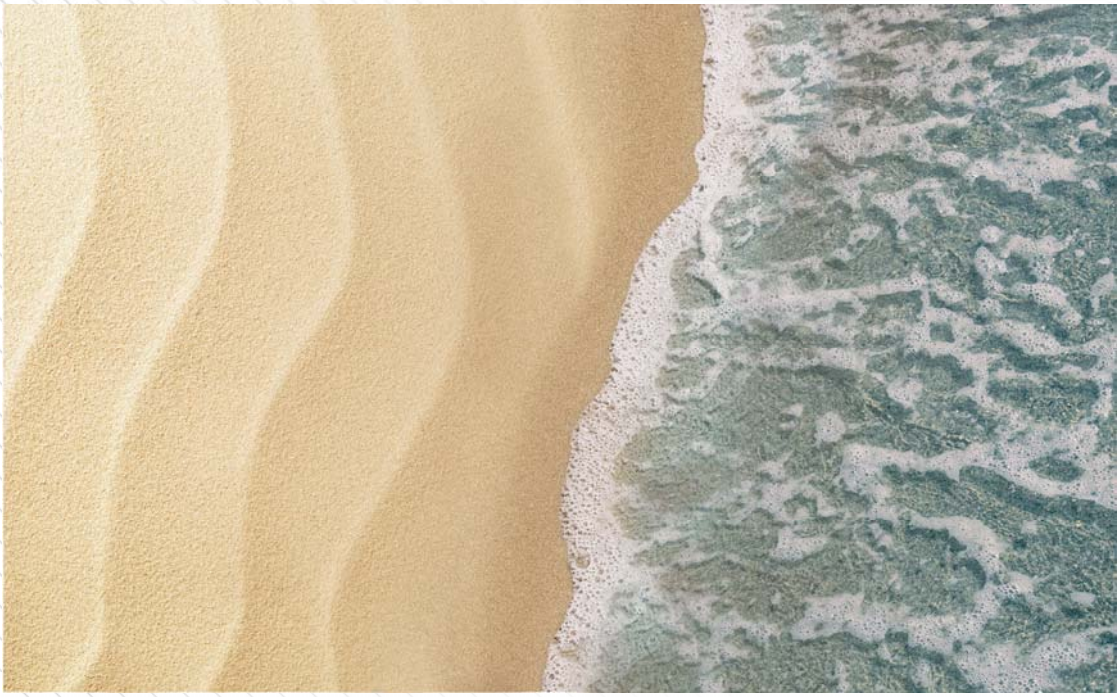
Technical Report



Product

SEACODE™

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An entire different world under the water

Aquatic ecosystems are critical components of the global environment as they are essential contributors to biodiversity and ecological productivity. **Marine ecosystems** cover **over 70% of the surface of the Earth** and contain approximately **97% of the water of the entire planet**. The habitats that form this vast ecosystem, which generates 32% of the world's net primary production, range from the nearshore regions to the barren ocean floor. **Oceans are home to some of the most diverse life forms** going from tiny planktonic organisms that comprise the base of the marine food net (like phytoplankton and zooplankton) to large marine mammals, fish species and invertebrates (sponges, crustaceans, molluscs,...), which rely on marine ecosystems for food and protection.

One of the basic **differential features** of marine ecosystems is the presence of **dissolved compounds** in their seawater. They are mainly **salts** with generally 85% of Sodium and Chlorine, whose total gram weight in one kg of seawater is referred to as salinity. These dissolved elements are responsible for the distinctive "salty" taste and **affect the development and composition of sea inhabitants**.

Just like any other, marine ecosystems require **light and nutrients** to produce food and energy, but both are found to be **limiting factors**. The amount of light penetrating the ocean surface tends to decrease with increasing water depth, so photosynthesis can only take place within a small superficial band of water, and nutrient availability significantly varies from place to place. Other factors like high pH (around 8), seawater salinity (35 ppt on average), tides, waves, currents and a gradient of temperature, oxygen and light contribute to make **life in marine ecosystems not generally easy for their inhabitants**.

