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Review

Exopolysaccharides Synthesized by Thermophilic Microorganisms Isolated from Bulgarian Hot Springs

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Abstract

High temperature in thermophilic niches determines unusual metabolic processes based on an ability of thermophilic microorganisms to synthesize unusual compounds. One of the strategies for active growth of this type of extremophiles is a synthesis of exopolysaccharides (EPSs). EPSs from thermophiles are characterized by unknown before composition of their molecules and correspondingly unusual physic-chemical properties. Several thermophilic EPS producers were isolated from Bulgarian hot springs and three of them belonging to different genera were chosen for further work, namely *Geobacillus tepidamans* V264, *Aeribacillus pallidus* 418, and *Brevibacillus thermoruber* 423. Cultivation medium and conditions were optimized for each EPS producer. Influence of agitation and aeration rates on the bacterial growth and polymer synthesis were investigated in laboratory fermentors for some of them. Biosynthesis in continuous cultures revealed low increase of the yield and higher degree of purity of the EPS synthesized by *A. pallidus* 418. Productivity of one of the strains, *B. thermoruber* 423 was comparable with that of mesophilic bacteria. Investigations of their molecules revealed unknown before composition, high molecular weight, and thermostability. Physico-chemical analyses of their properties revealed an unique rheology like viscous solutions at low concentrations, good emulsifying properties, stability of emulsions, and good synergism with other hydrocolloids. EPS from *G. tepidamans* V264 expressed biological activity against cytotoxic compounds, while that from *B. thermoruber* 423 showed good compatibility with monkey fibroblast cell lines. The demonstrated functional properties and biological activities determine their possible future applications in cosmetics and medicine.

Keywords: thermophilic bacteria, bacterial exopolysaccharides, polysaccharide production, EPS composition; biotechnologically potential

Резюме

Високата температура, характерна за термофилни ниши определя необичайните метаболитни процеси основани на способността на термофилните микроорганизми да синтезират необичайни съединения. Една от стратегиите за активен растеж на този тип екстремофили е синтезата на екзополизахариди (ЕПЗ). ЕПЗ от термофилите се характеризират с непознат преди състав на техните молекули и съответно с необичайни физико-химични свойства. Няколко термофилни продуцента бяха изолирани от български горещи извори и три от тях, принадлежащи към различни родове, бяха избрани за по-нататъшна работа, а именно *Geobacillus tepidamans* V264, *Aeribacillus pallidus* 418 и *Brevibacillus thermoruber* 423. Средите и условията за култивиране бяха оптимизирани за всеки ЕПЗ продуцент. Влиянието на скоростите на разбъркване и аериране върху бактериалния растеж и синтеза на полимери бяха изследвани в лабораторни ферментатори. Биосинтезата в непрекъснати

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култури показа слабо повишаване на добива и по-висока степен на чистота на ЕПЗ, синтезирани от *A. pallidus* 418. ЕПЗ добив за един от щамовете, *B. thermoruber* 423, е сравним с този от мезофилните бактерии. Изследванията на техните молекули показа непознат преди състав, високо молекулно тегло и термостабилност. Физикохимичните анализи на техните свойства разкриват уникална реология като вискозни разтвори при ниски концентрации, добри емулгиращи свойства, стабилност на емулсиите, добър синергизъм с други хидроколоиди. ЕПЗ от *G. tepidamans* V264 демонстрира биологична активност срещу цитотоксични съединения, докато този от *B. thermoruber* 423 показа добра съвместимост с фибробластни клетъчни линии от маймуни. Установените функционални свойства и биологична активност определят възможността за тяхното бъдещо приложение в козметиката и медицината.

