

# Structural Peculiarities of Exopolysaccharides of Phototrophic Bacteria *Rhodobacter sphaeroides*

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**Abstract** For the first time, properties and structural peculiarities of exopolysaccharides produced by phototrophic bacteria *Rhodobacter sphaeroides* isolated from hydrocarbonate mineral waters in Armenia with a degree of mineralization in limits of 3.5-5.0g/l, pH 7.2 have been studied. It is noteworthy that despite membership of the isolated strains in one and the same genus, these strains are significantly different by the nature of the produced exooligosaccharide. The studies implemented show that phototrophic bacteria are able to synthesize exopolysaccharides with different compositions and structures: cyclic and linear, depending on the cultivation conditions.

**Keywords** Exopolysaccharide, NMR Spectrum, Phototrophic Bacteria

## 1. Introduction

Microorganisms belonging to various Genera and producing exopolysaccharides have been described [1-6]. The microbial polysaccharides are very important for cells in population, in the process of adhesion and also in protection of cells [7-11]. Many microbial polysaccharides are used in food production, pharmaceutical, cosmetic, textile industries and other fields. The structure and function of polysaccharides producing by different microorganisms have been studied [12-19].

Phototrophic microorganisms represent considerable interest for productions various biologically active compounds - vitamins, carotenoids, organic acids, enzymes, etc. However, the biosynthesis and properties of polysaccharides produced by phototrophic bacteria are not entirely studied and represent prospective areas of study.

The aim of these researches is to study the properties and structural peculiarities of exopolysaccharides produced by the strains of phototrophic bacteria *Rhodobacter sphaeroides*.

## 2. Materials and Methods

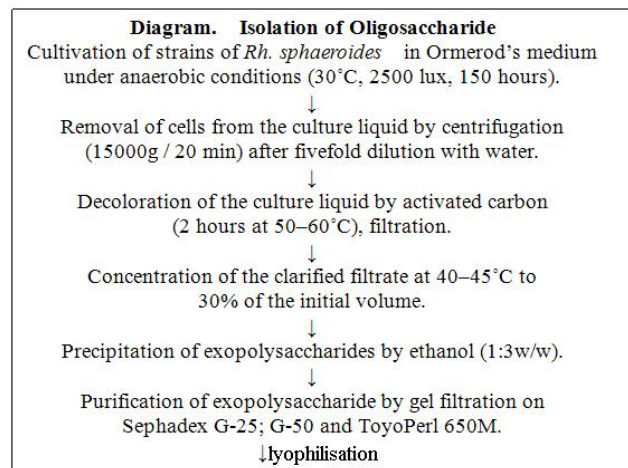
**Strains and media.** Phototrophic bacteria *Rhodobacter sphaeroides*, designated as A-1 and D-1 have been isolated from hydrocarbonate water sources of Armenia (Arzni and Jermuk) with a degree of mineralization of 3.5–5.0 g/l, pH 7.2. The strain of *Rh. sphaeroides* A1 was cultivated in the under stationary conditions with illumination intensity of 2500–3000 lux, at 30°C, during 8 days in slightly modified Ormerod's nutrition medium [20]: (g/l) KH<sub>2</sub>PO<sub>4</sub> – 0.6, K<sub>2</sub>HPO<sub>4</sub> – 0.9, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> – 0.5, MgSO<sub>4</sub> · 7H<sub>2</sub>O – 0.2, CaCl<sub>2</sub> · 2H<sub>2</sub>O – 0.08, FeSO<sub>4</sub> · 7H<sub>2</sub>O – 0.012, Na-acetate – 7.0, or succinat, biotin – 15 mg, microelements – 1 ml, pH – 7.0.

The total quantity of sugars was determined by anthrone method [21].

Antimicrobial activity of polysaccharides was identified by the agar diffusion methods [22].

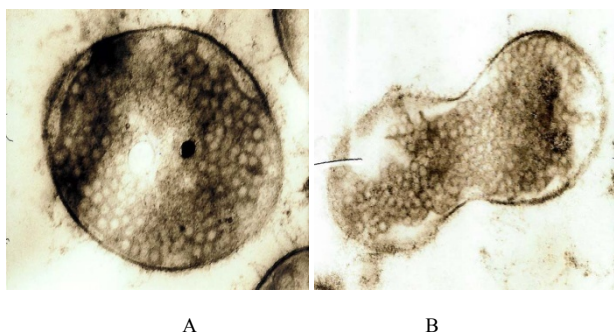
NMR spectra were registered on spectrometer MERCURY 300Vx (Varian) with resonance frequency of 300.077 and 75.465 MHz, on <sup>1</sup>H and <sup>13</sup>C nuclei, respectively. Heavy water was used as solvent. In the NMR spectrum, <sup>13</sup>C signal of methanol group (δ (CH<sub>3</sub>) 49 ppm) acted as the internal standard.