Moreover, there are **certain aquatic areas where life can be even harder** due to extra difficult conditions. **Intertidal zones** where high (flooding) and low tides (desiccation) generate local drastic water changes are a clear example, as well as **the Antarctica**, where extreme low temperatures and dramatic nutrient concentration are typical. In such difficult habitats, the living organisms need to adapt and develop special mechanisms to ensure its own survival and/or help their colony [1-3].

Marine ecosystems are an enormous source of diversity, where organisms need to adapt to the environment to survive.

Adaptation to extreme conditions

Bacteria, algae and fungi have the potential to **synthesise and secrete numerous Extracellular Polymeric Substances** (ECPS) to the surrounding media **in response to environmental stress**. Among them, **bacterial** ECPSs, basically polysaccharides, seem to be specifically interesting because they offer properties that are not available by plant polymers and their production is less influenced by pollution or climatic impact [1-2]. **In extreme conditions** where **bacteria** require specific mechanisms to survive, their **capability to produce functional ECPSs is higher** than any other bacteria [3].

Inhabitants need to develop useful bioactive compounds to **optimise their metabolic processes in the Antarctica** for instance, where there are prolonged periods of darkness and sub-zero temperatures (even under -40 ºC), freezing and defrosting twice a year that can last 100 days, and nutrients are scarce. Surprisingly, a diverse microbial diversity succeeds on surviving, being **psychrotrophs** a clear example [7, 8]. Such extremophile bacteria are perfectly adapted to temperatures below 5 °C: they live in sea ice and its underlying seawater. ***Pseudoalteromonas*** strains are **the most frequent** [9, 10].

Intertidal rocky shores are another clear case of difficult habitats due to its constant exposure to wave action and drastic changes in temperature, hydration, UV radiation and nutrients availability depending on tides, which become a **challenge for all their inhabitants** [11]. ***Pseudoalteromonas genus* is found** in these harsh areas too, where the severe physiological constraints are overcome due to the development of specific ECPSs [12].

Included in the ECPSs, **Exopolysaccharides** (EPS) are useful polysaccharides found external to the structural outer surface of the microbial cell. They contain **polymers of diverse composition**, although most of them present either uronic acids like D-glucuronic or ketal-linked pyruvate [4]. These glucidic polymers can **protect from extreme salinity, pressure and temperatures** by increasing hydration and nutrition, participating in intracellular processes, cell recognition, proliferation and migration, immunologic modulation, and helping in favourable adhesions to solid surfaces, possibly having the ability to interact with cell receptors to promote concrete activities too [1, 3, 5-6].

Bacterial ECPSs from harsh environments offer potentially useful properties for skin care.

ECPSs benefits on the skin due to resemblance

There is a wide variety of compounds that bacteria produce in order to get adapted to their surrounding conditions. Concretely, some of the Gram-negative bacteria belonging to the ***Pseudoalteromonas genus*** **are able to produce** active molecules, like **Glycoproteins** (GP) **and EPSs**, with specific properties to raise their survival in harsh environments [13].

GPs are essential compounds for humans, appearing in nearly all biological processes. They consist of **polypeptides covalently bonded to oligosaccharide chains** (glycans), where the carbohydrate can represent from 1% to 80% of the total mass and it is either O-linked or N-linked. Thus, GPs present a large diversity of properties and functions, playing a key role in cellular **proteins maintenance**, **stress recovery**, **cell-to-cell communication** and as **constituents of cell walls**.

Such macromolecules are often significant integral membrane proteins, where they influence cellular interaction. **GPs mediate the adhesion between cells**, which is essential for the development of functional tissues, as well as **cell-substrate unions** where they serve as receptors for adhesion ligands, as it occurs with fibroblasts and fibronectin [14]. This capacity has structural effects when binding cells with proteins like collagen for example, as it offers **strength and support to the matrix** [14].

