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NON-TRADITIONAL PRODUCERS OF MICROBIAL EXOPOLYSACCHARIDES

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Data on exopolysaccharides synthesis by psychrophilic fungi and bacteriae, halo- and thermophilic archaea and bacteriae, including those isolated from deep-sea hydrothermal vents — sources — were provided. Physiologic significance, physico-chemical properties and possible practical applications of exopolysaccharides from unusual sources were analyzed. Most of them have immunomodulating, antiviral, anticoagulant, antitumor, antioxidant activities promising for medical and pharmaceutical applications.

Meanwhile, based on the literature date, the conclusion follows about the urgent necessity to develop efficient technologies for synthesis of these exopolysaccharides by nontraditional producers, which currently lags far behind common techniques.

Key words: exopolysaccharides, thermophiles, psychrophiles, halophiles, hydrothermal vents.

Microbial exopolysaccharides (EPS) are high molecular hydrocarbonic exogenous products of microbial metabolism [1–3]. They are widely used in industry (food production, chemistry, oil production, etc.) due to their ability to gel, emulsify, flocculate, form suspensions and to change rheological parameters of aqueous systems [3–5].

Most of currently known microbial EPS have similar functional properties that determine their practical significance [2, 4]. Thus, it is not surprising that only a few of many isolated, described and studied polysaccharides of microbial origin (xanthan, gellan, alginate, dextran) are produced industrially [1, 4].

A polysaccharide must now have unique properties to enter the free niches of rapidly developing fields like medicine, pharmacy, cosmetics, and nature conservation.

Since late XX century, scientists actively study microorganisms living in habitats previously overlooked in the search for bioactive compounds-producing microorganisms (permafrost, hot springs, oceanic depths, salt marshes, etc.). Quite possibly, they survive in such places due to specific adaptive mechanisms and synthesis of protective compounds [5], including EPS with new properties.

Such organisms are known as extremophiles, or microorganisms isolated from extreme habitats [6, 7]. We argue that the terms “extremophile” and “extreme” are not quite applicable, since microbiology considers “extreme” conditions in which only specialized microorganisms survive and many other taxa perish. Therefore this review refers to them simply as “nontraditional”.

To date, a number of reviews have been published about synthesis of EPS by nontraditional producers [6–17]. However, the reviewers mostly paid attention to habitat description, physico-chemical properties and environmental significance of the synthesized polysaccharides and almost ignored the possibility of practical applications [13]. In addition, the reviews were devoted to a specific group of microorganisms (thermophilic [15], halophylic bacteriae [14, 16] and archaea [18], cryophilic yeast [19], sea microbes [9, 10, 17], and microorganisms isolated from hydrothermal vents [8]). Only a few papers reviewed several unusual producers at once [6, 7, 12]. The listed studies were published in 2010–2012 and include mostly summaries of specifics of EPS biosynthesis and their physico-chemical properties. A recent paper [11] discusses practical applications of several polysaccharides, synthesized by bacteriae isolated from hydrothermal sources.

This review aimed to summarize the available information on EPS synthesis by non-traditional producers (thermo-, cryo, halophilic microorganisms and bacteriae isolated from deep-sea hydrothermal vents), and properties of polysaccharides that support their potential practical application in medicine, pharmaceutical, food industries and nature conservation.

Thermophiles

The studies of thermophilic microorganisms started approximately in 1967 [20]. The paper briefly summarized the available knowledge about the microorganisms. In those days, attention was mostly paid to their environmental niche and the mechanisms enabling their survival at high temperatures.

One of those adaptive mechanisms is synthesis of microbial EPS. It should be noted that, unlike industrial mesophilic producers, using thermophils for the preparation of polysaccharides has a number of technological advantages, in particular, at elevated temperatures, the viscosity of the culture fluid and the possibility of the process infection are reduced, as well as mass exchange processes increase, etc. [21–25].

Archaea. The first reports of EPS synthesis by thermophilic archaea began to appear at the end of XX century [26–29]. In 1993, Nicolaus et al. [26] found out that the thermoacidophilic archaea *Sulfolobus solfataricus* MT4 and MT3, isolated from a hot acidic spring (Agnano volcanic crater, Italy) produced EPS at 75–88 °C.

The main disadvantage of those archaea as well as almost all other thermophilic producers of EPS is the low concentration of the target product (Table 1). This can be caused by low concentrations of the carbon and energy source (2–9 g/l) in the cultivation medium. Special attention was paid to the polysaccharide effect on physiology. Thus, several papers [27, 28, 30] presented data on synthesis of EPS by archaea linked to biofilm formation. Rinker et al. [27, 28] studied the growth of hyperthermophilic anaerobic organism *Thermococcus litoralis* DSM 5473. They established that biofilms formed on hydrophilic surfaces (polycarbonate filters) followed by accumulation of sulfated mannan (over 0.3 g/l EPS). Other researchers [30] studied the biofilm structure in thermoacidophilic archaea of the genus *Sulfolobus*.

Polysaccharides can perform other vital functions other than the formation of biofilms which protect the microorganisms from unfavorable factors and toxins. Thus, a hypothesis was formulated [29] that EPS of thermophilic methanogenic archaea *Methanosarcina thermophila* TM-1 can be an osmoprotectant.

Notably, researchers [26–30] did not try to intensify EPS synthesis by thermophilic archaea. Due to the low concentration of the target product, this microorganisms are hardly going to be industrially important in the near future. Another complication is the difficulty of culturing most thermophilic archaea that require complex media with a lot of vitamins, amino acids, etc. [27, 28].

Bacteriae. Simultaneously with studying EPS-synthesizing archaea, researchers turned to thermophilic bacteriae. Almost all of them belong to the family *Bacillaceae* (the genera *Bacillus* [31–33], *Geobacillus* [22, 34–36], *Anoxybacillus* [37], *Aeribacillus* [24]) and *Paenibacillaceae* (the genus *Brevibacillus* [23, 25]) with optimal growth temperature of 45–65 °C (Table 1). Notably, the first reports of EPS synthesis by thermophilic bacteriae also included representatives of these families. Thus, Manca et al. [35] in 1996 reported isolation of extremely thermophilic bacteriae *Geobacillus thermoantarcticus*, which at 65 °C synthesized up to 400 mg/l sulfated EPS from soil near the crater of Melbourne volcano (Antarctica).

Besides representatives of *Bacillaceae* and *Paenibacillaceae*, synthesis of polysaccharides is known for hyperthermophilic bacteriae of the genus *Thermotoga* (optimal temperatures 80–85 °C) [27] and thermophiles of the genus *Thermus* (optimal temperature 60 °C) [38].

All thermophilic bacteriae in the literature produce less than 1 g/l EPS [22–25, 32, 33, 35, 36, 38]. Recently, bacteriae *Anoxybacillus* sp. R4-33, able to produce 1.1 g/l polysaccharide and tolerant of high temperature and radiation were isolated from geothermal radon springs (China) [37].

Thermophilic obligate methanotroph *Methylococcus thermophilus* 111п synthesizes up to 5 g/l EPS [2] and thus is a much better choice. Those amounts were achieved after a complex investigation of pH, temperature, diluted oxygen concentration, gaseous methane to oxygen ratio conditions, and the pre-treatment of the inoculum. The exogenous addition of 0.5 g/l aspartic acid (obtained by transferring amino group to oxaloacetic acid) to the culture medium of strain 111п was followed by an almost two-fold increase in the polysaccharide biosynthesis rate [2].

