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SPECIFICITY OF LECTINS LABELED WITH COLLOIDAL GOLD TO THE EXOPOLYMERIC MATRIX CARBOHYDRATES OF THE SULFATE-REDUCING BACTERIA BIOFILM FORMED ON STEEL

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The studies of the carbohydrate composition of the sulfate-reducing bacteria (SRB) biofilms formed on the steel surface, which are a factor of microbial corrosion, are significant. Since exopolymers synthesized by bacteria could activate corrosive processes. ***The aim of the study*** *was to investigate the specificity of commercial lectins, labeled with colloidal gold to carbohydrates in the biofilm exopolymeric matrix produced by the corrosive-relevant SRB strains from man-caused ecotopes.* ***Methods.*** *Microbiological methods (obtaining of the SRB biofilms during cultivation in liquid Postgate B media under microaerophilic conditions), biochemical methods (lectin-binding analysis of 10 commercial lectins, labeled with colloidal gold), transmission electron microscopy using JEM-1400 JEOL.* ***Results.*** *It was shown using transmission electron microscopy that the binding of lectins with carbohydrates in the biofilm of the studied SRB strains occurred directly in the exopolymeric matrix, as well as on the surfaces of bacterial cells, as seen by the presence of colloidal gold particles. For detection of the neutral carbohydrates (D-glucose and D-mannose) in the biofilm of almost all studied bacterial strains PSA lectin was the most specific. This lectin binding in biofilms of Desulfotomaculum sp. K1/3 and Desulfovibrio sp. 10 strains was higher in 90.8 % and 94.4 %, respectively, then for ConA lectin. The presence of fucose in the SRB biofilms was detected using LABA lectin, that showed specificity to the biofilm EPS of all the studied strains. LBA lectin was the most specific to N-acetyl-D-galactosamine for determination of amino sugars in the biofilm. The amount of this lectin binding in D. vulgaris DSM644 biofilm was 30.3, 10.1 and 9.3 times higher than SBA, SNA and PNA lectins, respectively. STA, LVA and WGA lectins were used to detect the N-acetyl-D-glucosamine and sialic acid in the biofilm. WGA lectin showed specificity to N-acetyl-D-glucosamine in the biofilm of all the studied SRB; maximum number of bounded colloidal gold particles (175 particles/µm²) was found in the Desulfotomaculum sp. TC3 biofilm. STA lectin was interacted most actively with N-acetyl-D-glucosamine in Desulfotomaculum sp. TC3 and Desulfomicrobium sp. TC4 biofilms. The number of bounded colloidal gold particles was in 9.2 and 7.4 times higher, respectively, than using LVA lectin. The lowest binding of colloidal gold particles was observed for LVA lectin.* ***Conclusions.*** *It was identified the individual specificity of the 10 commercial lectins to the carbohydrates of biofilm matrix on the steel surface, produced by SRB. It was estimated that lectins with identical carbohydrates specificity had variation in binding to the biofilm carbohydrates of different SRB strains. Establishing of the lectin range selected for each culture lead to the reduction of the scope of studies and labor time in the researching of the peculiarities of exopolymeric matrix composition of biofilms formed by corrosive-relevant SRB.*

Keywords: lectins, labeled with colloidal gold, exopolymeric matrix, sulfate-reducing bacteria, biofilm.

Recently, a great importance is given to the study of microbial biofilms, since the basic processes take place inside them [1, 2]. Exopolysaccharides (EPS) produced by bacteria in the exopolymeric matrix play a key role in biofilm formation [3, 4]. Neutral carbohydrates, as well as uronic acids and aminosaccharides, which interact with carboxyl, amino groups and other components of the matrix and participate in the formation of the biofilm structure were identified in the EPS [5, 6]. Biofilms formed by sulfate-reducing bacteria (SRB) on the steel surface are factors of microbial corrosion [7, 8]. Exopolysaccharides produced by bacteria in the biofilm, due to their polyanionic properties, are able to bind metal ions and sulphides in the matrix, thus activating the corrosion process [8]. Therefore,

a wide range study of the carbohydrate composition of the exopolymeric matrix of biofilms by various methods is relevant.

Currently, along with biochemical also electron microscopy methods are used to study the properties of bacterial biofilms: fluorescent, laser confocal scanning and transmission microscopy using labeled lectins. The feature of lectins to appear the maximum affinity to oligosaccharides of a strictly defined structure makes it possible to identify carbohydrate components produced by bacteria during the biofilm formation. Using lectin-binding analysis for the estimating the biochemical characteristics of biofilms, it is necessary to know the spectrum of carbohydrate binding of a certain lectin i.e. its specificity. However, standard lectins (from the catalog) are tested by the manufacturer only for certain carbohydrates that are important for cell biological research, but are not necessarily present in exopolymers of natural biofilms [9].