Moreover, **GPs can act as vehicles** (for vitamins, hormones,...), as key **hormones** (erythropoietin), as **enzymes** (transferases, oxidoreductases and hydrolases), as **protecting and lubricating agents** (mucins or elements secreted by the lachrymal and sweat glands), as **cryoprotecting** molecules that guard from freezing by modifying or avoiding crystals formation, and as vital elements of the **immune system** (surface compounds of B or T cells with bacteria-binding properties or immunoglobulins) [14-15].

In addition, **GPs** are able to generate **Glycosaminoglycans** (GAG) and polysaccharides that compose the skin intercellular matrix, which keeps its cells and structure healthy.

As well as GPs, **EPSs** offer a varied range of potential skin benefits, including structural and anti-aging effects. Highly hygroscopic EPSs, for example, **can resemble GAGs and function like them in the Extracellular Matrix** (ECM). Usually, such long linear heterogeneous polysaccharides are covalently attached to a protein core forming proteoglycans, but **Hyaluronic Acid** (HA) is not [16-17]. This GAG is extremely hydrophilic due to its negative charges, being able to attract and **retain water up to 1000 times** its own weight. Thus, it **provides hydration and support, decreases epidermal water loss and raises water retention** into the dermis (skin replenishing effect), but it also participates in cellular migration, proliferation and wound healing [16, 18].

The fore above elements are essential compounds to keep the skin firm, tense and hydrated, their application could help when ECM is altered and there is a hydration, volume and firmness loss.

Fast aging evidences can be minimised

A nice and attractive smile embellishes any face, provides facial harmony, enlightens the look and transmits a lot about individuals. **Together with its closest skin, the mouth is one of the most attractive and magnetic zones**. It has the ability to catch everyone's attention and it is a key tool to express different attitudes and feelings, being also essential for many daily activities like talking, drinking, eating, laughing, crying or kissing.

When aging, alterations become visible in the delicate area surrounding the mouth in the form of fine lines or wrinkles. Perioral wrinkles are the visible lines around the mouth and the lips, which get worse with the passing years. Among the **fastest in appearing, the lines that etch vertically from the upper lip to the nose** are highlighted, known as **lipstick lines** (as lipstick colour gets diffused in them), smoker lines (since they are more prevalent in smokers) or bar code lines (due to its similar aspect).

Genetics play a major role in the undesired development of such lines, but certain facial movements like smiling, whistling, pouting, pursing or drinking with a straw have clearly negative effects on it as facial muscles have an extra work. **Smoking** is also a highly damaging habit for lipstick wrinkles: nicotine reduces the amount of oxygen locally delivered (slowing down the production of skin firming compounds), the markedly repeated gesture of mouth muscles while inhaling the cigarette and the harmful free radicals of cigarette smoke. Additionally, **stress, unhealthy diet** (poor in vitamins) and **environmental conditions** like wind, extreme temperatures and dryness **exacerbate their appearance** too.

Despite the proven extra stimulation that these factors have on lipstick lines formation, their development is mainly the consequence of the **deteriorating effects that aging has on the skin**. They include dermal **ECM alteration**, **collagen and elastin** fibres **damage and reduction,** and both **HA availability and** its **water-retaining** capacity **decrease** (as its degradation raises and its synthesis decreases), which all together cause a **loss** of skin **elasticity, firmness, thickness, volume and hydration** [18].

Adding the constant facial muscles use and movement, and the groove that it can cause beneath the skin surface with the passing years, the **appearance of such annoying aging signs is more than frequent when getting older**, sometimes including individuals in their **20s and 30s**.

The use of compounds with the properties to revert the aging negative skin alterations would help to minimise the visible wrinkles above the upper lip and get a younger aspect.

SEACODE™, marine eraser for aging lines

SEACODE™ is an active ingredient containing ECPSs obtained through **biotechnology** from *Pseudoalteromonas* bacteria inhabiting **extremely difficult marine environments**. As survival is harsh in areas like the Antarctica and intertidal rocky shores, organisms develop special features and bioactive compounds that offer multiple benefits once applied on the skin.