The EPS of several thermophilic and thermotolerant bacteriae were observed to

have antiviral [31, 34, 39] and immunomodulating [38] activities and to inhibit biofilm formation [40].

Treatment of mononuclear cells of human peripheral blood with polysaccharide solutions (300 μg/ml) of strains *Geobacillus thermodenitrificans* B3-72, *Bacillus licheniformis* B3-15 and T14 stimulated the production of IFN-γ, IFN-α, TFN-α, IL-12 and IL-18 and inhibited the replication of herpes simplex virus type 2 [31, 34, 39]. In the presence of EPS of strains B3-72, T14 and B3-15 the virus was inhibited by 67, 77 and 85%, respectively [39]. Notably, antiviral activity is usually seen in sulfated polysaccharides [41], and the compounds described in [31, 34, 39] did not contain sulfate groups.

Lin et al. [38] isolated from the biofilm of *Thermus aquaticus* YT-1 a polysaccharide that heightened immune response. That EPS was observed to act as an agonist of TLR2 receptor and helped induce synthesis of cytokines IL-6, TNF-α, and nitrogen monoxide (NO) by murine macrophages and human monocytes. That immunoregulatory activity supposedly was caused by galactofuranose in its structure [38].

Several thermophilic representatives of the genus *Bacillus* were also observed to synthesize polysaccharides with anticytostatic activity [22, 33]. Fraction 1 EPS *B. licheniformis* T14, consisting of fructose, fucose and glucose (1:0.75:0.28), at 500 ppm raised LD_{50} of avarol (a cytostatic agent) from 0.18 to 0.99 mg/ml [33], and EPS of *Geobacillus tepidamans* V264 raised it to 2.24 mg/ml [22].

Recently Spanò et al. [40] found that EPS of *B. licheniformis* T14 at 400 μg/ml inhibited biofilm formation by multiresistant strains *Escherichia coli* 463, *Klebsiella pneumoniae* 2659, *Pseudomonas aeruginosa* 445 and *Staphylococcus aureus* 210 by 74, 56, 54 and 60%, respectively. The researchers suggested that due to the emulsifying properties of the polysaccharide it is able to impact the hydrophobicity of bacterial cells and so prevent their primary adhesion to surfaces [40].

A summary of EPS biosynthesis by thermophilic and thermotolerant microbes is given in Table 1. Currently, the microbes are not considered promising due to low EPS synthesis ability. Meanwhile such polysaccharides have properties important for medicine and pharmacy (antiviral, immunomodulating, anticytostatic, etc.), which can stimulate work on intensifying their synthesis.

EPS-producing microbes from deep-sea hydrothermal vents. Deep-sea hydrothermal vents, characterized by high concentrations of toxic compounds (sulfides and heavy metals), sharp changes in temperature and pressure, are habitats of thermophilic bacteriae with various properties [8, 21, 42–44].

Since the first such vent was discovered in 1977 near the Galapagos, a great many other hydrothermal vents with various unique microorganisms were found [43, 44]. Thus, from the East-Pacific Rise (2600 m deep), EPS-synthesizing strains of bacteriae from the genera *Alteromonas* [45–48] and *Vibrio* [49] were isolated; at Mid-Atlantic Ridge (3500 m deep), bacteriae *Alteromonas macleodidi* subsp. *fijiensis* var. *medioatlantica* were found [50]; at Guaymas Basin and North Fiji Basin (2000 m deep), strains *A. macleodii* [43] and *Alteromonas infernus* [44] were isolated, respectively.

Despite the fact that these EPS-producing bacteriae were isolated from extreme habitats, most of them turned out to be mesophilic neutrophils with optimal growth temperature 25–35 °C and pH 6–8 [43–45, 46–50], and only a few of them were thermophiles (40–45 °C) [49].

The EPS-producing bacteriae isolated from deep-sea hydrothermal vents became a subject of active research in 1990s [42–44, 47–49]. In 1994, Guezennec et al. [42] published results of screening EPS-producing bacteriae isolated from hydrothermal vents. Almost all polysaccharides except for neutral monosaccharides contained sulfate moieties (to 21.5%) and glucuronic acids (to 7.9%), several had amino sugars (to 2.5%).

Interestingly, EPS-producing bacteriae are isolated not only from soil or water near hydrothermal vents [42], but from the surfaces of various organisms living there (shrimps, worms, etc. [45, 46, 48–50]). The strain *Alteromonas macleodii* subsp. *fijiensis* var. *medioatlantica* MS907, producing 9 g/l EPS after 72 hours of culturing was found on carapax of the shrimp *Rimicaris exoculata* [50].

The outer shell of a sea polychaete *Alvinella pompejana* (at the depth of 2600 m) yielded EPS-synthesizing bacteriae *Alteromonas* sp. HYD1545 and *A. macleodii* subsp. *fijiensis* biovar deepsane HYD657 [45, 48]. The strain HYD1545 after 120 hours of culturing produced 11 g/l of polysaccharide [48], and strain HYD657 produced 7 g/l EPS after

Table 1. **Synthesis of exopolysaccharides by thermophilic and thermotolerant microorganisms**

Microorganism	Culture temperature	Carbon source, g/l	EPS concentration, g/l	Physico-chemical properties of EPS		Physiological role, functional properties and prospects of EPS application	References
				content	Molecular mass, kDa		
EPS of thermophilic archaea							
Methanosarcina thermophila TM-1	45–55 °C	Trimethylamine, 4.8	–	Glucuronic acid (over 40%)	–	Osmoprotectant	[29]
Sulfolobus acidocaldarius	76 °C	–	–	Glucose, galactose, mannose, *N*-acetylglucosamine	–	Biofilm formation	[30]
Sulfolobus solfataricus MT3	75 °C	Glucose, 3	7.0 mg/l	Glucose, mannose, glucosamine, galactose (1.2:1.0:0.77:0.73). Sulfates 5–12%	–	–	[26]
Sulfolobus solfataricus MT4	88 °C	Glucose, 3	8.4 mg/l	Glucose, mannose, glucosamine, galactose (1.2:1.0:0.18:0.13). Sulfates 5–12%	–	–	[26]
Sulfolobus tokodaii	76 °C	–	–	Glucose, galactose, mannose, *N*-acetylglucosamine	–	Biofilm formation	[30]
Thermococcus litoralis DSM 5473	88 °C	Maltose, 2	0.18–0.32	Mannan, sulfates 1-2%	41	Biofilm formation	[27, 28]
EPS of thermophilic and thermotolerant bacteriae							
Aeribacillus pallidus 418	55 °C	Maltose, 9	0.17	Fraction 1: mannose, glucose, galactosamine, glucosamine, galactose, ribose (1:0.16:0.1:0.09:0.07:0.06:0.04) Fraction 2: mannose, galactose, glucose, galactosamine, glucosamine, ribose, arabinose (1:0.5:0.46:0.35:0.24:0.16:0.14)	Fraction 1: 700; Fraction 2: 1000	Emulgent	[24]
Anoxybacillus sp. R4-33	55 °C	Glucose, 10	1.1	Fraction 2: mannose, glucose (1:0.45)	1000	Adsorbs heavy metals	[37]
Bacillus licheniformis B3-15	45 °C	Glucose, 6	0.165	Fraction 1: mannose, glucose (1:0.3); Fraction 2: mannose; Fraction 3: glucose	600	Antiviral and immunomodulatory	[31, 32]

