https://dx.doi.org/10.22161/ijaers.4.11.10 *ISSN: 2349-6495(P) | 2456-1908(O)*

Peculiarities of a Colloidal Polysaccharide of Newly Isolated Iron Oxidizing Bacteria in Armenia

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Abstract*—Microorganisms belonging to different systematic and physiological groups produce various intra- and extracellular polysaccharides, which both play an important role in the life of microorganisms and have great practical application. Iron and sulfur oxidizing bacteria produce capsular (EPS) and colloidal polysaccharides. At present the properties and functional role of EPS are well studied. However, the properties of the colloidal polysaccharides produced by iron oxidizing bacteria have not been sufficiently explored. A new iron oxidizing bacteria Leptospirillum ferriphilium CC was isolated from sulfide ores of Armenia. Its morphological and physiological features have been studied. A colloidal polysaccharide has been isolated with the use of an original method developed by the authors, and its physical and chemical properties have been studied. It has been shown that the colloidal polysaccharide consists of three different monomers- glucose, fructose, mannose.. Investigations with a complex method of optical polarization microscopy and analytical programs allowed determining the size, shape change, perimeter, degree of hydratation and crystallization at 0.07% and 0.04% of polysaccharide concentration. It was shown that the size of a polysaccharide colloidal particle does not much depend on polysaccharide concentration, however, the number of identical colloidal formations is dependent on the concentration of polysaccharide.*

Keywords— cell morphology: colloidal polysaccharide: iron oxidizing bacteria.

I. INTRODUCTION

Microorganisms, like eukaryotes and prokaryotes produce polysaccharides, with different biological, chemical, physical properties and functional activity. Iron and sulfur oxidizing chemolithotrophic bacteria produce itra- and exopolysaccharides. The properties of the intracellular polysaccharides - lipopolysacchardes (LPS) of iron and sulfur oxidizing bacteria have been studied. It was revealed that the inter-specific diversity as well as the conditions of cultivation and sources of energy define various properties of LPS [1-7]. Generally the exopolysaccharides produced by the above-mentioned bacteria are subdivided into capsular and soluble/colloidal forms. Extensive research undertaken in the past decades has been focused on understanding the properties of the exopolysaccharides contained in the extracellular substances (EPS) including *Acidihiobacillus ferrooxidans, Leptospirillum ferriphilum, Leptospirillum ferrooxidans* and formig a capsule around the bacterial cell. EPS play an essential role for the formation of a biofilm, which mediates adhesion of cells to the minerals surface and form a cohesive three-dimensional polymer interconnecting and immobilizing cells in the process of bioleaching by iron and sulfur-oxidizing bacteria. An important role of capsular polysaccharides as a fundamental structural element of the EPS, determining the mechanical stability of biofilm was disclosed [8-12].

However, the properties of colloidal polysaccharides produced by iron and sulfur oxidizing chemolithotrophic bacteria remains understudied. The objective of the present study is to investigate the properties of a colloidal polysaccharide of the iron oxidizing chemolithotrophic bacteria *Leptospirillum ferriphilum CC* newly isolated in Armenia.

II. MATERIALS AND METHODS

Bacterial culture and media. The *Leptospirillum ferriphilum CC* was isolated from natural biotopes of sulfide ores in Armenia.Cultivation was carried out in 9K liquid medium with ferrous iron as a sources of energy at 37°C for 5-7 days.

Identification of strain. The isolated strain was identified both by morphological, physiological properties [14] and by the sequencing of 16S rRNA The primary analysis of the data was carried out using the BLAST program. The phylogenetic tree was constructed with the help of MEGA 6.06 and nighbor-joinig, and boot star programs.

Izolation of colloidal polysaccharide. The colloidal polysaccharide produced by *L. ferriphilum* has been

https://dx.doi.org/10.22161/ijaers.4.11.10 *ISSN: 2349-6495(P) | 2456-1908(O)*

isolated according to the method developed by the authors. The isolation protocol is summarized in Fig 3.