The fore above mentioned ingredient proved to ameliorate skin properties by highly enhancing the *in vitro* synthesis of essential dermal proteins (like collagen), that help to maintain cutaneous firmness and tonicity, in human dermal fibroblasts. *In vivo,* SEACODE™ offered a statistically significant effect in **improving skin roughness** after 1 and 4 weeks, visibly decreasing the upper lip wrinkles due to its replenishing effect.

Coming from marine biotechnology, SEACODE™ helps to evidently reduce the aging-associated wrinkles that rapidly appear above the upper lip, therefore rejuvenating facial look.

In vitro efficacy

TYPE I COLLAGEN INDUCTION

An Enzyme-Linked Immunosorbent Assay (ELISA) was performed on Human Dermal Fibroblasts (HDFa) to analyse the effect of SEACODE™ on the induction of type I collagen synthesis.

HDFa were grown until confluence in medium with specific growth factors. After cells were seeded into 24-well plates and incubated for 24 h at 37 ºC, fresh medium containing scalar dilutions of SEACODE™ (0.016%, 0.4% or 2%) was added and plates were incubated 48 h more. Then, well medium was collected and 50 µL of this medium or a standard curve prepared with type I collagen was analysed by an ELISA.

Absorbance values were read at 490 nm in a microtiter plate reader and collagen concentrations were determined using a linear regression of type I collagen standard curve.

Non-treated cells were used as controls.

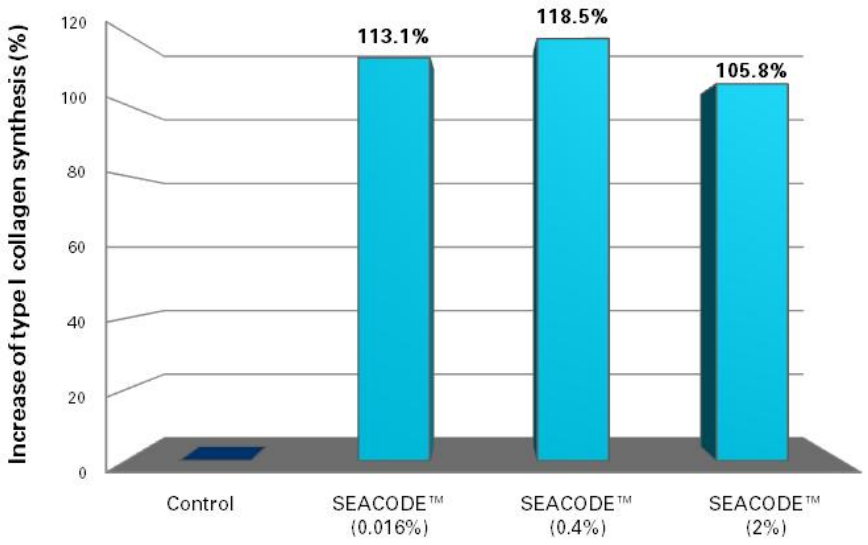

Fig.1. Raise of type I collagen synthesis induced by SEACODE™ versus non-treated cells.

At all tested concentrations, **SEACODE™ induced type I collagen synthesis** by **more** than **100%** in HDFa with respect to non-treated cells.

SEACODE™ provided a statistically significant increase of type I collagen synthesis.

In vivo efficacy

QUANTITATIVE EVALUATION OF SKIN ROUGHNESS

The purpose of this study was to evaluate the *in vivo* efficacy of SEACODE™ in improving one of the most visible signs of aging, the upper lip wrinkles or lipstick lines.

A panel of 20 female volunteers between 35-50 years old with healthy skin applied a cream containing 2% SEACODE™ on the facial skin above the upper lip twice a day for 4 weeks. The depth of the wrinkles (R_z) was examined before and after the treatment by means of PRIMOS optical 3D skin measurement.

This technique detects slight differences in the height of skin surface. Average values were represented and pictures were taken at the initial time and after 1 and 4 weeks.