Table 1. Continued

Microorganism	Culture temperature	Carbon source, g/l	EPS concentration, g/l	Physico-chemical properties of EPS		Physiological role, functional properties and prospects of EPS application	References
Bacillus licheniformis T14	50 °C	Sucrose, 50	0.366	Fraction 1: fructose, fucose, glucose and traces of galactosamine, mannose (1:0.75:0.28:traces:traces)	1000	Antiviral, immunomodulatory and anticytotoxic. Inhibits biofilm formation	[33, 39, 40]
Brevibacillus thermoruber 423	55 °C	Maltose, 18	0.897	Glucose, galactose, galactosamine, mannose, mannosamine (1:0.3:0.25:0.16:0.04)	–	–	[25]
Brevibacillus thermoruber 438	55 °C	Maltose, 18	78.1 mg/l	–	–	–	[23]
Geobacillus tepidamans V264	60 °C	Maltose, 30	111.4 mg/l	Glucose, galactose, fucose, fructose (1:0.07:0.04:0.02)	>1000	Anticytotoxic	[22]
Geobacillus thermoantarcticus	65 °C	Mannose, 6	0.4	Fraction 1: mannose, glucose (1:0.7); Fraction 2: mannose and traces of glucose Sulfated	Fraction 1: 300; Fraction 2: 300	Emulgent	[35]
Geobacillus thermodenitrificans B3-72	65 °C	Sucrose, 6	70 mg/l	Fraction 1: glucose, mannose (1:0.3); Fraction 2: mannose, glucose (1:0.2)	Fraction 2: 400	Fraction 2: antiviral and immunomodulating	[34, 36]
Methylococcus thermophilus 111п	40 °C	Methan	5	Fraction 1: mannose, galactose, glucose, fucose, xylose, rhamnose, glucuronic acid. Fraction 2: mannose, glucose, xylose, rhamnose	–	Intensification of oil production	[2]
Thermotoga maritima DSM 3109	88 °C	Maltose, 2	0.120	Glucose, ribose, mannose (1:0.06:0.03)	–	Flocculant	[27]
Thermus aquaticus YT-1	60 °C	–	–	Galactofuranose, galactopyranose, *N*-acetylglucosamine (1:1:2)	500	Immunomodulatory activity; adjuvant to vaccines	[38]

Note: «–» — no data available.

52 hours of culture [45]. Further research [51] of EPS of strain HYD657 established that they efficiently protect keratinocytes from inflammation agents. The protective effect was also found towards Langerhans cells, which are sensitive to the ultraviolet and play an important role in the system of human skin immune protection. Nowadays, cosmetic preparation Abyssine® was developed based on the polysaccharide (deepsane). It is recommended for soothing and protection against irritation of sensitive skin [52].

Notably, the polysaccharide of strain HYD657 has an unusual component, a residue of 3-*O*-(1-carboxyethyl)-*D*-glucuronic acid [45]. Currently, the compound was also found in EPS of the strain *Alteromonas* sp. HYD1644, isolated from the epidermis of the polychaete *Alvinella caudata* [46], and in drought-resistant cyanobacteriae *Nostoc commune* DRH-1 [53]. Helm et al. [53] suggested that this and other uronic acids with carboxyethyl moieties play a key part in providing survival in unfavorable conditions. For example, such functional groups can help EPS attach to adjacent chains of the polymer, organic (biofilms) or inorganic surfaces, etc.

The strain *Vibrio diabolicus* HE800^T was isolated from polychaete *Alvinella pompejana*. The strain produces a polysaccharide similar to hyaluronic acid [49]. The EPS is made up equally from glucuronic acid and hexosamines (*N*-acetylglucosamine and *N*-acetylgalactosamine) [54]. Treating damaged skullcap skin of Wistar rats with the EPS made the wound close sooner, while the trabecular and cortical anatomic structure of the defect fully recovered [55]. Zanchetta et al. [55, 56] suppose that the effect is caused by the ability of EPS to form extracellular matrix that helps direct adhesion of osteoblasts and pericytes, generally protect the damaged site while it heals, and to bind calcium.

Senni et al. [57] suggested that glycosoaminoglycan polysaccharide of strain HE800^T is a promising agent for various derivatives (heparan sulfate, chondroitin sulfate, etc.). Such depolymerization of native polysaccharide to molecular mass of 22 kDa with further deacetylation and sulfation (sulfate content 34%) resulted in a polymer similar to heparan sulfate. Those derivatives were observed to stimulate proliferation of dermal and gingival fibroblasts and inhibit secretion of matrix metalloproteinases [57].

The EPS of *Alteromonas infernus* GY785 after sulfation (sulfate content 40%) and controlled depolymerization by free radicals to molecular mass of 24 kDa substantially raised APTT (activated partial thromboplastine time) [58, 59]. The anticoagulant activity of the polysaccharide was on the level of calcium pentosan polysulfate though 2.5–6.5 times lower compared to heparin [58]. Notably, due to the low sulfate content in the native polysaccharide (5.5–10%) it did not have anticoagulant activity [58].

Recently the effect of depolymerized EPS of strains *V. diabolicus* HE800^T and *A. infernus* GY785 on the complement system was studies [60]. The low molecular (2.9 kDa) derivative of the polysaccharide of strain HE800^T to a large extent activated the system (60% activation at 50 μg EPS), while the depolymerized (molecular mass 23 kDa) and sulfated (sulfate content 37–42%) EPS of strain GY785, conversely, caused its significant inhibition (78% inhibition at 10 μg EPS). Due to those properties, the polysaccharides are promising for treating diseases caused by deregulation of immune system and over activation of the complement system.

Therefore, EPS of bacteriae isolated from hydrothermal vents can become widely accepted into medical, pharmaceutical and cosmetic industries due to anticoagulant, protectant, immunomodulatory and regenerative activities. Notably, such microorganisms can synthesize up to 11 g/l of the product, and some polysaccharides from hydrothermal-dwelling bacteriae are already mass-produced. For example, EPS of *A. macleodii* subsp. *fijiensis* biovar deepsane HYD657 is used for cosmetics (Abyssine®).

Data on EPS of bacteriae isolated from hydroterms are summarized in Table 2.

Psychrophiles

Cold environments are found from deep seas to snow-laden mountaintops, from Arctic to Antarctica. Temperature of almost 75–80% of the Earth surface is below 5 °C [60–62]. Cold habitats are characterized by frequent sharp changes in temperature (cycles of freezing and thawing, etc.), UV-radiation, nutrient concentration [63, 64]. Oceanic and sea waters also have pressure and salinity oscillations [21]. Evidently, microorganisms would not survive in such conditions without relevant adaptive mechanisms [62, 65, 66].