Determination of the chemical composition of the colloidal polysaccharide. Chemical composition of the colloidal polysaccharide was analyzed after hydrolysis by 2 N HCl at 100°C for 2 hours by high performance liquid chromatography (HPLC) on the Shimidzu 2010 C analyzer, column ULTKON , P5-80-H 2 x 250 mm, the mobile phase – 0.1 mM acetat buffer/acetonitril-1:5, pH 5.8, flow rate 1ml/min. Measurements were made by RI.

Properties of colloidal formation. Properties of colloidal formation of polysaccharide were studied by a complex method combining optical polarization microscopy and analytical programs LobViEW15 and WOVA [15, 16].

III. RESULTS AND DISCUSSION

Investigations of morphological properties of the isolated iron oxidizing bacteria by light and optical polarization microscopy and analytical programs disclosed that the cells of the their area is 7033 μm, the length is 125μm, perimeter is 462 μm and the shape of the cells is 0.0392.The observed scatter of the morphological parameters of *L. ferriphilum* is due to the non-synchronous growth of culture in the population (Fig.1,Tabl. 2).

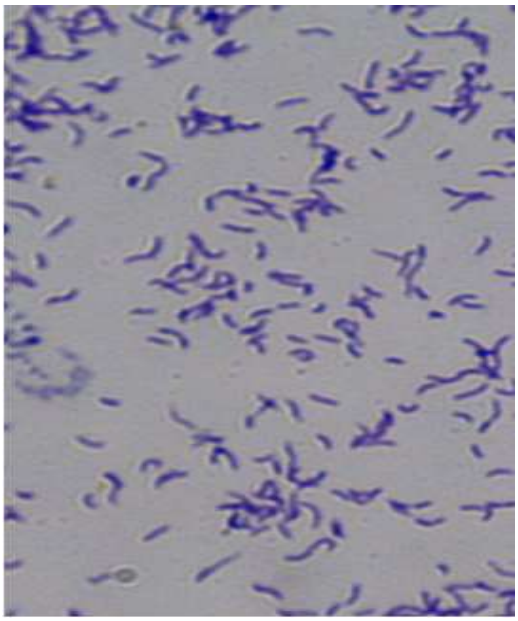

Fig.1: Microphotography of L. ferriphilum the investigated bacteria have a curve rod- shape,

Based on the morphological and physiological characteristics, as well as on the sequence of nucleotides in 16SrRNA, the isolated strain was identified as *L. ferriphilum* CC (Fig. 2). The strain was deposited at the Republican Center for the Depostion of Microorganisms, Armenia, MDC 7047.The results of sequencing are included in the data base of Geen Bank.

Table.1: Phenotypic characteristics of L. ferriphilum CC

	Area	Average Size	Length	Perimeter	
Unit	um*um	um	um	um	α
Ave-rage	7033.052	82.356	125.743	462.21	0.03292
SD	2469.569	15.824	17.899	93.828	
1	9438	97.149	137.693	521	0.03477
2	6860	82.825	119.406	500	0.02744
3	8182	90.454	125.342	445	0.041318
4	5977	77.311	125.907	443	0.030456
5	8952	94.615	149.862	602	0.024702
6	7044	83.928	114.885	420	0.039932
7	9320	96.54	132.468	467	0.042735
8	5361	73.218	125.02	395	0.03436
9	1946	44.113	86.44	280	0.024821
10	10984	104.804	149.862	647	0.026239
11	4095	63.992	98.943	333	0.036929
12	10017	100.084	140.789	531	0.035526
13	6534	80.833	115.542	470	0.029579
14	7953	89.179	135.714	604	0.0218
15	7183	84.752	130.189	448	0.035789
16	4414	66.437	115.982	392	0.028725
17	3450	58.736	94.509	321	0.033482
18	5279	72.656	141.431	458	0.025166
19	10639	103.145	149.141	505	0.041717

α- shows the shape of the bacterial cells, as determined by the formula

$\alpha = S/P^2$, where S is the area, P is the perimeter of cells. The value of α less than 0.06 means that the investigated object is rod-shaped.