Introduction

Microbial exopolysaccharides (EPS) a comparatively new class of microbial products with high-molecular-weight polymers composed of sugar residues. The interest toward them is determined by their natural, origin, non-toxicity, biocompatibility, biodegradability, and wide variety of their properties as a result of the variety in their composition. Microbial EPS have been used as stabilizers, thickeners, gelling agents, coagulants, emulsifiers, stabilizers in multiphase solutions, lubricants, flocculants or flavor enhancers in many industrial sectors like food, brewing, cosmetics, textiles, pharmacology, detergents, oil recovery, wastewater treatment (Schiraldi *et al.,* 2006). Although firstly reported in the 1880s (Whitfield, 1998), they appeared on the market comparatively recently, about three decades ago. Some of them, such as xanthan, dextran, alginate, gellan and curdlan, are already produced on an industrial scale. Among them xanthan and gellan are commercially most appreciated on the global market for hydrocolloids.

The EPSs exploration partially covers the deficiency of plant polysaccharides and prevents some destruction of perennial plants. They suggest variety of advantages in comparison with plant polysaccharides: their production is a matter of days while plants life cycles last months or years, being the production cycle usually seasonal; industrial wastes/byproducts such as glycerol, whey, molasses can be used as carbon substrates hydrocarbon residues; they can be produced under controlled conditions with consistent and reproducible yield and with a possibility for control of product composition (Kambourova *et al*., 2015). The major difference between bacterial and plant polysaccharides is the lack of significant branching in microbial polymers as well as their predominantly heteropolysaccharide nature (Sutherland, 1997). The number of different chemical structures of bacterial exopolysaccharides is very high and in general they consist in three to seven different monosaccharides often substituted with acetate, pyruvate, succinate, sulfates and phosphates. The monosaccharides frequently found in EPSs are hexoses, pentoses, uronic acids and amino sugars. Some EPSs are neutral macromolecules but most of them are polyanionic due to the incorporation of the substituents. By examining the chemical characteristics of these carbohydrate polymers, it is possible to understand their environmental role and to reveal their commercial future (Maugeri *et al.*, 2002; Poli *et al.,* 2004; Mancuso-Nichols *et al.,* 2005).

EPS producers have been found in both microbial kingdoms, Bacteria and Archaea. The majority of them are mesophilic microorganisms some of them being pathogenic. Isolation of non-pathogenic producers would allow their application in the food industry to be expanded and new biotechnological processes developed in the field of drug production, medical diagnosis, new biodegradable plastics. In recent years, there has been an increasing demand for an isolation of new microbial polysaccharides with properties superior to those of the existing polymers like higher viscosity for lower polymer concentrations and greater stability to a wide ranges of temperature, pH, solvents and ionic strength, higher emulsifying and flocculating activities; biological activity. Much hope for identification of novel polymers is oriented to isolation of new EPS producers.

Extremophiles are between the less known microorganisms and the number of the reports for EPS produced by them is insignificant in comparison with mesophiles. These microorganisms have developed unique metabolic and physiological capabilities to thrive in extreme habitats and produce novel metabolites which are not often present in microbes of modest environments and which represent a vast natural resource of commercial grade products (Rozanov *et al.,* 2014) one of which is EPS biosynthesis. Traditionally, extremophilic microorganisms offer products that are not pathogen-

ic, making them suitable for use in food, pharmaceuticals and cosmetics industry.

Thermophiles are a type of extremophiles able to grow at temperatures between 45 and 122°C (Takai *et al*., 2008). Thermophilic processes imply a number of advantages in EPS production such as short fermentation processes (lasting from several hours to several days); reduced viscosity of the culture fluid; reduced risk of contamination in thermophilic processes. EPSs of thermophilic origin have unique rheological properties because of their capability of forming very viscous solutions at low concentrations and their pseudoplastic nature, they have high molecular weight, stability of their molecules, good synergism with other hydrocolloids, biological activity against cytotoxic compounds, antiviral and immunostimulating activities that determine their possible potential future applications. Comparatively low levels of EPS synthesis by thermophilic microorganisms and correspondingly high production cost determine the interest for the development of microbial EPSs related to high-value market niches, where traditional polymers fail to comply with the required degree of purity or lack some specific functional properties (Kumar *et al.,* 2007).