EPS play a large role in it. Exopolymers, including polysaccharides, take part in

Table 2. **Exopolysaccharide synthesis by bacteriae isolated from deep-sea hydrothermal vents**

Microbial source*	Carbon source, g/l	EPS content, g/l	Physico-chemical properties of EPS		Physiological effect, functional properties and possible implementations of the EPS	References
			Chemical composition	Molecular mass, kDa		
Alteromonas infernus GY785	Glucose, 30	Fraction 1: 5.5 Fraction 2: 4.3	Fraction 1 (water-soluble): glucose, galactose, glucuronic and galacturonic acid (1.0:0.9:0.7:0.4). Sulfates 5.5–11%	Fraction 1: 1000	Anticoagulant, adsorbent	[44, 58, 59]
Alteromonas macleodii subsp. *fijiensis* ST716	Glucose, 30	6	Galactose, glucose, mannose, glucuronic and galacturonic acid (1.0:0.95:0.4:1.1:0.57). Sulfates 5%	330	Thickener	[43]
Alteromonas macleodii subsp. *fijiensis* biovar deepsane HYD657	Glucose, 30	7	Galactose, glucose, rhamnose, fucose, mannose, glucuronic, galacturonic and 3-*O*-(1-carboxyethyl)-D-glucuronic acids (1.0:0.43:0.86:0.5:0.43:0.5:0.5:0.5). Sulfates 7.5%	1100–1600	Protects keratinocytes and Langerhans cells from inflammation agents	[45, 51]
Alteromonas macleodii subsp. *fijiensis* var. *medioatlantica* MS907	Glucose, 30	9	Galactose, glucose, glucuronic and galacturonic acids (1.0:0.5:0.7:0.26)	1500	Thickener	[50]
Alteromonas sp. HYD1545	Glucose, 30	11	Glucose, galactose, mannose, glucuronic and galacturonic acids (1.0:0.55:0.04:0.24:0.14)	1800	–	[48]
Alteromonas sp. HYD1644	Fructose, 40	Fraction 1: 7.5 Fraction 2: 5.0	Fraction 1 (water-soluble): galactose, glucose, rhamnose, mannose, glucuronic, galacturonic and 3-*O*-(1-carboxyethyl)-D-glucuronic acids (1.0:0.74:0.7:0.13:0.4:0.19:0.23)	Fraction 1: 5000	Thickener	[46, 47]
Vibrio diabolicus HE800^T	Glucose, 40	2.5	Glucuronic acid, *N*-acetylglucosamine, *N*-acetylgalactosamine (1:0.5:0.5)	800–850	Raw material to obtain glycosaminoglycan derivatives. Fastens bone fusion	[49, 54–57]

aggregation, adhesion to surfaces and other microorganisms, biofilm formation, nutrient storage, etc. in marine bacterial communities [66–68]. Often aggregates of salty drops remain unfrozen after the sea water freezes, and the microbes are trapped in salt canals [63, 66]. Then, EPS are cryoprotectants and protectants from high salinity [62, 65, 66].

The majority of microorganisms, able to survive at low temperature, are yeasts and bacteriae [8]. Notably, phylogenetic research also registers a lot of representatives of *Archaea* [61], although they have not been cultured.

Fungi. EPS synthesis by fungi at relatively low temperatures is a novel approach. The first report of polysaccharide production by cryotolerant mycelial fungi appeared only at the beginning of XXI century. In 2002, Selbmann et al. [69] established the ability of *Phoma herbarum* CCFEE 5080 cultured on medium containing sorbitol (60 g/l) to produce 13.4 g/l 7412 kDa glucan. Due to cryoprotectant properties of the polysaccharide, strain CCFEE 5080 is able to grow at 0–5 °C (optimal temperature 28 °C) [70].

Another glucan-producing fungus is strain *Thelebolus* sp. IITKGP-BT12 [68]. Unlike the strain CCFEE 5080, at 18 °C it synthesizes only 1.94 g/l EPS. Experiments have shown that the glucan has significant antiproliferative effect on cells of skin cancer in B16-F0 mice. IC_{50} (the concentration at which maximal inhibition occurred) of the EPS was 275.4 μg/ml. The polysaccharide had almost no effect on normal fibroblasts of the L929 line (at the concentration of 187.5–1500 μg/ml cytotoxicity was almost absent) [67].

Recently, isolation of EPS-synthesizing cryotolerant yeasts of the genera *Sporobolomyces* [71] and *Cryptococcus* [72–74] was reported from Livingstone Island. Cultivation in medium with sucrose (40–50 g/l) and ammonium sulfate (0.25%) at 22–24 °C resulted in 4.6–6.4 g/l of polysaccharides (Table 3).

Research of economically valuable properties of EPS of yeasts from the Livingstone Island confirmed their possible use in cosmetics, food industry [73, 75, 76] and medicine [78]. EPS of strain *Cryptococcus laurentii* AL_{100} exhibited high emulgent activity, significantly enhanced by other polysaccharides (xanthan, guar gum, cellulose, etc.) [73].

Other researchers showed that cosmetic emulsions with 2% EPS *Sporobolomyces salmonicolor* AL_1 remained stable for a month at –10 °C and for 3 months at 22 and 45 °C [75, 76]. To achieve similar results, concentration of synthetic emulgent Arlacel 165 or Rofetan N/NS was 5% [75]. Besides that, EPS of *S. salmonicolor* AL_1 has anticytostatic activity. At 5 ppm it changed LD_{50} of (cytostatic) avarol from 0.18 to 0.10 ppm [77].

EPS of cryotolerant fungi can be used as emulgents and thickeners in food and cosmetic practices at low temperatures. They are promising for medicine and pharmacy due to antitumor and anticytostatic activities.

Bacteriae. Reports of EPS synthesis by cryophilic and cryotolerant bacteriae started shortly after the first study about polysaccharides of cryotolerant fungi [69].

Polysaccharides of cryotolerant bacteriae isolated from free ice and marine aggregates in the Antarctic ocean, with *in situ* temperature of 4 °C were described in 2005 [78]. Six of the studied isolates belonged to the genus *Pseudoalteromonas*, three to the genera *Shewanella*, *Polaribacter*, and *Flavobacterium*. A strain $CAM030^T$ represented the family *Flavobacteriaceae*, later it became a new taxon *Olleya marilimosa* [79]. Most cryophilic bacterial producers isolated after 2005 belong to the genera *Pseudoalteromonas*, *Polaribacter* and *Flavobacterium* (Table 3).

By their monosaccharide content, the polysaccharides of cryophilic bacteriae are similar to EPS of marine bacteriae (Table 2).

Lowering the growth temperature from 20 to 10, or to –2 °C caused an almost 30-fold rise in EPS-producing ability of strain *Pseudoalteromonas* sp. CAM025 (up to 99.9 and 97.2 mg EPS/g biomass, respectively), and a changed monosaccharide ratio [80].

Cryoprotectant properties of EPS of *Pseudoaltermonas* sp. SM20310 were studied in [63]. At 30 mg/ml EPS the number of living cells of strain SM20310 and *E. coli* DH5α was 7 to 18 times as high as in the control group (without EPS) after three cycles of freezing-thawing. Other researchers [68] report that adding the polysaccharide of cryotolerant bacteriae *Flavobacterium* sp. ASB 3–3 at 50 mg/ml led to a four times increase in the number of living cells of strains ASB 3–3 and *E. coli* DH5α after two cycles of freezing-thawing compared to the cultures without EPS.

Cryotolerant bacteriae *Pseudoalteromonas elyakovii* ArcPo 15 isolated from Chukchi Sea were observed to synthesize 1.7 MDa EPS with high cryoprotectant activity [81]. Adding the

EPS (0.5%) to a suspension of *E. coli* DH5α resulted in 94.2% survival of the cells after five cycles of freezing-thawing. Adding 20% glycerin resulted in 54.1% survival of the cells.

