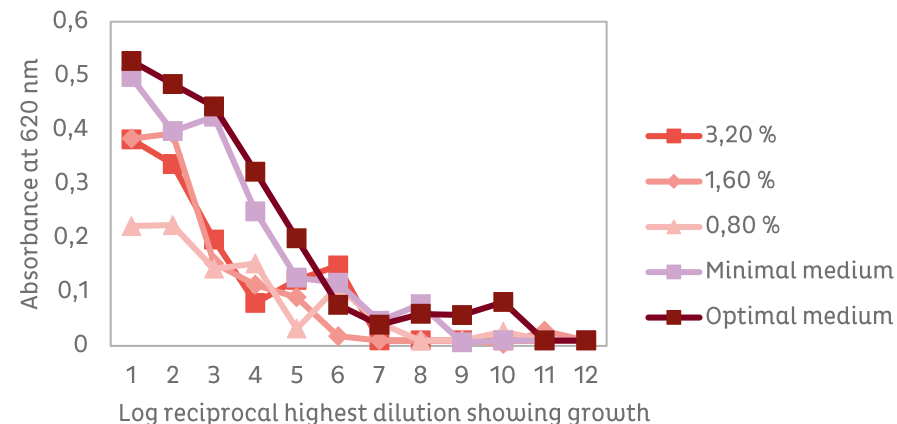
Malassezia furfur: strain involved in seborrheic dermatitis

Growth of *C. acnes* in the Calybiota® Bio presence

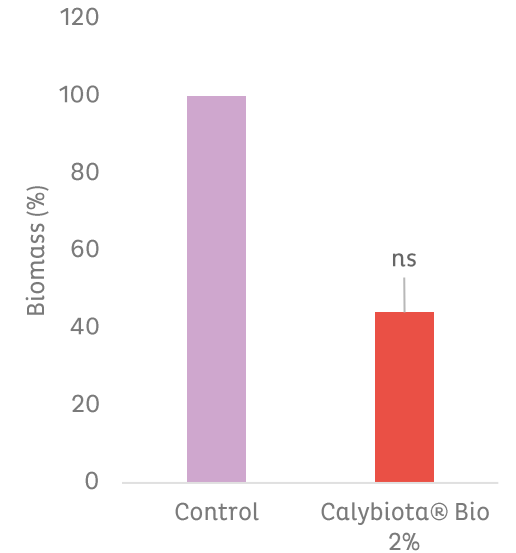


RHE incubation with Calybiota® Bio at different concentrations + strains
Growth study by counting bacteria or by measuring absorbance (yeast)

Growth of *M. furfur* in the Calybiota® Bio presence



C. acnes RT4 biofilm



Incubation 24h, 48h
and 72h in microplate
Biofilm study by crystal
violet technique



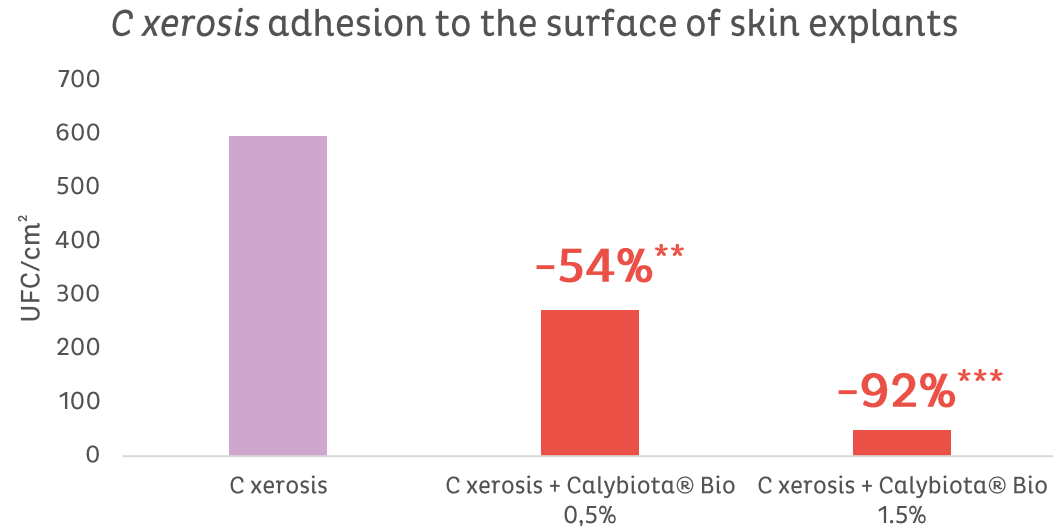
Calybiota® Bio inhibits *M. furfur* & *C. acnes* growth and tends to limit the biofilm formation of acne strain

Microbiota and axillary odors

Calybiota® Bio effect on *C xerosis* adhesion

Science &
Conscience
NATURALLY®

Corynebacteria (especially *C xerosis*) and **Staphylococci** are the main bacterial species responsible for axillary odors



* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$ us Control

C. xerosis adhesion to the surface of skin explants (incubation 2h + 24h)
incubated with Calybiota® Bio (0,5% and 1,5%)

Axillary odors development is linked to the transformation of odorless secretions into volatile odoriferous molecules by the commensal flora microorganisms.

Many factors have an influence on the secretion's quality and quantity and on the bacteria present: **age, sex, genetic factors, environmental factors, hygiene, cosmetics...**

Calybiota® Bio significantly inhibits the *C xerosis* adhesion to the skin surface



Microbiota and axillary odors

Anti-odour effect of Calybiota® Bio

In vitro **sniff test** against 2 main bacterial species responsible for axillary odors. Each strain has a typical olfactory signature, resulting from the metabolization of sweat components:

Viability and bacteria odour in reconstituted sweat

***p<0,01 – ANOVA test vs Control*

	<i>S epidermidis</i>			
	6h		24h	
	Viability (Log10 CFU/ml)	Odour (Sniff)	Viability (Log10 CFU/ml)	Odour (Sniff)
Control (reconstituted sweat)	8,91 ± 0,21	intense	8,69 ± 0,09	intense
Chlorhexidin	0,00 ± 0,00**	absent	0,00 ± 0,00**	absent
Calybiota® Bio 0,05%	8,80 ± 0,08	moderate	8,64 ± 0,30	moderate-intense
Calybiota® Bio 0,3%	8,87 ± 0,07	moderate-absent	8,87 ± 0,15	moderate-absent
Calybiota® Bio 1%	8,73 ± 0,34	moderate	8,78 ± 0,18	moderate-absent

