Technical Report

Product

HYANIFY™

Date

September 2012

Revision

3

Pol. Ind. Camí Ral C/ Isaac Peral, 17
08850 Gavà Barcelona (Spain)
Tel. +34 93 638 80 00
www.lipotec.com
commercial@lipotec.com

Lipotec Group

hyanify™ Biointec™

Contents

Harsh environments produce unique molecules

Being almost completely surrounded by sea, the **Brittany region in north-western France** has more than 2700 km of coast and the highest number of islands in France (more than 790). **This Finistère department receives the influence of tides and streams of water, creating a frontier zone which potentiates varied and special ecosystems**.

In this occidental French region, there are estuaries and bays protected from open sea conditions but also rocky zones and sandy beaches influenced by tides. Abers are inlets of sea which penetrate into the coastline, where both **salty water from the sea and water from rivers and rain join together**; making these zones rich in nutrients and phytoplankton. Aber Wrac'h is an arm of the Atlantic Ocean that extends inland to meet the Wrac'h River forming the **Aber Wrac'h estuary**, an example of enriched area located in the occidental coast of Brittany between Landéda and Plouguerneau.

High salinity and pH (around 8), currents, tides and waves, as well as a gradient of temperature, oxygen and light are some of the typical conditions of marine habitats. Generally, fresh water from rivers and rain present other features like lower salinity and more homogeneous oxygen and light levels, due to its minor depth. When both differentiated environments come together, its inhabitants need to develop special survival tactics that contribute to create a varied and unique ecosystem.

On the shores of this estuary, ***Laminaria*** can be found among other marine species. These macroalgae have marine γ-proteobacteria strains on their surfaces, which have been found to show a high capacity to **produce Exopolysaccharides (EPS). EPS are specific compounds to mainly protect, fix or nourish the benthonic community.**

Due to the high density of microorganisms inhabiting these communities but its unusual eutrophication phenomena, the communication within the niche is thought to be essential [1]. Thus, **chemicals with intercellular and intracellular signalling functions are supposed to be found** to keep the ecological balance.

Aber Wrac'h estuary is an extreme environment which induces the production of functional EPS.

Fig. 1. Aber Wrac'h estuary in Finistère (Brittany).

Exopolysaccharides to survive

Marine ecosystems are a well-known valuable source of diversity and inhabiting microorganisms. **Bays and estuaries are especially difficult environments** due to its combination of salt and fresh water, which determine the local living conditions. Therefore, microorganisms of both habitats can be found in these zones, presenting **differentiated structures and mechanisms to increase their survival and help the colony** [2-4].

In natural environments, **bacteria**, algae and fungi have the potential to synthesise numerous intra- and extra-cellular polysaccharides, including multifunctional **exopolysaccharides**. EPS are glucidic biopolymers **naturally secreted to the surrounding media as a response to environmental stress**. The benefits of microbial EPS include constant chemical and physical properties, a stable supply and a model to explore how microorganisms can stabilise themselves in extreme conditions [3, 4].

Bacterial polysaccharides are considered highly interesting because they offer an extensive range of properties which seem not to be available by plant polymers and their production is less subject to variability due to marine pollution or climatic impact [2, 3]. In extreme conditions, like estuaries, **bacteria need special mechanisms and bioactive compounds to survive**, so they present better capability to produce functional EPS than any other bacteria [4]. These polymers can protect both colonies and microorganisms from extreme salinity, pressure and temperatures. They can also raise microorganism survival by increasing hydration and nutrition, acting in intracellular processes, immunologic modulation, cell recognition, proliferation and migration, and assisting in favourable adhesions to solid surfaces [2, 4-6]. **Thus, EPS can be thought to interact with cell receptors promoting beneficial activities, like the production of structural compounds for a better survival.**

Exopolysaccharides can have a highly varied composition, which implies many chemical and physical properties. Most EPS present either uronic acids like D-glucuronic acid or ketal-linked pyruvate, which make them polyanionic. Their diverse composition creates multiple possible industrial applications such as stabilising, film-forming, suspending, thickening, gelling, coagulating or water retaining agents [3]. For this reason, there is a sincere **growing interest in identifying new EPS** and their practical applications [4].

