Cosmetic Applications of Exopolysaccharides

Subjects: Biotechnology & Applied Microbiology

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Bacteria are well-known to synthesize high molecular weight polysaccharides excreted in extracellular domain, which constitute their protective microenvironment. Several bacterial exopolysaccharides (EPS) are commercially available for skincare applications in cosmetic products due to their unique structural features, conferring valuable biological and/or textural properties.

marine bacterial exopolysaccharides biotechnology cosmetic industry

1. Introduction

Polysaccharides are complex polymers composed of monosaccharides linked by glycosidic bonds, forming large branched or linear molecular structures. These high molecular weight polymers are classified into two groups based on their osidic composition, either homopolysaccharides, containing only one type of monosaccharides, or heteropolysaccharides, consisting of different monosaccharides. The polysaccharide's primary structure depends not only on chain length (molecular weight can vary significantly from 10,000 to several millions g/mol [1][2][3][4]) or the type of monosaccharides, but also on the linkages between monosaccharides, their sequence and branching pattern, and on substituents [5].

Polysaccharides are ubiquitously found in every living organism from plants to animals, including microorganisms. Regarding microorganisms, two main types of polysaccharides depending on their cellular localization are identified, i.e., intracellular and extracellular polysaccharides, the latter include capsular polysaccharides (CPS), tightly associated with the cell surface and forming a capsule, slime layer loosely associated to cell surface and exopolysaccharides (EPS), excreted by microorganisms into their surrounding environment. EPS can form a slime which remains loosely linked to the cells and can also be dissolved into the extracellular environment. Various microorganisms can produce EPS, including Gram-negative and Gram-positive bacteria, archaea, fungi, and microalgae. Microbial EPS are secondary metabolites, which create a microenvironment around the cells, whose physico-chemical characteristics can balance environmental conditions (pH, salinity, chemicals) that in some cases can be harsh (e.g., deep-sea hydrothermal vents). EPS play a key role in cell protection against dehydration, heavy metals, and other external stress, and may also be involved in aggregation of cells, adhesion onto biotic and abiotic surfaces, biofilm, and nutrient uptake [6]. EPS production by bacteria is an energy-intensive process that accounts for up to 70% of the carbon investment. Despite this high energy cost, EPS benefits are significantly higher, as bacterial growth and survival are increased in their presence [6][7].

2. Non-Marine Bacterial EPS in Cosmetics

Over the past 20 years, the number of new cosmetic products containing bacterial EPS increased significantly, as demonstrated by a statistical study retrieved from the Mintel's Global New Products Database (GNPD) on skincare products on three markets (France, USA, China). Five specific INCI names were targeted, corresponding to bacterial EPS: levan, gellan gum, dextran, xanthan gum, and hyaluronic acid.

Xanthan gum is the most extensively used non-marine bacterial EPS in cosmetics since its discovery and first commercial development by Kelco [8]. Xanthan is an anionic high-molecular weight (0.4–15 × 10^6 g/mol) heteropolysaccharide secreted by *Xanthomonas* sp. strains, usually industrially obtained from *X. campestris*. It is composed of a pentasaccharide repeating unit with a cellobiose backbone and a trisaccharide side chain containing one glucuronic acid between two mannose residues, substituted by pyruvyl and acetyl groups [9][10]. Side chains account for 65% of the molecular weight of xanthan and play a significant role in the molecular conformation [11]. Xanthan undergoes conformation transitions from helix to random coil depending on stimuli such as pH, ionic strength, temperature and shear [12]. At low concentrations and low shear rate (pseudoplastic behavior), xanthan displays the unusually high viscosities important to its suspension-stabilizing properties. Low temperature and high salt concentration favor ordered helix forms, while high temperature and low salt concentration favor disordered coil shapes [13]. Due to its outstanding solution properties, xanthan gum is widely commercially used for a wide range of applications in the food, pharmaceutical, and cosmetic industries [1][14].