Due to the cryoprotectant ability of bacterial EPS we suggest using them as alternative cryoprotectant agents for long-term storage of suspended cultures [82, 83].

According to Carrión et al. at 10% EPS of *Pseudomonas* sp. ID1, survival of *E. coli* ATCC 10536 after freezing and storing for seven days at −20 and −80 °C was 36 and 64%, respectively [82]. Cell survival decreased at lower EPS concentrations. After similar freezing of EPS-synthesizing strain ID1, the cell survival rates were 75 and 94%, respectively. Another study [84] showed that EPS of cryophilic *Colwellia psychrerythraea* 34H are a better cryoprotectant agent for freezing cells at −80 °C than 10% glycerin solution.

Notably, cryoprotectant properties of polysaccharides are not limited to merely the protection of microbial cells. Sun et al. [84] reported that, survival rate of human dermal fibroblasts after 20 hours at 4 °C reached 76.1% with 500 μg/mg EPS of *Polaribacter* sp. SM1127, while without the polysaccharide it was only 44.2%.

In the native environment, other physico-chemical factors besides temperature can induce EPS synthesis, such as pressure and salinity [63, 83]. For example, culturing *C. psychrerythraea* 34H at high hydrostatic pressure (up to 400 atm) resulted in EPS content increasing 4.5–7.5 times.

Polysaccharides of cryophilic and cryotolerant bacteriae can also hold moisture [84, 85], emulsify [82, 68, 86], flocculate [68, 86] and adsorb metal [86, 87].

Research of EPS of bacterial strains *Polaribacter* sp. SM1127 and *Zunongwangia profunda* SM-A87 [84, 85] showed that after 72 hours of incubation with silica gel (relative humidity 43%) the polysaccharide of strain SM1127 retained 76% moisture, which is higher than for hyaluronic acid, glycerin, sodium alginate. This is possibly due to not only a lot of glucuronic acid and *N*-acetylglucosamine (components of hyaluronic acid), but also fucose, which has moisturizing properties, in EPS [84]. The polysaccharides also have antioxidant activity [84, 85]. Thus, the level of neutralization of 2,2-diphenyl-1-picrylhydrazyl radical radical (DPPH·), hydroxyl radical (·OH) and superoxide anion (O_2·) at 10 mg/ml of EPS of SM1127 and SM-A87 10, was 27.2–55.4%. Further research [87] established the ability of EPS of strain SM-A87 to adsorb Cu^{2+} and Cd^{2+} (48 and 39.75 mg/g EPS, respectively).

After optimization of the culture medium [88] in the fed-batch culture [85], the concentration of EPS of strain *Z. profunda* SM-A87 increased to 17 g/l, which is 1.93 times higher compared to the initial.

Recently Sathiyanarayanan et al. [68, 86] isolated cryotolerant *Flavobacterium* sp. ASB 3–3 and *Pseudomonas* sp. PAMC 28620 (AS-06/29) from the soil of Svalbard Arctic glacier fore-field. The optimal carbon and energy source for those bacteriae, unlike other microbial sources of EPS (Table 3) is glycerin. At the medium with 30 g/l of this substrate, the bacteriae produced 7.25 g/l EPS with flocculant and emulgent properties.

In kaolinite suspension (0.5%), flocculant activity of 40 mg/l EPS for strains PAMC 28620 and ASB 3-3 70 was 71.2 and 91.3%, respectively [68, 86]. The polysaccharide of strain ASB 3-3 emulsified *n*-hexane (emulsification index 66.3%) and *n*-hexadecane (64.3%) just as efficiently as sodium dodecyl sulfate [68]. EPS of strain PAMC 28620 efficiently emulsified toluene (67.2%) and methyl octanoate (66.7%) [86]. Besides that, polysaccharide of strain PAMC 28620 expediently adsorbed Fe^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+} (approximately 99%), and Mn^{2+}, Ca^{2+} (92%) [86].

Unlike thermophilic and thermotolerant sources (Table 1 and Table 2), cryophilic and cryotolerant microorganisms synthesize more EPS (up to 17 g/l; Table 3), and their polysaccharides have cryoprotectant, emulsifying properties, retain moisture and adsorb heavy metals. That, consequently, makes the polysaccharides potentially attractive for various fields from food industry (foodstuffs storage) and cosmetics (production of protective cosmetics) to environment-friendly technology (purification of waste waters).

Halophiles

Halophiles are organisms able to survive in briny habitats, whose development requires salt. The salt in question is generally NaCl, while many researchers in their experiments on halophilic cultures use sea salt which contains not only NaCl but also comparatively small amounts of other salts of two- and monovalent metals [89].

Table 3. **EPS synthesis by cryophilic and cryotolerant microorganisms**

Microbial source	Incubation temperature	Carbon source, g/l	EPS concentration, g/l	Physico-chemical properties of EPS		Physiological effect, functional properties and possible avenues of implementation of EPS	References
				Chemical composition	Molecular mass, kDa		
EPS of cryotolerant fungi							
Cryptococcus flavus AL_{51}	24 °C	Sucrose, 50	5.75	Mannose, glucose, xylose, galactose (1:0.47:0.17:0.03:0.08)	1010	–	[72]
Cryptococcus laurentii AL_{62}	22 °C	Sucrose, 40/50	4.73/4.6	Xylose, mannose, glucose (1:0.74:0.41)	8	–	[74]
Cryptococcus laurentii AL_{100}	22 °C	Sucrose, 40	6.4	Arabinose, mannose, glucose, galactose, rhamnose (1:0.25:0.2:0.1:0.05)	4.2	Emulgent	[73]
Phoma herbarum CCFEE 5080	28 °C	Sorbitol, 60	13.4	Glucan (glucose 100%)	7412	Cryoprotectant	[69]
Sporobolomyces salmonicolor AL_1	22 °C	Sucrose, 50	5.2–5.6	Mannose, glucose, galactose (1:0.1:0.08)	>1000	Thickener, emulgent	[71, 75–77]
Thelebolus sp. IITKGP-BT12	18 °C	Glucose, 50	1.94	Glucan (glucose 100%)	500	Antiproliferative activity	[67]
EPS of cryophilic and cryotolerant bacteriae							
Flavobacterium sp. *ASB 3-3*	25 °C	Glycerin, 30	7.25	Glucose, galactose (1:0.43). Sulfates were found	–	Emulgent, flocculant, cryoprotectant	[68]
Polaribacter sp. SM1127	15 °C	Glucose, 30	2.11	*N*-acetylglucosamine, mannose, glucuronic acid, galactose, fucose, glucose, rhamnose (1:0.84:0.76:0.62:0.26:0.06:0.03)	220	Cryoprotectant, moisture-retention agent, antioxidant	[84]
Pseudoalteromonas elyakovii ArcPo 15	15 °C	Glucose, 20	1.64	Mannose, galacturonic acid (3.3:1.0)	17000	Cryoprotectant	[81]
Pseudoalteromonas sp. CAM025	10 °C	Glucose, 30	99.9 mg/g biomass	Glucose, galactose, rhamnose, mannose, fucose, arabinose, ribose, glucuronic acid (1:0.64:0.61:0.31:0.25:0.12:0.05: 0.26). Sulfates 5%	5700	Cryoprotectant	[80]

