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# Screening of Sulfate-Reducing Bacteria According to Their Ability to Synthesize Lectins.

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**ABSTRACT**

We screened 10 species of sulfate-reducing bacteria in order to find the active producers of lectin. We determined that all tested bacterial strains are able to synthesize endo- and exo-lectins of different activity rate, wherein the activity of exogenous lectins was significantly higher for most species of sulfate-reducing bacteria than the activity of lectins isolated from cell lysates. The highest activity of both endogenous and exogenous lectins was determined in the strains of *Desulfotomaculum nigrificans* ECM - 1492 and *Thermodesulfobacterium mobile* ECM-1128. An exception was the original strain *Desulfotomaculum P-2 spp*., which had the highest activity of exogenous lectins, while the activity of endogenous lectins was the same as in other strains. Lectins of different species of sulfate-reducing bacteria differ from each other in the carbohydrate specificity. Nevertheless, carbohydrates such as D-galactose, D- and L-glucose, arabinose, mannitol and fructose have inhibited haemagglutination reaction in all strains of sulfate-reducing bacteria studied. While carbohydrates such as sucrose, xylose, ribose, arabinose and cellobiose have blocked haemagglutination reaction of lectins only in certain strains of microorganisms.

**Keywords:** sulfate-reducing bacteria, lectins, activity, carbohydrate specificity.

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## INTRODUCTION

Lectins are non-immune (glycol-) proteins, which agglutinate cells and/or precipitate complex carbohydrates [19]. They are found in living organisms of different organization degree, ranging from viruses to a human [8]. The most studied are plant lectins and their symbionts - microorganisms [5], [12], [31]. It was found that lectins play role in the adhesion of microorganisms on the tissue surface, perform the migration of lymphocytes into lymphoid organs out of the blood, and participate in the differentiation processes, embryogenesis, proliferation and other processes [16], [22], [23].

Despite the considerable interest the scientists show to the lectins of microorganisms, most of the work on the isolation and study of their properties have been performed in aerobic cultures. Lectins of anaerobic microorganisms are understudied.

There are some works in the literature dedicated to the adhesive properties of lectins of intestinal microorganisms that are associated with dysbacteriosis [11], [14]. There is evidence of lectin role in the infectious process in *Mycoplasma smegmatis* [18]. Lectins of several anaerobic bacteria are involved in the formation of films [26]. At the same time, there is no data in the literature on the ability of sulfate-reducing bacteria to form lectins. However, they occur widely in nature and play a significant role in the transformation of organic substances [4], [30]. Sulfate-reducing bacteria have also been found in a human body [2]. We may assume that the cells of these bacteria are capable of forming lectins ensuring their adhesion, either on the mineral particles or on the tissue surface when performing the various functions.

Objective of this study was to investigate the ability of sulfate-reducing bacteria of different genera and species to synthesize endogenous and exogenous lectins.

## MATERIALS AND METHODS

We used cultures of sulfate-reducing bacteria, obtained from the All-Russian Collection of Microorganisms RAS (Pushchino): *Desulfovibrio desulfuricans* ECM – 1799*, Desulfovibrio vulgaris* ECM – 1760*, Desulfomicrobium macestii* ECM – 1598*, Desulfomicrobium baculatus* ECM – 1378*, Desulfotomaculum nigrificans* ECM – 1492*, Desulfotomaculum kuznetsovii* ECM – 1805*, Thermodesulfobacterium mobile* ECM – 1128*,* as well as strains of sulfate-reducing bacteria isolated from the soil of oil fields of the Republic of Tatarstan.

The bacteria were grown in Postgate medium B [24]. Cultivation was carried out in 500 ml sealed vials. Cultivating temperature 37$^{o}$C.

The ability of sulfate-reducing bacteria to grow under experimental conditions was assessed by the protein buildup using Lowry assay interpreted by Gorina and Iakovleva [3]. Both protein and the activity of lectins were determined on the third day of cultivation. The experiments were performed in triplicate.

During the studies, we monitored purity of sulfate-reducing bacteria by using phase-contrast microscopy (KF-4) and inoculations on glucose-peptone medium for anaerobic and aerobic heterotrophic bacteria.