Another example of EPS largely used in cosmetic products constitutes gellan gum. This linear anionic high-molecular weight (0.24–2.2 × 10^6 g/mol) heteropolysaccharide, being a part of the "sphingan" polymer family, is produced by *Pseudomonas* sp. and *Sphingomonas* sp. bacterial strains, and is composed of rhamnose, glucose and glucuronic acid, substituted by acetyl groups [15]. Gellan has interesting functional properties due to its ability to form a transparent gel in the presence of divalent cations, resistant to acid and heat [16]. Slightly acylated gellan forms hard and brittle gels, while highly acylated gellan forms soft and elastic gels [17]. In cosmetic products, it is used as thickening agent and emulsion stabilizer [18].

Hyaluronic acid (HA) or hyaluronan is another well-known anionic high-molecular weight (2 × 10^6 g/mol) polysaccharide belonging to GAG family used in cosmetics. It was firstly discovered in the vitreous humor of the eye [19]. HA is recognized as an important moisturizer due to its high water retention capacity, being able to bind 1000 times its volume in water [20]. It is mainly used in cosmetic products as skin conditioning and viscosity increasing agent [21]. This linear polymer is based on the repeating disaccharide unit composed of glucuronic acid and *N*-Acetylglucosamine [22]. Several bacteria are also able to produce HA, amongst *Streptococcus* sp. [23], a genus which unfortunately comprises pathogenic bacteria [24]. To encounter this issue, heterologous production was achieved in bacteria belonging to 'generally recognized as safe' (GRAS) group. *B. subtilis*, a GRAS bacterium, was thus engineered for HA production [25]. In cosmetics, HA is often used as an anti-aging or anti-wrinkle agent [26], promoting skin hydration and elasticity [27]. HA is widely used as a dermal filler and replaced collagen-based dermal fillers [20][28].

Several other EPS have been described for cosmetic applications, additional examples including cellulose, dextran, Fucogel (Solabia), FucoPol, GalactoPol and levan are given in **Table 1**. Even though cellulose and dextran have the same osidic composition (glucose), their glycosidic linkages differ, as monosaccharides are linked through β-1,4 linkages and through α-1,6 linkages, respectively. In consequence, these EPS display distinct structural and conformational features. Cellulose is known for its crystalline appearance and insolubility in aqueous solvents, bacterial cellulose is known for its higher water holding capacity, higher crystallinity and higher purity compared to plant derived cellulose [29], while dextran is highly soluble in water. Concerning high fucose containing EPS, Fucogel (Solabia) displays interesting bioactivities such as anti-aging properties, probably arising from its anionic charges, its linear structure and its lower molecular weight (4×10^4 g/mol) compared to other presented EPS. On the other hand, FucoPol has a branched structure, possesses anionic charges and a higher molecular weight ($2–6 \times 10^6$ g/mol) conferring functional and bioactive properties. Otherwise, GalactoPol is a linear and anionic high molecular weight EPS ($>1 \times 10^6$ g/mol), mainly composed of galactose and three other neutral sugars (mannose, glucose and rhamnose), substituted with three anionic groups (succinate, pyruvate, acetate) exhibiting functional properties. Finally, levan is a linear homopolysaccharide composed of fructose, which can be linear or branched depending on the producing strain, of high molecular weight generally around 2×10^6 g/mol, with rheological and film forming properties, as well as some bioactivities.

Table 1. Non-marine bacterial EPS extensively used in cosmetics: bacterial EPS, producing strain, EPS composition (charges, ramifications, monosaccharides and substituting groups), molecular weight (Mw) and functional properties.

Bacterial EPS	Bacterial Strain	EPS Composition	Mw (g/mol)	Functional Properties	Ref.
Xanthan	*Xanthomonas* sp.	Anionic, branched Glc, Man, GlcA, Pyruvate, acetate	0.4–15 × 10^6	Hydrocolloid, binder, emulsion stabilizer, viscosity enhancer, thickening agent Skin conditioning agent	[1][8][9][18]
Gellan	*Sphingomonas* sp.	Anionic, linear Glc, Rha, GlcA Acetate, glycerate	0.24–2.2 × 10^6	Hydrocolloid, emulsion stabilizer, viscosity enhancer	[15][18][30][31]
Hyaluronic acid (HA)	*Streptococcus* sp.	Anionic, linear GlcA, GlcNAc	2 × 10^6	Viscosity enhancer, high water retention capacity Skin conditioning agent Bioactive: anti-wrinkle, moisturizing, skin elasticity enhancer, dermal filler	[20][22][23][26][27][32][33]

