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EXPERIMENTAL ARTICLES

On the Hemagglutination Reaction in Methanotrophic Bacteria

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Abstract—The reaction of hemagglutination with trypsin-treated rabbit erythrocytes was used to reveal lectins on the cell surface of methanotrophic bacteria and in their culture liquids. By this method, no lectins were detected on the cell surface of *Methylococcus capsulatus* IMV B-3001 and *Methylomonas rubra* IMV B-3075 or in the culture liquid of any of the species studied. With intact cells of *Methylocystis parvus* IMV B-3491, the positive hemagglutination reaction observed was nonspecific and most probably occurred due to the high cell surface hydrophobicity characteristic of this species.

Key words: methanotrophic bacteria, lectins, hemagglutination reaction, hydrophobicity

Lectins are proteins of a nonimmunoglobulin nature. They specifically recognize and reversibly bind to glycoconjugates or polysaccharides without changing their covalent structure. Lectins exhibit high mitogenic and interferonogenic activity. They can, therefore, find application in medicine, biochemistry, and other fields [1]. Finding new lectin sources would allow researchers to study lectin functions in detail and probably obtain lectins with previously unknown properties.

One of the most important lectin functions is their participation in the process of the specific adherence of microorganisms to plant, animal, and human tissues [1, 2]. This role of lectins is the most prominent in pathogenic microorganisms [3] and in bacteria that establish symbiotic relationships with plants [4].

Unlike other microorganisms, methanotrophic bacteria inhabit, as a rule, mineralized substrates [5–7]. The isolation of methanotrophic bacteria from living organisms and tissues was described in a few reports [8]. Note that lectins were revealed in bacteria of different physiological groups [1]. We supposed, therefore, that methanotrophic bacteria may also contain lectins.

In this work, we examined methanotrophic bacteria for the presence of lectins.

MATERIALS AND METHODS

This study used the methanotrophic bacteria *Methylococcus capsulatus* IMV B-3001, *Methylocystis parvus* IMV B-3491, and *Methylomonas rubra* IMV B-3075 from the culture collection of the Institute of Microbiology and Virology, National Academy of Sciences of Ukraine. Bacteria were cultivated in mineral medium containing (g/l) $K_2HPO_4 \cdot 3H_2O$, 0.25; KH_2PO_4, 0.25; KNP_3, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.02; and NaCl, 0.3. The solution of microelements described by Leadbetter and Foster was also added to the medium [9]. *M. capsulatus* was grown in the presence of 0.5 g/l $(NH_4)_2SO_4$, which served as the nitrogen source.

Bacteria were cultivated in hermetically closed 500-ml flasks containing 50 ml of the medium. The flasks were partially evacuated and refilled with methane to obtain a methane–air ratio of 3 : 7.

The methanotrophic bacteria were grown. Cultivation was performed in a batch mode on a shaker (240 rpm) for 48–72 h at 28°C (*M. parvus* and *M. rubra*) or at 37°C (*M. capsulatus*). The cells were sedimented by centrifugation at 2400 *g* for 30 min, washed three times with 0.15 M phosphate buffer, and resuspended in the same buffer to a certain optical density.

To reveal lectins in the strains studied, the conventional hemagglutination reaction was conducted in standard plates with U-shaped wells [2], using a 2% suspension of rabbit erythrocytes pretreated with trypsin and fixed with glutaraldehyde. The hemagglutination reaction (RH) was scored as RH titer^{-1} from the maximum dilution of the studied cell suspension at which the erythrocyte agglutination was still observed. The reaction was conducted at 20°C; RH titers were determined after the reaction proceeded for 90–120 min.

The carbohydrate specificity of the lectins was determined from the inhibition of their hemagglutinating activity. The minimal concentration of carbohydrate that completely inhibited lectin activity was determined using a series of carbohydrate dilutions [2].

We used the following carbohydrates: D-glucose, L-fucose, glucose-6-phosphate, phosphogluconic acid, D-fructose, turanose, L-altrose, L-arabinose, dulcitol, α-methyl-D-mannitol, D-allose, D-ribose, α-methyl-

Reaction of the hemagglutination (RH) of *M. parvus* IMV B-3491 cells as dependent on the biomass content of the suspension

Biomass, g/l	RH titer^{-1}					
	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64
0.071	–	–	–	–	–	–
0.25	+	–	–	–	–	–
0.41	+	+	–	–	–	–
0.89	+	+	+	–	–	–
2.49	+	+	+	+	–	–
6.02	+	+	+	+	+	+
23.01	–	–	–	–	–	–
CL	–	–	–	–	–	–
Control	–	–	–	–	–	–

Note: "–" denotes negative RH; "+" denotes positive RH. Control wells contained 2% trypsin-treated erythrocytes in buffered physiologic saline. CL denotes culture liquid free of cells.

