

LactoSporin® is a non-living microbial cell product having biological activity. It is purified from the culture supernatant of probiotic strain, *B. coagulans* MTCC 5856 (LactoSpore®) with an INCI name - *Bacillus Ferment Filtrate Extract*.

Skin microbiome

The normal human skin is a microbiome colonized by 10,000 – 1,000,000 beneficial microorganisms, especially bacterial units/cm² that prevent the growth of pathogenic organisms and maintain the immunity of the skin and nourish the skin optimally with an established safety profile.

Skin microbiome protect the skin by :

- They act as a protective shield by preventing pathogenic microorganisms from provoking an immune reaction
- They produce antimicrobial metabolites to combat pathogenic bacteria
- They boost the skin's functionality in dealing with external damaging factors including free radicals, sun, and pollution
- They help the skin stay hydrated
- Restore a healthy pH balance in the skin

These functions creates a calming environment for the skin, help to reduce inflammation, flare ups and prevents premature aging.

Next level postbiotics

These are the water soluble, non-living microbial cell products, fractions, lysates or metabolites obtained from microorganisms possessing biological activity (Patel and Denning 2013). It has various advantages over probiotics (Aguilar-Toalá et al., 2018).

- Biological activity in non-viable state
- Lower chance for microbial translocation
- Lower chance for microbial infection
- Improved inflammatory defence
- Favourable physiochemical properties (solubility)
- Favorable pharmacokinetic properties (absorption, distribution, metabolism, and excretion)

LactoSporin®

- A standardized antimicrobial product
- It's an extracellular protein metabolite obtained from the supernatant of probiotic strain, *B. coagulans* MTCC 5856 (LactoSpore®)
- Pale yellow to brown color clear to hazy liquid with characteristic odor
- Completely soluble in water
- It acts against other pathological organisms
- It can be a potent next-generation probiotic in skin care application. Thus, eliminating the usage of live microorganisms in various biological streams
- Patents EP : "Method of producing partially purified extracellular metabolite products from *Bacillus coagulans* and biological applications thereof"

Anti-aging effects of LactoSporin®

Anti-collagenase activity : UV radiation induces the collagen degradation by enhancing the activity of collagenases, a type of matrix metalloproteases (MMPs) (Sanches Silveira and Myaki Pedroso 2014).

LactoSporin® inhibited the collagenase activity (percentage inhibition) in a dose-dependent manner with an IC₅₀ of 1.8% v/v. Thus, LactoSporin® can preserve the extracellular matrix of the skin and prevent wrinkle formation and skin sagging.

Antioxidant activity : Reactive oxygen species (ROS), the major contributor for ageing is generated from mitochondria (Rinnerthaler et al., 2015). It acts as the major factor in developing the oxidative stress that exceeds the antioxidant defence of the target cell. UV radiations are the major extrinsic factor that generates the ROS leading to sun-induced skin ageing (photo-ageing) (Poljšak and Dahmane 2012; Xu et al., 2017).

LactoSporin® showed dose-dependent DPPH scavenging effect with an IC₅₀ of 0.43%. This result emphasises the role of LactoSporin® as a potent antioxidant in cosmetic preparations.

Anti-glycation effect : Glycation is the process of undesired cross-linking of collagen proteins with sugar molecules, eventually impairing the function of collagen fibres, making the skin hard and aged in appearance (Chaudhuri et al., 2018). The anti-glycation effect of LactoSporin® was performed using ribose sugar and bovine serum albumin.

LactoSporin® was found to inhibit the formation of AGEs by 50% with an IC₅₀ of 0.7% v/v. Thus, inhibition of AGE formation by the retardation of the collagen fibres glycation provides the skin with youthful and aesthetically pleasant appearance (83% Inhibition at 2%, 56% @1%, 36% @0.5%)

UV protection effect : Humans are mostly affected by UV-A followed by UV-B. UV-B being shorter in wavelength and higher power radiation can easily penetrates deeper into the tissues of the skin. Thus, causing structural damage to the DNA and induce apoptosis. Whereas, UV-A radiation is mostly responsible for the generation of ROS such as superoxide anion, hydrogen peroxide and singlet oxygen, causing ROS-mediated inflammatory skin damage (Sanches Silveira and Myaki Pedroso 2014). The phototoxic (UV-A and UV-B light-induced toxicity) preventive effects of LactoSporin® was studied using mouse fibroblast cell line (Balb/C 3T3).

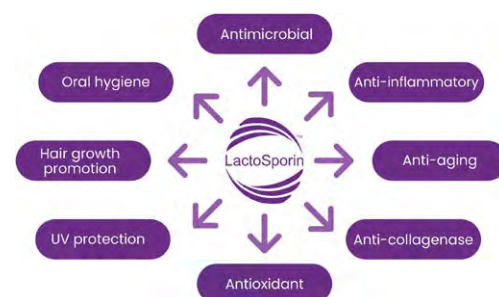
LactoSporin® showed notable protection from UV-A as well as UV-B-induced cell death (protection against UV radiation-induced toxicity).