Bacterial EPS	Bacterial Strain	EPS Composition	Mw (g/mol)	Functional Properties	Ref.
Cellulose (β-glucan)	*Aliivibrio* sp., *Agrobacterium* sp., *Gluconacetobacter* sp., *Komagataeibacter* sp., *Pseudomonas* sp., *Rhizobium* sp.	Neutral, linear Glc	1×10^6	Insoluble in aqueous solvents, highly crystalline, high degree of hydration, emulsion stabilizer Bioactive: moisturizer	[29] [34] [35] [36] [37] [38] [39] [40]
Dextran	*Lactobacillus* sp., *Leuconostoc* sp., *Pediococcus* sp., *Streptococcus* sp., *Weissella* sp.	Neutral, linear Glc	$2–40 \times 10^6$	Binder, bulking agent Bioactive: skin smoothing, brightening agent, anti-inflammatory	[41] [42] [43] [44] [45] [46] [47]
Fucogel	*Klebsiella* sp.	Anionic, linear Fuc, Gal, GalA Acetate	4×10^4	Skin conditioning agent Bioactive: skin moisturizing, anti-aging	[48] [49]
FucoPol	*Enterobacter* A47	Anionic, branched Fuc, Gal, Glc, GlcA Succinate, pyruvate, acetate	$2–6 \times 10^6$	Hydrocolloid, emulsifying, flocculating and film-forming agent Bioactive: antioxidant, wound healing, photoprotection	[50] [51] [52] [53] [54] [55]
GalactoPol	*Pseudomonas* sp.	Anionic, linear Gal, Man, Glc, Rha Succinate, pyruvate, acetate	$1–5 \times 10^6$	Hydrocolloid, emulsifying, flocculating and film-forming agent	[56] [57]
Levan	*Aerobacter* sp., *Bacillus* sp., *Halomonas* sp., *Pseudomonas* sp., *Streptococcus* sp., *Zymomonas* sp.	Neutral, linear or branched Fru	2×10^6	Water-soluble, strongly adhesive, film former, viscosity enhancer Skin conditioning agent Bioactive: anti-inflammatory, cell proliferative	[2][18] [58] [59] [60] [61] [62] [63]

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3. Marine Bacterial EPS in Cosmetics

With ocean representing 70.8% of the Earth surface and the existence of a large number of niches characterized by specific conditions, this wide ecosystem still remains underexplored and represents a source of new biodiversity and chemical diversity. Many industry fields exhibit great interest to these potentially new compounds, including bacterial EPS. In particular, marine bacteria constitute a rich source of innovative EPS, that are explored for their skincare effects in cosmetic products. The main described bacteria are Gammaproteobacteria belonging to the

genera *Alteromonas*, *Pseudoalteromonas* and *Vibrio* [3][64][65][66][67][68][69][70]. *Alteromonas* and *Pseudoalteromonas* species usually produce highly branched anionic EPS composed of both neutral sugars and uronic acids substituted with sulfate, pyruvate and/or lactate groups. In contrast, *Vibrio* species synthesize linear anionic EPS containing mainly uronic acids and hexosamines, and can also be substituted with acetyl and/or lactate groups as well as amino acids [71][72][73][74]. The presence of anionic monosaccharides and different negatively charged substituting groups is determinant for functional properties of marine EPS.

A non-exhaustive list of marine EPS with cosmetic applications and their characteristics is presented in **Table 2**. These data were extracted from a patent review made on Orbit Express database (Questel©), focusing on patents deposited by cosmetic companies or suppliers. Some other data for these patented EPS were also extracted from articles published in peer-reviewed scientific journals. Presented patents are granted alive to date (database accessed on March 2023), except for patents related to the HYD657 (Abyssine^{TM} PF) EPS produced by *A. macleodii* subsp. *fijiensis* biovar deepsane CNCM I-1285, which have expired. Eleven EPS are shown, including data information on producing bacterial strain, EPS composition and molecular weight, as well as scopes of cosmetic actions and summarized bioactivities. It appears that marine-derived EPS are only used as active ingredients. The eleven presented polymers encompass two strains of *Alteromonas* sp., one *Cobetia marina* strain, two strains of *Halomonas* sp., one *Pseudoalteromonas* sp., and four strains belonging to *Vibrio* genus (**Table 2**). The strain *C. marina* CNCM I-4353 is outlined twice in the table as its EPS is patented in native form and as depolymerized derivative, depicting different molecular weights and bioactivities [75][76]. Another depolymerized derivative from *V. alginolyticus* CNCM I-4151 is also discussed [77].