	<i>C striatum</i>			
	6h		24h	
	Viability (Log10 CFU/ml)	Odour (Sniff)	Viability (Log10 CFU/ml)	Odour (Sniff)
Control (reconstituted sweat)	8,51 ± 0,08	moderate	8,64 ± 0,19	moderate
Chlorhexidin	0,00 ± 0,00**	absent	0,00 ± 0,00**	intense
Calybiota® Bio 0,05%	8,59 ± 0,31	moderate	8,38 ± 0,11	moderate
Calybiota® Bio 0,3%	8,66 ± 0,32	absent	8,57 ± 0,11	moderate-absent
Calybiota® Bio 1%	8,28 ± 0,06	absent	8,31 ± 0,28	absent

- ***Staphylococcus epidermidis***:
Valerianic and propionic acids
- ***Corynebacterium striatum***:
Sulphanyl alcohols



Calybiota® Bio inhibits odors produced by *S epidermidis* et *C striatum* without altering the bacteria viability
Potential for deodorant applications



Clinical study

Clinical study

Effectiveness evaluation of Calybiota® Bio for intimate care/hygiene

Science &
Conscience
NATURALLY®



POPULATION

Women - Phototypes I to IV

60 women divided into 5 groups:

- Post-Vaginosis n=11
- Post-Mycosis n=12
- Irritation n=13
- Active post-menopause n=12
- Post-Menopause control n=12



PROTOCOL

Products formulated with **Calybiota® Bio 1,25%** (except for the Post-Menopause group Control): product without the active ingredient

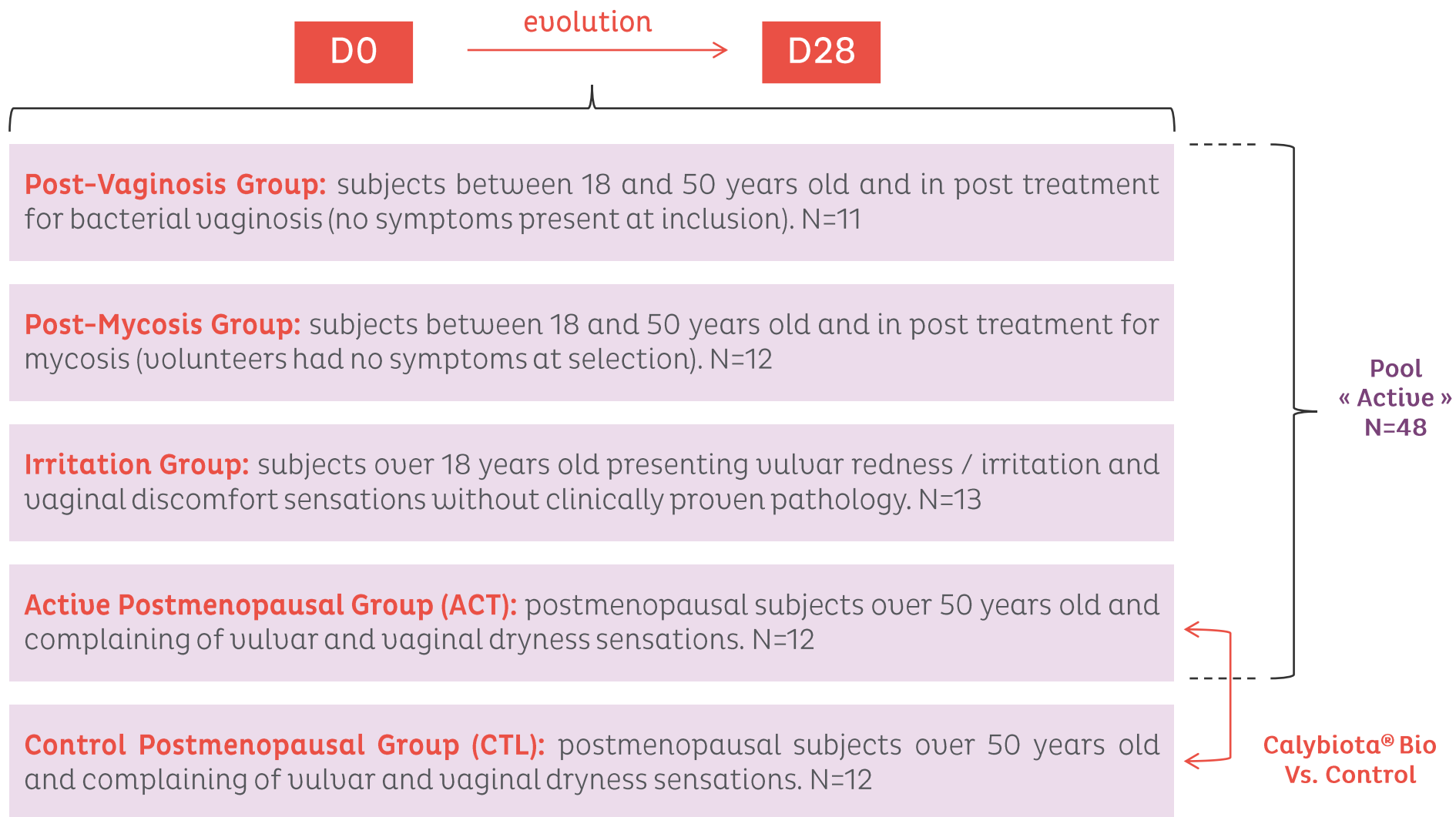
Combination of a cream and a cleanser:

- **Cream:** 2x / day after a hygiene routine or at any time of the day.
Massage until completely absorbed.
- **Cleansing gel:** 1x / day. Use as a liquid soap, for external use on the intimate area (not for the whole body). Rinse with clear water.