The most common method is the fluorescent lectin-binding analysis. Lectins labeled with fluorescent dyes in combination with confocal laser scanning microscopy are used as markers for studying the spatial localization of glycopolymers produced *in situ* in model biofilms [10–13]. Using these studies, it is possible to evaluate exopolysaccharide-specific glycoconjugates and even differentiate several of their types [14]. However, this method is not always available. So, we previously studied the possibility of detecting and identifying carbohydrates in the EPS of the biofilm exopolymeric matrix using transmission electron microscopy with lectins labeled with colloidal gold [15]. Using this method, carbohydrate components of biofilms formed by mono- and associative bacterial cultures *Desulfovibrio* sp. 10, *Bacillus subtilis* 36, *Pseudomonas aeruginosa* 27, isolated from a corrosive microbial community were studied. Neutral carbohydrates (D-glucose, D-mannose) and aminosaccharides (N-acetyl-D-glucosamine, N-acetyl-D-galactosamine) were detected in the monosaccharide composition of exopolymers. It has been shown that limensis beans agglutinin (LBA) lectin is most specific for detecting N-acetyl-D-galactosamine in *Desulfovibrio* sp. 10 and *B. subtilis* 36 biofilms and wheat germ agglutinin (WGA) lectin is for detecting of N-acetyl-D-glucosamine in *B. subtilis* 36 and *P. aeruginosa* 27 biofilms. *Pisum sativum* agglutinin (PSA) lectin was the most specific for visualization of neutral carbohydrates in the biofilms of the studied strains [15].

We also determined the composition of the biofilm exopolymer matrix of the above mentioned mono- and associative bacterial cultures by gas-liquid chromatography. In the monosaccharide composition of EPS biofilms formed by the sulfidogenic microbial association and monocultures of *P. aeruginosa* 27, *B. subtilis* 36, *Desulfovibrio* sp. 10 were found neutral carbohydrates (glucose, galactose, mannose, rhamnose, fucose, xylose), uronic acids (galacturonic and glucuronic), as well as aminosaccharides (galactosamine and glucosamine) [16]. Therefore, the results of biochemical studies were comparable to the data of lectin-binding analysis. Thus, it was proved that lectins labeled with colloidal gold can be used as a rapid method for identifying and localizing of carbohydrates in the glycopolymers of biofilms matrix of corrosive-relevant bacteria.

The composition of carbohydrates in the exopolymeric matrix of a biofilm formed on steel by various SRB strains has not been studied. Given the above, the aim of our work was to determine the specificity of a range of commercial lectins labeled with colloidal gold relative to carbohydrates produced in the exopolymeric matrix of the biofilms formed by corrosive-relevant SRB individual strains, isolated from man-caused ecotopes.

Materials and methods. In this study we used the collection SRB strains that we have previously isolated and identified from various man-caused ecotopes: gas pipelines, city heating systems and reinforced concrete structures. *Desulfovibrio desulfuricans* DSM642 (UCM B-11501), *Desulfovibrio vulgaris* DSM644 (UCM B-11502) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) collection, *Desulfovibrio* sp. 10 (UCM B-11503) [17], *Desulfovibrio* sp. TC2 (UCM B-11504), *Desulfotomaculum* sp. TC3 (UCM B-11505), *Desulfomicrobium* sp. TC4 (UCM B-11506) [18] and *Desulfovibrio* sp. K2, *Desulfotomaculum* sp. K1/3 [19] were isolated and stored in Ukrainian Collection of Microorganisms (UCM) and in the collection of the Department of General and Soil Microbiology of the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine.

Cultivation methods. Bacterial cultures were grown in 50 mL flasks in liquid Postgate B medium [16] inoculated with SRB strains in the log-phase of growth. The amount of inoculation material was 10 % vol/vol, the initial bacterial cells titer was 10^7 cells/mL. The determination of bacterial amount were performed by the method of serial dilutions

in liquid Postgate B medium with subsequent calculation (in cells per mL) using of the MacCrady tables. A biofilm was formed on the low-carbon steel samples from St-3 brand. Treatment of steel samples were carried out according to the technique described in [16], then the samples were put in flasks with inoculated nutrient medium and closed hermetically with rubber plugs. Cultures were incubated at 28° C for 10 days. The data are shown for three independent experiments.

Commercial preparations of lectins labeled with colloidal gold ("Lectintest", Lviv), used in the study, are shown in Table 1.

The lectins were selected according to a preliminary biochemical and electron microscopic studies [15, 16], as well as using literature data [4–6, 9]. The lectins were used as sol containing 0.02 % of sodium azide, 10–20 % of ethylene glycol in 0.01 M phosphate buffer solution (pH 6.5–8.5) as a preservatives. The size of the colloidal gold particles was 8-12 nm. The lectin concentration was A_{520} = = 5.0±0.2. Lectin solutions were prepared according to the manufacturer's recommendations.

Preparation of samples for electron microscopy. Biofilm samples for electron microscopy were obtained by the print method [15]. After exposure, steel samples on the surface of which a bacterial biofilm was formed were removed from the flasks. Next, copper grids for electron microscopy covered with a formvar film (Sigma-Aldrich, USA) were put on the bacterial biofilm surface. After 1 minute of exposure, the grids with the biofilm prints were carefully taken and dried in the air. The grids with the samples of biofilms were covered with corresponding lectin (labeled with colloidal gold) solution and kept for 1 hour for lectin binding with carbohydrates of the exopolymeric matrix. After exposure, the grids were washed three times with distilled water to avoid traces of lectin solutions and dried.

