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Results of the Study of Mutagenic Effects of Microbial Polysaccharides

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Abstract

The article presents the results of a study of mutagenic effects of *Pseudomonas alcaligenes* polysaccharides. Pseudomonas genus – non-fermentative ubiquitous bacteria, having specific metabolic cycles and unique physical, chemical and biological properties was used as a producer of natural exopolysaccharides. In an experiment using the Ames test, three variants of test compounds were studied: 1. a compound of the *Pseudomonas alcaligenes* biofilm, 2. exopolysaccharide matrix and the microorganism cell wall compound, and 3. actually the microbial exopolysaccharide. In all cases the lack of mutagen action of polysaccharides of *Pseudomonas alcaligenes* is proved that make them perspective for use as nanomaterials of new generation – alternative wound coverings.

Keywords: *Pseudomonas alcaligenes*, polysaccharides, exopolysaccharides, Ames test, mutagenic effect, wound coverings.

1. Introduction

The majority of microbic polysaccharides have the unique specific structure or serological group and, as a rule, represent mix of molecules of different molecular weight, but of an identical chemical structure (Belyaev, 2000). They are divided on intracellular, localized in cytoplasm, and extracellular – an extracellular layer, capsules or covers. Free hydroxyl groups of exopolysaccharides (EPS) are acylated and alkylated, glycosidic bonds can be hydrolyzed by acids and specific enzymes (Arkadieva et el., 1989). Besides, in microbic glycans earlier unknown monosaccharides which do not occur neither in animals, nor in plants often are found (Malashenko et al., 2001; Kumar et al., 2007). Unique chemical, biological and rheological properties of microbial EPS long ago attracted attention of experts in various branches of science and engineering as the promising biologically active compounds important for the solution of a number of practical and theoretical tasks in the field of biomedicine and biotechnology. Following companies are considered to be full-fledged leaders in their study and production: «Rhone Poulenc» (France), «Statoil» (Norway), «Kelco» (USA). Through their activity the annual increase in production of polysaccharides of microbial origin in the world is on average 10 % (Sutherland, 2009). Microbial EPS are widely used in the oil and mining industries, in paints, in medicine - as a matrix for tissue engineering, producing surgical and non-woven materials, elements for osteosynthesis, drug delivery systems, the preparation of food technology and more (Greenberg,

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1991; Obraztcova, Sidorova, 2014; Perok et al., 2002). At the Microbiology Department, Institute of Medicine of Petrozavodsk State University the possibility of using *Pseudomonas alcaligenes* exopolysaccharide to develop measures to prevent infection of primary and secondary tension wounds was successfully investigated. For this physicochemical and biological properties of pseudomonads exopolysaccharides were studied (Voronov, Sidorova 2015). Opposing activity of lytic enzymes and microbial cellular wall components was investigated (Sidorova, Komkova, 2015). In *Pseudomonas alcaligens* hydrolysates 5 compounds of monosaccharide structure were revealed: galactose, arabinose, xylose, rhamnose, galacturonic acid (GA); in the structure of core oligosaccharides, except rhamnose, α-alanine is revealed, being typical aliphatic amino acid. Thanks to joint cultivation of *Pseudomonas alcaligens* with laboratory cultures of *Staphylococcus aureus* and *Escherichia coli* data on antagonistic properties of polysaccharides are obtained. By means of methods of direct antagonism it is established that variability on percentage suppression of test cultures determined by antibiotic effects of EPS. The listed biological properties of exopolysaccharides of pseudomonads do them perspective for development of an alternative wound covering capable not only to restore structure of a tissue, but also to use the antimicrobial potential of microbial polymer for prevention of infections and associated with them complications of various genesis. In the context of the above, the purpose of the present work was to study the mutagenic effect of *Pseudomonas alcaligenes* exopolysaccharide.

2. Materials and methods

Mutagenic activity of biological products based on *P. alcaligenes* metabolites was investigated according to the Ames test, described in the «Manual on experimental (preclinical) study of new pharmacological substances» (Khabriev, 2005) and recommended by Pharmacological State Committee of the Russian Ministry of Health for testing products at the stage of pre-clinical toxicological study. The test is used to evaluate the mutagenic activity of drugs on the basis of *Pseudomonas* and consists of two components: the registering and activating. The recording part is a set of indicator strains of *Salmonella typhimurium*, w~~hose~~hich characteristics are given in Table 1.

