

Protecting the Skin from Environmental Stresses with an Exopolysaccharide Formulation

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ABSTRACT: *A polysaccharide with a repetitive unit of 11 glycosidic residues and a relative molecular mass of $1.8 \cdot 10^3$ kDa has been found to exert properties such as skin repair, restructuring and protection against inflammatory processes, in a described formulation.*

An increasing number of hyperthermophilic and mesophilic bacteria have been isolated from deep-sea hydrothermal vents throughout the past 20 years and found to be an extraordinary source of innovative molecules with unusual biological properties. These properties have developed as defense mechanisms enabling the marine organisms to adapt themselves to extreme conditions of temperature, pressure and darkness that prevail in the world's seas.

Among these, bacteria belonging to the genus *Alteromonas*, and precisely to the species *A. macleodii*, were identified as being able to produce large amounts of biologically active exopolysaccharides (EPS).¹ A close investigation of some of these biopolymers revealed

that they could be of powerful value in the cosmetic industry by providing a new way to protect the skin from environmental injuries. The best example is a polysaccharide produced by *A. macleodii*, strain HYD657^a (EPS657) that is the sole bioactive polymer in a described *Alteromonas* ferment extract formulation^b (SEPS657). The present review will focus on this ferment extract with particular emphasis on its properties as exemplified by a number of laboratory and clinical studies.

Innovative EPS of Biotechnological Interest in Cosmetics

Recently, a new EPS produced by a strain of *A. macleodii* (HYD657), a bacterium collected on the dorsal integument of the polychaete annelid *Alvinella pompejana* in an active hydrothermal vent of the East Pacific Rise, was characterized using monosaccharide analysis, methylation analysis, β -elimination studies and NMR spectroscopy.² The HYD657 strain was selected on the basis of its ability to exhibit a swarming mucoid phenotype when grown on Okutani medium.³ According to cytological, physiological, biochemical and molecular investigations, strain HYD657 of *A. macleodii* was described as a motile (single polar flagellum), encapsulated gram-negative bacterium that appeared nonpigmented, nonluminescent and nonfermentative. Optimal growth temperatures on artificial medium were estimated to be 30°C to 35°C and optimal pH between 6.5 and 7.5. The bacterium was found to produce catalase and cytochrome oxidase, and to secrete a complex EPS, or EPS657.

Chemical characterization of EPS657 revealed that neutral sugars (glucose, galactose, fucose, rhamnose and mannose) in the polymer accounted for 58%, and uronic acids (glucuronic and galacturonic acids) for 30% of the molecule. Interestingly, this exopolymer differed from all other EPS extracted from *Alteromonas* sp. by the presence of an unusual sugar identified as a 3-O-(1 carboxyethyl)-D-glucuronic acid. This originality of EPS657 relied also in the replacement of a glucuronic acid residue by a lactate group in

^a Deepspan is a trade name of Atrium Biotechnologies.

^b Abyssine 657 (INCI: Water (aqua) (and) *Alteromonas* ferment extract (and) butylene glycol) is a product of Atrium Biotechnologies.

position 3 (see the EPS originality in **Figure 18.1**). EPS657 has a repetitive unit of 11 glycosidic residues and a relative molecular mass of $1.8 \cdot 10^3$ kDa (see **Figure 18.2**).

Even though the biological function of EPS657 is not fully elucidated yet, one may suggest that, in nature particularly, it contributes to the formation of the filamentous mats found in the microenvironment of the annelid *Alvinella pompejana*. Such bacterium-produced mats are thought to help the annelids to fix on the hydrothermal sulfide-chimney and to protect them against the high concentration of toxic compounds such as heavy metals. It is this latter property that incited further investigation into the potential value of this polymer in skin protection against injuries and inflammatory reactions.

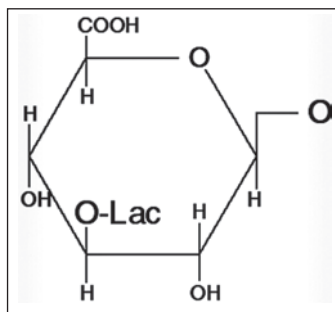


Figure 18.1. This EPS' originality: A glucuronic acid residue replaced in position 3 by a lactate group.

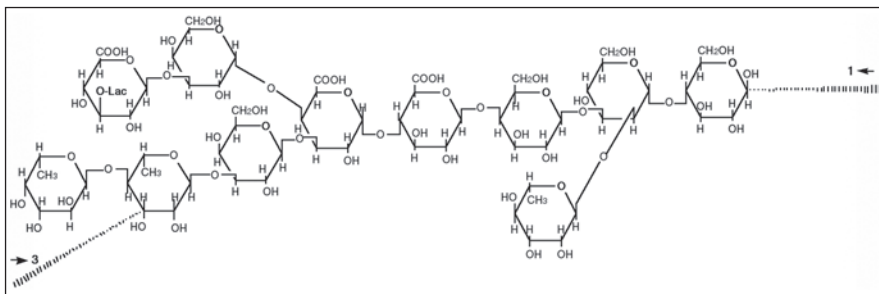


Figure 18.2. Hypothetical structure of the repeating unit of the EPS ($n = 900$ to 100)

Skin as Primary Target of the Inflammation Process

In the epidermis, the principal cell types found are the keratinocytes, the melanocytes and the Langerhans cells. In response to injury, keratinocytes produce cytokines and expressing molecules from the Major Histocompatibility Complex and adhesion molecules called ICAM-1 (Intercellular Adhesion Molecule). In most inflammatory skin reactions, the expression of ICAM-1 in the epidermis initiates

a series of events leading to interactions between leukocytes and keratinocytes.⁴ Under normal conditions, the level of expression of ICAM-1 by leukocytes is quite low. However, this level can be amplified markedly upon exposure to pro-inflammatory agents, contact allergens, histamine, enterotoxins, pollutants and other chemical or physical irritants.