Microscopic studies. The prepared samples were studied using the transmission electron microscope JEM-1400 ("JEOL", Japan) on the basis of the Center for Collective Use of Devices of the National Academy of Sciences of Ukraine. The research was conducted at an acceleration voltage of 80 kV and total instrumental magnification of 3000–12000 times. Using the randomized selection method no less than 20 fields of vision were viewed. The number of colloidal gold particles associated with carbohydrate components of the exopolymeric matrix of the biofilm was calculated on 1 μm^2 of the field of view using the Image J ver. 143u program (http://rsb.info.nih.gov./ij).

Statistical processing of the obtained results was performed using the standard deviation for amount of bonded colloidal gold particles in the field of view using the MSExel 2010 program.

Results. It was shown using transmission electron microscopy that the binding of lectins with carbohydrates in the biofilm of the studied bacterial cultures occurred directly in the exopolymeric matrix, as well as on the surfaces of bacterial cells, as seen by the presence of colloidal gold particles. Lectins labeled with colloidal gold were visualized in the images as small rounded electron-dense particles of 8–12 nm size (Fig. 1).

To determine neutral carbohydrates in the biofilm, we used specific labeled lectins: pea lectin (PSA) and concanavalin A (ConA) which are specific to D-glucose and D-mannose and golden rain bark lectin (LABA) which is specific to fucose (Fig. 2).

Binding of PSA and ConA lectin gold particles with biofilm components was observed for all the studied SRB cultures. However, when using PSA in comparison with ConA, a higher amount of colloidal gold particles were detected in the biofilm formed by *D. desulfuricans* DSM642, *Desulfovibrio* sp. 10, *Desulfovibrio* sp. TC2, *Desulfovibrio* sp. K2, *Desulfotomaculum* sp. K1/3, *Desulfomicrobium* sp. TC4 strains. Thus, the maximum number of colloidal gold particles of PSA lectin bound in the *Desulfovibrio* sp. 10 and *Desulfotomaculum* sp. K1/3 biofilms was 94.4 and 90.8 % higher, respectively, when using ConA lectin. On the other hand, in *D. vulgaris* DSM644 and *Desulfotomaculum* sp. TC3 biofilms, the amount of ConA bound lectin with D-glucose and D-mannose compared to PSA was 47.3 and 37.1 % higher, respectively (Fig. 2).

The presence of fucose in the SRB biofilm was detected using LABA lectin. This lectin showed specificity to the biofilm EPS of all the studied SRB strains, but more amounts of bounded colloidal gold particles (35–44 particles/μm^2) were detected in the biofilms of *D. vulgaris* DSM644, *Desulfovibrio* sp. 10, *Desulfovibrio* sp. K2 and *Desulfotomaculum* sp. TC3. Only 11–16 colloidal gold particles per μm^2 associated with LABA lectin fucose were found in the biofilm of the other SRB strains.

Labeled lectins of beans (LBA), soy (SBA), peanuts (PNA), and black elderberry (SNA) with appropriate carbohydrate specificity were used to determine amino sugars in the biofilm, in particular N-acetyl-D-galactosamine. Based on the obtained results, LBA lectin was the most acceptable for

Table 1

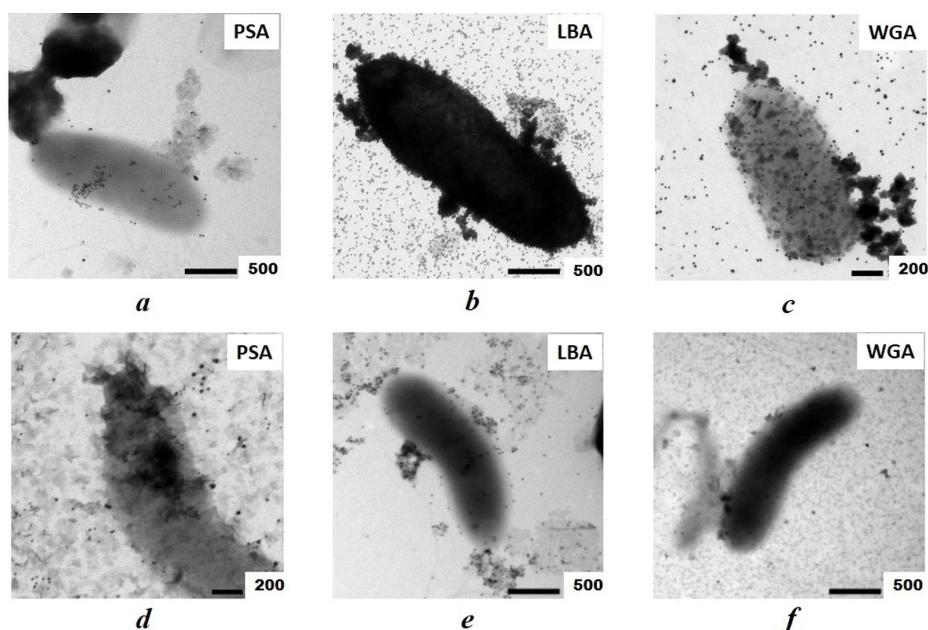
Characteristics of the lectins used in the study (“Lectintest”, Lviv)