Table 3. End

Microbial source	Incubation temperature	Carbon source, g/l	EPS concentration, g/l	Physico-chemical properties of EPS		Physiological effect, functional properties and possible avenues of implementation of EPS	References
				Chemical composition	Molecular mass, kDa		
Pseudomonas sp. ID1	11 °C	Glucose, 20	–	Glucose, galactose, fucose (1:0.5:0.48), uronic acids are present	2000	Cryoprotectant, emulgent	[82]
Pseudomonas sp. PAMC 28620	25 °C	Glycerine, 30	7.24	Rhamnose, galactose, glucose, fucose, mannose, ribose (1:0.32:0.25:0.07:0.07:0.03), sulfates detected	–	Emulgent, flocculant, adsorbent	[86]
Pseudoaltermonas sp. SM20310	15 °C	Glucose, 30	0.567	Mannose, glucose, galactose, rhamnose, xylose, *N*-acetylglucosamine and *N*-acetylgalactosamine (1:0.15:0.13:0.03:0.01:0.06:0.02)	2000	Cryo- and osmoprotectant	[63]
Zunongwangia profunda SM-A87	9.8 °C	Whey (60.9%, v/v)	12–17.2	Glucose, mannose, galactose, xylose, fucose, glucuronic acid, not indentified carbohydrate (1:0.84:0.29:0.29:0.05:0.06:0.21)	3760	Moisture-retention agent, antioxidant, adsorbent	[85, 87]

As to salinity, halophiles can be halotolerant (upper salinity limit 15%), weak (NaCl content of 2–5%), moderate (5–5%) and extreme halophiles (20–30%) [16].

Usually, they can be found in various saline habitats such as salt lakes, salt evaporation ponds, saline soils, mines, food products, etc. [21, 90]. Traditional halophilic sources are salterns, which usually have high salt content, intensive sunlight and low oxygen levels [90–95].

Archaea. Main papers on polysaccharide synthesis by halophilic archaea include research on isolation of new producers [94, 96], EPS structure [97–99], and the possibilities of their practical application [96].

In 1988, Antón et al. [96] established that extremely halophilic archaea *Haloferax mediterranei* ATCC 33500 cultured on a medium with 1% glucose and 25% sea salt produced 3 g/l of sulfated high molecular polysaccharide. Viscosity of EPS solutions was stable in wide ranges of pH, temperature and salinity. Hence EPS of strain ATCC 33500 can be utilized in increasing oil production from wells with high salt content. Later, researchers established the structure of repeating sequences of EPS strain ATCC 33500 [98] and other EPS-synthesizing archaea, in particular *Haloferax gibbonsii* ATCC 33959 [97] and *Haloferax denitrificans* ATCC 35960 [99].

At the end of the twentieth century, for new producers of polyhydroxyalkanoates and EPS, Nicolaus et al. [94] isolated three obligate halophilic strains T5, T6 and T7, which synthesized 35–370 mg/l EPS, from the salt works of Tunisia. The isolates belonged to the genus *Haloarcula*. Among halophilic EPS-synthesizing archaea is strain *Halobacterium volcanii* 1539, which produces 300 mg/l sulfated polysaccharide [100].

There have been no new studies on EPS synthesis by halophilic archaea after that, until a recent

report of EPS-synthesizing archaea *Haloterrigena turkmenica* DSM-5511, isolated from briny soil (Turkmenistan) [101]. The polysaccharide has high emulsifying (emulsification index of sunflower and olive oils are 62.2 and 59.6%, respectively) and antioxidant activity (68.2% neutralization of DPPH· at 10 mg/ml EPS). The EPS also better than hyaluronic acid and sodium alginate retained moisture.

Similar properties were found in certain polysaccharides of cryophilic bacteriae [85, 86] (Table 3). However, the level of target product is too low (at least now) in strain *H. turkmenica* DSM-5511 to consider it a marketable EPS source.

Bacteriae. Polysaccharides of halophilic bacteriae induced scientific interest almost simultaneously with the first reports of EPS synthesis by halophilic archaea. The most studied bacteriae belonged to the genera *Halomonas* [90–92, 95, 102–110], *Idiomarina* [111], *Alteromonas* [111], *Salipiger* [93] and *Halobacillus* [112].

Those bacteriae are moderately halophilic, their optimum salt content is 2.5–13%, usually 7.5% (Table 6). Most of them survive increased salinity (up to 20–25%) [64, 91, 113], and therefore are halotolerant microorganisms.

In early 1990s, reports were published on the synthesis of sulfated polysaccharide (2.8 g/l) by moderately halophilic bacteriae *Volcaniella eurihalina* F2-7 [104, 109] (now *Halomonas eurihalina* [114]).

Soon, wide-scale screening of possibly halophilic producers isolated from solar salterns in Morocco was published [92]. Thirty two isolates of the genus *Halomonas* were selected for a more detailed analysis out of more than 500 isolates. Only four of them accumulated over 2 g/l polysaccharide, and the highest amount (2.8 g/l) was produced by strain S-30. According to phylogenetic analysis, the strain and isolates S-7, S-31^T and S-36 were combined into a new species *Halomonas maura* [115]. Further optimization of the cultivation medium (reducing sea salt concentration, instead adding 2.5% NaCl and 0.05% $MgCl_2 \cdot 6H_2O$) increased EPS production of strain S-30 to 3.8 g/l [103].

Strain *Halomonas xianhensis* SUR308, isolated from soil of a solar saltern (India) [90, 91], on a medium with glucose (1%) and NaCl (10%) produced 2.56 g/l EPS [91]. Further increase of glucose content to 3% and decrease of NaCl to 2.5% was followed by increased EPS production to 7.87 g/l [90]. The polysaccharide was not toxic for Huh7 human hepatocytes. Also, the polymer had high antioxidant activity: the level of neutralization of DPPH· was 72% at 1 mg/ml EPS 72% [91].

Poli et al. [95] reported isolating a moderately halophilic bacteria *Halomonas* sp. AAD6^T from Turkish salterns. Later it was identified as the typical strain of a new species *Halomonas smyrnensis* [113]. It produced levan (a fructose homopolysaccharide). Adding 50 мМ boric acid, 0.8 mg/l thiamine and trace quantities of salts of Mn, Zn, Fe and Cu to the culture medium resulted in a five times increase in levan concentration (up to 8.84 g/l) compared with the initial medium [116].

Further studies aimed to lower the production cost of the target product by using various molasses instead of sucrose in the EPS biosynthesis medium [105]. EPS concentration reached 7.56 g/l (12.4 g/l after 210 hours of cultivation) in culture medium with beet pre-treated with calcium phosphate, sulfate acid and activated carbon. In culture medium with likewise pre-treated starch molasses (a side product of manufacturing dextrose from starchy materials) it was 4.38 g/l. Using starch molasses as a substrate resulted in levan with high emulgent activity [117]. Levan of strain AAD6^T was shown to be useful in targeted delivery of drugs, in particular, of antibiotic vancomicyn [118]. It also increased LD_{50} of avarol from 0.18 ppm to 10 ppm [95]. Anticoagulant activity of artificially sulfated derivatives of that EPS was studied in [119].