Bacterial EPS from harsh environments present special properties, which can be beneficial to improve skin health and appearance.

Extracellular matrix and skin aging evidences

One of the most relevant effects as a **result of getting older** is the **degradation** of the dermal Extracellular Matrix (**ECM**), leading to undesired skin alterations. Various macromolecules are found in this 3D mesh, including proteins and Glycosaminoglycans (GAGs). Hyaluronic Acid (**HA**) **belongs to the GAG family**, being a major compound of the ECM.

GAGs consist of long linear heterogeneous polysaccharides with a changeable number of repeating disaccharide units. Normally, they are covalently attached to a protein core to create bigger structures known as proteoglycans [7]. Due to their highly negative charges, **GAGs are hydrophilic**, and are able to **attract water inside** the tissue and affect its physical properties [8].

Contrary to other GAGs, **HA** is not involved in the formation of proteoglycans so it is mainly **found as a free molecule** [9]. Half of its total body content is found in the skin, where it **provides hydration, support and volume, participating in cellular migration, proliferation and wound healing** [9]. Moreover, HA retains water up to 1000 times its own weight, turning it into a key agent for skin moisturisation. This non-sulphated GAG **decreases epidermal water loss while increasing water retention** into the deeper dermis, both effects **leading to a plumping effect** [10].

In normal skin conditions, there is a **dynamic equilibrium between HA synthesis and degradation**, which allows the skin to have a stable quantity of available HA, although its short life span. Unfortunately, **aging alters this balance** increasing HA union to tissues, diminishing its synthesis and increasing its degradation, due to specific glycosyl hydrolases known as Hyaluronidases (HYAL). These hydrolytic enzymes increase their catalytic activities when aging, reducing even more the quantity of available HA. By catalysing the hydrolysis of HA, HYALs lower its viscosity and stimulate its dispersion. This reduction in HA availability can generate further water loss, **resulting in dehydration and volume loss**, which leads to **wrinkles appearance**.

Besides, aging and UV exposure contribute to exacerbate skin alterations by degrading collagen and elastin, and inducing matrix disorganisation and tissue modifications.

Facial morphology becomes highly affected by aging consequences, losing volume in key areas related to young appearance. The nasolabial area (between the nose and the upper lip) is a delicate zone susceptible to be affected even by small volume changes. The **nasogenian folds are considered one of the most clear and visible marks of aging, being a main anti-aging cosmetic target**.

The aging-induced reduction of HA availability in skin results in dehydration and volume loss, which is translated in wrinkles and nasolabial folds. Inducing HA increase, volume would be recovered obtaining a younger appearance.

HYANIFY™: replenishing effect from the sea

HYANIFY™ is an **exopolysaccharide** obtained via biotechnological fermentation of a marine γ-proteobacteria strain**, isolated from the surface of a *Laminaria* alga in the Aber Wrac'h estuary**. This area has both the influence of salt and fresh water (rivers and rain), so the inhabiting microorganisms had to develop special structures and mechanisms to survive, including the production of functional EPS.

The EPS produced by the members of this harsh ecosystem can present many properties, including the capacity to interact with cell receptors to induce the production of beneficial compounds, which can provide support and volume.

HYANIFY™ induces HA synthesis offering a restructuring and replenishing effect, improving wrinkles appearance as a result. Several in *vitro* and *in vivo* tests were performed in order to support its efficacy.

HYANIFY™ demonstrated to highly stimulate HA synthesis *in vitro* in human dermal fibroblasts. It also had an important *in vivo* effect offering a clearly improvement of the depth, circumference, volume and area of the nasolabial fold after 14 days. Moreover, it obtained statistically significant results on these parameters after 28 days.