Lectin	Lectin source	Name	Carbohydrate specificity	Molecular mass (kDa)	Number of subunits in a molecule	Number of carbohydrate-binding centers
Concanavalin A	Canavalia ensiformis	Con A	α-D-glucose α-D-mannose	102	4a	4
Lectin from peas	Pisum sativum	PSA		48	4ab	–
Lectin from golden rain bark	*Laburnum anagyroides* agglutinin	LABA	L-fucose	106	2a	–
Lectin from soybeans	Glycine max	SBA	N-acetyl-D-galactosamine	110	4a	2
Lectin from peanut	Arachis hypogace	PNA	N-acetyl-D-galactosamine	120	2	2
Lectin from beans	Phaseolus limensis	LBA		127	4	–
Lectin from sambucus nigra	*Sambucus nigra* agglutinin	SNA	N-acetyl-D-galactosamine	115	4	2
Lectin from wheat germs	Triticum vulgaris	WGA	N-acetyl-D-glucosamine, sialic acid	36	2a	2
Lectin from spring white flower	Leucoju svernus	LVA	N-acetyl-D-glucosamine	48	2a2b	2
Lectin from potatoes	Solanum tuberosum	STA		105	2	–

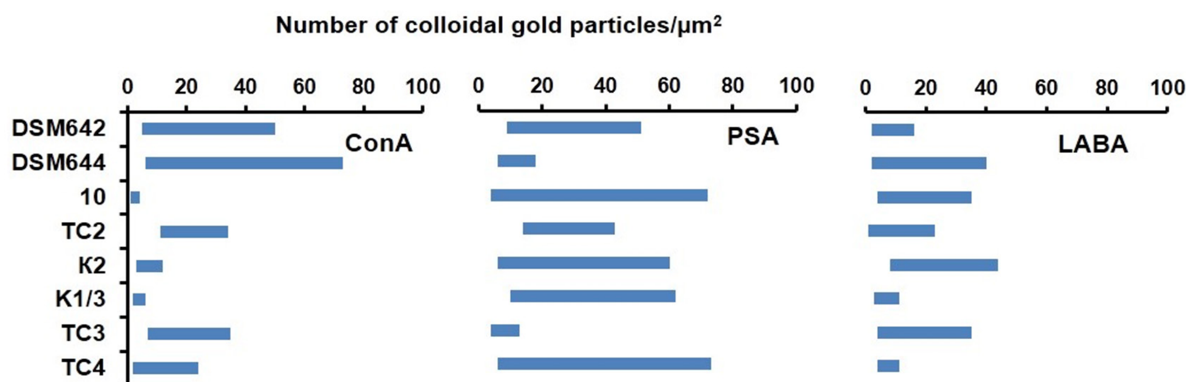
Legends: “– “no data available.

detecting N-acetyl-D-galactosamine in the SRB's biofilm, as evidenced by the presence of bounded colloidal gold particles (Table 2).

The amount of the above-mentioned lectin associated with N-acetyl-D-galactosamine significantly prevailed in the biofilm of all the studied SRB cultures excluding *D. desulfuricans* DSM642, where LBA binding to carbohydrates was not detected. The maximum number of colloidal gold particles of LBA lectin was detected in *D. vulgaris* DSM644 biofilm – in 30.3, 10.1 and 9.3 times more compare to SBA, SNA and PNA lectins, respectively. In *Desulfotomaculum* sp. TC3 biofilm also detected a significant binding of LBA lectin, which was in 2.9, 7.6 and 12.5 times higher compared to SBA, SNA, and PNA lectins, respectively. These lectins are more applicable for detecting N-acetyl-D-galactosamine in *D. desulfuricans* DSM642 biofilm. However, the number of bounded colloidal gold particles in this biofilm was insignificant (15–21 particles/μm^2). It is possible that *D. desulfuricans* DSM642 strain produce a low amount of N-acetyl-D-galactosamine in the biofilm. For *Desulfovibrio* sp. TC2 strain the most specific for detecting of the N-acetyl-D-galactosamine was SBA lectin, which showed 25.0 and 33.3 % more attachments to biofilm glycoconjugants, than LBA and PNA lectins, respectively.