Ruiz-Ruiz et al. [110] studied antitumor properties of polysaccharides of halophilic bacteriae *Halomonas stenophila* B100 and N12^T. Artificially sulfated EPS (sEPS) of strains B100 and N12^T (sulfate content 23 and 17%, respectively) efficiently decreased proliferation of T-cells of acute lymphoblast leukemia line Jurkat (500 μg/ml sEPS of strain B100 resulted in 100% inhibition of cell proliferation). Only sEPS of strain B100 induced apoptosis of tumour cells (lines CEM, MOLT-4, HPB-ALL, etc.), while healthy T-cells resisted the apoptosis induction [111]. Authors considered that antitumor effect to directly depend on the concentration of sulfates. It was suggested that sulfates change the charge of polymer molecule to negative and affect its structure, increasing the interaction between EPS and the target cell surface [110].

Bacteriae of the genus *Halomonas* are not only moderately halophilic producers of polysaccharides. A strain isolated from the hypersaline soil of solar saltern (Spain), *Salipiger mucosus* A3^T (sEST 5855^T) cultured

for 72 hours in a medium with 1% glucose and 7.5% sea salt produced 1.35 g/l EPS [93]. Approximately the same amount of EPS (1–1.5 g/l) was obtained from strains *Idiomarina fontislapidosi* F23^T, *Idiomarina ramblicola* R22^T and *Alteromonas hispanica* F32^T isolated similarly from hypersaline habitats [111]. Unlike these bacteriae, strain *Halobacillus trueperi* AJSK produced almost 13 g/l EPS on an optimized medium [112].

Many polysaccharides of moderate halophilic organisms can adsorb cations of various metals [93, 103, 106, 111, 117] (Table 4), emulsify carbohydrates, vegetable and mineral oils [91, 93, 102, 103, 106, 111, 117] (Table 5). Besides that, EPS of *Halomonas*

Table 4. **Adsorption of metal cations by polysaccharides of halophilic bacteriae**

EPS-producing microbe	Adsorption rate, mg/g EPS			References
	Cu^{2+}	Pb^{2+}	Co^{2+}	
Alteromonas hispanica F32^T	6.95	30	4	[111]
Halomonas almeriensis M8^T	19.2	24.5	10	[106]
Halomonas anticariensis FP35^T	26.6	26.3	10.5	[117]
Halomonas anticariensis FP36	28.1	25.15	10.5	[117]
Halomonas maura S-30	4.24	46.4	0.72	[103]
Halomonas ventosae A112^T	12	24.8	2.5	[117]
Halomonas ventosae A116	27.6	25.7	10	[117]
Idiomarina fontislapidosi F23^T	16.3	40	8	[111]
Idiomarina ramblicola R22^T	26.25	44.65	10	[111]
Salipiger mucosus A3^T	15.7	43.5	8.7	[93]

Table 5. **Emulsifying properties of polysaccharides of halophilic bacteriae**

EPS-producing microbe	Emulsifying index,%						References
	Oil			Tetra-decane	Octane	Kero-sene	
	sunflower	olive	mineral				
Alteromonas hispanica F32^T	55	40	50	50	55	67.5	[111]
Halomonas almeriensis M8^T	65	67.5	67.5	62.5	65	65	[106]
Halomonas anticariensis FP35^T	47.5	40	47.5	45	45	–	[117]
Halomonas anticariensis FP36	37.5	42.5	50	55	42.5	–	[117]
Halomonas stenophila HK30	70	85	55.8	41	56.7	80	[102]
Halomonas ventosae A112^T	51	42.8	35.5	57.5	57.5	–	[117]
Halomonas ventosae A116	60	55	62.5	60	60	–	[117]
Halomonas xianhensis SUR308	–	71.3	–	80.3	76.3	–	[91]
Idiomarina fontislapidosi F23^T	65	60	62.5	45	60	55	[111]
Idiomarina ramblicola R22^T	60	65	62.5	55	60	62.5	[111]
Salipiger mucosus A3^T	70	60.3	71	75	70	70	[93]
Control							
Triton X-100	62.5–67.5	60–62.5	60–67.5	62.5–65	60–62.5	60–62.2	
Tween 80	62	61.5–62.5	60–70	60–62.5	60	60	[91, 93, 102, 106, 111, 117]

Table 6. **Synthesis of exopolysaccharides by halophilic and moderately halophilic microbes**

Microbial source	Salt content	Carbon source, g/l	EPS content g/l	Physico-chemical properties of EPS		Physiological effect, functional properties, possible avenues of implementation of EPS	References
				Chemical composition	Molecular mass, kDa		
EPS of halophilic archaea							
Haloarcula sp. T6	NaCl, 200 g/l	Glucose, 6	0.045	Mannose, galactose and glucose (1:0.2:0.2)	–	–	[94]
Haloarcula sp. T7	NaCl, 200 g/l	Glucose, 6	0.035	Mannose, galactose and glucose (1:0.2:0.2)	–	–	[94]
Haloarcula japonica T5	NaCl, 200 g/l	Glucose, 6	0.37	Glucuronic acid, mannose and galactose (3:2:1)	–	–	[94]
Halobacterium volcanii 1539	NaCl, 156 g/l	Galactose, 10	0.3	Mannose. Hexuronic acids present. Sulfates 0.6%	–	–	[100]
Haloferax mediterranei ATCC 33500	Sea salt, 25%	Glucose, 10	3	Mannose, glucose, galactose. Sulfates 6%	>100	Thickener, intensification of oil production	[96]
Haloterrigena turkmenica DSM-5511	NaCl, 200 g/l	Glucose, 10	0.207	Glucose, glucosamine, glucuronic acid, galactose, galactosamine (1:0.65:0.24:0.22:0.02). Sulfates 2.8%	Fractions 1–3: 801.7; 206; 37.6	Emulgent, antioxidant, moisture retention agent	[101]
EPS of moderately halophilic bacteriae							
Alteromonas hispanica F32^T	Sea salt (7.5%)	Galactose, 10	1.25	Mannose, glucose, xylose (1:0.29:0.11). Sulfates 0.25%	19000	Biofilm formation. Emulgent, adsorbent	[111]
Halobacillus trueperi AJSK	NaCl (61.56 g/l)	Glucose, 22,2	12.93	–	–	–	[112]
Halomonas alkaliantarctica CRSS	NaCl (100 g/l)	Sodium citrate, 3	2.9 g EPS/g biomass	Mannose, xylose, glucose, galactosamine, fructose, rhamnose, not indentified component (1:0.7:0.3:0.2:traces:traces:0.3)	–	Thickener	[64, 108]
Halomonas almeriensis M8^T	Sea salt (7.5%)	Glucose, 10	1.7	Fraction 1: mannose, glucose, rhamnose (1:0.38:0.01); Fraction 2: mannose, glucose (1:0.97). Sulfates 1.4%	Fraction 1: 6300; Fraction 2: 15	Emulgent, adsorbent	[106]
Halomonas anticariensis FP35^T	Sea salt (7.5%)	Glucose, 10	345.5 mg/l	Mannose, galacturonic acid, glucose (1:0.82:0.33). Sulfates 0.73%	20	Biofilm formation. Emulgent, adsorbent	[117]
Halomonas anticariensis FP36	Sea salt (7.5%)	Glucose, 10	0.386	Mannose, galacturonic acid, glucose (1:0.87:0.4). Sulfates 1.16%	46	Biofilm formation. Emulgent, adsorbent	[117]
Halomonas eurihalina F2-7	Sea salt (7.5%)	Glucose, 10	2.8	Glucose, mannose, rhamnose (molar ratio 2.9:1.5:1). Sulfates 2.7%	–	Thickener, emulgent, intensification of oil production	[104, 109]