Bulgaria is a country rich in thermal springs with different geotectonic origin and correspondingly different mineral composition, temperature and pH of water suggesting variety of isolates. Scientists from the Laboratory of Extremophilic Bacteria, Institute of Microbiology, Bulgarian Academy of Sciences were able to isolate perspective thermophilic producers of EPSs with interesting properties and biotechnological potential.

Optimization of the fermentation conditions for EPS synthesis

Among the tested about 400 strains isolated from water, soil and algobacterial mat samples from different hot springs in Bulgaria twelve were chosen as exopolysaccharide producers due to mucoid consistence of their colonies. The strain V264 was isolated from Mizinka hot spring, Velingrad, Central Bulgaria and referred to the species *Geobacillus tepidamans* based on 16SrRNA gene sequence analysis (Kambourova *et al*., 2009). The strain grew in pH range between 6-9 with an optimum at pH 7.0 and in temperature range between 40-65°C with an optimum at 60°C. Optimal conditions for EPS synthesis were established to be the same as those for growth. Another thermophilic EPS producer, *Aeribacillus pallidus* 418 was isolated from a hot spring located at Rupi basin, South-West Bulgaria and represented the first EPS producer from this genus (Radchenkova *et al.,* 2013). The strain was strict aerobe growing in the area 35-72°C, highest polymer production was observed at 55°C and pH 7.0. *Brevibacillus thermoruber* strain 423 was isolated from a hot spring close to the village Gradechnitsa, Blagoevgrad region, South-West Bulgaria (Yasar Yildiz *et al.,* 2014). It grew in the range 34-58°C; optimal temperature for EPS synthesis was 55°C and pH 6.5.

Maltose and $(NH_4)_2HPO_4$ were chosen as best carbon and nitrogen sources for EPS production by *G. tepidamans* V264 in corresponding concentrations of 30 g/l and 3 g/l. The highest EPS production by *A. pallidus* 418 was registered in a medium containing maltose as a carbon source and NH_4Cl as a nitrogen source in a relative ratio 4.5:1 (w/w). Utilization of inorganic nitrogen source for EPS production by our isolates is an important advantage for further industrial design of cost effective medium. Highest EPS production levels by *B. thermoruber* 423 were reached in the presence of maltose and peptone in ratio 18:1. The enhanced production in abundance of carbon source and minimal nitrogen source reaching values up to 18:1 is typical for EPS production processes (Banik *et al.,* 2000). EPS synthesis was growth associated for the investigated strains - it began in the early exponential phase and continued during the early stationary phase.

EPS synthesis by *G. tepidamans* V264 followed in a bioreactor (Bioflo, New Brunswick, Co Inc., USA) with working volume of 0.35 l showed the highest yield at 300 rpm - 111.4 mg/l (106.6 mg/l at 200 rpm, 60.5 mg/l at 400 rpm and 58.0 mg/l at 100 rpm). Time course of growth and EPS production at 300 rpm are presented in Fig.1. The polysaccharide synthesis begun in the middle of the exponential phase of growth and maximum quantity was reached at stationary phase after only 8 h of cultivation.

Similar short lasting processes were observed for *A. pallidus* 418 (18 h) and *B. thermoruber* 423 (12 h). Despite the commonly observed lower EPS yield short fermentation process is animportant advantage of thermophilic synthesis in comparison with mesophilic production lasting several days. Thermophilic producers of the species *Bacillus thermoantarcticus*, *B. licheniformis* and *G. thermodenitrificans* were isolated from shallow water springs (Manca *et al*., 1996; Nicolaus *et al.,* 2002). Although EPS production is less than that reported for *B. thermoantarcticus* (Manca *et al*., 1996),