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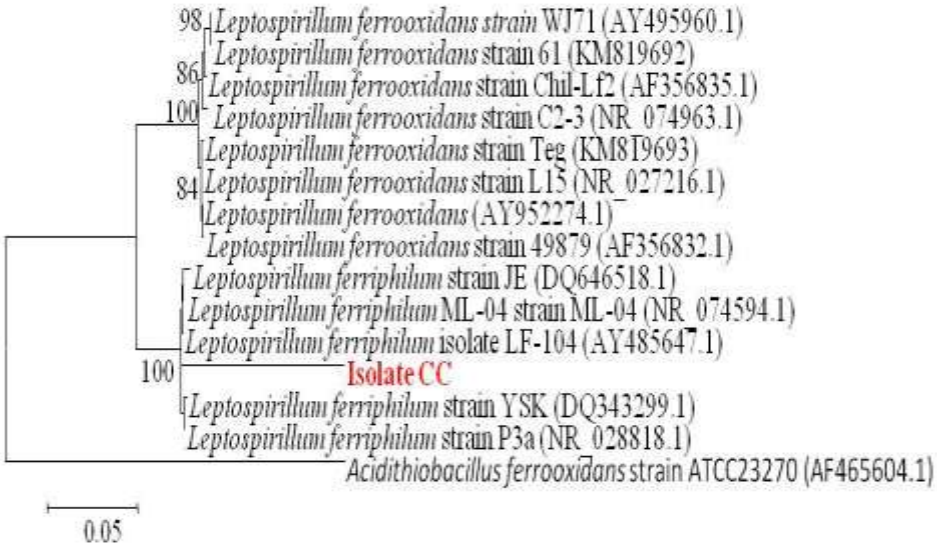

Fig. 2: Dendrogram of Leptospirillum ferriphilum CC

Previously, we have studied the peculiarities of bioleaching of sulfide minerals by the above-mentioned bacteria, the adhesion on the surface of the mineral, the capsular EPS and the properties of immobilized cells on natural carriers [11, 18-21]. In continuation of these studies, we set out to study the characteristics of the colloidal polysaccharide produced by *L. ferriphilum.*

Isolation and chemical composition of the colloidal polysaccharide. *L. ferriphilium* was cultivated in a reactor in a Macintosh medium [22], under aeration and stirring for 120 hours. Then, after harvesting the biomass by centrifugation ferrous sulfate was precipitated from the solution by addition of NaOH up to pH 7.5-8.0. After removal of pellets by centrifugation, the centrifugate was concentrated to 15-20% of the initial volume at 40°C in a rotary evaporator. The polysaccharide was precipitated with ethanol (1:3v/v).The precipitate of polysaccharide was dissolved in 50 ml of distilled water and then the traces of proteins were removed and the solution was desalted by adsorption and gel chromatography using columns with ToyoPerl 650 M and Sephadex G25, respectively. The polysaccharide was then re-precipitated with ethanol and dried at 25-30°C in vacuum (Fig. 3).