Table 6. End

Microbial source	Salt content	Carbon source, g/l	EPS content g/l	Physico-chemical properties of EPS		Physiological effect, functional properties, possible avenues of implementation of EPS	References
				Chemical composition	Molecular mass, kDa		
Halomonas maura S-30	Sea salt / NaCl (2.5%)	Glucose, 10	3.8	Mannose, galactose, glucose, glucuronic acid (1:0.4:0.84:0.63). Sulfates 6.5%	4700	Emulgent, thickener; adsorbent	[92, 103]
Halomonas smyrnensis AAD6^T	NaCl (137.2 g/l)	Sucrose, 50	1.84–8.84	Fructose (levan)	>1000	Flocculant [118]; targeted drug delivery [119], anticoagulant [120]; anticytotoxic activity	[95, 116]
	NaCl (137.2 g/l)	Processed beet molasses (30 g/l carbohydrates)	12.4	Fructose, glucose (traces)	>1000		[105]
Halomonas stenophila B100	Sea salt (7.5%)	–	–	Glucose, galactose, mannose (1:0.91:0.34). Sulfates 7.9%	375	Antitumor activity	[110]
Halomonas stenophila HK30	Sea salt (5%)	Glucose, 10	3.89	Glucose, glucuronic acid, mannose, fucose, galactose, rhamnose (1:0.3:5.5:0.23:0.19:0.05:0.002)	Fraction 1: 1400; Fraction 2: 82	Biofilm formation. Thickener, emulgent; flocculant	[102]
Halomonas stenophila N12^T	Sea salt (7.5%)	–	–	Glucose, mannose, fucose (1:0.52:0.53). Sulfates 2.45%	250	Antitumor activity	[110]
Halomonas ventosae A112^T	Sea salt (7.5%)	Glucose, 10	283.5 mg/l	Mannose, glucose, galactose (1:0.43:0.25). Sulfates 1.09%	53	Biofilm formation. Emulgent, adsorbent	[117]
Halomonas ventosae A116	Sea salt (7.5%)	Glucose, 10	289.5 mg/l	Mannose, glucose, galactose (1:0.42:0.22). Sulfates 0.71%	52	Biofilm formation. Emulgent, adsorbent	[117]
Halomonas xianhensis SUR308	NaCl (10% / 2.5%)	Glucose, 10 / 30	2.56 / 7.87	Glucose, galactose, mannose, xylose, ribose (1:0.74:0.39:0.04:0.02)	–	Thickener, emulgent, antioxidant	[90, 91]
Idiomarina fontislapidosi F23^T	Sea salt (7.5%)	Glucose, 10	1.45	Fraction 1: mannose, glucose, galactose, xylose (1:0.61:0.32) Fraction 2: mannose, glucose, galactose, xylose (1:1:0.5:traces). Sulfates 0.65%	Fraction 1: 1500; Fraction 2: 15	Biofilm formation, emulgent, adsorbent	[111]
Idiomarina ramblicola R22^T	Sea salt (7.5%)	Glucose, 10	1.5	Fraction 1: mannose, glucose, rhamnose (1:0.37:0.1); Fraction 2: mannose, glucose, galacturonic acid, rhamnose, xylose (1:0.35:0.47: traces: traces). Sulfates 0.5%	Fraction 1: 550; Fraction 2: 20	Biofilm formation. Emulgent, adsorbent	[111]
Salipiger mucosus A3^T	Sea salt (2.5–7.5%)	Glucose, 10	1.35	Mannose, galactose, glucose, fucose (1:0.97:0.58:0.39). Sulfates 0.9%	250	Emulgent, adsorbent	[93]

stenophila HK30 have high flocculant activity: 72.06% EPS in a suspension of kaolinite (0.5%) at 20 mg/l EPS [102].

Table 6 summarizes information on EPS synthesis by halophilic archaea and moderately halophilic bacteriae.

Thus, studies of EPS from non-traditional sources (cryophilic fungi and bacteriae, halo- and thermophilic archaea and bacteriae, including those from deep-sea hydrothermal vents) is a novel field which began to develop rapidly at the end of the twentieth century. Many of those isolated microorganisms produce polysaccharides. The physiological effect, physico-chemical properties and possibilities of industrial application of those EPS are studied. Those substances due to their immunomodulating, antiviral, anticoagulant, antitumor, antioxidant activities can be widely employed, in medicine and pharmacy, etc.

Meanwhile the practical implementation of polysaccharides is limited by the low efficiency of production. Non-traditional sources produce EPS in much lower concentrations than the traditional ones. In our opinion, solving this problem is only a question of time, because various approaches to metabolic and gene engineering for microbial synthesis intensification are already developed [88, 112, 120–122].

EPS biosynthesis by non-traditional sources currently requires expensive carbohydrate materials (glucose, fructose, sucrose, and maltose) (Tables 1–3, 6). At the same time, many new studies aim to substitute carbohydrate substrates with cheap industrial wastes (whey, crude glycerin, oil-containing wastes, and agricultural wastes) in culturing traditional producers of polysaccharides. Those approaches to microbial polysaccharide production are reviewed in [123]. We demonstrated that it is possible to obtain microbial EPS ethapolan using fried vegetable oil [124] and its mixture with molasses [125].

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НЕТРАДИЦІЙНІ ПРОДУЦЕНТИ МІКРОБНИХ ЕКЗОПОЛІСАХАРИДІВ

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Наведено дані літератури щодо синтезу екзополісахаридів психрофільними грибами, гало- і термофільними археями та бактеріями, зокрема й виділеними з глибоководних гідротермальних вентів — джерел. Проаналізовано фізіологічну роль, фізико-хімічні властивості та можливі галузі практичного використання екзополісахаридів, синтезованих нетрадиційними продуцентами. Більшості з них притаманна імуномодулювальна, противірусна, антикоагулянтна, протипухлинна, антиоксидантна активність, що робить їх перспективними для застосовування у медицині та фармацевтиці.

Водночас аналіз літератури засвідчив необхідність розроблення ефективних технологій одержання таких полісахаридів, оскільки показники їх синтезу нетрадиційними продуцентами є значно нижчими порівняно з традиційними.

Ключові слова: екзополісахариди, термофіли, психрофіли, галофіли, гідротермальні венти.

НЕТРАДИЦИОННЫЕ ПРОДУЦЕНТЫ МИКРОБНЫХ ЭКЗОПОЛИСАХАРИДОВ

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Представлены данные литературы о синтезе экзополисахаридов психрофильными грибами, гало- и термофильными археями и бактериями, в частности выделенными с глубоководных гидротермальных вентов — источников. Проанализированы физиологическая роль, физико-химические свойства и возможные отрасли практического использования экзополисахаридов, синтезированных нетрадиционными продуцентами. Большинство из них обладает иммуностимулирующей, противовирусной, антикоагулянтной, противоопухолевой, антиоксидантной активностью, что делает их перспективными для применения в медицине и фармацевтике.

В то же время анализ литературы показал необходимость разработки эффективных технологий получения таких полисахаридов, поскольку показатели их синтеза нетрадиционными продуцентами значительно ниже по сравнению с традиционными.

Ключевые слова: экзополисахариды, термофилы, психрофилы, галофилы, гидротермальные венты.