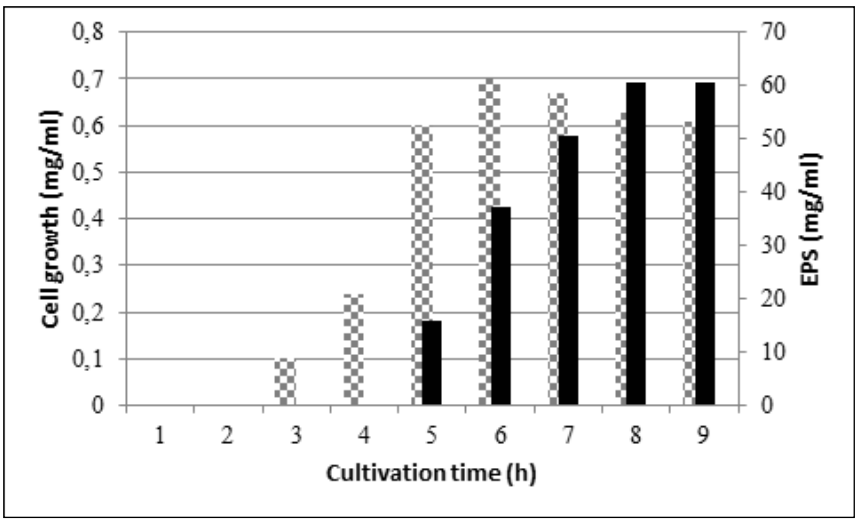

Fig. 1. Time course of growth and EPS production by *G. tepidamans* V264. ▩, growth; ■, EPS synthesis.

the unusual short fermentation processes referred the strains as perspective EPS producers. The established production although low in comparison with industrial exploited microbial producers, is not less than reported for other thermophilic producers. EPS production in the range 55-165 μg/ml was reported for flask cultures of thermophilic producers (Nicolaus *et al*., 2003) and higher for *B. thermoantarcticus* (400 mg/l max production). EPS concentration for *B. thermoruber* 423 in fermenter showed highest EPS (897 mg/l) production at early stationary phase. The bioreactor cultures were found to reach two times higher yields and three times higher productivities when compared with other thermophilic producers and comparable with these from mesophiles (Yasar Yildiz *et al*., 2014). Essential genes associated with EPS biosynthesis were detected by genome annotation, and together with experimental evidences, a hypothetical mechanism for EPS biosynthesis was generated (Yasar Yildiz *et al*., 2013; 2015). The performed analysis of the full genome of *B. thermoruber* strain 423 is the first genome analysis for a thermophilic *Brevibacillus* species.

Oxygen transfer is often the rate-limiting step in the aerobic bioprocess due to its low solubility in the medium especially at enhanced temperatures of thermophilic fermentations. Optimization of agitation and aeration conditions in thermophilic processes for EPS production has not been reported before our investigations. The strong influence of agitation on EPS production and strain growth was clearly demonstrated by comparing the results obtained in 24 h fermentation runs of *A. pallidus* 418 at different agitation speeds of 100, 200, 600, 900 and 1100 rpm (revolutions per minute) and aeration rate of 0.5 vvm (Radchenkova *et al*., 2014). An improvement in EPS yield was observed when agitation rate increased from 100 to 900 rpm. Referring to the lowest levels of agitation (100 and 200 rpm), the polymer concentration and bacterial growth sharply differed from other results, i.e. EPS production at these rates was insignificant (Fig. 2A) and growth was respectively eight and four fold less than the ones observed at 900 rpm. Further increase in agitation speed contributed to greater growth and better EPS production by *A. pallidus* 418 as a result of the enhanced amount of dissolved oxygen and dispersion of macromolecules in the medium. Dissolved oxygen concentration was found to have a significant effect on growth because noticeable changes in biomass and EPS formation were observed. The processes were under oxygen limitation at four of the tested five agitation rates (100, 200, 600 and 900 rpm). Oxygen deficiency stopped culture development at low agitation (100 and 200 rpm). Although an oxygen limitation was also observed at moderate agitation (for 10 h at 600 rpm and 2 h at 900 rpm) the dissolved oxygen was enough for good growth and its deficiency stimulated EPS production. Maximum EPS yield of 170 μg/ml was registered in a single impeller mono-agitator system type Narcissus at agitation speed 900 rpm and aeration rate 0.5 vvm. Evidently, the limitation provoked polymer production as a mechanism for cell adaptation to unfavorable conditions (Nicolaus *et al*., 2010).