Application on the intimate zone for 28 days



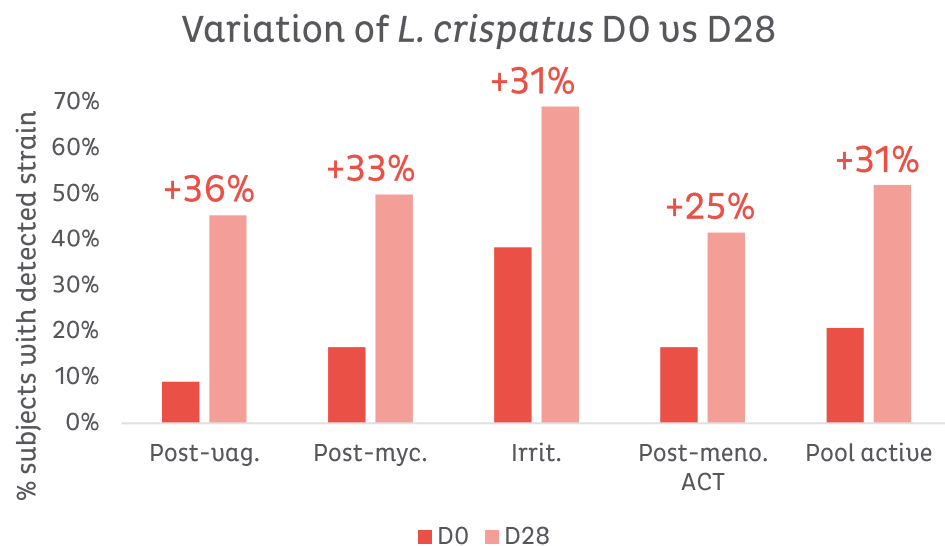
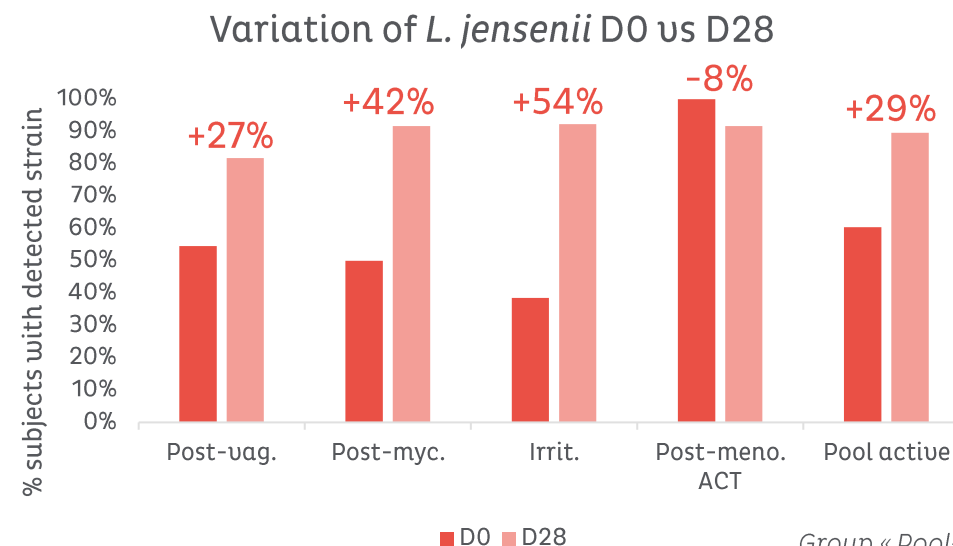
5 groups of women



Bacteriological analysis of *L. crispatus* and *L. Jensenii*

L. crispatus and *L. jensenii* are lactobacillus species present in the vaginal ecosystem, they are considered as protectors and biomarkers of the healthy vaginal tract.

Population of *L. crispatus* and *L. Jensenii* increase for all groups and in particular for the groups using Calybiota® Bio.



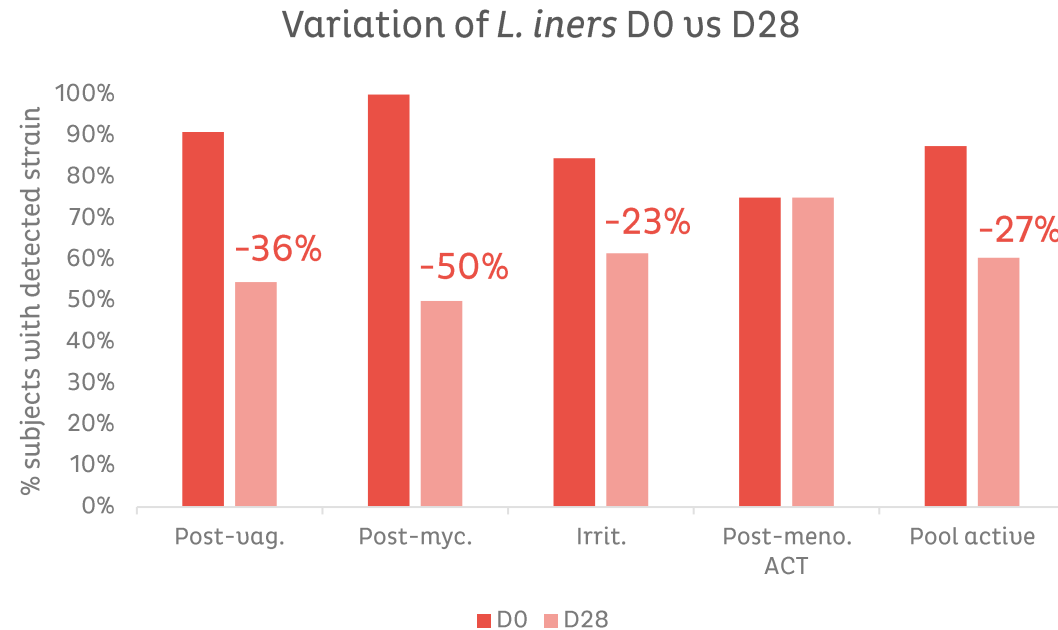
Group « Pool-active » n=48
(Post-Vaginosis + Post-Mycosis
+ Irritation + Postmenopause)