Table 2. Marine bacterial EPS with cosmetic applications from literature and patent review: bacterial strain, EPS composition (charges, monosaccharides and substituting groups), molecular weight (Mw) (* depolymerized EPS), scopes of action and bioactivities.

Bacterial Strain	EPS Composition	Mw (g/mol)	Scopes of Action	Bioactivities	Refs.
A. macleodii subsp. *fijiensis* biovar deepsane HYD657 CNCM I-1285	Anionic Gal, Glc, Rha, GlcA, GalA, Man, Fuc Sulfate, lactate, pyruvate	1×10^6	Soothing Irritation	Soothing effect; reduction of sensitive skin irritation by chemical, mechanical and UVB aggression; promotion of skin repair.	[66] [78] [79] [80] [81] [82]
Alteromonas sp. CNCM I-4354	Anionic GlcA, Glc, Gal, GalA, Man	1×10^6	Wrinkles	Wrinkle depth reduction; collagen fibers contraction inducing a tensing effect.	[83]
C. marina CNCM I-4353	Anionic Glc, Rha, Gal, GlcA, GalA Sulfate	1×10^6	Soothing Inflammation	Inhibition and prevention of inflammation.	[76]

Bacterial Strain	EPS Composition	Mw (g/mol)	Scopes of Action	Bioactivities	Refs.
C. marina CNCM I-4353	Anionic Glc, Rha, GlcNAc, GalA, Gal Sulfate 2 amino acids (threonine and serine)	2×10^5 *	Barrier function Skin appearance Aging	Improvement of barrier function and moisturizing of the skin in the treatment of aged skin; improvement of skin repair kinetics against external aggressions.	[75]
H. anticariensis LMG P-27891	Neutral or anionic Man, Rha, Glc Optional: GalA, Xyl	1×10^4	Inflammation Aging Wrinkles Skin firming	Treatment of cellulite; reduction of skin lipid accumulation; stimulation of lipolysis and collagen synthesis; reduction of the amount of nocturnin in cells.	[84]
H. eurihalina LMG P-28571	Neutral or anionic Glc, GlcN, Man, Rha, Gal Optional: Fuc, GlcA Sulfate	1×10^4	Aging Wrinkles Skin firming	Promotion of collagen synthesis and connexins levels.	[4]
Pseudoalteromonas sp. CNCM I-4150	Anionic Glc, Gal, GlcA, GlcNAc, GalA, Man	8×10^5 *	Aging Wrinkles	Improvement of skin moisturizing due to the water retention capacity.	[85]
V. alginolyticus CNCM I-4151	Anionic GalA, GlcNAc 2 amino acids (alanine and serine)	2×10^5 *	Aging Inflammation Acne	Reduction of inflammation reduced; improvement of quality of the superficial layers of the epidermis; degradation of the extracellular matrix reduced.	[77]
V. alginolyticus CNCM I-5035	Anionic Gal, GlcNAc, GulNAcA	5×10^5	Barrier function Acne	Improvement of physical and chemical barriers function by increasing the keratinocyte differentiation and epidermal renewal. Increasement of immune defense against pathogens involved in acne.	[86]
Vibrio sp. CNCM I-4239	Anionic GlcNAc, GlcA, GalNAc	1×10^5–1×10^6 *	Hydration Inflammation	Promotion of the healing process; inhibition of neuronal exocytosis (inflammation; acne; wrinkle reduction).	[87]
Vibrio sp. CNCM I-4277	Anionic GlcA, GlcNAc,	1×10^6	Aging Wrinkles	Increase of hyaluronic acid synthesis.	[88]

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as-Meyer [91]. Abyssine™ PF is an anionic heteropolysaccharide of high-molecular weight (1×10^6 g/mol) composed of neutral sugars (galactose, glucose, mannose, fucose), uronic acids (glucuronic acid, galacturonic acid), and