Isolation of exopolysaccharids was carried out according to the following method developed by as (diagram).



### 3. Results and Discussion

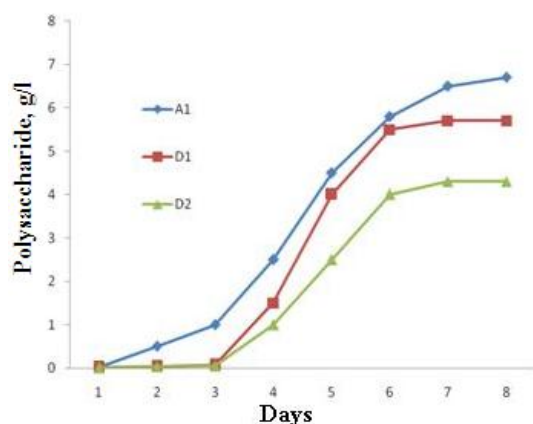
Studies of morpho-physiological and biochemical characteristics of *Rh. sphaeroides* strains A-1, D-1 have revealed that they are Gram-positive and can grow in nutrient medium containing organic substrates under anaerobic conditions in the light, as well as under aerobic conditions in the dark. Electron microscopic studies have shown that the cells of the studied bacteria are of spherical form (2.0 – 2.5 to 1.3-1 mc) (Figure 1).



**Figure 1.** Electron microphotography of *Rh. sphaeroides* A-1 (A) and division (B), (x15600)

The mentioned cultures grew well in the cultivation medium, containing the salts of organic acids – acetate, piruvate, lactate, malate, fumarate, succinate as well as glucose, fructose, maltose, saccharose and other sugars. Microscopic studies have shown that bacterial cells form a mucous capsule.

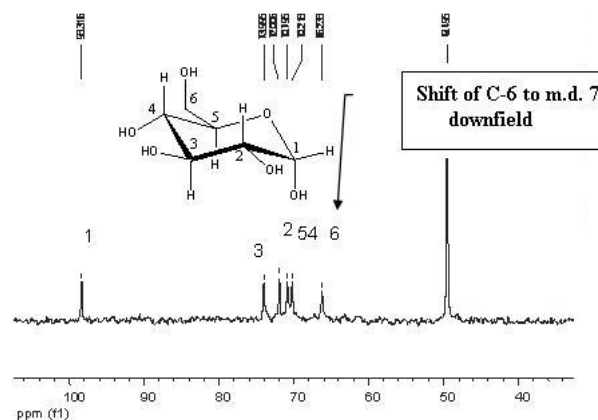
Studies of synthesis of exopolysaccharides have demonstrated that their content reaches maximum in the 6-7 days of cultivation, which coincides with the maximum of culture growth. Under further cultivation the level of sugars is not changed, as the case with the biomass (Figure 2).



**Figure 2.** Synthesis of exopolysaccharids by *Rh. sphaeroides* (A1, D1 in the medium with acetate and D2- with succinate)

Microbial polysaccharides are known to be biologically active [2, 11, 18, 22]. Our studies of the effect of the isolated exopolysaccharides on microorganisms – *E. coli*, *B. subtilis*, *Candida albicans* – have not indicate any antimicrobial activity.

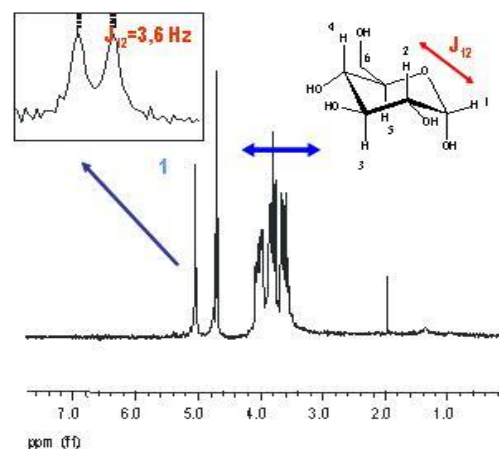
Structural peculiarities of the noted exopolysaccharide have been studied by means of nuclear-magnetic resonance (NMR) spectroscopy of hydrogen and carbon atom nuclei. The  $^{13}\text{C}$  NMR spectrum of the exopolysaccharide from *Rh. sphaeroides* strain A-1 contains six absorption signals from six non-equivalent carbon atoms (Figure 3).



**Figure 3.**  $^{13}\text{C}$  NMR spectrum of exopolysaccharide of *Rh. sphaeroides* A-1

The J-coupling values for proton NMR of polysaccharide of *Rh. sphaeroides* A-1 are:  $\delta$  (C<sub>1</sub>) 98.3 ppm,  $\delta$  (C<sub>2</sub>) 72.0 ppm,  $\delta$  (C<sub>3</sub>) 74.01 ppm,  $\delta$  (C<sub>4</sub>) 70.22 ppm,  $\delta$  (C<sub>5</sub>) 70.80 ppm,  $\delta$  (C<sub>6</sub>) 66.24 ppm.