ICAM-1 is a transmembrane glycoprotein molecule of the immunoglobulin superfamily. Each molecule is characterized by five distinct immunoglobulin-like domains, a transmembrane domain and a cytoplasmic tail.⁴ While the final protein is 505 amino acids long, the molecule weighs between 80 and 114 kDa, depending on the level of glycosylation that varies among cell types and environments. ICAM-1 expression is regulated through four primary pathways: protein kinase (PKC), AP-1 and MAP kinase, JAK/STAT and interferon- γ (IFN- γ), and NF κ B.⁵ IFN- γ has a signaling effect on the transcriptional control of ICAM-1. The pathway appears to be prompted by pro-inflammatory cytokines which, in turn, activate a signaling cascade leading ultimately to the transcription of ICAM-1.⁵

Histamine is another compound known for its capacity for regulating the expression of inflammatory molecules. While histamine is produced mainly by different leukocyte populations, it also is produced by keratinocytes upon UV irradiation.⁶ Thus, ICAM-1 is produced abundantly by skin keratinocytes in cutaneous inflammatory reactions⁷ and is up-regulated on the surface of many cell types by pro-inflammatory mediators such as IFN- γ and interleukin-1 α . Expression of ICAM-1 by keratinocytes has been correlated with accumulation of lymphocytes in the skin epidermis. Lymphocyte-keratinocyte interactions play an important role in immunological defense reactions and also in the pathogenesis of several mucocutaneous disorders such as *Candida albicans*-incited dermatitis.

In view of the predominant role played by ICAM-1 in cutaneous inflammatory reactions, one can argue that any molecule able to reduce the expression of ICAM-1 by keratinocytes represents a promising tool for cosmeceutical formulations that may have the potential to protect irritable, hyperreactive or simply sensitive skin from inflammation.

Effect of SEPS657 on the Expression of ICAM-1 by Keratinocytes

As mentioned in the preceding section, ICAM-1 is a fundamental component in many immune-related processes including skin inflammatory processes.⁸ For example, the topical application of molecules causing allergic contact dermatitis has the ability to initiate cutaneous inflammation by directly influencing the transduction signal that leads to the expression of ICAM-1 in the keratinocytes. This expression takes place during the pre-erythematous phase preceding the appearance of T lymphocytes and the clinical symptoms of allergic dermatitis, and is very intense during the amplification phase that coincides with dermal and epidermal infiltration of T cells. Lymphokine INF γ then is released and this amplifies the original stimulus.

In an attempt to demonstrate that SEPS657 could reduce ICAM-1 production by human keratinocytes, experiments were performed by using cultured human keratinocytes exposed to IFN- γ , a natural inducer of ICAM-1. In these experiments, human keratinocytes were cultured at 37°C in a serum-free medium. Culture suspensions were seeded in 96-well plates and incubated at 37°C for 72 hours (h) before being exposed to the test product (5% SEPS657). After incubation of the cultured keratinocytes at 37°C for 24 h, stimulation of ICAM-1 was performed by using interferon γ (INF γ). Twenty-four hours later, ICAM-1 was measured by immunocytochemistry at the surface of keratinocytes. Controls consisted of human keratinocytes treated with INF γ only. The results clearly demonstrated that pre-treatment of keratinocytes with SEPS657 drastically reduced the level of ICAM-1 produced upon stimulation by INF γ (see **Table 18.1**).

Effect of SEPS657 in Stimulating Keratinocytes Proliferation

Skin cell renewal is strongly dependent on the presence of active keratinocytes in the epidermis. In order to determine whether SEPS657 could have a beneficial effect on skin cell renewal, human

Table 18.1. Effect of EPS657 and SEPS657 on the production of ICAM-1 by keratinocytes

	Control		SEPS657—5 %	
INF- γ (U/ml)	0	100	0	100
ICAM-1 (DO/well)	0.17	1.20	0.14	0.29
Proteins (μ g/well)	7.53	7.30	7.34	7.29
ICAM (ua/ μ g prot.)	23.12	164.71	18.72	39.68
Production ICAM-1		141.59		20.96

epidermal keratinocytes were cultured in single layers at 37°C in a calcium-poor SFM medium and in the absence of any complement. The keratinocytes were labeled by incorporating tritiated thymidine in the DNA. SEPS657 (1.7%) was added to the culture medium and the culture suspension was seeded in 96-well plates before being incubated at 37°C for 24 h. Labeled keratinocytes were detected by liquid scintillation count. The experiment was replicated six times and included epidermal growth-factor in addition to pituitary extract (EGF) as a positive reference.

EPS657 at a concentration of 50 μ g/ml (equivalent to 1.7% SEPS657) strongly stimulated the incorporation of tritiated thymidine (163%) into cultured keratinocytes (see **Table 18.2**), thus suggesting that it could exert a positive effect on skin cell renewal.

Table 18.2. Effect of SEPS657 (1.7 %) on the percentage of tritiated thymidine incorporation in the keratinocytes

Treatment	n=number of replicates	% incorporation	p=significance
Control	6	100	/
EGF Reference	6	147	< 0.01
1.7% SEPS657	6	163	< 0.01