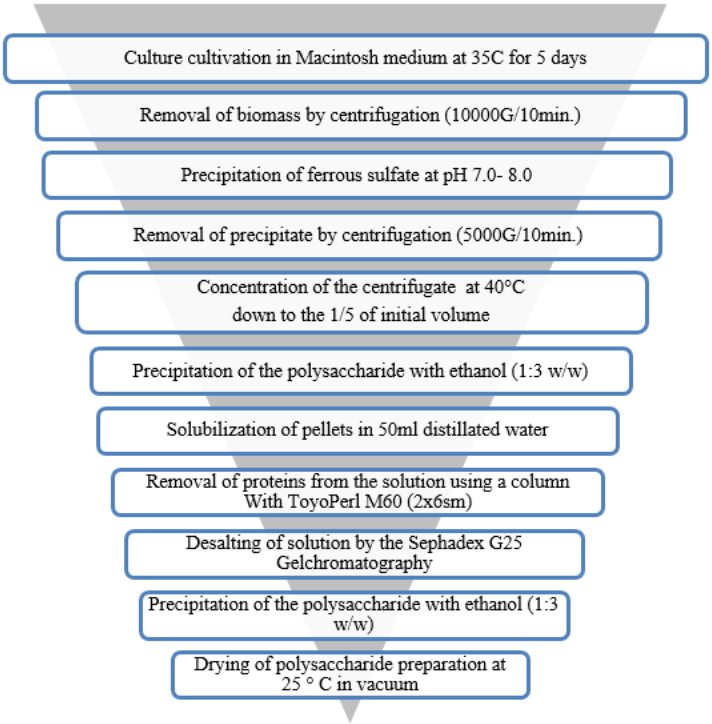

Fig.3: General procedure for isolation of colloidal polysaccharide

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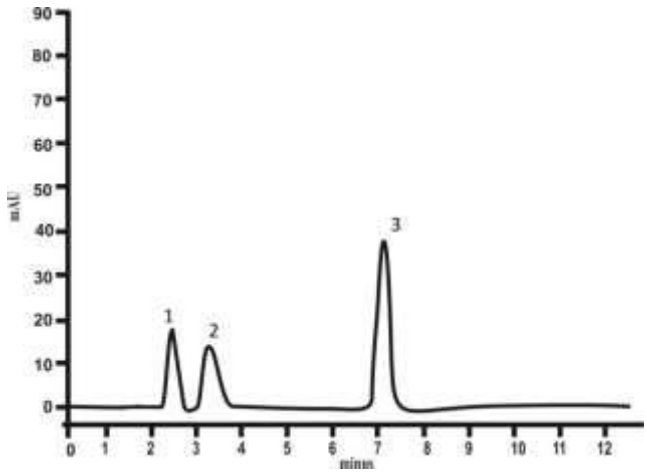

Fig.4: Chromatogram (HPLC) of chemical composition of colloidal polysaccharide of L. ferriphilum CC (1 - glucose, 2 - fructose, 3 - mannose)

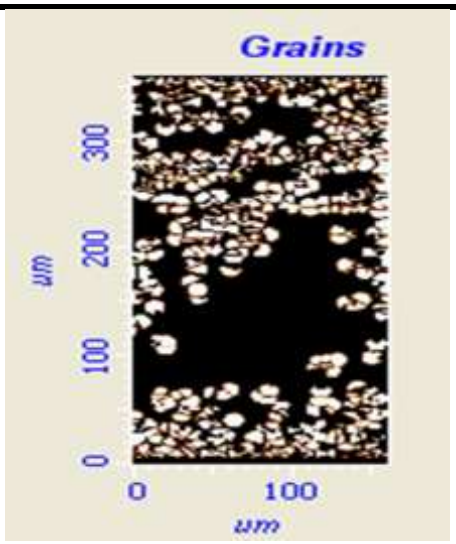

Fig. 5: Microphotography of colloidal formations at the concentration of the polysaccharide in solution of 0.07%.

Studies of the chemical composition of isolated colloidal polysaccharides show the presence of various monomers (Fig. 4).

The physico-chemical properties of the isolated exopolysaccharide were also studied with a complex method developed by the authors and based on optical polarization microscopy (MEIJI) as well as the analytical programs LabVIEW-15 and WISION [15,16]. The obtained results of microscopy studies were transformed in accordance with the NOVA program, which allows identifying the size, shape changes, the degree of hydratation, and the crystallization of colloidal formations of the exopolysaccharide in solution.

It was shown that at 0.07% concentration of the polysaccharide the dimensions, average area, shape and perimeters of the colloidal particles are 19773, 0.079 and 14, 077μm respectively (Fig. 5, 6, Tab.2). It has also been explained that the degree of crystallization of colloidal formations is 82.79 (Fig. 7).