Bacterial Strain	EPS Composition	Mw (g/mol)	Scopes of Action	Bioactivities	Refs.
	Glc, Fuc Sulfate				[79]
V. diabolicus CNCM I-1629	GlcA, GlcNAc, GalNAc	[85] 1 × 10^6	Skin regeneration	Collagen structuring and extracellular matrix establishment by dermal fibroblasts	[4] [65] [89] [90]

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decorated with amino acids (threonine and serine) [75]. The native high-molecular weight (1 × 10^6 g/mol) EPS exhibits anti-inflammatory properties, whereas its low molecular weight derivatives (2 × 10^5 g/mol) possess anti-aging properties and improve skin appearance, and barrier functions.

EPS synthesized by *Vibrio* sp. are linear anionic polysaccharides devoid of sulfate mainly composed of uronic acids and hexosamines that may also be substituted with amino acids. For instance, a high molecular weight (1 × 10^6 g/mol) EPS, diabolican, produced by *V. diabolicus* CNCM I-1629 displays original structural features close to those of hyaluronic acid as it is composed of glucuronic acid and *N*-Acetylglucosamine as well as *N*-Acetylgalactosamine [65]. The EPS produced by *V. alginolyticus* CNCM I-5034 is composed of glucuronic acid, *N*-Acetylglucosamine, galactose and galacturonic acid and it is substituted with alanine and lactate groups [73]. Epidermist 4.0™ produced by *V. alginolyticus* CNCM I-5035 is a linear EPS composed of galactose, *N*-Acetylglucosamine and *N*-Acetylguluronic acid, with 30% of acetyl groups [86].

To modulate the EPS bioactivities, depolymerization can be performed to reduce the molecular weight of the native polymer. Several techniques are used to decrease the molecular weight of the polymer, such as chemical depolymerization using free radicals or acids [92][93], as well as mechanical [94] or ultrasonic degradations [95]. Free-radical depolymerization with hydrogen peroxide and copper, used as a metal catalyst, is reproducible and can be controlled through pH regulation and adjustment of hydrogen peroxide concentration, leading to low molecular weight derivatives between 20,000 and 100,000 g/mol [93][96]. Two patented derivatives were obtained using radical depolymerization of the native EPS from *Pseudoalteromonas* sp. CNCM I-4150 and *Vibrio* sp. CNCM I-4239 strains [85][87]. Even if depolymerization using acids or free radicals is suitable to decrease the molecular weight of polysaccharides, this method is not specific and may lead to the loss of substituents or sugar residues. Enzymatic hydrolysis remains more specific and more sustainable for polysaccharide depolymerization. However, it is highly challenging due to specific EPS structures, which implies the use of appropriate enzymes. Indeed, it was shown that enzymatic depolymerization of infernan (GY785 EPS) produced by the deep-sea hydrothermal vent bacterium *A. infernus* was not effective although various commercially available enzymes were tested. Only intracellular protein extract of this bacterium, containing a polysaccharide lyase, was able to depolymerize the EPS that it produces [97][98]. Another patented depolymerization process which was successfully applied to prepare low molecular weight derivatives is based on supercritical fluid-accelerated hydrothermolysis [99]. It allows partial depolymerization without altering monosaccharide pattern, decorating amino acids and sulfate groups. This technique using carbon dioxide heated to 200 °C and high pressures up to 250 bars was applied to depolymerize native EPS from *C. marina* CNCM I-4353 [75][76] and *V. alginolyticus* CNCM I-4151 [77].

4. EPS-Producing Extremophilic Bacteria

EPS-producing marine bacteria were isolated from different environments, including atypical ones presenting extreme conditions such as the Antarctic marine environment, sea ice, marine snow, microbial mats, hypersaline environments, shallow and deep-sea hydrothermal vent environments [67][72][100]. Some of the marine bacteria producing the EPS presented in Section 6.2 were isolated from extreme environments, such as *A. macleodii* subsp. *fijiensis* biovar deepsane (HYD657 CNCM I-1285) [66] and *V. diabolicus* CNCM I-1629 [65], both isolated from the polychaeta annelid *Alvinella pompejana* living close to hydrothermal vents located on the East Pacific Rise [101].