We determined endogenous lectins of sulfate-reducing bacteria in lysates of biomass of the studied bacteria. For this purpose, we precipitated the cells by centrifuging them at 10000 g, for 5 minutes, with subsequent three-fold flushing with 20 mM phosphate buffer (pH 7.3).

The resulting biomass was re-suspensed in 20 mM phosphate buffer (pH 7.3) at 1:1 ratio and sonicated with the use of ultrasonic homogenizer UP100H. Sound frequency – 30 kHz, the total processing time - 4 minutes [28]. To remove cell debris biomass, we centrifuged the biomass extract at 15000 g, for 30 minutes, and used it to analyze the protein content and the presence of lectins.

We determined exogenous lectins of sulfate-reducing bacteria in the culture broth after separating the cells by centrifugation at 15000 g, for 30 minutes.

To determine the activity of lectin we used agglutination reaction with trypsinized and native "O" type human red blood cells [15]. Native erythrocytes for hemagglutination were obtained by using the method by Lutsyk et al. [9]. To increase the sensitivity of the hemagglutination reaction, we trypsinized native red blood cells [27].

The specificity of lectins was determined by their interaction with various sugars. The assay was carried out in immunological U-shaped plates by inhibiting the hemagglutination reaction using 2% suspension of red blood cells and native solutions of carbohydrates. We used the following solutions as agglutination inhobitors: L-glucose, D-glucose, D-sucrose, L-arabinose, D-arabinose, D-xylose, D-ribose, D-lactose, D-galactose, D-maltose, D-fructose, D-mannitol, cellobiose and starch, produced by "Fluka" (Germany). For analysis, a series of two-fold successive dilutions of carbohydrate - inhibitors of 0.02 ml each, 200 mM concentration for simple sugars and 5 mg/ml for polymeric carbohydrates - were prepared in the wells. We added equal volume of the extract of micromycetes with specific titers into each well. We carried out incubation for 60 minutes at room temperature, further added the double amount of 2% erythrocyte suspension, and allowed the mixture to stand for 30-40 minutes at 4°C. Lack of agglutination of red blood cells in the wells indicated the specific interaction between carbohydrate and lectin. The minimum inhibitory concentration (MIC) was considered as the lowest concentration of carbohydrate capable to completely inhibit the hemagglutination reaction [23].

## RESULTS

The main property of all known lectins isolated from various natural objects is their ability to give hemagglutination reaction. This common reaction allows determining lectins in crude protein extracts [20], [21], [29].

When studying the lysate of biomass consisting of 10 species of sulfate-reducing bacteria, and their culture fluid (Desulfovibrio desulfuricans BKM-1799, Desulfovibrio vulgaris ECM – 1760, Desulfovibrio gigas ECM – 1382, Desulfomicrobium macestii ECM – 1598, Desulfomicrobium baculatus ECM – 1378, Desulfotomaculum nigrificans ECM– 1598, Desulfotomaculum kuznetsovii ECM– 1805, Thermodesulfobacterium mobile ECM – 1128, Desulfovibrio E-2 spp., Desulfotomaculum P-2 spp.) , we found that all the strains studied could synthesize both endogenous and exogenous lectins (Tables 1-2). Screening of sulfate-reducing bacteria for their ability to synthesize endogenous lectins showed that the highest activity of endogenous lectins (274 ± 0.40 units, 255 ± 0.37 units, 240 ± 0.35 units) was in strains of *Desulfotomaculum nigrificans* ECM – 1492, a strain of *Desulfobacterium macestii* ECM – 1598, *Desulfovibrio gigas* ECM – 1382, respectively.