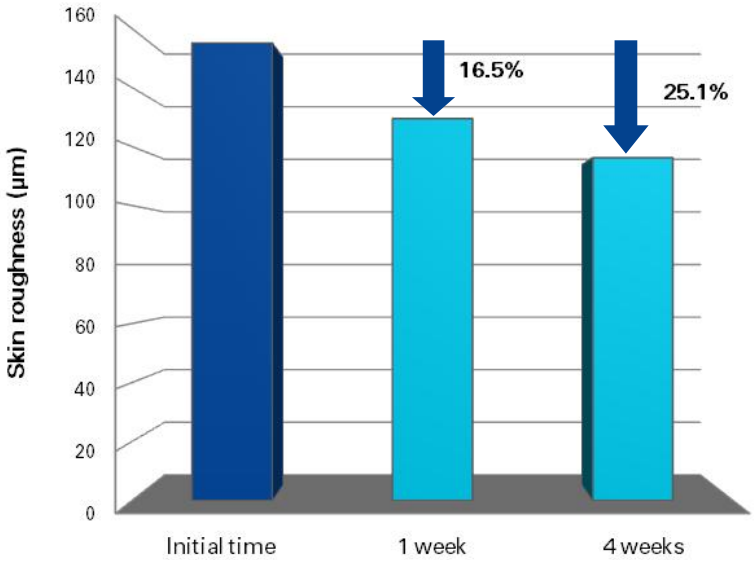

Fig. 2. Skin roughness average values after 1 and 4 weeks.

Results showed that **SEACODE™** clearly **reduced roughness** after its application **on the skin above the upper lip**. The maximum decreases recorded were 19.8% after 1 week and 29.5% after 4 weeks, compared to the initial roughness.

SEACODE™ offered a perceptible and statistically significant effect ameliorating skin roughness by 16.5% and 25.1% after 1 and 4 weeks respectively, versus the initial time.

DERMATOLOGICAL EVALUATION

The efficacy of SEACODE™ in reducing upper lip wrinkles or lipstick lines was evaluated in a panel of 10 female volunteers between 35-50 years old that applied a cream containing 2% SEACODE™ on the facial skin above the upper lip twice a day for 4 weeks. Lipstick lines were examined by taking pictures with a digital camera before and after 1 and 4 weeks of treatment.

Fig. 3. Images of two volunteers at the initial time (left), after 1 week (middle) and after 4 weeks (right) of treatment with SEACODE™.

The real images of the volunteers after the treatment confirmed that **SEACODE™** had a positive effect **reducing visible lipstick lines** in 1 week, and even more in 4 weeks.

SEACODE™ reduced skin lipstick lines, helping minimise aging signs and obtain a younger look.

Cosmetic properties

SEACODE™:

- new ingredient containing ECPSs designed to eliminate some of the fast-appearing wrinkles induced by aging, especially those located above the upper lip (lipstick lines), and rejuvenate the skin look.
- induced **type I collagen synthesis** by **more** than **100%** in HDFa cultures, contributing to ameliorate skin structural properties.
- provided a statistically significant improvement of the **skin above the upper lip** by **decreasing** its **roughness by 16.5%** and **25.1%** after 1 and 4 weeks of *in vivo* treatment respectively, leading to a **reduction** of **lipstick lines.**
- **improved lipstick lines**, visibly contributing to reduce skin aging effects and rejuvenating facial appearance.

Cosmetic applications

SEACODE™ is the ideal ingredient for facial products willing to **minimise lipstick lines due to the aging-induced deterioration** of the skin.

Additionally, due to its firming, smoothing and anti-wrinkle effects, this ingredient can be incorporated in facial formulations for any age but especially for mature skin (restructuring, nourishing, hydrating treatments,...) to improve its properties and rejuvenate its appearance.

Technical data

INCI NAME OF THE ACTIVE INGREDIENT

Active ingredient	INCI name
SEACODE™	Pseudoalteromonas Ferment Extract

PRESENTATION AND PRESERVATIVES

Solution containing 25.625% of active ingredient.

Code	Product presentation	Preservatives
BI040	SEACODE™	Salicylic Acid, Sodium Salicylate

Application data

PROCESSING

SEACODE™ needs to be incorporated in the aqueous phase. In case of emulsions, it should be added once the emulsion is formed and at temperatures below 40ºC.