Table 1. Characteristics of indicator strains of *Salmonella typhimurium*

Strains	Mutations			Plasmid pKM 101	Type of registered mutations
	histidine auxotrophy	rfa	uvrB		
TA 100	G-46	+	+	+	Bases replacement
TA 98	D-3552	+	+	+	Frameshift

Indicator strains are able to record the action of mutagens that cause a replacement of base pairs in DNA molecule (TA 100), and frameshift mutations (TA 98). Under the mutagen action his gene is able to revert to the wild type, and the appearing revertant bacteria become histidine prototrophs. For the analysis of the obtained results mutagenic index (MI) is used, which is calculated as the ratio of the number of colonies of *Salmonella* in the experiment in relation to the control cultures grown in the presence of a mutagen.

To increase mutants sensitivity to mutagens action in indicator bacteria genome additional mutations are introduced which allow to obtain widely the used strains. Deletion of galbiouvrB captures biotin operon, the part of the galactose operon and uvrB gene. Last defect causes a disturbance of the excision repair system, which further enhances the sensitivity of the test strains to the action of a number of mutagens. Rfa mutation increases the permeability of the cell wall due to defects in the polysaccharide layer. The strains TA 100 and TA 98 carry pKM 101 plasmid, through which these strains are more sensitive to the action of a number of substances than the original plasmidless strains. The activating part consists of postmitochondrial supernatant of rat liver homogenate (S-9 fraction) and cofactors that are used for the normal functioning of microsomal oxidation systems. For forming activation mixture usually C-9 fraction is taken, NADP and glucose 6-phosphate. The latter are NADPH-generating system: NADPH serves as an electron donor, which is transferred to the cytochrome P-450. Rat liver S9 was used, which had been previously pretreated with inducers of microsomes. As inductor is used phenobarbital, 3-methylcholanthrene, polychlorinated biphenyls, Aroclor 1254 or sovol. To determine the mutagenic activity of the drug

into the molten semisolid starvation agar at 43–45°C 0,1 ml of the *Salmonella typhimurium* (2×10^8 cells) suspension is put in, 0.1 ml and 0.5 ml of a biological product activation mixture, after it the tube contents is rapidly mixed and layered on the lower selective agar. Plates are incubated at 37°C for 48 hours, the number of colonies are counted – histidine auxotrophy revertants to prototrophy. If a substance exhibits mutagenic activity, the number of revertants in the experimental plates exceeds the number of revertants in the control. During the experiment formulation positive and negative controls were considered. As a negative control, sterile water was used in an amount of 0.5 ml and 0.1 ml of an overnight culture of *Salmonella typhimurium* TA 100 with the addition of 0,1 ml of molten agar and histidine. For negative control spontaneous mutation in bacterial strains was used – separately for each version of the experiment. As positive control was used 0,1 ml of sodium azide, which was added to 0,1 ml of an overnight culture of *Salmonella typhimurium* TA 100 with 0,1 ml of histidine and molten agar solution. The experiment was performed times. The experimental results are accounted in accordance with the guidelines. As a positive, the result was considered, when there was an increase in the frequency of mutations in more than 2 times compared to the control.

3. Results and discussion

There were three variants of biological specimens used in this work:

1. Exopolysaccharide native biofilms *Pseudomonas alcaligenes* and in dilution $10^{-1} – 10^{-4}$ (n = 3);

2. Exopolysaccharide fractions and components *Pseudomonas alcaligenes* native cell wall and in dilution $10^{-1} – 10^{-4}$ (n = 3);

3. *Pseudomonas alcaligenes* native exopolysaccharides and in dilution $10^{-1} – 10^{-4}$ (n = 3).

For each experimental variant there was found a rate of geometric mean number excess of revertants in experimental and control values and then compared with critical value given in the guidelines.