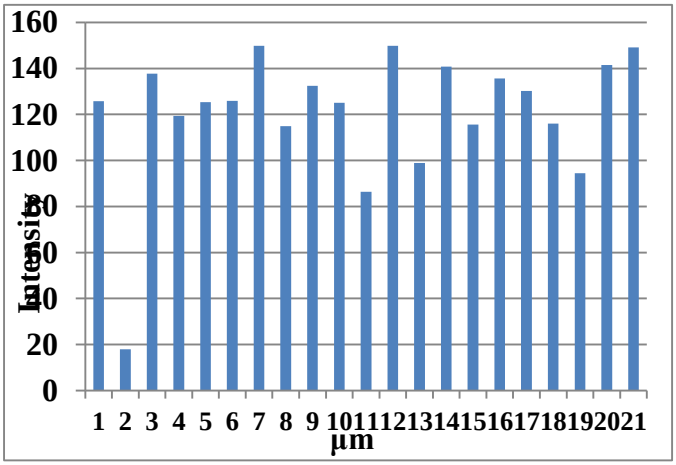

Fig. 6: Histogram of the sizes of colloidal formations obtained as a result of processing of microscopy at the sugar concentration of 0.07%

Table 2. The average of the size, area, perimeter and forms of colloidal formations at the polysaccharide concentration 0.07%

	Area	Average S	Peremeter	α
Unit	um*um	um	um	
Average	19.893	4.028	14.9777	0.088685
SD	17.586	1.913	9.867	0.180736

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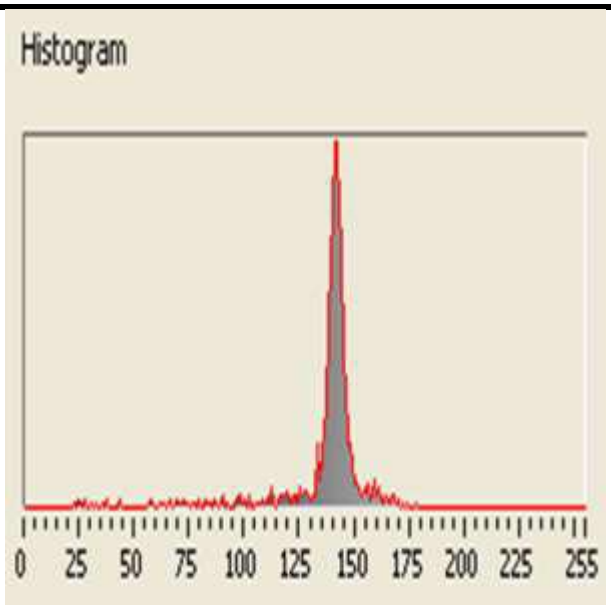

Fig. 7: Histogram of the micrometry of colloidal formations at the concentration of the polysaccharide at 0.07%.

It has been shown that reducing polysaccharide concentration by half (0.04%), the sizes of colloidal particles increase on average by 5.079, and their average area is 29.61. The shape and perimeters of the colloidal formations significantly increase, to 0.073 and 20.175μm, respectively (Fig 8,9, Tab. 3). At a low concentration of the polysaccharide, the degree of crystallization of the colloidal particles is also significantly lower (Fig. 10).

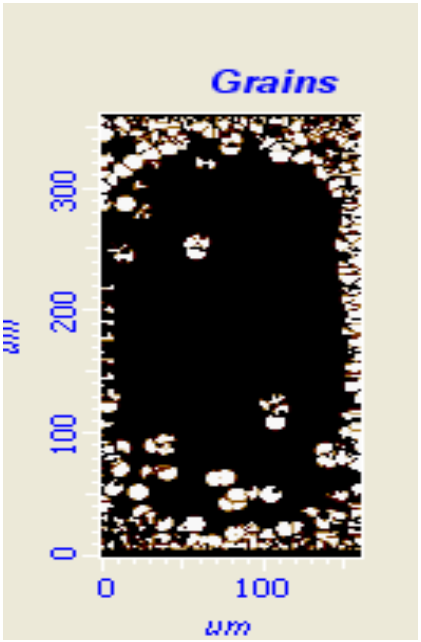

Fig.8: Microphotography of colloidal formations at the concentration of the polysaccharide at 0.04%.