SEACODE™ is stable at a pH range between 3.0 and 7.5.

INCOMPATIBILITIES

Strong oxidants and electrophiles.

SOLUBILITY

SEACODE™ is soluble in water.

DOSAGE

A dosage of 2% of SEACODE™ is recommended in final cosmetic formulations.

References

1. Raguénès GHC, Peres A, Ruimy R, *et al. Alteromonas infernus* sp. nov., a new polysaccharide-producing bacterium isolated from a deep-sea hydrothermal vent. *J App Microbiol*. 82: 422-430, 1997.

2. Guezennec J. Deep-sea hydrotermal vents: A new source of innovative bacterial exopolysaccharides of biotechnological interest? *J Ind Microbiol Biotechnol.* 29: 204-208, 2002.

3. Chi Z, Fang Y. Exopolysaccharides from Marine Bacteria. *J Ocean Univ China*. 4(1): 67-74, 2005.

4. Sutherland, IW. Biotechnology of microbial exopolysaccharides. London: Cambridge University Press. Cambridge studies in biotechnology. Vol 9:1-6, 1990.

5. Zanchetta P, Lagarde N, Guezennec J. Systemic effects on bone healing on a New Hyaluronic acid-like bacterial exopolysaccharide. *Calcif Tissue Int*. 73: 232-236, 2003.

6. Hsu HY, Hua KF, Lin CC, *et al*. Extract of reishi polysaccharides induces cytokine expression via TLR4-modulated protein kinase signaling pathways. *J Immunol*. 173: 5989-5999, 2004.

7. Castellví J. De cómo España abrió su ruta para la investigación antártica. *ARBOR Ciencia, Pensamiento y Cultura.* CLXXXIII 727: 739 – 747, 2007.

8. Tindall BJ. Prokaryotic diversity in the Antarctic: the tip of the iceberg. *Microb Ecol.* 47(3): 271 - 283, 2004.

9. Hamamoto T, Kaneda M, Horikoshi K, Kudo T. Characterization of a Protease from a Psychrotroph, Pseudomonas fluorescens 114. *Appl Environ Microbiol*. 60(10): 3878 – 3880, 1994.

10. Bowman JP. Pseudoalteromonas prydzensis sp. nov., a psychrotrophic, halotolerant bacterium form Antarctic sea ice. *Int J Syst Bacteriol*. 48 Pt 3: 1037 – 1041, 1998.

11. Decho AW. Microbial biofilms in intertidal systems: an overview. *Continental Shelf Research.* 20: 1257 – 1273, 2000.

12. Ortega-Morales BO, Santiago-García JL, Chan-Bacab MJ, *et al*. Characterization of extracellular polymers synthesized by tropical intertidal biofilm bacteria. *J Appl Microbiol.* 102(1): 254-264, 2007.

13. Mikhailov VV, Romanenko LA, Ivanova EP. The genus *Alteromona*s and related Proteobacteria. *The Prokaryotes: a Handbook on the Biology of Bacteria*. 6: 597 – 645, 2006.

14. Ivatt RT. The Biology of Glycoproteins. Plenum Press: New York, 1984. Kornfeld R, Kornfeld S. Assembly of Asparagine-Linked Oligosaccharide. *Annual Review of Biochemistry.* 54: 631-664, 1985.

15. Gottschalk A. Glycoproteins. Their composition, structure and function. *Elsevier Publishing Company*. New York, 1972.

16. Souza-Fernandes AB, Pelosi P, Rocco PR. Bench-to-bedside review: The role of glycosaminoglycans in respiratory disease. *Crit Care*. 10(6): 237, 2006.

17. Stern R. Review: Devising a pathway for hyaluronan catabolism: are we there yet? *Glycobiology*. 13(12): 105R-115R, 2003.

18. John HE, Price RD. Perspectives in the selection of hyaluronic acid fillers for facial wrinkles and aging skin. *Patient Prefer Adherence*. 3: 225-230, 2009.