Anti-inflammatory effect : LactoSporin® tested at various concentrations showed a dose-dependent anti-inflammation activity, so potentiality of LactoSporin® against the skin inflammation induced by pro-inflammatory environmental insults (inhibition of Lipopolysaccharide induced secretion, TNF-α on human cell line THP-1).

LactoSporin® is a non-living microbial cell product having biological activity : Antimicrobial activity, antiageing effects and hair growth promotion

Beneficial effects of LactoSporin®

LactoSporin® has wide applicability starting from oral health to UV protection, anti-collagenase, and anti-inflammatory:



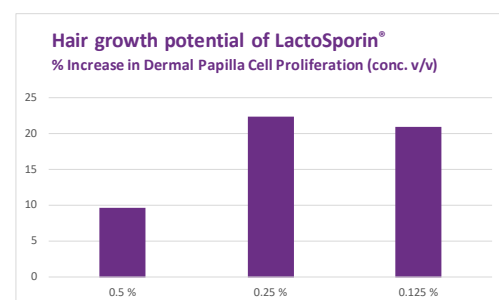
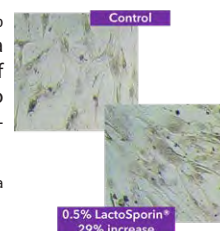
Hair growth promotion with LactoSporin®

Hair is one of the fastest growing tissues of the human body. Hair follicles undergo repetitive regenerative cycles consisting of anagen (rapid growth, active stage), catagen (regression, physiological involution stage), and telogen (resting stage). Hair proliferation is controlled by androgen hormones (e.g. testosterone). Androgens shorten the anagen phase by targeting dermal papilla cells (DPCs) of the hair follicle. The 5α-dihydrotestosterone (potent active metabolite generated by 5α-reductase) has greater affinity towards androgen receptors in DPCs. Thus, extending the anagen phase is an effective treatment for the prevention of hair loss.

LactoSporin® capability of hair growth promotion was assessed by estimating the proliferation of Human Follicle Dermal Papilla cells (HFDPC) in vitro.

LactoSporin® induced 29.7% proliferation of dermal papilla cells and 16.2% inhibition of 5α-reductase activity in vitro at 0.5% v/v LactoSporin® concentration.

(picture : proliferation of dermal papilla cells with LactoSporin®)





LactoSporin®

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Antimicrobial activity

According to Riazi et al., 2012, LactoSporin® might exert antimicrobial action by targeting the plasma membrane of sensitive microbial cells and forming transient pores followed by leakage of intracellular contents and dissipation of pH gradient component of proton motive force (PMF). The PMF is important for physiological processes such as ATP generation, ion transport as well as motility, thus affecting the sustenance of the organism.

Results : LactoSporin® was found to be highly active against the important skin pathogens such as *Staphylococcus aureus*, *Streptococcus epidermis*, acne-causing bacterium – *Propionibacterium acne*, as well as against an opportunistic pathogen – *Pseudomonas aeruginosa*.

It exhibits a broad spectrum antimicrobial activity against both **Gram-positive** and **Gram-negative** bacteria. The minimum inhibitory concentration (MIC) against various pathogenic groups was found to be in the range of 0.5 to 4.0% v/v. LactoSporin® was effective in killing the tested harmful bacteria at varied pH (2-5) and temperature ranges (70 – 90 °C). Also, LactoSporin® retained the antimicrobial efficacy in varied storage conditions (Stable & Accelerated condition).

Oral Hygiene

The surface of the tooth is covered by a bio-film of microorganism, especially bacteria. Tooth decay or dental caries results from the acids secreted by certain bacteria. *Streptococcus mutans* are the common type of acid-producing oral pathogen that is present mainly in the mouth, pharynx and intestine. It decays the tooth by secreting glucose polysaccharides known as glucans (Forssten et al., 2010).

LactoSporin® was evaluated for the effect of oral hygiene by assessing its impact on the growth and drop in pH level of *S. mutans*.

A significant reduction (for about 12 hours) in the growth, rate of pH drop (prevention from detrimental acidic environment) and glucan production was observed in *S. mutans* within 5 minutes of the LactoSporin® treatment.

Biofilm inhibitory potential of LactoSporin®

Biofilm, the extracellular matrix on living or non-living surfaces are less susceptible to antimicrobial agents. Thus, these associations of microbial cells are difficult to target and treat (Donlan 2000). LactoSporin® exhibited a concentration-dependent biofilm inhibitory effect against *S. mutans*, *S. aureus*, *P. aeruginosa* and *E. Coli* (broth micro-dilution method).