Calybiota® Bio clinically confirms its prebiotic effect

Bacteriological analysis of *L iners*

L. Iners is a lactobacillus also present in the vaginal flora, more likely to promote **dysbiosis** conditions.

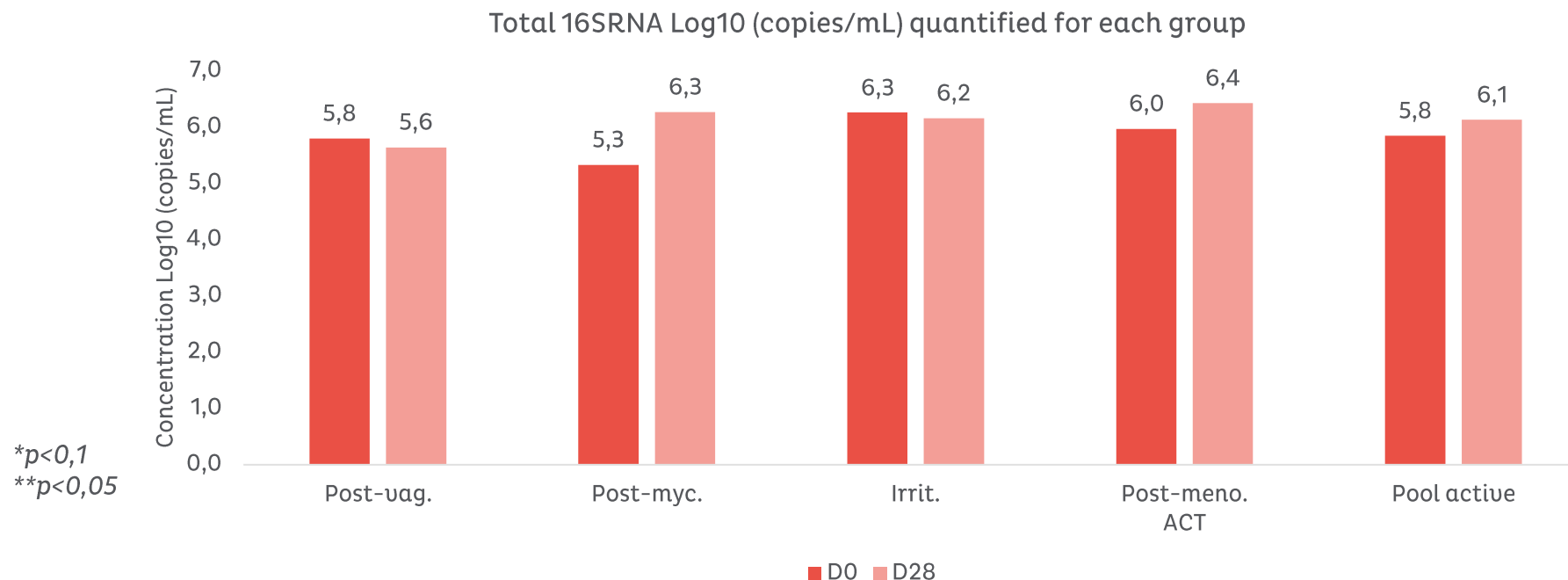
Population of *L iners* **decreases** for all groups



Calybiota® Bio clinically confirms its prebiotic effect

Bacteriological analysis of the total flora

Calybiota® Bio has no impact on the total amount of strains present in the vaginal flora

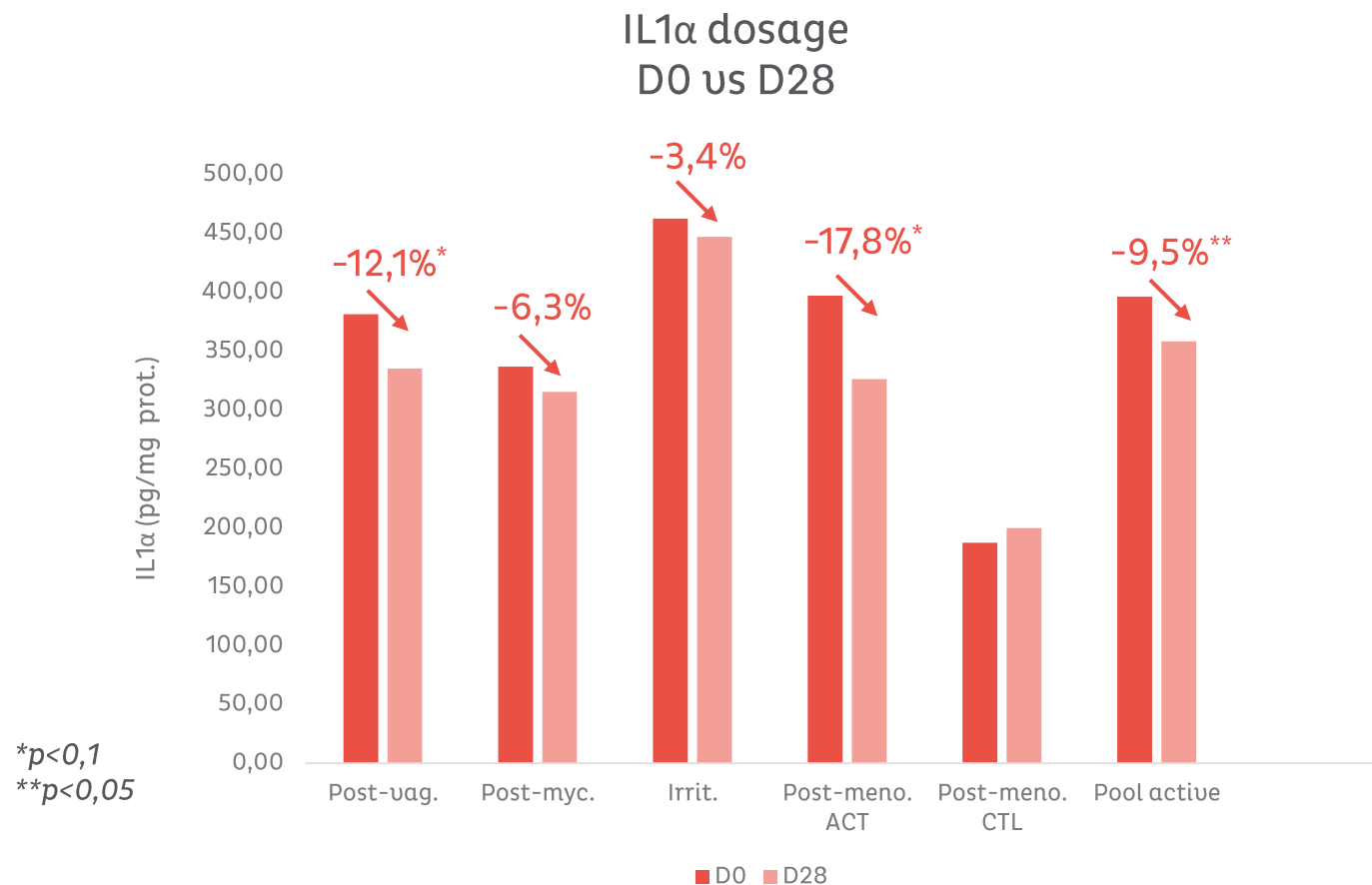


Calybiota® Bio respects the women's intimate flora

Calybiota® Bio decreases a strong inflammation maker

Science &
Conscience
NATURALLY®

Dosage of IL1- α , strong inflammatory marker (swab method)