Investigation of the influence of the agitation speed on exopolysaccharide production at four aeration rates, namely, 0.25 vvm (0.6 l/min), 0.5 vvm (1.2 l/min), 1 vvm (2.4 l/min), and 1.5 vvm (3.6 l/min) showed a variation between 150 and 170

μg/ml (Fig. 2B). Comparatively small differences in growth and exopolysaccharide production at the four aeration rates suggested that the effect of aeration on polymer production was more weakly expressed in comparison with the influence of agitation. Similar relationship has been found by other authors (Borges *et al*., 2008) reporting for strong enhance of EPS production level with increase of agitation speed and weak effect of increased air flow rate. Oxygen limitation was observed at 0.25 vvm (for 7 h), and 0.5 vvm (for 2 h). Similarly to runs with different agitation, the process with shortest oxygen limitation provided the highest EPS production.

The advantages of continuously growing aerobic thermophiles are connected with determined our interest toward development of continuous processes for EPS production. Such advantage is connected with elimination of the inherent down time for cleaning and sterilization of the vessel and the comparatively long lag phases before the organisms enter a brief period of high productivity. Additional advantage is sharply decreased viscosity at high temperature. Although continuous cultivation of several mesophilic bacteria for EPS production has been known (Mende *et al.,* 2012; Gogola-Kolling *et al.* 2014) investigations of EPS synthesis in continuous thermophilic cultures have not been reported before our work. Development of a constant system for EPS production by keeping of the thermophilic culture in a steady state ensure higher levels of EPS production than that in batch culture escaping the need in reactor handling after each cycle. The synthesized polymer did not increased viscosity of the culture liquid and correspondingly did not hinder mass transfer due to the high temperature of the