In vitro efficacy

EVALUATION OF HYALURONIC ACID INDUCTION ON HUMAN DERMAL FIBROBLASTS

An ELISA test was performed on **human dermal fibroblasts to observe the effect of HYANIFY™ on the induction of HA**, as these cells are its main producers of HA [11].

After incubating the fibroblasts at 37 ºC during 24 h, deprivation medium was added and wells were incubated again for 24 h. Subsequently, 1 mg/mL **HYANIFY™ was added**, cells were incubated during 48 h and well medium was collected. Finally, 100 µL of the **collected well medium or HA standard solutions** was analysed in a competitive ELISA assay where the amount of HA present in the sample is inversely proportional to the colorimetric signal. **Absorbance** values were read at 405 nm in a microtiter plate reader.

Non-treated cells were used as the negative control and cells treated with Platelet-Derived Growth Factor (PDGF-BB) were used as the positive control.

HA concentrations were determined using a linear regression of the HA standard solutions curve, calculating the percentage of induction with respect to the negative control.

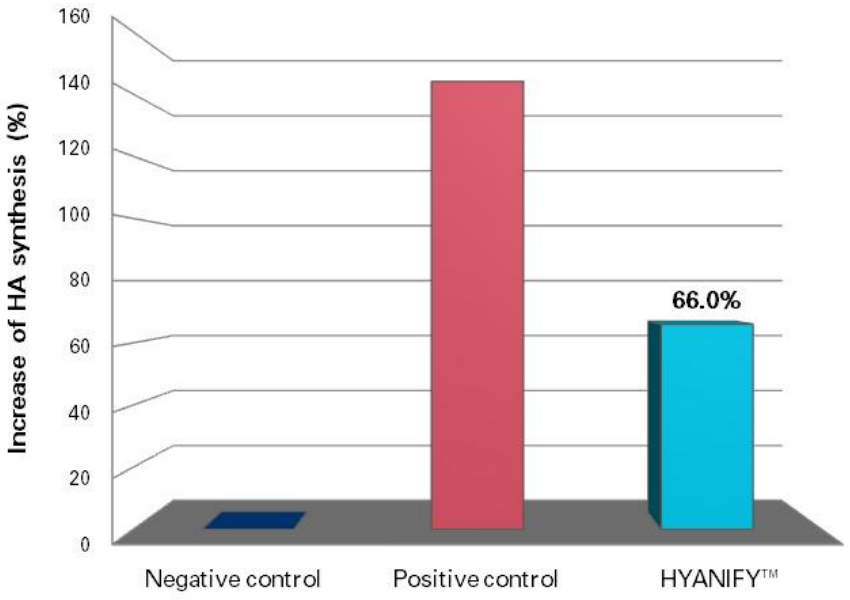


Fig. 2. Percentage of HA synthesis induced by HYANIFY™ with respect to non-treated cells.

Results showed that **HYANIFY™ efficiently induced HA synthesis by 66.0%** compared to the negative control.

HYANIFY™ produced a statistically significant induction of HA synthesis (66.0%) on human dermal fibroblasts.

In vivo efficacy

EFFICACY ON THE NASOGENIAN FOLD VOLUME RECOVERY

The aim of this study was to **assess the *in vivo* efficacy on the nasolabial fold volume recovery of HYANIFY™** by measuring physical parameters related to skin topography using the **FOITS technique,** before its application and after 2 and 4 weeks of treatment.

A panel of 19 volunteers between 44-56 years old, with nasolabial fold of moderate intensity and II-III Fitzpatrick phototype, applied a cream with 1% **HYANIFY™ SOLUTION twice a day** on the face, insisting on the nasogenian fold.

Skin topography was evaluated before the first application and **after 14 and 28 days** by FOITS, but also by taking photographs. Fringe projection gave 3D images where the **maximum and average depth, circumference, area and volume** were calculated.

The maximum depth represents the distance between the skin basal height and the top bottom of the cavity, and the average depth is the mean of all the possible depths of the cavity. The volume is referred to the volume of the cavity created in the skin, the circumference is the circumference of the cavity at the basal height and the area is the surface corresponding to the cavity.