Calybiota® Bio reduces inflammation and soothes the intimate area

Clinical scoring

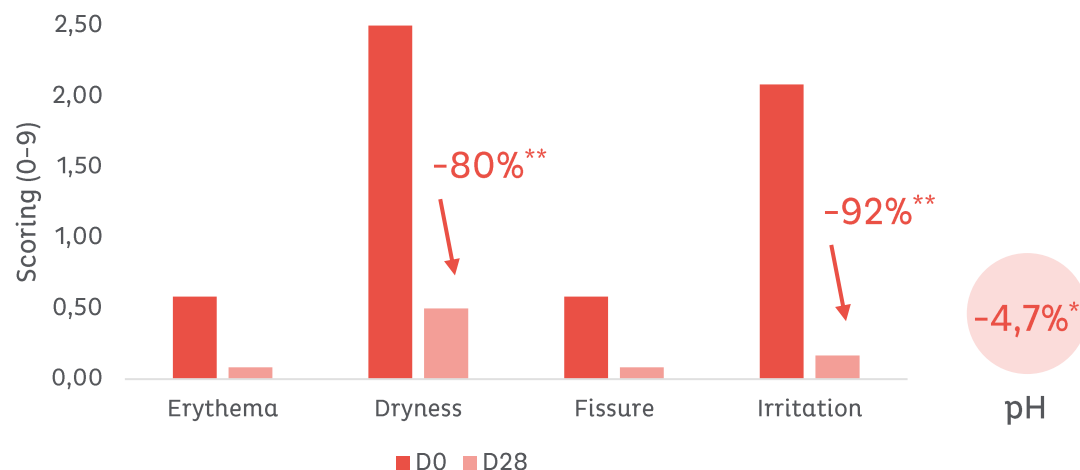
Post-Menopause Group Calybiota® Bio and Control

Science &
Conscience
NATURALLY®

Significative decrease: ↓ **dryness**, ↓ **irritation**, ↓ **pH**

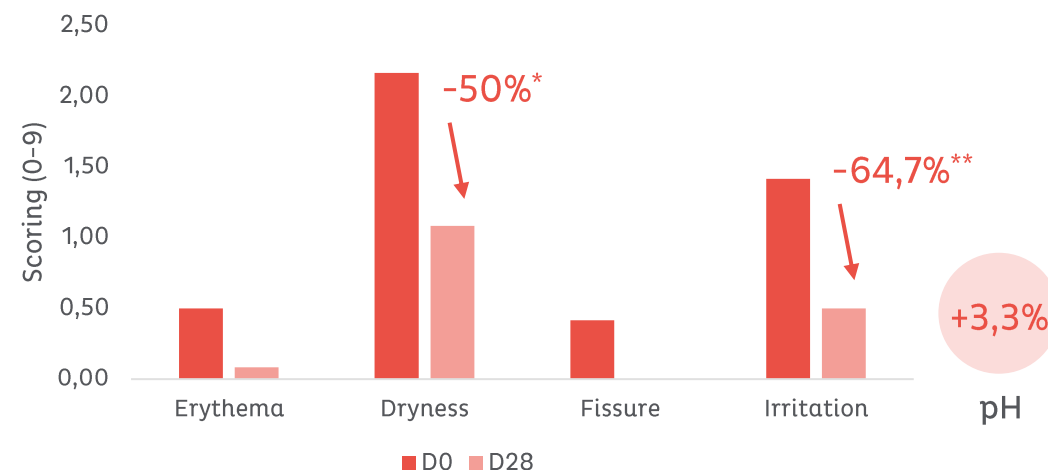
Clinical scoring (D0 vs D28)

Post-Menopause group Calybiota® Bio



* $p < 0,1$
** $p < 0,05$

Post-Menopause group Control



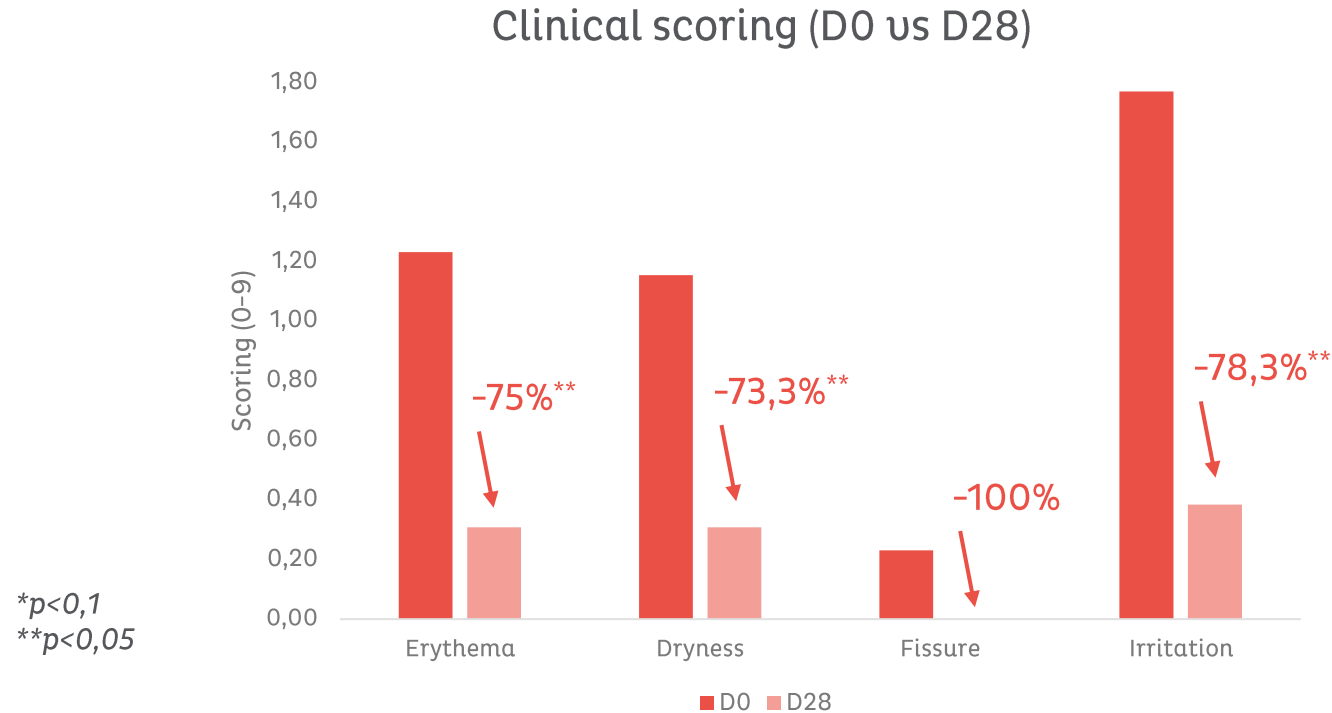
Calybiota® Bio soothes intimate area and provides moisture for the postmenopausal women

Clinical scoring

Irritation Group Calybiota® Bio D28 vs D0

Science &
Conscience
NATURALLY®

Significative decrease: ↓ **dryness**, ↓ **irritation**, ↓ **erythema**



Calybiota® Bio soothes intimate area and provides moisture for all the women suffering of irritation