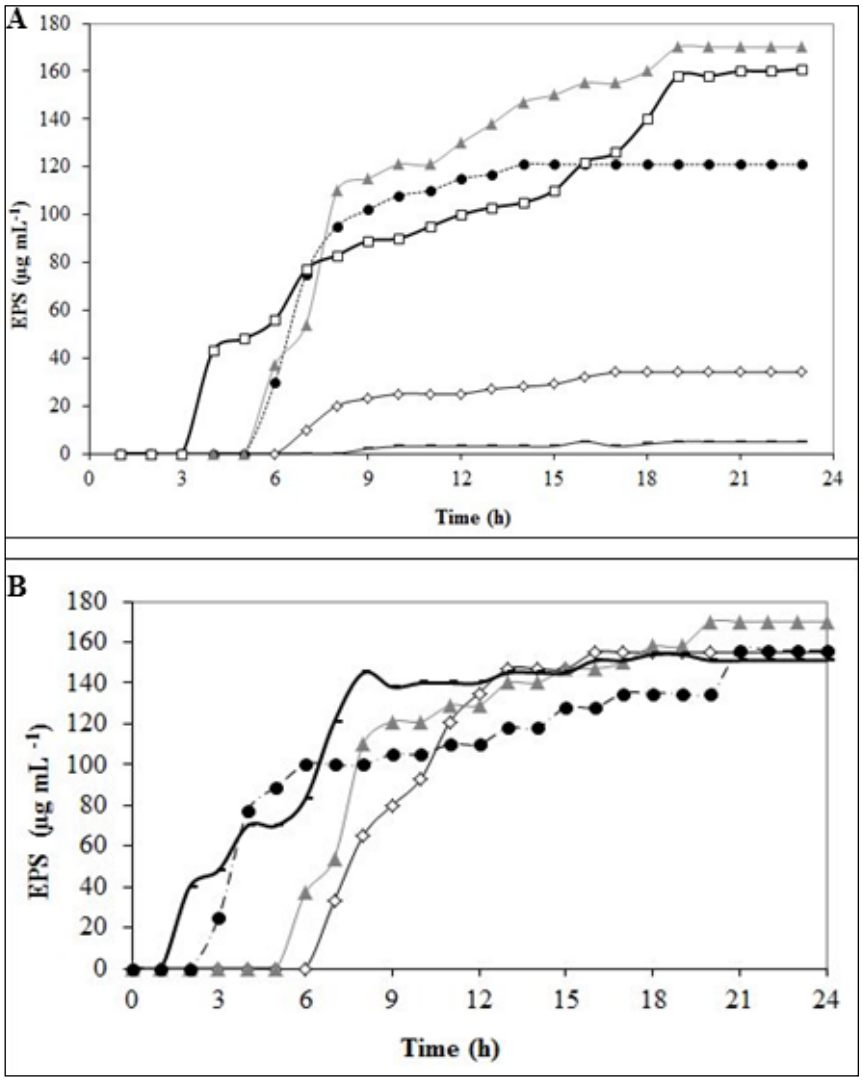

Fig. 2. Effect of different agitation (A) and aeration (B) rates on EPS production during the cultivation of *A. pallidus* 418 in a stirred-tank fermenter. Symbols: (▬) 100 rpm; (◊) 200 rpm; (●) 600 rpm; (▲) 900 rpm; (□) 1100 rpm.

Table 1. EPS production by *Aeribacillus pallidus* 418 in batch and continuous cultures

Type of cultivation	Dilution rate (D)	Maximum biomass (mg/ml)	EPS (μg/ml)	Specific production (EPS/X)	Productivity - biomass (X.D)	Productivity - product (EPS.D)
Batch	-	1.40	160	114.0	-	-
Continuous	0.06	1.30	170	130.8	0.078	10.0
	0.1	0.76	131	172.4	0.076	13.1
	0.2	0.57	91	159.6	0.114	18.2

process in which polysaccharide solubility significantly increased. The highest quantity of the EPS synthesized by *A. pallidus* 418 (180 μg/ml) was established at low dilution rates (0.06), however the highest productivity was established at high dilution rate of 0.2 (Table 1).

Lower degree of substrate utilization determined the fermentation process at higher dilution rate economically unfavorable. Although the received value for EPS (180 μg/ml) is not significantly higher than that measured in batch culture (170 μg/ml), the ability to use the advantages of continuous cultivation suggest a good possibility for large scale production of EPS by *A. pallidus* 418 in continuous cultivation process. The deal of the concomitant protein decreased with more than 15% after cultivation of *A. pallidus* 418 in continuous cultures in comparison with batch cultures as EPS represented 80.5% in the pellet collected after batch cultivation while it was 95.3 and 97% at D = 0.06 and 0.2 respectively, after continuous cultivation. The suggested approach facilitates the process of purification as content of the contaminating polymers significantly decreases due to keeping of culture in exponentially phase and correspondingly escaping of cell lysis and accompaning release of cell proteins and nucleic acids. The system showed high operation stability - the level of the synthesized EPS did not changed after 8 days and slightly decreased after 10 days.

Monosaccharide composition and properties of EPSs molecules

The chemical-physical analyses of EPS from *G. tepidamans* V 264 revealed 98% carbohydrate and low presence of protein (1.8%) and nucleic acid (0.9%). Presence of uronic acid (0.2%) was also registered. Analysis of the ethanol precipitate by *A. pallidus* 418 showed a presence of carbohydrates (90.5%), protein (9%) and nucleic acids (0.5%). Elution profile after Sepharose DEAE CL-6B purification revealed a presence of two polysaccharides in the polymer fraction, one of them (EPS 1) was eluted with water and another one was negatively charged and eluted at 0.4 M NaCl (EPS 2) (Fig. 2). The ratio between both EPSs was 3:2.2.