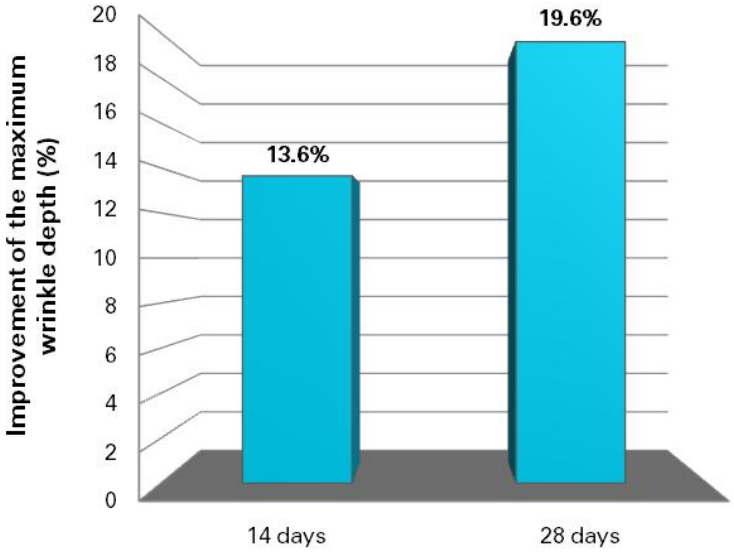
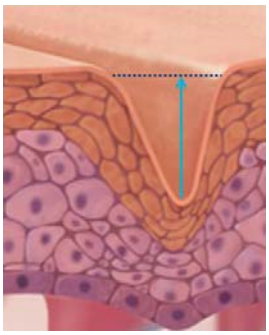


Fig. 3. Improvement of the maximum wrinkle depth.

HYANIFY™ significantly improved the maximum depth of the nasogenian fold a mean of 13.6% and 19.6% after 14 and after 28 days of treatment.

Maximum reductions of 64.7% and 70.6% were recorded for a volunteer after 14 and 28 days, respectively.



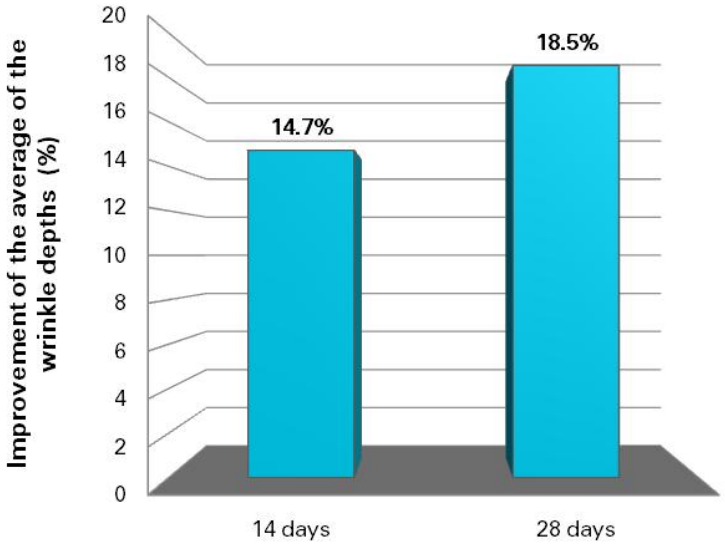
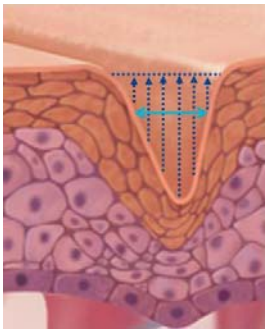


Fig. 4. Improvement of the average of all the depths of the wrinkle.

The average of all the depths of the nasogenian fold was reduced by 14.7% and 18.5% thanks to HYANIFY™, after 14 and 28 days of treatment, respectively. Both improvements were statistically significant.