F i g. 1. Binding of lectins with carbohydrates in *Desulfovibrio* sp. 10 (a, b, c) and *D. vulgaris* DSM644 (d, e, f) biofilms: the length of the scale bar a, b, e, f is 500 nm; c, d is 200 nm



F i g. 2. Binding of lectins specific to neutral monosaccharides (glucose, manose, fucose) in SRB biofilms: DSM642 – *D. desulfuricans* DSM642; DSM644 – *D. vulgaris* DSM644; 10 – *Desulfovibrio* sp. 10; TC2 – *Desulfovibrio* sp. TC2; K2 – *Desulfovibrio* sp. K2; K1/3 – *Desulfotomaculum* sp. K1/3; TC3 – *Desulfotomaculum* sp. TC3; TC4 – *Desulfomicrobium* sp. TC4

Table 2

Binding of lectins with aminosaccharides in SRB biofilms formed on steel

Bacterial culture	Number of bounded colloidal gold particles (min – max), particles/μm^2						
	N-acetyl-D-galactosamine				N-acetyl-D-glucosamine		
	SBA	PNA	LBA	SNA	WGA	LVA	STA
D. desulfuricans DSM642	3–20	12–15	–	3–21	13–30	4–30	5–34
D. vulgaris DSM644	4–8	4–26	20–242	3–24	6–26	2–14	6–18
Desulfovibrio sp. 10	2–12	2–36	6–40	4–88	2–42	2–8	2–14
Desulfovibrio sp. TC2	2–36	3–24	7–27	–	9–22	–	–
Desulfovibrio sp. K2	3–8	3–10	25–84	2–10	10–70	3–10	10–29
Desulfotomaculum sp. K1/3	9–39	3–9	14–81	5–25	9–66	2–15	7–36
Desulfotomaculum sp. TC3	5–48	4–11	17–137	2–18	9–175	2–15	6–138
Desulfomicrobium sp. TC4	3–13	8–143	16–81	4–59	1–12	5–21	7–156

Legends: "–"no colloidal gold particles were detected.

To detect the N-acetyl-D-glucosamine and sialic acid in the biofilm, potato (STA), wheat germs (WGA) and spring white flower (LVA) lectins were used. According to the obtained results, these lectins were bound variously with the carbohydrates of the biofilms of the studied SRB strains. WGA lectin showed specificity to N-acetyl-D-glucosamine in the biofilm of all the SRB strains, while in *Desulfovibrio* sp. TC2 biofilm bonded only with WGA lectin but not with LVA and STA lectins. The maximum number of bounded colloidal gold particles (175 particles/μm^2) was found in *Desulfotomaculum* sp. TC3 biofilm. STA lectin was interacted most actively with N-acetyl-D-glucosamine in *Desulfotomaculum* sp. TC3 and *Desulfomicrobium* sp. TC4 biofilms. The number of bounded colloidal gold particles was in 9.2 and 7.4 times higher, respectively, than using LVA lectin. The lowest binding of colloidal gold particles was observed for LVA lectin.

Discussion. Due to transmission electron microscopy, it was shown that the binding of lectins to carbohydrates in the biofilm of the studied bacterial cultures occurred directly in the exopolymeric matrix, as well as on the surfaces of bacterial cells, as seen by the presence of colloidal gold particles. According to the literature [6, 21], the structure of the biofilm widely depends on the content of neutral carbohydrates in the polysaccharides, charged monosaccharide units, i.e. uronic acids (galacturonic, glucuronic), as well as aminosaccharides (galactosamine and glucosamine). Thus, we paid attention to the content of these carbohydrates in the exopolymeric matrix of the SRB biofilms.

Based on the specificity of lectins labeled with colloidal gold, in the biofilm exopolymeric matrix of the studied bacterial strains neutral carbohydrates (D-glucose, D-mannose, and fucose) and aminosaccharides (N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, and sialic acid) were visualized. Studies had shown that lectins with the same carbohydrate specificity differed in the degree of interaction with carbohydrates produced in biofilms formed on steel by different SRB strains.

The most specific for detecting of the D-glucose and D-mannose was PSA lectin by which in *Desulfovibrio* sp. 10, *Desulfotomaculum* sp. K1/3 and *Desulfomicrobium* sp. TC4 biofilms were visualized the significantly higher number of colloidal gold particles compared to ConA lectin. It should be noted that ConA is the most common and widely used lectin in biological research [9]. However, using it for estimating the carbohydrate composition of SRB biofilm exopolymers, we found that ConA lectin was showed less affinity for SRB carbohydrates. Perhaps, there was a few targets exactly for this lectin in the studied bacterial biofilms.

For the detection of N-acetyl-D-galactosamine, LBA lectin was the most specific for all the SRB strains. For example, 30.3 and 10.1 times more bindings of LBA lectin were revealed in *D. vulgaris* DSM644 biofilm compared to SBA and SNA lectins, respectively. The exception was *D. desulfuricans* DSM642 strain, in whose biofilm no binding to LBA lectin was detected.

For the detection of N-acetyl-D-glucosamine in the EPS from biofilm, the most specific was WGA lectin, in contrast to LVA and STA lectins, which showed specificity for all the studied SRB strains.

This points to the need for study the presence of amino saccharides in the biofilm matrix of the SRB with the most specific for them lectins as LBA and WGA lectins.