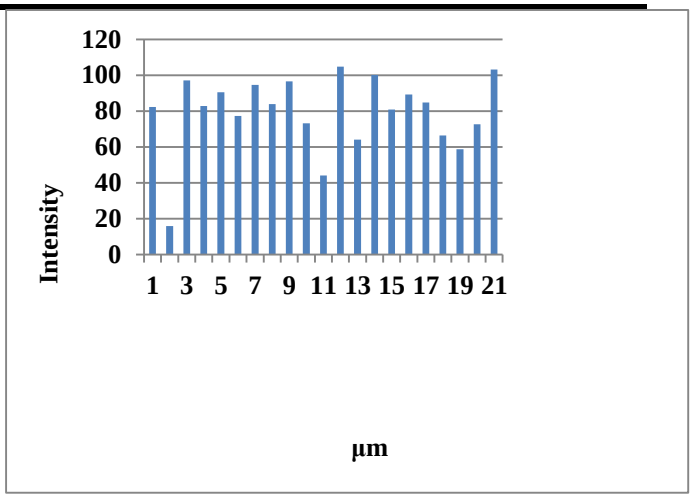

Fig.9: Histogram of the sizes of colloidal formations obtained as a result of processing of microscopy at the sugar concentration of 0.04%.

Table.3: The average of the size, area, perimeter and forms of colloidal formations at the polysaccharide concentration of 0.04%.

Area	Average S	Peremeter	α
um*um	um	um	
29.671	5.079	20.175	0.072896
20.448	1.967	9.93	0.207373

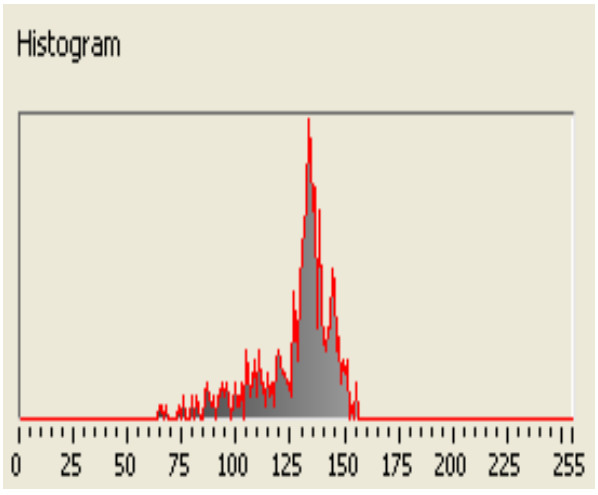

Fig. 10: Histogram of the sizes of colloidal formations (μm), obtained as a result of processing of microscopy at the sugar concentration of 0.04%.

IV. CONCLUSION

Iron oxidizing chemolithotrophic bacteria have been isolated from the sulfide ores and identified as *L. ferriphilum* CC on the bases of their morphological and physiological characteristics, as well as on the sequences of nucleotides in 16SrRNA. It has been shown that in addition to the EPS *L. ferriphilum* produces a colloidal polysaccharide. Isolation of the above-mentioned

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polysaccharide was carried out according to a method developed by the authors, and its chemical and physical properties were studied. Studies of the chemical composition showed that the colloidal polysaccharide synthesized by *L. ferriphilum* CC consists of three monomers- glucose, fructose,mannose. The crystallization degree of colloidal particles and the shape parameters basically depended on polysaccharide concentration, which were conditioned by the hydration degree of a particle. A comparison of histograms (Fig.5 and Fig. 8) indicates that an increase in polysaccharide concentration does not lead to bigger colloidal particle formation, but contributes to increasing the quantity of shaped particles. This means that the dispersion of colloidal particles (size) does not much depend on polysaccharide concentration. However, the number of identical colloidal formations basically depend on the concentration of polysaccharide.

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