Maximal decreases of 65.8% and 71.4% after 14 and 28 days were observed in a volunteer.



After applying HYANIFY™ for 14 days the nasolabial fold noticeably diminished its average volume (15.4%), area (6.7%) and circumference (1.9%), improving even more after 28 days, with statistically significant effects.

Maximal improvements at the end of the treatment of 93.5% in the volume, 77.8% in the area and 79.3% in the circumference were recorded for a volunteer.

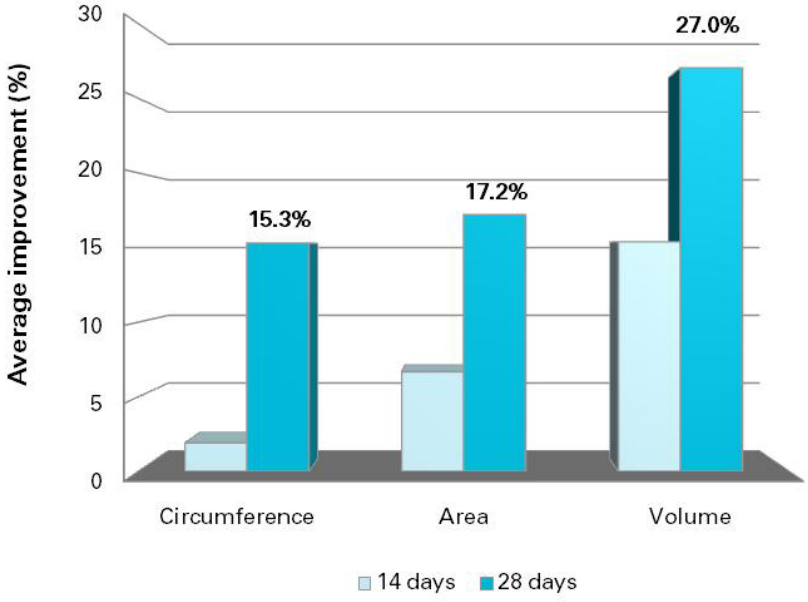


Fig. 5. Average volume, area and circumference improvement.

HYANIFY™ provided a statistically significant reduction in the maximum and average wrinkle depth, and significantly improved the volume, area and circumference of the nasogenian fold after 28 days.

The **pictures** taken at the beginning of the *in vivo* treatment and after 28 days clearly demonstrated the **positive evolution of the nasolabial fold**, visible and clearly improving its appearance. The silicon patterns also showed that the nasolabial fold decreased significantly.