Table 2. Evaluation of the potential mutagenic activity of *Pseudomonas alcaligenes* EPS and their complexes using the Ames test without metabolic activation

Bio-preparations variants	Dilutions	TA 100		
		$\bar{x}$	σ	$\overline{x0}/\overline{xk}$
1	native	104,2	6,3	1,50
	10^{-1}	93,1	5,4	1,28
	10^{-2}	101,9	6,9	1,64
	10^{-3}	98,4	5,1	1,21
	10^{-4}	92,1	6,4	1,52
«+» control		907,8	11,4	
«–» control		32,1	4,2	
2	native	108,4	6,7	2,16
	10^{-1}	98,5	5,9	1,90
	10^{-2}	98,2	5,9	1,90
	10^{-3}	101,1	6,8	2,19
	10^{-4}	98,7	5,2	1,67
«+» control		1010,5	10,3	
«–» control		24,9	3,1	
3	native	101,2	6,7	2,31
	10^{-1}	97,3	5,9	2,03
	10^{-2}	98,4	5,2	1,79
	10^{-3}	90,1	5,6	1,93
	10^{-4}	98,1	5,7	1,96
«+» control		1009,2	10,1	
«–» control		21,2	2,9	

Notes: $\bar{x}$ – the arithmetic average of the number of colonies, σ – the standard deviation,

$\overline{x0}/\overline{xk}$ - the ratio of the average number of revertants in an experiment to «–» control.

As can be seen from the data presented in Table 2, bacterial strains reduced the number of revertants under the action of substances taken as negative controls without metabolic activation by increasing dilution. Thus, the experimental data the potential mutagenic activity of EPS *Pseudomonas alcaligenes* and their complexes, in Ames test are objective.

Table 3. Evaluation of potential mutagenic activity of *Pseudomonas alcaligenes* EPS and their complexes with Ames test at metabolic activation

Bio-preparations variants	Dilutions	TA 100		
		$\bar{x}$	σ	$\overline{x0}/\overline{xk}$
1	native	108,6	6,7	1,76
	10^{-1}	98,9	5,9	1,55
	10^{-2}	96,4	5,4	1,42
	10^{-3}	102,1	6,1	1,60
	10^{-4}	98,7	5,8	1,52
«+» control		1020,6	10,4	
«–» control		52,3	3,8	
2	native	106,3	6,3	1,70
	10^{-1}	102,1	6,1	1,64
	10^{-2}	101,2	6,8	1,83
	10^{-3}	98,9	5,9	1,59
	10^{-4}	95,4	5,7	1,54
«+» control		1010,5	10,1	
«–» control		35,2	3,7	
3	native	104,9	6,2	1,44
	10^{-1}	97,7	6,0	1,39
	10^{-2}	101,2	5,8	1,34
	10^{-3}	100,1	6,3	1,46
	10^{-4}	99,1	6,2	1,44
«+» control		1017,4	10,4	
«–» control		61,6	4,3	

When analyzing the potential mutagenic activity of *Pseudomonas alcaligenes* EPS and their complexes with metabolic activation (Table 3), it was found that the ratio of the mean number of revertants to the negative control is lower compared with the results obtained in the experiment of evaluating the potential mutagenic activity of *Pseudomonas alcaligenes* EPS and complexes without metabolic activation (Table 2).

4. Conclusion

From the above data it can be concluded that *Pseudomonas alcaligenes* EPS and their complexes in concentrations of 0.1 - 1000 μg/plate causes no increase in the number of revertants among strains of *Salmonella typhimurium* TA98, TA100, and thus does not exhibit mutagenic effects and hence bio-inert wound covering being developed based on *Pseudomonas alcaligenes* EPS can be recommended for clinical trials as an alternative for the treatment of wounds of various etiologies.

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Результаты исследования мутагенного эффекта полисахаридов микробного происхождения

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Аннотация. В статье приводятся результаты исследования мутагенного эффекта полисахаридов *Pseudomonas alcaligens*. В качестве природных продуцентов экзополисахаридов использованы представители рода *Pseudomonas* – неферментирующие убиквитарные бактерии, обладающие специфическими метаболическими циклами и

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уникальными физико-химическими и биологическими свойствами. В эксперименте с использованием теста Эймса изучено 3 варианта тестируемых соединений: 1. в составе биопленки *Pseudomonas alcaligenes,* 2. в составе экзополисахаридного матрикса и компоненты клеточной стенки микроорганизма и 3. собственно – микробный экзополисахарид. Во всех случаях доказано отсутствие мутагенного действия полисахаридов *Pseudomonas alcaligenes,* что делает их перспективными для использования в качестве наноматериалов нового поколения – альтернативных раневых покрытий.

Ключевые слова: *Pseudomonas alcaligenes*, полисахариды, экзополисахариды, тест Эймса, мутагенный эффект, раневые покрытия.