The obtained results most likely could be explained both by the lectins properties and the structure of the exopolymeric matrix in which carbohydrates were the target in the studied system. Previously, researchers [20, 22, 23] showed that the interaction of lectins with carbohydrates depends on the structure of the latter: from the conformation of the pyranose and furanose forms of the monosaccharide residues comprising the carbohydrate portion of the biopolymers, from the configuration of the monosaccharide anomeric center (D- or L-forms of the pyranose ring), from α- or β-position of the glycoside bond at the C_1-position of the monosaccharide. Therefore, in the interaction of lectins with carbohydrates the most important factors are stereo specificity, the presence or absence of coordinating bonds, hydrogen bonds, as well hydrophobic bonds and Van der Waals interactions. In addition, the authors in the study of glycoconjugates of EPS biofilm *in situ* found using fluorescent lectin-binding analysis that the lectin specificity depends on the applied dye used for lectin labeling. It is shown that the specificity of lectins is affected by both the presence and nature of fluorochrome [10, 11]. During the using of the fluorescent lectin-binding analysis, researchers found that the composition of EPS produced by cyanobacteria contains neutral carbohydrates such as fucose and galactose, aminosaccharides (N-acetyl-D-glucosamine, N-acetyl-D-galactosamine), as well as sialic acids. Using fluorochrome-labeled lectins, it was shown that the biofilm formed by *Deinococcus geothermalis* on stainless steel intensely interacted with WGA lectin, which indicated the presence of N-acetyl-D-glucosamine and N-acetyl sialic acid [13].

Fluorescent lectin-binding analysis is widely used in the analysis of bacterial biofilm glycoconjugates in combination with other fluorochromes, for example, specific for nucleic acids [13, 24]. In addition, their multi-valence provides highly affine binding to the cell surface and biofilm structures containing various glycoconjugates. However, since EPS consists of many types of macromolecules, it is not possible to study their complexity by only one staining method. Even for such glycoconjugates, it is necessary to use several lectin probes [26]. Thus, the structure of EPS biofilms formed on pyrite and elemental sulfur by archaea *Ferroplasma acidiphilum* DSM28986, *Sulfolobus metallus* DSM6482T and *Acidianus* sp. DSM29099 was studied. Using fluorescent lectin binding analysis 75 commercially available lectins were tested. The researchers showed that only 22 lectins bound to archaea strains in the pyrite biofilm, and 21 lectins bound to *Acidianus* sp. DSM29099 in the biofilm on the sulfur surface. Thus, the authors showed the necessity for screening the lectins library to find those that bind with glycoconjugates in a certain biofilm [13, 27, 28]. The authors' conclusion can also be applied to the results of our research. In particular, firstly, we have shown the spectrum of lectins for effective detection of carbohydrates in SRB biofilms. Additional interferences for using of lectin-binding analysis firstly are the complex nature of the matrix of individual bacterial biofilm, the probability of the absence of a target for the used lectin, and the huge variety of potential combinations of carbohydrates. To avoid a number of uncertainties, each biofilm system must be tested with a set of different lectins. At the same time, in contrast to the biochemical method, lectin-binding analysis allows us to study fully hydrated (native) matrixes in mono- and associative cultures of bacterial biofilms to identify the qualitative carbohydrate composition without isolating individual carbohydrate components.

Thus, lectin-binding analysis can be used *in situ* to study the distribution of glycopolymers in microbial communities. Using the carbohydrate specificity of lectins labeled with colloidal gold in the examination of carbohydrates produced by bacteria is the most convenient, affordable and fast test system, but it does not give a complete description of the exopolymeric matrix composition. Our obtained results indicate that during the selection of the lectins for visualization of carbohydrates in the exopolymeric matrix produced by SRB, it is necessary to take into account not only the carbohydrate specificity of lectin, but also based on biochemical studies of exopolymers and features of bacterial cultures. Comparing the results from biochemical studies exopolymeric matrix produced by components of the sulfidogenic microbial community [16] with data of the lectin-binding analysis, we can state that the use of lectins labeled with colloidal gold, can be effective for the initial identification and localization of carbohydrates in the composition of glycopolymers of SRB biofilm exopolymeric matrix. In this aspect, currently available lectins can be a valuable tool for evaluating the

distribution of glycopolymers in complex biofilm systems.

Conclusions. It was identified the individual specificity of 10 commercial lectins to the carbohydrates of biofilm matrix, produced by SRB of different genera on the steel surface. It was estimated that lectins identical in specificity were bound variously to carbohydrates produced in the biofilm of different SRB strains. It was proved that it is necessary to study the matrix of corrosive-relevant SRBs with lectins that are most specific for each bacterial culture. Establishing of the lectin range selected for each SRB culture lead to the reduction of the scope of studies and labor time in the researching of the peculiarities of exopolymeric matrix composition of biofilms formed by corrosive-relevant SRB.