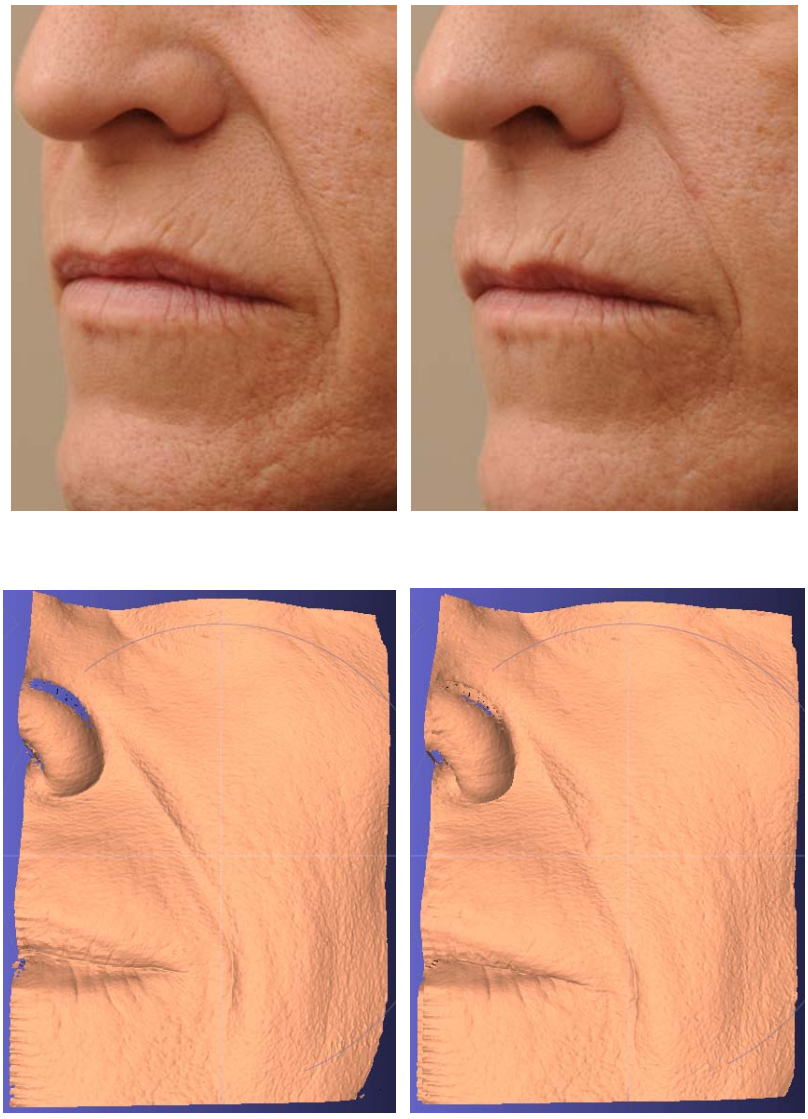


Fig. 6. Images and silicon patterns of a volunteer at the beginning (left) and after 28 days of treatment with a cream containing HYANIFY™ (right).