Both were heteropolysaccharides composed mainly of mannose. According to Sutherland (1999) microbial EPSs are rich in hexoses like glucose and galactose whereas relatively high content of rhamnose, mannose and xylose are typical for phytoplankton (Hoagland *et al*., 1993). However, concerning thermophilic EPSs, mannose seems to be a frequent component of the molecule together with glucose and galactose. EPS synthesized by *G. tepidamans* V 264 is glucan, in which glucose represented 88% of its content; galactose, fucose and fructose represented together about 12% (Kambourova *et al*., 2009). Similarly, glucose was more than 90% in the polymer produced from *Thermotoga maritima*, however additional sugars were ribose and mannose (Vanfossen *et al*., 2008). EPSs isolated from two *Geobacillus* sp. strains contained glucose, galactose and mannose in different proportions and the third strain from the same genus contained glucosamine and arabinose together with galactose and mannose (Nicolaus *et al*., 2003). GC-MS analysis of EPS fraction from *A. pallidus* 418 revealed that EPS 1 consists of mannose/trehalose/galactosamine/glucosamine/galactose/glucose/ribose (69.3/7.8/6.3/5.4/4.7/3.4/2.9). EPS 2 was constituted by similar sugars, however in different proportions: manose/galactose/glucose/galactosamine/glucosamine/ribose/arabinose (33.9/17.9/15.5/11.7/8.1/5.3/4.9). Such high number monosaccharide components haven't been reported previously. The presence of uronic acids was registered in the molecule of EPSs, 1.9% for EPS 1 and 6.5% for EPS 2. Uronic acids are desirable for cosmetics as they are good hydrating agents. The increased content of uronic acids in EPS 2 is in a coincidence with its anionic nature. Chemical characterization of EPS from *B. thermoruber* strain 423 by TLC, GC-MS, FT-

IR and NMR suggested a heteropolymer structure with the follow sugar composition (%): glucose/galactose/mannose/galactosamine/mannosamine (57.7/16.3/9.2/14.2/2.4).

Although the molecular mass of the most microbial EPSs varied in the range 10 to 30 kDa (Bhaskar and Bholse, 2005) EPSs from thermophiles are characterized by high molecular mass. Molecular mass of exo-polysaccharide from *G. tepidamans* V 264 was higher than $1x10^6$ Da. Both molecules synthesized by *A. pallidus* 418 displayed high molecular mass with values of 700 kDa for EPS1 and over 1000 kDa for EPS 2. The observed high molecular masses were among the highest reported for microbial EPS and could offer high viscosity in industrial products by comparatively low quantity of the polymer in them. A molecular mass higher than 300 kDa was reported for EPSs from thermophilic bacilli (Nicolaus *et al.*, 2003). Another advantage of thermophilic EPSs is the rigidity of their molecules proved by its decomposition at unusually high temperature that facilitates its handling and storing. Thermogravimetrical analysis of EPS from *G. tepidamans* V 264 revealed that the bio-polymer was very stable and started to decompose at about 280°C. Transition maximum of the decomposition process for EPSs from *A. pallidus* 418 was observed at 176°C for EPS 1 and 226°C for EPS 2. Stability of polymer molecules seems to be a common property for thermophilic EPS (Arena *et al*., 2006; Spanò *et al*., 2013).