The comparison of the abovementioned spectrum with  $^{13}\text{C}$  NMR spectrum of  $\alpha$ -glucose has revealed good coincidence of signals chemical shifts for carbon atoms in positions 2 – 5, whereas carbon atom signals in positions 1 and 6 were significantly different. This indicates that  $\alpha$ -glucose is the base of the oligosaccharide, and glycosidic residues are connected in positions C1 and C6 (Figure 4).

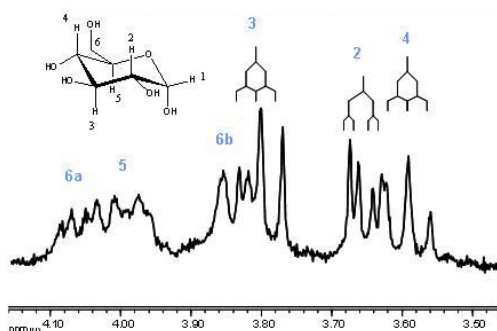


**Figure 4.**  $^1\text{H}$  NMR spectrum of the exopolysaccharide of *Rh. sphaeroides* A-1

Analysis of the proton spectra of the oligosaccharide in question agrees well with the abovementioned (Figure 5 and Table 1).

The doublet signal of anomeric hydrogen atom with the spin-spin interaction constant  $J = 3.6$  Hz indicate the evidences of  $\alpha$ -configuration of the monosaccharide.

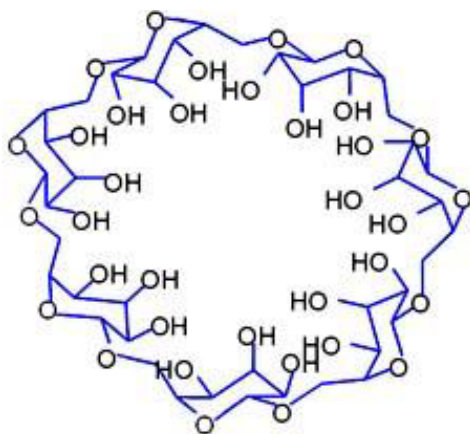
Secondly, detailed analysis of multiplet structure and determination of the spin-spin interaction constant between nuclei 1 and 2, 2 and 3, 3 and 4, 4 and 5 indicates that  $\alpha$ -glucose is the monosaccharide residue, and 7 molecules are connected in positions C1 and C6, forming a cyclic structure with certain elements of symmetry and molecular mass 1135 (Figure 6).



**Figure 5.**  $^1\text{H}$  NMR spectrum of the exopolysaccharide of *Rh. sphaeroides* A-1, proton regions 2-6

**Table 1.** NMR  $^1\text{H}$  spectrum for polysaccharide of *Rh. sphaeroides* A-1

Proton	$\delta$ ppm	Multiplet	J
H <sub>1</sub>	5.05	d	$J_{1,2} 3.6$
H <sub>2</sub>	3.65	dd	$J_{1,2} 3.6, J_{2,3} 9.8$
H <sub>3</sub>	3.80	t	$J_{2,3} 9.7, J_{3,4} 9.8$
H <sub>4</sub>	3.59	t	$J_{3,4} 9.2, J_{4,5} 9.8$
H <sub>5</sub>	3.99	m	
H <sub>6a</sub>	4.06	m	
H <sub>6b</sub>	3.84	m	

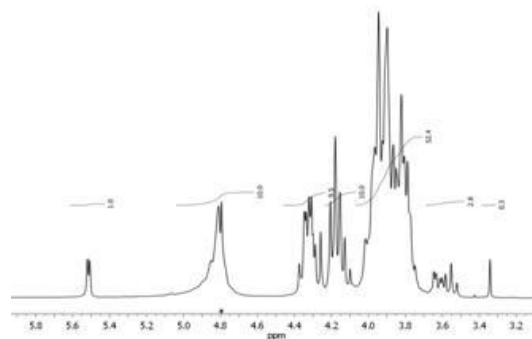


**Figure 6.** Cyclic structure of the 1,6 oligosaccharide of *Rh. sphaeroides* A-1

It is important to note that spectra of oligosaccharide with linear structure and, therefore, without symmetry elements, would have appeared to be much more complex than the observed spectra. In particular, the  $^{13}\text{C}$  NMR spectrum of such oligosaccharide should have consisted of several tens of signals.