**Table 1: Determination of hemagglutinating activity of endogenous lectins of sulfate-reducing bacteria**

| SRB strains | Cell lysate | |
|---|---|---|
| | Native red blood cells | Trypsinized red blood cells |
| *Desulfovibrio desulfuricans* ECM – 1799 | 125±0.10 | 500±0.30 |
| *Desulfovibrio vulgaris* ECM –1760 | 105±0.10 | 210±0.27 |
| *Desulfovibrio gigas* ECM-1382 | 240±0.35 | 480±0.70 |
| *Desulfobacterium macestii* ECM – 1598 | 255±0.37 | 255±0.37 |
| *Desulfomicrobium baculatus* ECM –1378 | 139±0.20 | 558±0.70 |
| *Desulfotomaculum nigrificans* ECM – 1492 | 274±0.40 | 549±0.60 |
| *Desulfotomaculum kuznetsovii* ECM – 1805 | 150±0.12 | 150±0.11 |
| *Thermodesulfobacterium mobile* ECM – 1128 | 121±0.12 | 969±0.10 |
| *Desulfovibrio E-2 spp.* | 109±0.10 | 436±0.23 |
| *Desulfotomaculum P-2 spp.* | 100±0.09 | 400±0.10 |

The data is expressed as specific activity ($L_{sa}$) shown in units of hemagglutinating activity (GAU), in 1 mg protein in 1 ml sample.

Works of several authors studying the hemagglutination that involves lectins showed that the trypsinized red blood cells increase the sensitivity of the reaction [1], [8], [17]. In the course of our experiments we found that the trypsinization of red blood cells increased the hemagglytinating activity of lysates in most cells of sulfate-reducing bacteria in 2-8 times. A strain of *Thermodesulfobacterium mobile* ECM

– 1128 had the highest hemagglytinating activity (969±0.10 units) As exception were strains of Desulfobacterium macestii ECM – 1598 and Desulfotomaculum kuznetsovii ECM – 1805, which lectin activity remained at the same level. It is known that the trypsinization of red blood cells opens areas for interaction with lectins, from the one side, and reduces the content of sialic acids on their surface, from the other side [1], [10]. Increased activity of endogenous lectins of most sulfate-reducing bacteria, is, presumably, due to the increased number of interaction receptors on the erythrocyte surface. Lectins of strains of *esulfobacterium macestii* ECM – 1598 and *Desulfotomaculum kuznetsovii* ECM – 1805 have, apparently, a high specificity and interact with a limited number of receptors of both native and trypsinized red blood cells.

Investigation of the culture fluid of sulfate-reducing bacteria also revealed the presence of lectins therein, but the level of activity differed significantly in various strains. The strains of *Desulfotomaculum P-2 spp., Thermodesulfobacterium mobile* ECM – 1128, *Desulfotomaculum nigrificans* ECM – 1492 had the activity of exogenous lectins much higher than the same of endogenous lectins. However, the strains of *Desulfobacterium macestii* ECM – 1598, *Desulfomicrobium baculatus* ECM – 1378, *Desulfovibrio desulfuricans* ECM – 1799 showed insignificant activity of exogenous lectins. Previously, the ability to synthesize highly exogenous lectins was detected in a significant number of species of aerobic, spore-forming bacteria of the genus *Bacillus* [6], [13]. Our experiments also showed high activity of exogenous lectins of spore-forming strains.

**Table 2: Determination of hemagglutinating activity of exogenous lectins of sulfate-reducing bacteria**

| SRB strains | Culture liquid | |
|---|---|---|
| | Native red blood cells | Trypsinized red blood cells |
| *Desulfovibrio desulfuricans* ECM – 1799 | 31±0.06 | - |
| *Desulfovibrio vulgaris* ECM –1760 | 834±0.74 | 26±0.08 |
| *Desulfovibrio gigas* ECM-1382 | 246±0.5 | 15±0.04 |
| *Desulfobacterium macestii* ECM – 1598 | 16±0.08 | - |
| *Desulfomicrobium baculatus* ECM –1378 | 17±0.02 | - |
| *Desulfotomaculum nigrificans* ECM – 1492 | 1994±2.7 | 15±0.06 |
| *Desulfotomaculum kuznetsovii* ECM – 1805 | 300±0.35 | 18±0.07 |
| *Thermodesulfobacterium mobile* ECM – 1128 | 3657±2.80 | 28±0.06 |
| *Desulfovibrio E-2 spp.* | 872±0.72 | 27±0.06 |
| *Desulfotomaculum P-2 spp.* | 6400±5.0 | 25±0.04 |

The data is expressed as specific activity ($L_{sa}$) shown in units of hemagglutinating activity (GAU), in 1 mg protein in 1 ml sample.