Physical properties determining their industrial potential

Exploration of EPSs as emulsifying and stabilizing agents is one of their most perspective applications. Investigation of the emulsifying properties of EPS from *A. pallidus* 418 showed its ability to form stable emulsions with oil (Radchenkova *et al*., 2015). Stable oil-in water emulsions, low level of separated water phase and high dispersion stability were established for all EPS concentrations tested (Table 2). The synthesized polymer showed a good lipophylic effect due to its adsorption on the boundary phase oil/water of the emulsion system. The fact that the added oil was firmly connected in the emulsion and an oil phase was not separated is an important property of this EPS preventing oil oxidation and unpleasant smell in future cosmetic products. Like other EPSs (Yun and Park, 2003; Denev *et al.,* 2005; Pavlova *et al.,* 2011) hydrophilic nature is more weakly presented and quantity of the separated water decreased from 42 to 26% with increasing the quantity of the used polymer. Light penetration of the emulsions (T) varied between 82 and 61% reflecting some instability of the dispersion system at lower EPS concentration. Unlike other two EPSs this from *B. thermoruber* 423 synthesized EPS with characteristics of a typical Newtonian fluid and its shear stress increased linearly with increasing shear rate.

Due to variety of polysaccharide properties and their ability to show a synergistic mechanism of action, a combination of two or more of them is often applicable in trade products. The hydrocolloids used could influence in different ways the interaction in the boundary oil/water phase based on their difference in monosaccharide composition, structural conformation of the chain, and molecular weight. The effective synergism between different EPS was reported by several authors (Shatwell *et al.,* 1991; Kang and Pettitt, 1993). The results from the influence of four plant and microbial hydrocolloids largely used in food and cosmetic industries, on the emulsion received with EPS from *A. pallidus* 418 are presented in Table 2. Enhanced synergism

Table 2. Stability of oil/water emulsions (1:1) received with 0.5% commercial polysaccharides separately and in combination with 1% EPS synthesised by *A. pallidus* 418

Polymer concentration (% in water medium)	Separated phase,%		
	Oil	Water	Emulsion
guar gum	50	0	50
EPS + guar gum	5	0	95
cellulose gum	23	33	44
EPS + cellulose gum	trace	40	60
xanthan gum	58	38	4
EPS + xanthan gum	2	0	98
Na-alginate	3	45	55
EPS + Na-alginate	trace	40	60

between the *A. pallidus* 418 synthesized EPS and various commercially used hydrocolloids were observed, superiority being achieved by xanthan gum. The results obtained clearly demonstrated the synergism between the EPS synthesized by *A. pallidus* 418 and commercially used hydrocolloids.

Biological activity

The exopolysaccharide synthesized by *G. tepidamans* V264 showed a strong inhibition of cytotoxic effect produced by avarol in brine shrimp bioassay, which gives results that correlate well with cytotoxicity in cancer cell lines such as KB, P388, L5178y and L1210 (Crispino *et al*., 1989). Avarol shows strong toxicity (LD_{50} 0.18 g/ml) (Minale *et al*., 1974) The bio-polymer isolated from *G. tepidamans* V264 was found to increase the value of LD50 of this compound more than twelvefold from 0.18 g/ml up to 2.24 g/ml. A possible reason for its action as anti-cytotoxic agent is the adhesion of toxic compounds to the surface of the polysaccharide. EPSs synthesized by thermophilic bacilli exhibit interesting cytotoxic, antiviral, anti-inflammatory and immunoregulatory properties (Arena *et al*., 2006; 2009). Unlike other agents with anti-cytotoxic activity, polysaccharides are much more neutral to normal metabolism of living organisms and their use in pharmacy could suggest significant priority in development of novel class of anti-cytotoxic drugs.

Considering the fact that biocompatibility is one of key factors for health-related applications purified EPS from *B. thermoruber* 423 was subjected to viability tests using monkey kidney fibroblast cell line Cos-7. The viability of cells increased in the large EPS concentration area after incubation for 24 h and only in the highest concentration of 500 μg/ml insignificantly decreased.

Fast productivity, high level of polymer synthesis, high biocompatibility of pure EPS fractions and the advantages of its nonpathogenicity suggest potential use of this EPS in biomedical applications as scaffolds or matrices in tissue engineering, drug delivery and wound dressing. Some polysaccharides have the ability to scavenge free radicals, induce differentiation of cancer cells and enhance animal or human antitumor ability via the activation of various immune responses in the host (Liu *et al*., 2010).

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