Clinical scoring

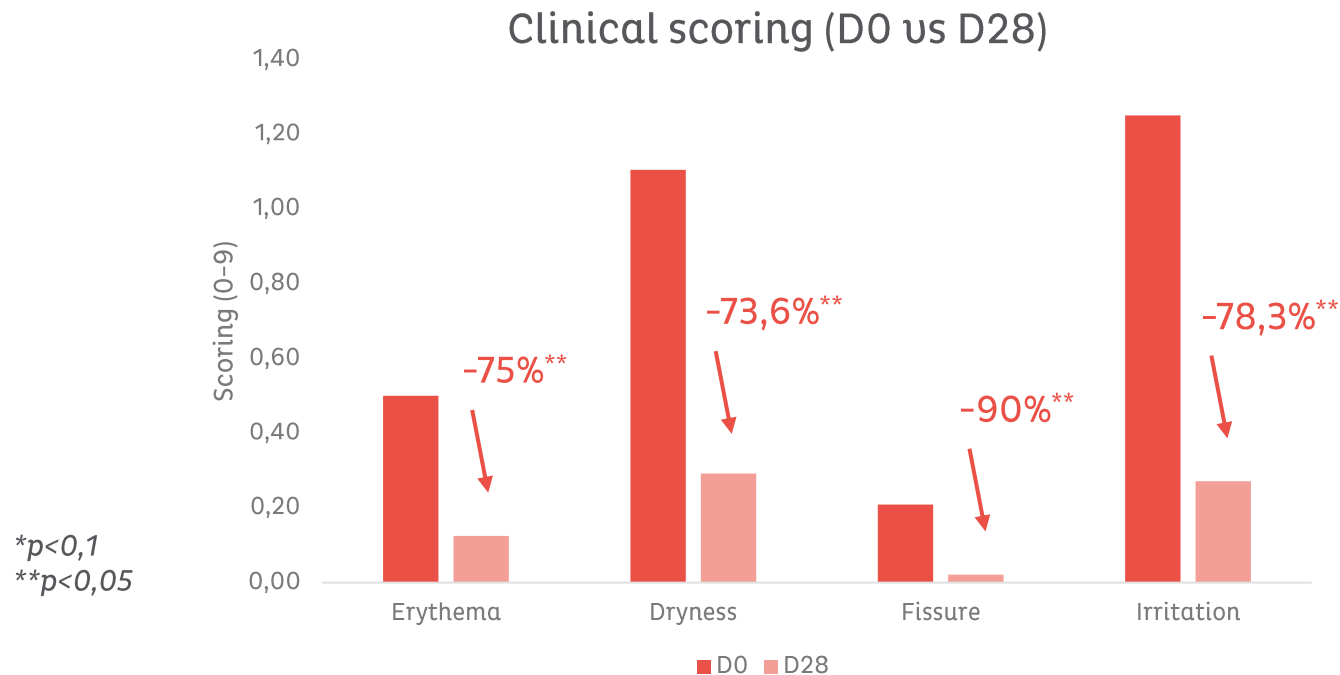
Pool-active Calybiota® Bio vs D0

Science &
Conscience
NATURALLY®

Group « Pool-active » n=48 (Post-Vaginosis + Post-Mycosis + Irritation + Post-Menopause)

Significant decrease : ↓ **dryness** ; ↓ **irritation** ; ↓ **erythema** ; ↓ **fissure**

No pH variation



**Calybiota® Bio soothes women's intimate area
and provides moisture**

Auto-scoring

Post-Vaginosis and Irritation Groups Calybiota® Bio vs D0

Science &
Conscience
NATURALLY®

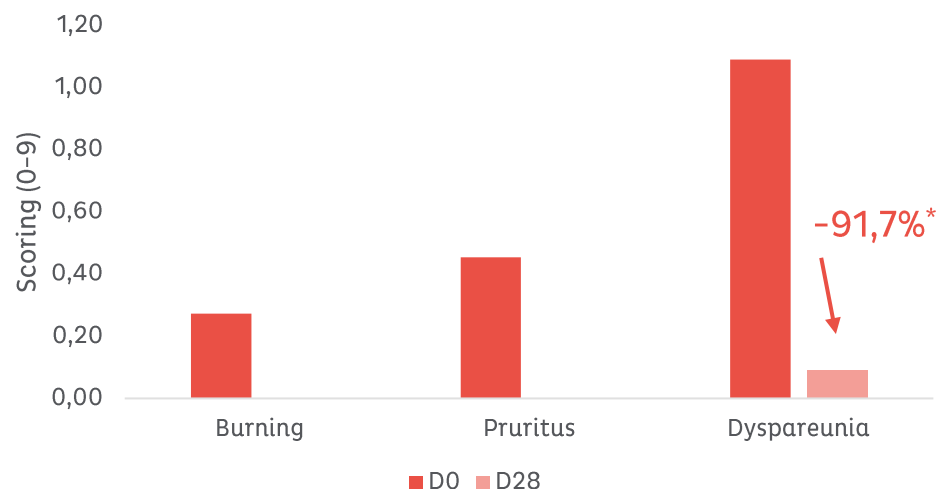
Post-Vaginosis Group:

Significant decrease ↓ **dyspareunia**

Irritation Group:

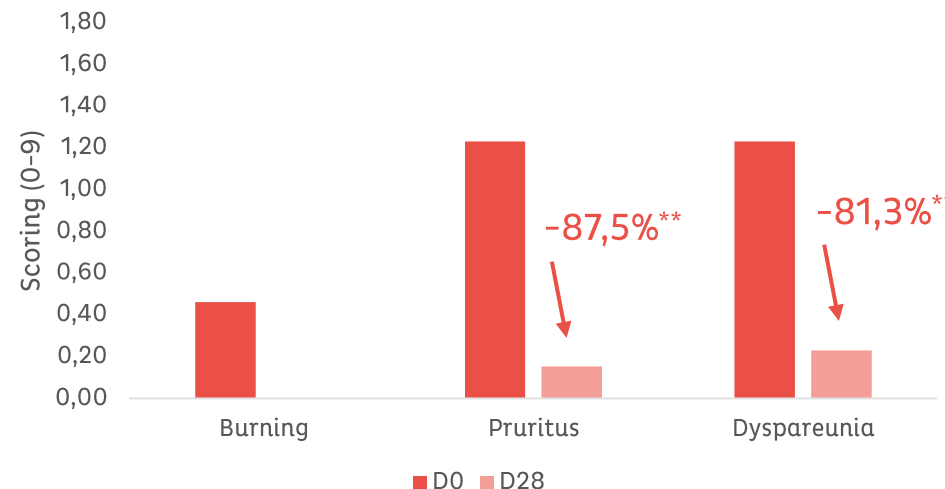
Significant decrease ↓ **dyspareunia** ; ↓ **pruritus**