Adding the trypsinized red blood cells to the hemagalutination reaction resulted in a sharp decrease in the activity of lectins and even in complete disappearance in specific cases. Control of the protein content in the culture liquid after adding trypsinized and untreated erythrocytes showed its amount remaining the same. Thus, the reduced activity of exogenous lectins does not result from adding the residual trypsin, but, apparently, is due to the removal of sialic acids from the erythrocyte surface.

One of the important properties of lectins is their ability to react selectively and reversibly with carbohydrates and carbohydrate rich polymers, which gives them a number of unique properties [9].

To determine the carbohydrate specificity of lectins of sulfate-reducing bacteria we used endogenous and exogenous lectins of microbial strains (*Thermodesulfobacterium mobile* ECM – 1128*, Desulfotomaculum nigrificans* ECM – 1492, *Desulfotomaculum kuznetsovii* ECM – 1805, *Desulfovibrio gigas* BKM – 1382), with different hemagglutinating activity. We determined their specificity in relation to a wide range of carbohydrates (Tables 3-4).

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**Table 3: Inhibition of hemagglutination reaction of endogenous lectins and red blood cells with different carbohydrates**

| Inhibitors | Strains of sulfate-reducing bacteria | | | |
|---|---|---|---|---|
| | ECM-1128 | ECM-1382 | ECM-1492 | ECM-1805 |
| D-arabinose | >200 mM | NI | >50 mM | >100 mM |
| D-galactose | >100 mM | >100 mM | >200 mM | >200 mM |
| D-glucose | >200 mM | >50 mM | >100 mM | >200 mM |
| D-lactose | NI | NI | NI | NI |
| D-xylose | NI | NI | NI | >100 mM |
| D-ribose | NI | NI | >200 mM | >25 mM |
| D-cellobiose | NI | >50 mM | NI | NI |
| L-arabinose | >25 mM | >25 mM | >50 mM | >50 mM |
| L-glucose | >6.25 mM | >25 mM | >100 mM | >25 mM |
| Starch | NI | NI | NI | NI |
| Maltose | NI | NI | NI | NI |
| Mannitol | >200 mM | >200 mM | >50 mM | >50 mM |
| Sucrose | NI | NI | NI | NI |
| Fructose | >200 mM | >200 mM | >200 mM | >200 mM |

NI - no inhibition. Data on exogenous and endogenous lectins of 72-hour cultures of sulfate-reducing bacteria.

Test on specific carbohydrate binding of lectins, leading to inhibition of hemagglutination of erythrocytes and lectins by different carbohydrates showed that both endogenous and exogenous lectins of the studied strains of sulfate-reducing bacteria are characterized by the inhibition of agglutination with carbohydrates: D-galactose, D- and L-glucose, arabinose, mannitol at various concentrations, and fructose at a concentration of >200 mM for endogenous lectins and >12.5 mM for exogenous lectins. Lack of agglutination inhibition in endogenous and exogenous lectins of all strains studied was observed when adding a solution of D-lactose, starch and maltose. Sucrose had an inhibitory effect on the agglutination reaction only when added with exogenous lectins of the strain of Thermodesulfobacterium mobile ECM - 1128.

**Table 4: Inhibition of hemagglutination reaction of exogenous lectins and red blood cells with different carbohydrates**

| Inhibitors | Strains of sulfate-reducing bacteria | | | |
|---|---|---|---|---|
| | 1128 | 1382 | 1492 | 1805 |
| D-arabinose | >6.25 mM | NI | >50 mM | >100 mM |
| D-galactose | >200 mM | >100 mM | >200 mM | >200 mM |
| D-glucose | >200 mM | >50 mM | >100 mM | >200 mM |
| D-lactose | NI | NI | NI | NI |
| D-xylose | NI | NI | NI | >100 mM |
| D-ribose | NI | NI | >200 mM | >25 mM |
| D-cellobiose | NI | >50 mM | NI | NI |
| L-arabinose | >200 mM | >200 mM | >100 mM | >100 mM |
| L-glucose | >12.5 mM | >25 mM | >100 mM | >25 mM |
| Starch | NI | NI | NI | NI |
| Maltose | NI | NI | NI | NI |
| Mannitol | >100 mM | >200 mM | >100 mM | >100 mM |
| Sucrose | >3.125 mM | NI | NI | NI |
| Fructose | >12.5 mM | >12.5 mM | >12.5 mM | >12.5 mM |

NI - no inhibition. Data on exogenous and endogenous lectins of 72-hour cultures of sulfate-reducing bacteria.