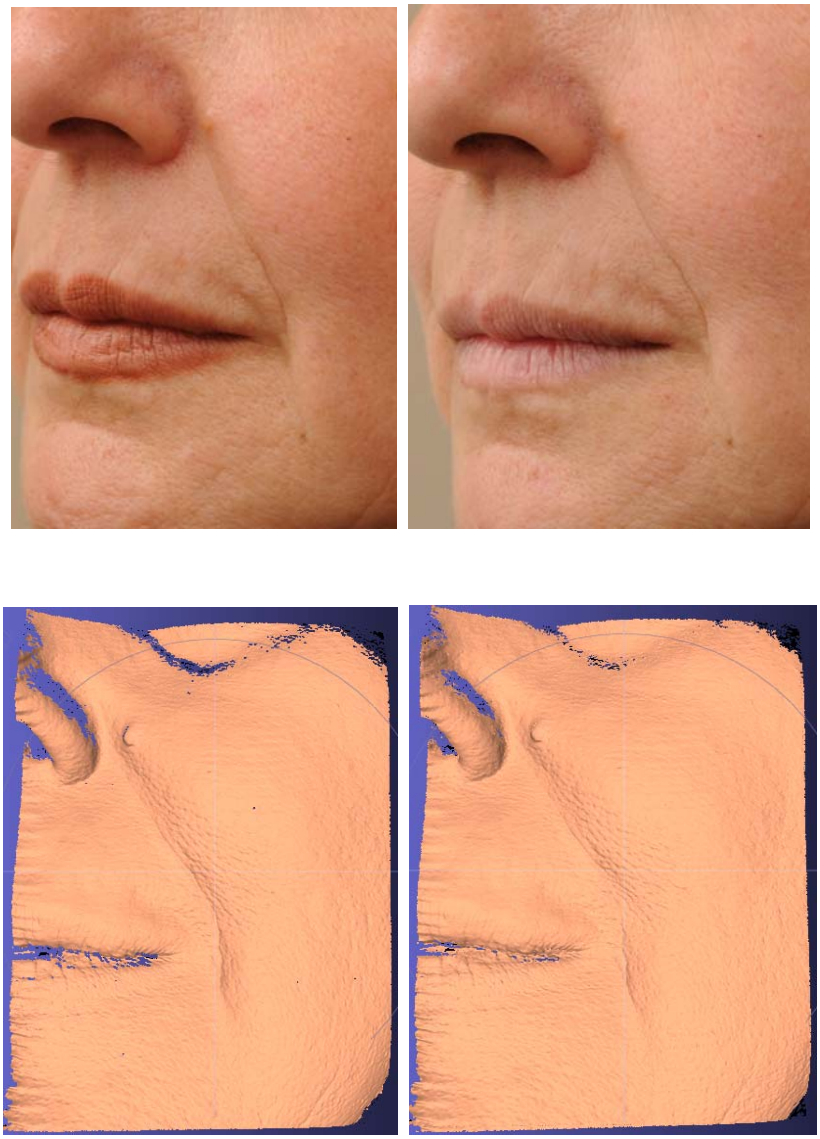


Fig. 7. Pictures and 3D images from a volunteer before (left) and at the end of the treatment (after 28 days) with a cream containing HYANIFY™ (right).

HYANIFY™ clearly improved the nasolabial wrinkles and provided a visible replenishing effect on the area, which rejuvenated the appearance of the skin.

Cosmetic properties

HYANIFY™:

- efficiently **induces *in vitro* HA synthesis by 66.0% on human dermal fibroblasts**, having a statistically significant effect.
- provides an *in vivo* **statistically significant decrease of the maximum and average wrinkle depth, obtaining statistically significant improvements of 13.6% and 14.7%** in the **nasogenian folds only after 14 days**, showing even better mean results after 28 days (19.6% and 18.5% respectively).
- visibly **reduces the average volume, area and circumference** of wrinkles as it was seen in the **nasogenian fold after 14 days**, providing *in vivo* **statistically significant improvements after 28 days** (27.0%, 17.2% and 15.3% respectively).

Cosmetic applications

HYANIFY™ can be incorporated in facial **formulations to reduce the visible signs of aging and rejuvenate the skin**. It decreases wrinkles such as the nasolabial folds, and minimises shadows, so it can be used as a complement in daily facial products for mature skin (hydrating, nourishing, whitening or firming) as well as in specific anti-aging treatments.

As it helps to increase water content, it is perfect for sun care formulations, as well as for preventive cosmetics products, to avoid dehydration and flaccidity. It could be also incorporated into specific formulations for hands care, with the same function.

Technical data

INCI NAME OF THE ACTIVE INGREDIENT

Active ingredient	INCI name
HYANIFY™	Saccharide Isomerate

PRESENTATION AND PRESERVATIVE

Gel containing 0.75% of active ingredient.

Code	Product presentation	Preservative
BI030	HYANIFY™ SOLUTION	Preservative free

Application data

PROCESSING

HYANIFY™ SOLUTION can be formulated in the aqueous phase. In case of preparing an emulsion, it should be added once the emulsion is formed and at temperatures below 40 ºC.

HYANIFY™ SOLUTION is stable over a pH range between 5.5 and 8.0, being the optimal pH 7.0.

INCOMPATIBILITIES

Strong oxidants.

SOLUBILITY

HYANIFY™ SOLUTION is soluble in water.

DOSAGE

A dosage of 1% of HYANIFY™ SOLUTION is recommended in final cosmetic formulations.

References

1. Hily C. Is the activity of benthic suspension feeders a factor controlling water quality in the Bay of Brest? *Mar Ecol Prog Ser*. 69: 179-188, 1991.

2. 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.

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

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

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

8. House M, Kaplan DL, Socrate S. Relationships between mechanical properties and extracellular matrix constituents of the cervical stroma during pregnancy. *Semin Perinatol.* 33(5): 300-307, 2009.

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

10. 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.

11. Li L, Asteriou T, Bernert B, *et al*. Growth factor regulation of hyaluronan synthesis and degradation in human dermal fibroblasts: importance of hyaluronan for the mitogenic response of PDGF-BB. *Biochem J*. 404(2): 327-336, 2007.