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СПЕЦИФІЧНІСТЬ МІЧЕНИХ КОЛОЇДНИМ ЗОЛОТОМ ЛЕКТИНІВ ДО ВУГЛЕВОДІВ ЕКЗОПОЛІМЕРНОГО МАТРИКСА БІОПЛІВКИ, СФОРМОВАНОЇ НА СТАЛІ СУЛЬФАТВІДНОВЛЮВАЛЬНИМИ БАКТЕРІЯМИ

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Резюме

Дослідження вуглеводного складу біоплівок, сформованих сульфатвідновлювальними бактеріями на сталі, що є факторами мікробної корозії, важливе, оскільки екзополімери, синтезовані бактеріями, здатні активізувати корозійні процеси. **Мета.** Дослідити специфічність ряду комерційних лектинів, мічених колоїдним золотом, відносно вуглеводів, що продукуються в екзополімерному матриксі біоплівки окремими штамами корозійноагресивних сульфатвідновлювальних бактерій, виділених із різних техногенних екотопів. **Методи**. Мікробіологічні (отримання біоплівки на поверхні зразків сталі за культивування сульфатвідновлювальних бактерій у рідкому середовищі Постгейта В за мікроаерофільних умов), біохімічні (лектинзв'язуючий аналіз із використанням 10 комерційних препаратів лектинів, мічених колоїдним золотом), трансмісійна електронна мікроскопія за допомогою приладу JEM-1400 JEOL. **Результати**. За використання трансмісійної електронної мікроскопії було показано, що зв'язування лектинів із вуглеводами біоплівок досліджених бактеріальних культур відбувалось безпосередньо як у екзополімерному матриксі, так і на поверхні бактерій, що видно за наявністю часточок колоїдного золота. Для більшості досліджених штамів сульфатвідновлювальних бактерій найбільш специфічним для виявлення в біоплівці нейтральних вуглеводів, зокрема D-глюкози і D-манози, був лектин PSA. Зв'язування цього лектину у біоплівках, утворених штамами *Desulfotomaculum* sp. K1/3 і *Desulfovibrio* sp. 10 було на 90.8 % і 94.4 % вищим, відповідно, аніж за використання лектину ConA. Наявність фукози у біоплівці сульфатвідновлювальних бактерій виявляли за допомогою лектину LABA, який проявляв специфічність до біоплівкових екзополісахаридів усіх досліджених штамів бактерій. Для виявлення у біоплівках аміносахаридів, зокрема N-ацетил-D-галактозаміну, найбільш специфічним виявився лектин LBA, кількість зв'язувань якого в біоплівці *D. vulgaris* DSM644 була у 30.3, 10.1 та 9.3 рази більше порівняно з лектинами SBA, SNA та PNA відповідно. Для виявлення у біоплівці N-ацетил-D-глюкозаміну та сіалової кислоти було використано лектини STA, LVA та WGA. Лектин WGA проявив специфічність до N-ацетил-D-глюкозаміну у біоплівках усіх досліджених штамів бактерій. Максимальне число зв'язаних колоїдних часток золота (175 часток/мкм^2) було виявлено у біоплівці *Desulfotomaculum* sp. TC3. Лектин STA найбільш активно взаємодіяв із N-ацетил-D-глюкозаміном у біоплівках *Desulfotomaculum* sp. TC3 та *Desulfomicrobium* sp. TC4. Кількість зв'язаних часток колоїдного золота була у 9.2 та 7.4 рази вищою, відповідно, аніж за використання лектину LVA. Найменше зв'язування часточок колоїдного золота спостерігали для лектину LVA. **Висновки**. Ідентифіковано індивідуальну специфічність 10 комерційних лектинів до вуглеводів матриксу біоплівок, синтезованих окремими штамами сульфатвідновлювальних бактерій різних видів на поверхні сталі. Встановлено, що ідентичні за специфічністю лектини по-різному зв'язувались

з вуглеводами біоплівки різних штамів сульфатвідновлювальних бактерій. Встановлення спектру лектинів, обраного для кожної культури, дозволяє скоротити об'єм досліджень і робочий час при дослідженнях особливостей і складу екзополімерного матриксу біоплівок, сформованих корозійно агресивними сульфатвідновлювальними бактеріями.

Ключові слова: лектини, мічені колоїдним золотом, біоплівка, сульфатвідновлювальні бактерії, екзополімерний матрикс.