In addition, *C. marina* strains, psychrophilic bacteria evolving in cold water, were isolated from coastal sea samples [102] and mussels [103]. *C. marina* KMM 296 could even grow in the wide range of temperatures from 4 to 42 °C [103][104]. *H. eurihalina* is a moderately halophilic bacterium which can spread in diverse saline environments, such as solar salterns, intertidal estuaries, hydrothermal vents, hypersaline lakes and open ocean [104][105]. *H. eurihalina* MS1 isolated from saline soil in Alicante (Spain) was shown to be an EPS producing strain [106][107]. Another *Halomonas* sp., *H. anticariensis* strains FP35 and FP36 isolated from saline soils (Spain) also produced EPS [106][107]. *Pseudoalteromonas* strains are only found in marine environments, they possess environmental adaptation capacities as they can survive in extreme habitats, such as hydrothermal vents and polar areas [67][69]. Moreover, *V. alginolyticus* is a halophilic bacterium growing in the ocean or estuary environment [108]. These marine bacteria can grow in harsh conditions, and EPS production is important for their survival. Although isolated from extreme environments, these strains' physiological requirements and tolerances are compatible with classical conditions of production, i.e., mesophilic temperature and neutral pH. These production conditions can be kept upon scaling up of an industrial process.

Commercial production of marine bacteria relies on the ability of the strain to grow in classical conditions of production, as high or low temperatures induce high energy demand and require specifically designed bioreactors, and salt concentration needs to be decreased to limit equipment corrosion. Bacteria growing at extreme values of pH require the use of acids and alkalis that induce corrosion and are risky to handle at industrial scale. Moreover, the use of piezophiles cannot be considered as it requires specific and costly equipment associated with high risks due to high pressure. However, among marine extremophiles, only thermophilic and psychrophilic bacterial strains have been reported to produce EPS. None of them are currently commercialized or have been considered as actives for cosmetics.

EPS production yield in marine thermophiles is lower than mesophilic marine strains [109]. Requirement of high temperature also imposes shake flasks as bioreactors are not developed for such high temperature. Fermentation for psychrophiles is longer than mesophiles. All of these specificities may hinder development of EPS from extremophiles unless more efforts are conducted for optimizing fermentation process conditions including further developments for scale-up production of EPS in bioreactor.

EPS are produced in response to biotic and abiotic stresses, and their secretion is one of the mechanisms used to tolerate harsh conditions including cold stress and ice crystal damage for psychrophiles, high temperature for thermophiles eventually exposed to large temperature gradients, high salts for halophiles, and acidic or toxic metal-containing environments for acidophiles or metal resistant microorganisms [110][111][112]. Some microorganisms also have the ability to thrive in environments with multiple extreme conditions, such as deep-sea hydrothermal vents with intermittent extreme physical and chemical gradients between vent fluids and surrounding seawater, or Kopara that are Polynesian microbial mats found in pools of Polynesian atolls and subjected to variations in salinity, dessication and sun exposure. This protective role of EPS relies on the formation of a mucous slime around bacterial cells and is claimed in the bioactivities of final ingredient, emphasizing their biomimetic action on skin, including protection against low-temperature for psychrophiles [113][114], chelation of trace metals and binding of heavy metals [115][116] although activity features of EPS from extremophiles were not studied for cosmetics. However, extremophilic bacteria represents a new biodiversity source of EPS, strengthening their chemical diversity.

5. Bioactivity Evaluation of Marine EPS

Marine-derived EPS presented in Section 6.2 exhibit interesting bioactivities for cosmetic applications. They are further detailed in this section. Examples of assays used to demonstrate these bioactivities are also presented, they can be applied to various active candidates depending on molecular weight, solubility and targeted bioactivities.

Due to regulatory requirements and safety assessments, EPS bioactivities need to be demonstrated on skin models for their commercial development. The EPS presented in **Table 1** possess interesting biological activities, which were assessed using in vitro and ex vivo techniques. Examples of targeted bioactivity assays among selected EPS are shown in **Table 3**. These EPS possess anti-aging [84], anti-inflammatory [77] and anti-acne [77] properties as well as moisturizing [88] and slimming effects [84]. They promote vascularization [75] and improve skin barrier function [77][87]. The first step in assessing the EPS bioactivities is to determine the cytotoxicity of the compound on the selected cell culture or skin model, to verify that the tested compound can be applied at a suitable concentration without adverse effects. Cytotoxicity data are not systematically given in patents. However, cell viability is often assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) or AlamarBlue assays.