Post-Vaginosis group (D0 vs D28)



* $p < 0,1$
** $p < 0,05$

Irritation group (D0 vs D28)



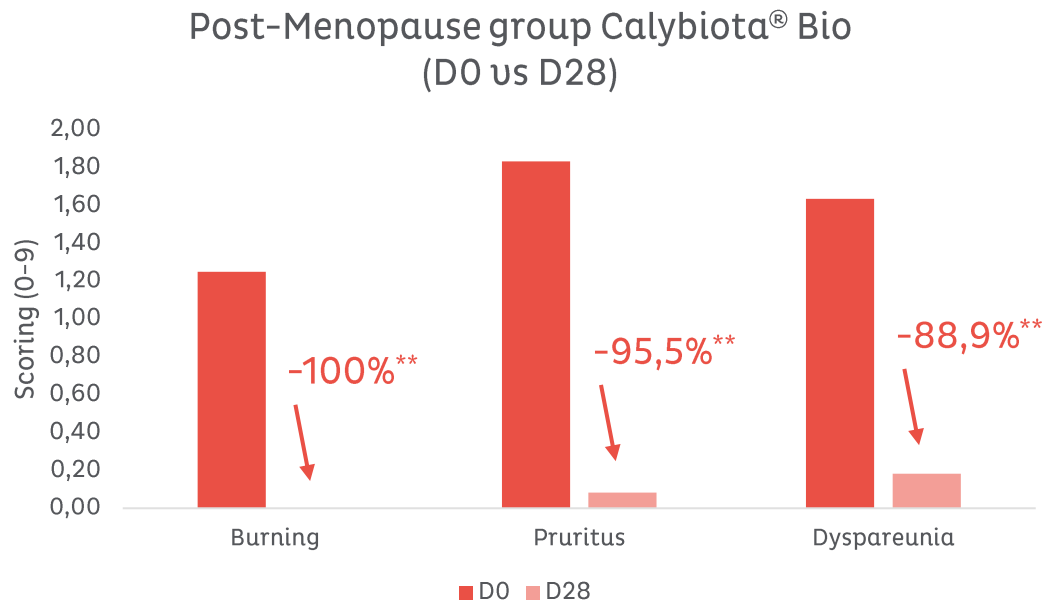
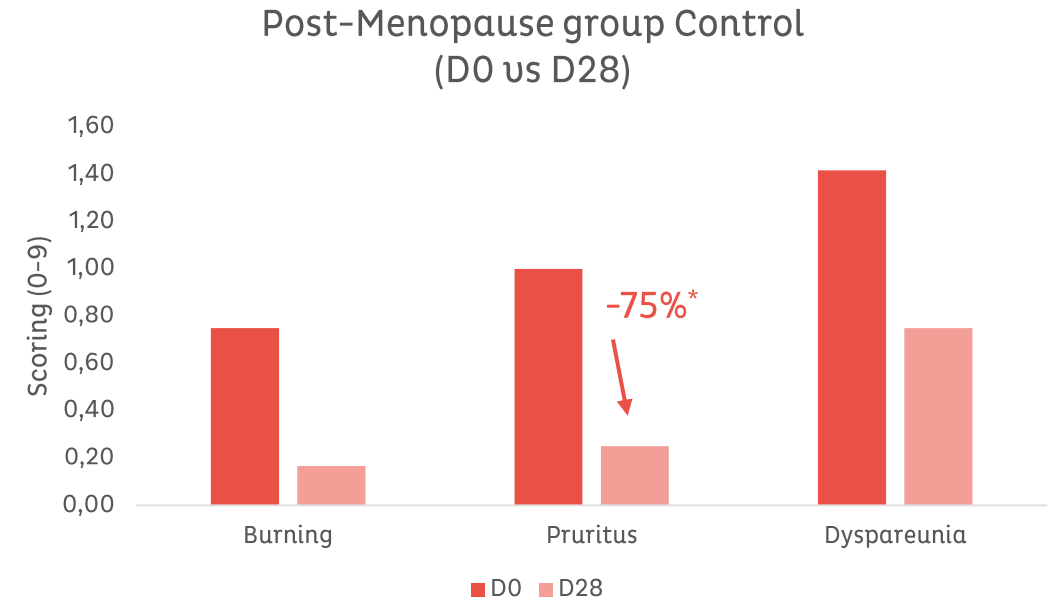
Calybiota® Bio improves the sexual comfort for post-vaginosis and women experiencing irritations

Auto-scoring

Post-Menopause Group Calybiota® Bio and Control

Science &
Conscience
NATURALLY®

Significant decrease for the active group ↓ **dyspareunia**, ↓ **burning**, ↓ **pruritus**