Lectins of *Desulfotomaculum kuznetsovii* ECM - 1805 also have the specificity to D-xylose and D-ribose. However, the inhibition of agglutination with xylose is typical only of this strain.

Specific inhibition of agglutination was also observed in the strain of *Desulfovibrio gigas* ECM - 1382 when adding a solution of D-cellobiose, and no reaction was observed when adding D-arabinose.

Adding a solution of D-ribose resulted in agglutination inhibition in strains of *Desulfotomaculum nigrificans* ECM - 1492, *Desulfotomaculum kuznetsovii* ECM - 1805, while other strains remain unaffected by this carbohydrate.

Comparative characteristics of the carbohydrate specificity of endogenous and exogenous lectins of specific strains of sulfate-reducing bacteria showed their ability to interact with the same carbohydrates, which is the main difference in the concentration of carbohydrates that block the interaction between lectins and red blood cells.

## CONCLUSION

Screening of 10 species of sulfate-reducing bacteria, including strains of 8 museum strains and 2 strains isolated from natural sources (oil) showed that all of them are able to synthesize endo- and exo-lectins, wherein the activity of exogenous lectins was significantly higher for most species of sulfate-reducing bacteria than the activity of lectins isolated from cell lysates. The most active endogenous and exogenous lectins were obtained when cultivating the strains of Desulfotomaculum nigrificans ECM - 1492 and Thermodesulfobacterium mobile ECM1128. A common property of these strains is their resistance to high temperatures [24], therefore we may assume that the thermophilic and thermotolerant strains are able to synthesize more active lectins compared to strains that grow at low temperatures.

We obtained interesting results for a strain of Desulfotomaculum P-2 spp., isolated from a natural source. This microorganism synthesized exogenous lectins with the highest specific activity (6400±5.0 units), but the activity of its endogenous lectins was at the same level as the activity of other strains. The ability of this strain to synthesize extracellular active lectins is consistent with the findings that the natural strains often have higher activity of lectins than the museum strains [13].

One of the methods for increasing the sensitivity of the hemagglutination of lectins and red blood cells and, consequently, their activity is the trypsinization of the latter. In our experiments we found an increase in the activity of endogenous lectins in 2-8 times in most strains of sulfate-reducing bacteria. We may assume that the increased activity of endogenous lectins is associated with the expansion of open areas on the surface of red blood cells containing receptors for their interaction. However, the activity of exogenous lectins for this treatment did not increase, but decreased sharply. As we know from the literature, the trypsinization of red blood cells reduces the content of sialic acids on their surface [1], [10], apparently, these compounds are a part of active receptors of exogenous lectins of most sulfate-reducing bacteria.

The main property of lectins is their ability to react selectively and reversibly with carbohydrates and carbohydrate rich polymers, without changing their structure. Our experiments showed that lectins of sulfate-reducing bacteria have carbohydrate species specificity. Thus, carbohydrates such as sucrose, xylose, ribose, arabinose and cellobiose have blocked haemagglutination reaction of lectins only in certain strains of microorganisms. At the same time, carbohydrates such as D-galactose, D- and L-glucose, arabinose, mannitol and fructose have inhibited haemagglutination reaction of lectins and red blood cells, however, at different concentrations of carbohydrates, which indicates the possibility of common ways of biosynthesis of lectins in different species of sulfate-reducing bacteria. This assumption is also confirmed by the carbohydrate specificity of endogenous and exogenous lectins, which hemagglutination reactions are blocked with the same carbohydrates but at different degrees of activity.

## SUMMARY

Screening of sulfate-reducing bacteria showed that these anaerobic organisms can be a source of new, specific lectins required for different practical use, and the strains of *Desulfotomaculum P-2 spp*. and *Thermodesulfobacterium mobile* ECM-1128 are promising biological agents for their production.

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