Table 3. Examples of biological activities of marine bacterial EPS: bacterial strain, claims, cell culture models (two-dimensional, 2D and three-dimensional, 3D) as well as assessed activity and the method used.

Strain	Claims	Cell Culture Model	Activity and Analysis	Refs.
C. marina CNCM I-4353	Vascularization	Co-culture of human dermal fibroblasts (NHDF) and human umbilical vein endothelial cells (HUVECs) infected with a lentivirus	Quantification of angiogenesis (fluorescence levels expressed by HUVECs)	[75]

Strain	Claims	Cell Culture Model	Activity and Analysis	Refs.
		that express green fluorescent protein		
H. anticariensis LMG P-27891	Anti-aging	Human dermal fibroblasts (2D)	Type I collagen synthesis (ELISA assay)	[84]
H. anticariensis LMG P-27891	Slimming	Human subcutaneous pre-adipocytes in a complete differentiation medium (2D)	Reduction of the lipid accumulation "adipogenesis" (fluorescence assay)	[84]
V. alginolyticus CNCM I-4151	Anti-inflammation	Skin explants inflamed by lipopolysaccharides addition	Interleukin production quantification (IL-8 levels of expression)	[77]
V. alginolyticus CNCM I-4151	Anti-inflammation Anti-acne	Inflamed reconstructed human skin (3D)	Inflammation level studied by metalloproteinase expression (MMP3 mRNAs levels of expression)	[77]
V. alginolyticus CNCM I-4151	Barrier function	Reconstructed aged human skin (3D)	Late Cornified Envelop Proteins (LCEs) proteins of the stratum corneum (gene expression of LCE3)	[77]
Vibrio sp. CNCM I-4277	Moisturizing	Human dermal fibroblasts (2D)	Hyaluronic acid synthesis (ELISA assay)	[88]
Vibrio sp. CNCM I-4239	Barrier function	Human keratinocytes (2D)	Healing test (microscopic observations of cells compared before and after treatment on the scrap region)	[87]
Vibrio sp. CNCM I-4239	Cytotoxicity	Human dermal fibroblasts (2D)	Proliferation assay to measure cell viability (fluorescence assay)	[87]
V. diabolicus CNCM I-1629	Promotion of fibroblast proliferation	Dermal equivalent matrices with human dermal fibroblasts (3D)	Proliferation and migration of fibroblasts and production of an extracellular matrix [84]	[89] [90]

ssess the in 2D cell s used to demonstrate moisturizing properties of the EPS from *Vibrio* sp. CNCM I-4277, which was shown to increase HA synthesis [88]. 2D cell culture of keratinocytes was selected to assess the barrier function of the skin upon treatment with EPS from *Vibrio* sp. CNCM I-4239 [87]. A particular example of 2D cell culture of subcutaneous pre-adipocytes was used to assess the potential slimming effect, i.e., the decrease of lipid accumulation, in the presence of EPS produced by *H. anticariensis* LMG P-27891 [84]. 2D cell co-cultures composed of dermal fibroblasts and vein endothelial cells were used to study more complex processes, such as the formation of blood vessels (angiogenesis), to trigger the vascularization stimulation effect of EPS from *C. marina* CNCM I-4353 [75].

More complex 3D cell culture models were also used to demonstrate EPS properties, such as reconstructed human skin. Specific reconstructed human skin with induced inflammation or aging was used to assess the potential anti-inflammatory activities of the EPS produced by *V. alginolyticus* CNCM I-4151. Inflammation was studied by measuring the expression of metalloproteinase 3 (MMP3), the level of which increases in the case of acne lesions. EPS from *V. alginolyticus* CNCM I-4151 on this model led to decreased MMP3 levels. Moreover, the effect of this EPS on reconstructed aged human skin improved barrier function by increasing the expression of proteins of the stratum corneum [77].