D-glucoside, L-sorbose, D-gulose, α-D-glucose-1-phosphate (sodium salt), methyl-β-D-glucopyranoside, D-trehalose, L-sorbitol, D-ribulose, D-ribose-5-phosphate, D-ribulose-1,5-diphoshate (sodium salt), α-D-glucose-6-phosphate (sodium salt), D-galactose, D-galactosamine HCl, *N*-acetyl-D-galactosamine, lactose, melibiose, raffinose, L-ribose, D-lyxose, talose, D-glucosamine HCl, D-mannose, maltose, cellobiose, D-arabinose, L-xylose, mannitol, inositol-1-hydrate, sorbitol, L-rhamnose, L-mannose, D-xylose, D-glucuronic acid, D-galacturonic acid, D-gluconic acid (calcium salt), 2-deoxy-D-glucose, α-methyl-D-mannoside, α-glucose-1,6-diphosphate, methyl-α-D-glucopyranoside, and fructose-1,6-diphosphate (calcium salt). The carbohydrates were from Chemapol, Serva, Fluca, Reanal, and Ferak.

The hydrophobicity of the cells of methanotrophic bacteria was assessed from their distribution in the microbial suspension–*n*-hexadecane biphasic system [10].

RESULTS AND DISCUSSION

Intact cells of *M. capsulatus* and *M. rubra* were established to cause no agglutination of trypsin-treated rabbit erythrocytes. In a wide range of cell biomass concentrations in the reaction mixture (from 0.036 to 29.5 g/l), negative RH was observed.

No free lectins unbound to the cell surface were detected in the culture liquids of any of the methanotrophs studied: the culture media of no methanotrophic species caused any agglutination of trypsin-treated rabbit erythrocytes (table).

The cells of *M. parvus*, however, produced a positive hemagglutination reaction (table). Even at a concentration of cell biomass of 0.25 g/l, the RH titer^{-1} was 1 : 2. As the content of the cells increased in the reaction mixture, the RH titer also increased (table).

A conclusion could be inferred from these results that lectins are present on the surface of *M. parvus* cells. However, we found no specificity of these lectins to any carbohydrate. None of the carbohydrates studied inhibited the reaction of hemagglutination induced by *M. parvus* cells. Some other bacteria were also previously reported to exhibit no carbohydrate specificity of the lectins detected on their cells by positive RH [1].

A more detailed analysis of the results obtained revealed that the cells of *M. parvus* formed an umbrella-like pellet both in the control, where erythrocytes were absent, and in the experiment. Thus, it became evident that hemagglutination caused by *M. parvus* was not due to the presence of lectins on the bacterial cell surface but to some other cell-surface properties of this bacterial species.

Comparison of the hydrophobicity of the methanotrophic species studied showed that it was the highest, up to 86.6%, for the *M. parvus* cell surface, whereas the cells of *M. rubra* and *M. capsulatus* exhibited a hydrophobicity of 48.7% and 9.5%, respectively.

The hydrophobicity of microorganisms is known to be one of the most important factors determining their interaction with various objects [11]. Owing to this property, bacteria can flocculate, i.e., bind to each other to form a three-dimensional network structure. When sedimenting, the cells of the reaction mixture could entrap erythrocytes into this network to form a precipitate similar to that characteristic of the reaction of hemagglutination. When the pink-colored cells of another methanotrophic species, *M. rubra,* were introduced into the suspension of *M. parvus* cells, a precipitate typical of the hemagglutination reaction was obtained, although *M. rubra* cells caused no agglutination of rabbit erythrocytes. Hence, the hemagglutinating activity of *M. parvus* observed with trypsin-treated rabbit erythrocytes was due to the high hydrophobicity of the cell surface of this bacterium, which caused cells to flocculate, entrapping erythrocytes: this process cannot be considered indicative of the presence the lectins in this bacterial species. The umbrella-like precipitates with *M. parvus* cells were also obtained when cells of the collection strains of the genus *Bacillus* were added to the reaction mixture instead of rabbit erythrocytes.

Thus, our results indicate that the reaction of hemagglutination observed between intact *M. parvus* cells and trypsin-treated rabbit erythrocytes was nonspecific. The observed positive reaction seems to occur due to hydrophobic interactions between *M. parvus* cells. Various cells, such as erythrocytes or bacterial cells of different species, can be entrapped into the three-dimensional network formed by *M. parvus* cells.

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