1. Watnick P, Kolter R. Biofilm, city of microbes. J. Bacteriol. 2000; 182(10): 2675–79.
2. Davey ME, O'Toole GA. Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev. 2000; 64(4):847–67.
3. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. Molecules. 2009; 14(7): 2535−54.
4. Flemming HC, Neu TR, Watnick D. The EPS matrix: The "House of biofilm cell". J. Bacteriol. 2007; 189(22):7945−47.
5. Lewandowski Z. Structure and function of bacterial biofilms. Biofilms: Recent Advances in Their Study and Control. (ed L.V. Evans). Harwood: Acad. Publishers. 2000; 21–79.
6. Sutherland IW. Biofilm exopolysaccharides: a strong and sticky framework. Microbiology. 2001; 147(1):3–9.
7. Hamilton WA. Microbially influenced corrosion as a model system for the study of metal microbe interactions: a unifying electron transfer hypothesis. Biofouling. 2003; 19(1):65−76.
8. Beech IW, Summer J. Biocorrosion: towards understanding interactions between biofilms and metals. Current Opinion Biotechnol. 2004; 15(3):181−6.
9. Lahtin V.M. [Lektiny v issledovanii uglevodnoi chasti glycoproteinov I drugih prirodnyh glicoconjugantov]. Biochemistry (Biokhimiya). 1995; 60(2):187–217. Russian.
10. Neu TR, Swerhone DW, Lawrence JR. Assessment of lectin-binding analysis for in situ detection of glycoconjugates in biofilm systems. Microbiology. 2001; 147:299-313.
11. Neu TR, Manz B, Volke F, Dynes JJ, et al. Advanced imaging techniques for assessment of structure, composition and function in biofilm systems. FEMS Microbiol Ecol. 2010; 72(1):1–21.
12. Lawrence JR, Swerhone GDW, Leppard GG, Araki T, Zhang X, West MM, Hitchcock AP. Scanning transmission X-ray, laser scanning, and transmission electron microscopy mapping of exopolymers matrix of microbial biofilms. Appl Environ Microbiol. 2003; 69(9):5543−54.
13. Zippel B, Neu TRB. Characterization of glycoconjugates of extracellular polymeric substances in tufa-associated biofilms by using fluorescence lectin-binding analysis. Appl Environ Microbiol. 2011; 77(2):505–16.
14. Lawrence JR, Swerhone GDW, Neu TR. *In situ* evidence for microdomains in the polymer matrix of bacterial microcolonies. Can J Microbiol. 2007; 53(3):450–458.
15. Purish LM, Asaulenko LG, Abdulina DR, Voychuk SI, Iutynskaya GA. Lectin-binding analysis of the biofilm exopolymeric matrix carbohydrate composition of corrosion-aggressive bacteria. Appl Biochem Microbiol. 2013; 49(5):458–63.
16. Purish LM, Asaulenko LG, Abdulina DR, Vasil'ev VN, Iutinskaya GA. Role of polymer complexes in the formation of biofilms by corrosive bacteria on steel surfaces. Appl Biochem Microbiol. 2012; 48(3):262–9.
17. Asaulenko LG, Abdulina DR, Purish LM. [Taxonomic position of certain representatives of sulphate-reducing corrosive microbial community]. Mikrobiol Z. 2010; 72(4):3–10. Ukrainian.
18. Purish LM, Asaulenko LG, Abdulina DR, Iutinskaia GA. [Biodiversity of sulfate-reducing bacteria growing on objects of heating systems]. Mikrobiol Z. 2014; 76(3):11–17. Russian.
19. Abdulina DR, Purish LM, Iutynska GO. Microbial communities and sulfate-reducing bacteria in soils near main-gas pipeline. Mikrobiol Z. 2018; 80(5):3–14.
20. Elgavish S, Shaanan B. Lectin-carbohydrate interactions: different folds, common recognition principles. Trends Biochem Sci. 1997; 22(12):462–67.

21. Beech I, Zinkevich V, Tapper R, Gubner R, Avci R. Study of interaction of sulphate-reducing bacteria exopolymers with iron using X-ray photoelectron spectroscopy and time-of-flight secondary ionisation mass spectrometry. J Microbiol Methods. 1999; 36(1–2):3–10.
22. Liener IE, Sharon N, Goldstein IJ. The Lectins: properties, functions and applications in biology and medicine. Orlando: Acad Press. 1986; 600 p.
23. Weiss WI, Drickamer K. Structural basis of lectin-carbohydrate recognition. Ann Rev Biochem. 1996; 65:441–473.
24. Castro L, Zhang R, Munoz JA, Gonzalez F, Blazquez ML, Sand W, Ballester A. Characterization of exopolymeric substances (EPS) produced by *Aeromonas hydrophila* under reducing conditions. Biofouling. 2014; 30(4):501–11.
25. Neu TR, Lawrence JR. Advanced techniques for in situ analysis of the biofilm matrix (structure, composition, dynamics) by means of laser scanning microscopy. In Microbial Biofilms: Methods and Protocols, Methods. In Molecular Biology. (ed. Donelli, G.). New York, USA: Springer, 2014:43–64.
26. Neu TR, Kuhlicke U. Fluorescence lectin bar-coding of glycoconjugates in the extracellular matrix of biofilm and bio aggregate forming microorganisms. Microorganisms. 2017; 5(1):1–13.
27. Bennke CM, Neu TR, Fuchs BM, Amann R. Mapping glycoconjugate- mediated interactions of marine *Bacteroidetes* with diatoms. Syst Appl Microbiol. 2013; 36:417–25.
28. Zhang RY, Neu TR, Bellenberg S, et al. Use of lectins to in situ visualize glycoconjugates of extracellular polymeric substances in acidophilic archaeal biofilm. Microb Biotechnol. 2015; 8(3):448–61.

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