Skin explant, an ex vivo skin model, was also used to demonstrate EPS bioactivities. For example, skin explants exhibiting induced inflammation by the addition of LPS were used to study the anti-inflammatory activity of the EPS from *V. alginolyticus* CNCM I-4151. An inflammatory cytokine known to stimulate sebum production in the skin, Interleukin-8 (IL-8) was quantified, its production level decreased after treatment with the studied EPS [77]. Furthermore, a high molecular weight EPS produced by the deep-sea hydrothermal vent bacterium *V. diabolicus* CNCM I-1629 was shown in a dermal equivalent model (composed of collagen I and EPS and containing living human dermal fibroblasts) to promote both collagen structuring and fibroblast colonization (migration and proliferation) with an extracellular matrix synthesis by the cells [89][90]. As an active ingredient, Epidermist 4.0™ improves skin barrier functions and increases skin defense against pathogens involved in acne [86]. These examples emphasize the interesting bioactivities of some marine bacterial EPS and demonstrate their value as cosmetic active ingredients.

6. Structure-Function Relationship

It was shown through the cited examples that bacterial EPS present a wide diversity not only in terms of structural features but also of molecular weights, which further determine their functional properties relevant for cosmetic products. Anionic high molecular weight xanthan and gellan gums are mainly used as functional ingredients providing textural properties to cosmetic formulations, while HA constitutes a multifunctional ingredient as it possesses both textural and bioactive properties. Marine bacterial EPS are mainly used for their various biological activities, which make these polymers good candidates as active ingredients. However, structure-function relationships of EPS for cosmetic applications are not easy to identify due to important diversity of their compositions and structures (if known), their molecular weights and molecular conformations. Nevertheless, some hypotheses can be proposed. Due to their anionic nature resulting from the presence of negative charges of uronic acids and sulfate, acetate or pyruvate groups, EPS can efficiently bind positively charged components (e.g., proteins, growth factors) through ionic interactions. Such a role is largely known for non-sulfated (hyaluronic acid) and sulfated (heparan, heparan sulfate, chondroitin sulfate) GAG of mammalian tissues. Indeed, through interactions with multiple proteins, GAG regulate cellular processes (adhesion, migration, proliferation, differentiation) and are thus involved in physiological processes [117]. These interactions have been shown important for skin repair or regeneration activities, where the presence of EPS was shown to stimulate the synthesis of the extracellular matrix rich in HA and collagen by the cells.

Polysaccharides in cosmetic formulations are also used to maintain skin structural integrity and health due to their moisturizing, soothing, and anti-wrinkle activities, as well as whitening action and UV protection. Since polysaccharides are highly hydrophilic polymers, their moisturizing properties result from their high-water binding capacity due to the hydrogen bond formation between their multiple polar groups and water molecules, which confers moisturizing activity and further prevents from the loss of water from the skin surface [118]. The presence of different functional groups in the EPS structure (hydroxyl, sulfate, carboxyl, carbonyl, secondary amine) also provides metal chelating activity, an important property for protective effect against pollution [119][120]. Skin whitening is an important cosmetic market in Asia and is mainly due to inhibition of tyrosinase activity involved in melanin biosynthesis. This property has been discussed in relation of mannose presence in polysaccharide extracts [121]. Polysaccharides endowed with anti-oxidant, in particular through reactive oxygen species (ROS) scavenging [118], or anti-inflammatory activities are valuable in anti-aging application, and UV protection of skin. Besides its whitening action, mannose was also shown to inhibit inflammatory damage in skin [121]. However, the underlying structural basis of action mechanisms is still limited.

Biological activities of bacterial EPS on skin also depend on the molecular weight of polymers, i.e., the length of the polysaccharide chain, and its molecular conformation. Similarly to other polysaccharides from plant and animal origin, bacterial EPS are highly flexible macromolecules adopting helical conformations in solution [122][123][124]. With increasing length, polysaccharide chains display tendency to high entanglement reinforced by hydrophilic and ionic interactions. Therefore, low molecular weight polymers can easier cross the skin barrier and activate various biological pathways (e.g., anti-aging), compared to high molecular weight native polymers, acting at the epidermis level through moisturizing, barrier function and anti-wrinkle smoothing effects. Regarding human skin, hyaluronic acid penetration was shown to depend on the molecular weight, as HA of high molecular weight (1000 to 1400 kDa) remained at the surface of the stratum corneum, and HA of low molecular weight (20 to 300 kDa) was able to crosse the stratum corneum [125].

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