* $p < 0,1$
** $p < 0,05$



Calybiota® Bio improves the sexual comfort for Post-menopausal women

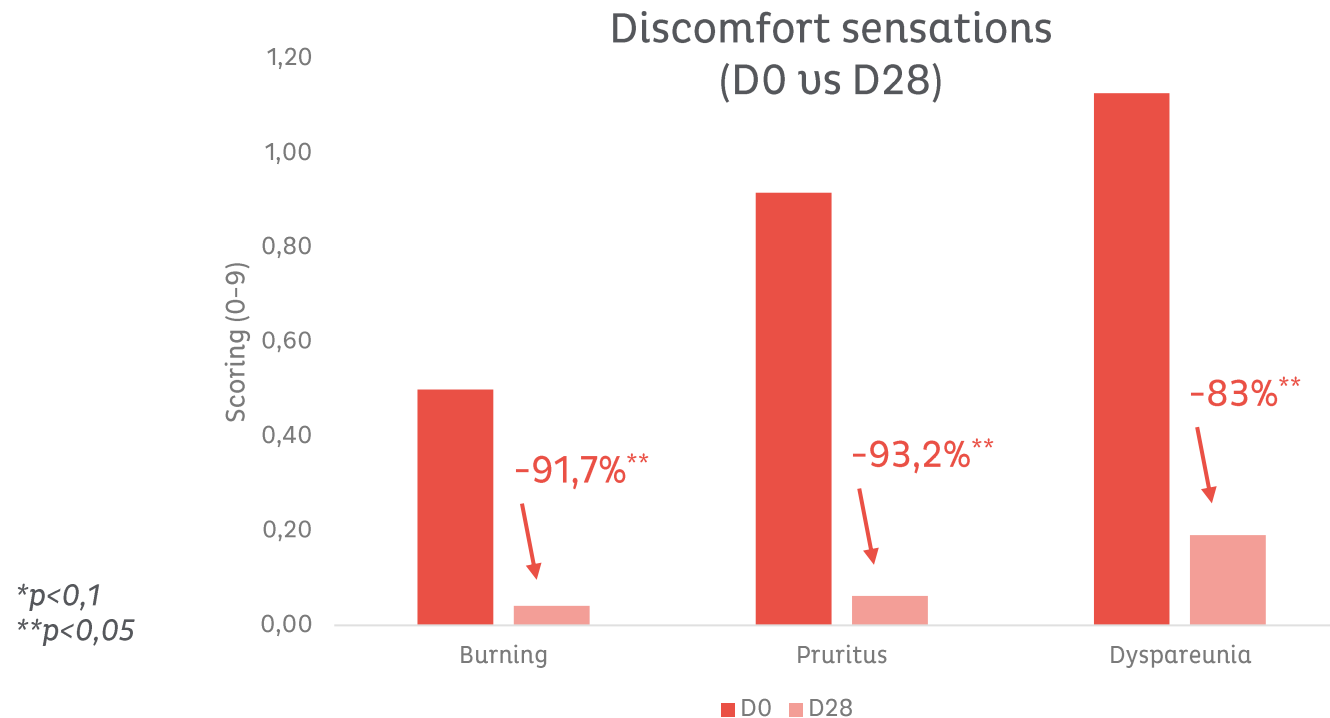
Auto-scoring

Pool-active Calybiota® Bio vs D0

Science &
Conscience
NATURALLY®

Group « Pool-active » n=48 (Post-Vaginosis + Post-Mycosis + Irritation + Post-Menopause)

Significant decrease: ↓ **dyspareunia**, ↓ **burning**, ↓ **pruritus**



Calybiota® Bio makes intimate discomfort disappear

What do women think of Calybiota® Bio?

Science &
Conscience
NATURALLY®

Group « Pool-active » n=48 (Post-Vaginosis + Post-Mycosis + Irritation + Post-Menopause)

81%

of women
have seen an
improvement of
their sexual life

83%

of women find
that the product
reduces the itching
sensations

89%

of women find
that the product
reduces the
irritation sensations

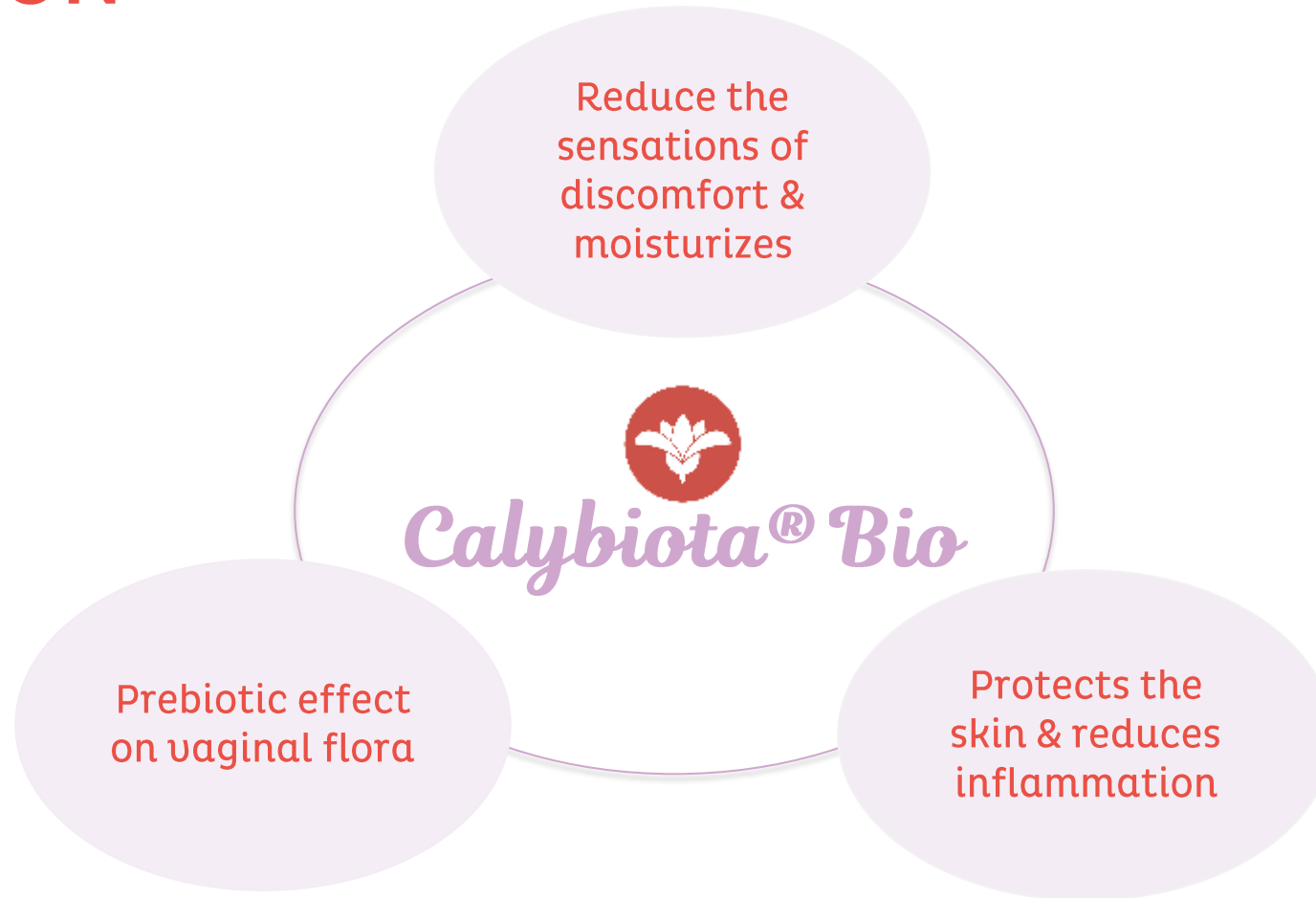
87%

of women find that
the product reduces
dryness



CONCLUSION

Science &
Conscience
NATURALLY®



By rebalancing the microbiota, Calybiota® Bio helps women feel better and improves their sexual life!

For further information

Science &
Conscience
NATURALLY®

Laboratoires Expanscience

1, place des Saisons

92048 Paris La Défense Cedex

www.expanscience-ingredients.com