The influence of different sources of carbon on the

peculiarity of exopolysaccharide produced by *Rh. sphaeroides* D-1 has been carried out. Investigations of  $^{13}\text{C}$  NMR spectrum of exopolysaccharide of phototrophic bacteria *Rh. sphaeroides* D-1 grown in acetate containing medium as the only source of carbon revealed the presence of numerous different intensity signals (Figure 7).

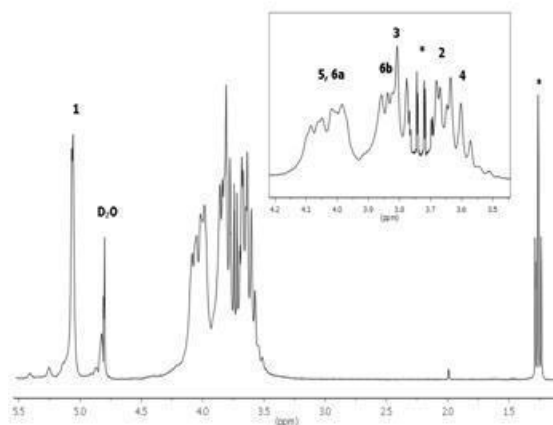


**Figure 7.**  $^{13}\text{C}$  NMR spectrum of the polysaccharide *Rh. sphaeroides* D-1 grown on acetate medium

The results obtained indicate that the investigating polysaccharide consists of two different monomers of glucose and galactose. The same results have been obtained by proton NMR spectrum. It should also be mentioned that the exopolysaccharide of *Rh. sphaeroides* D-1 is a linear heteropolysaccharide.

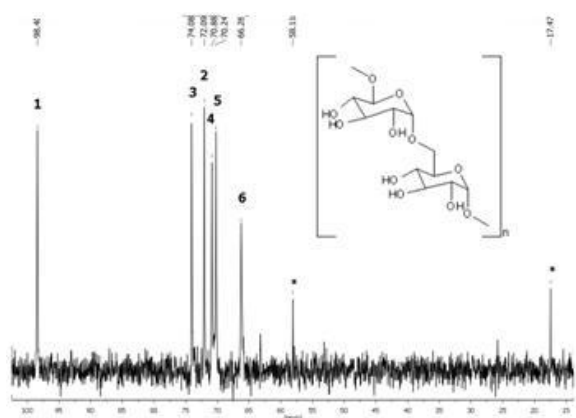
In literature, it is assumed that the monomeric composition and structure of synthesized polysaccharide by microorganisms depend on the cultivation conditions, particularly from source of carbon [7, 12, 14].

In order to study the possibility of changing the monomer composition and structure of exopolysaccharide of phototrophic bacteria of *Rh. sphaeroides* D-1, the cultivation has been carried out on Ormerud medium with succinate as the only source of carbon, instead of acetate. The studies of structural properties of isolated exopolysaccharide by  $^{13}\text{C}$  NMR spectrum have shown the presence of six identical carbon signals which have proved that the polysaccharide is composed of identical monomers (Figure 8).



**Figure 8.**  $^{13}\text{C}$  NMR spectrum of the polysaccharide *Rh. sphaeroides* D-1 grown on succinate medium

In the proton NMR spectrum of investigated polysaccharide is observed 5.06mm doublet swallowing with constant spin-spin interacts 3.8 Hz, indicating that the polysaccharide is a part of the monomer  $\alpha$ -glucopyranoside (Figure 9).



**Figure 9.** Proton NMR spectrum of the exopolysaccharide *Rh. sphaeroides* D-1 grown in succinate medium

Simultaneously, the chemical deviation of C6 atom in the weak magnetic field direction of 5.0mm indicates that monosaccharide residues have been connected by C1-C6 links. The presence of weak signal in proton-5.2mm spectrum of polysaccharide shows a linear nature of polymer. Thus, it has been revealed that in exopolysaccharide of *Rh. sphaeroides* D-1 cultivated in the succinate medium as the only source of carbon the repeating unit of the polymer is 6)  $\alpha$ -D-Glucopyranoside (1-6)- $\alpha$ -D-Glucopyranoside (1).

## 4. Conclusion

Strains of phototrophic bacteria have been isolated from mineral hydro carbonic water (Armenia). The studies of morphological, physiological and biological properties allowed identifying them as strains of *Rhodobacter sphaeroides*. The isolated bacteria are gram positive and can utilize organic substrates: acetate, piruvate, lactate, fumarate, succinate as well as glucose, fructose, maltose, saccharose and other sugars as in anaerobic condition in the light and in aerobic conditions in the dark.

For the first time it has been shown that the phototrophic bacteria (*Rh. sphaeroides* A1, D1) produce exopolysaccharides and it is noteworthy that in despite that isolated bacteria belonging the same genera can produced exopolysaccharides significantly differ by the structure and composition: in our study thr cyclic and linear forms have been described.

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