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SHORT COMMUNICATIONS

Formation of Aggregated Structures by Luminescent Bacteria in the Presence of Carbohydrates

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Capacity for aggregation, formation of higher-level structures (colonies, biofilms) is a common quality of microorganisms. The process of surface-colonization by bacteria consists of several stages: cell attachment to the surface (adhesion); growth of these cells on the surface with the formation of an attached monolayer; formation and confluence of microcolonies; and formation and functioning of a mature biofilm [1]. The formation of microcolonies by bacteria (in particular, by *Pseudomonas aeruginosa* and *Vibrio cholerae*) is caused by the presence of mannose-sensitive pili of the type IV, and the confluence of microcolonies is caused by intense exopolysaccharide synthesis at this stage of biofilm development. Specific adhesion allows microorganisms to inhabit certain ecotopes according to their requirements. The capacity of bacterial cells for adhesion is caused by the presence of adhesin macromolecules on their surface. Adhesins may be fimbrial (fimbriae, or pili), typical of gram-negative bacteria; or afimbrial, more typical of gram-positive bacteria. Many of the fimbrial adhesins are carbohydrate-binding proteins, i.e. lectins. An assumption was made that lectins participate in intercellular interactions in the course of microcolony and biofilm formation by cultures of luminescent bacteria. The inhibition of microcolony and biofilm formation in the presence of carbohydrates could be an indication of the participation of lectins in these processes. Inhibition of biofilm formation could also occur at the stage of their formation from microcolonies due to the inhibition of the synthesis of exopolysaccharides.

The goal of the present work was to determine the possible participation of lectins in the formation of aggregative communities by luminescent bacteria of the species *Vibrio harveyi*, *Vibrio fischeri*, *Photobacterium phosphoreum*, and *Photobacterium leiognathi*.

The objects of investigation were as follows: 18 cultures of *P. phosphoreum*; 3 of *P. leiognathi*; 3 of *V. fischeri*; and 16 of *V. harveyi*. The strains were obtained from the Luminous Bacteria Collection of the Institute of Biophysics Siberian Division, Russian Academy of Sciences (IBSO 836). The biofilm formation was observed through one week of incubation on the surface of a liquid semisynthetic medium containing (g/l): NaCl, 30; $MgSO_4 \cdot 7H_2O$, 0.2; $KH_2PO_4 \cdot 12H_2O$, 1; Na_2HPO_4, 6; $(NH_4)_2HPO_4$, 0.5; peptone, 5; glycerol, 3 ml; distilled water, up to 1 l. The formation of microcolonies was observed visually and via a binocular loupe through one month of incubation in a semiliquid semisynthetic medium with 2 g/l agar. To investigate the effect of carbohydrates on the formation of aggregative communities, the following carbohydrates (Sigma) were added to the culture media in 1% concentrations: sucrose, fructose, mannose, mannitol, galactose, maltose, glucose, arabinose, lactose, *N*-acetyl-D-galactosamine, and *N*-acetyl-D-glucosamine.

The cells of luminescent bacteria are known carry lectins of different carbohydrate specificity on their surface [2]. Unlike luminescent bacteria *P. leiognathi, V. fischeri*, and *V. harveyi*, bacteria of the species *P. phosphoreum* form granules both under natural (luminescent organs of marine animals) and simulated conditions (microcolonies in semiliquid agarized media) [3]. The effect of carbohydrates on the formation of microcolonies in semiliquid agarized medium by luminescent bacteria *P. phosphoreum* of different ecological groups was examined (Table 1). All the carbohydrates were found to inhibit microcolony formation by the commensal strain 1883 (diffuse growth was observed on carbohydrate-containing media, while in the control, microcolonies were formed). A low intensity of colony formation was observed during the cultivation of strain 491, isolated from water, and of the symbiotic strain 1856. At the same time, the symbiotic strain 1909 formed microcolonies very actively in all variants of media except for the media with sucrose, galactose, or *N*-acetyl-D-galactosamine. The type strain 1 (NCMB 1282) also actively formed microcolo-

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Table 1. Formation of microcolonies by cultures of *Photobacterium phosphoreum* in the presence of carbohydrates

Carbohydrate	Strain				
	1883 (commensal)	491 (free-living)	1856 (symbiont)	1909 (symbiont)	1 (type)
Control	+	±	+	+	+
Mannose	–	–	+	+	+
Glucose	–	±	–	+	–
Maltose	–	±	–	+	–
Arabinose	–	+	+	+	+
Galactose	–	–	–	–	±
N-acetyl-D-glucosamin	–	±	–	–	+
Sucrose	–	+	±	–	+
Lactose	–	–	–	+	+
Mannitol	–	±	+	+	+
Fructose	–	±	–	+	+

nies, except on the media with maltose, glucose, and galactose. Thus, the inhibition of microcolony formation was most intense in media with galactose; lectins with galactose specificity can therefore be expected to play a role in microcolony formation by luminescent bacteria *P. phosphoreum*.

The formation of biofilms by luminescent bacteria was observed on the surface of the liquid semisynthetic medium. The inhibition of biofilm formation by *N*-acetyl-D-glucosamine, glucose, mannose, galactose was revealed for all the investigated cultures of different ecological groups irrespective of their species (Table 2); the effect of other carbohydrates was strain-specific. The data obtained may indicate the participation of lectins with an appropriate specificity in the process of biofilm formation or the inhibition of the synthesis of polysaccharides of the extracellular matrix by specific carbohydrates in the course of biofilm formation from microcolonies. We have previously suggested that lectins with *N*-acetyl-D-galactosamine specificity take part in the initiation of the symbiosis of luminescent bacteria with marine animals [2]. The inhibition of microcolony formation in media with galactose is evidence of the possible participation of lectins with galactose specificity in microcolony formation (and, perhaps, also in the process of biofilm formation) by luminescent bacteria *P. phosphoreum*. Since mannose did not inhibit the formation of microcolonies of symbiotic strains, but counteracted the biofilm formation, the participation of mannose-specific lectines can be hypothesized at the stage of formation of biofilms from microcolonies. Thus, lectins with various carbohydrate spec-

Table 2. The formation of biofilms by luminescent bacteria in the presence of carbohydrates

Carbohydrates	Free-living								Commensals			Symbiont
	P. leiognathi			*V. fischeri*	*V. harveyi*			*P. phosphoreum*	*V. fischeri*		*V. harveyi*	*P. phosphoreum*
	54	208	554	1231	1212	72	178	491	2089	1920	1175	1909
Mannose	–	–	±	–	–	–	–	–	–	–	–	±
Glucose	–	–	–	–	–	–	–	–	–	–	–	±
Maltose	+	+	+	+	+	–	–	+	–	–	+	–
Arabinose	+	+	–	–	–	+	+	–	–	–	+	No growth
Galactose	–	±	–	–	–	±	–	–	–	–	–	–
N-acetyl-D-glucosamine	–	±	–	–	–	–	–	–	–	–	–	±
Sucrose	+	+	+	+	+	+	+	+	–	–	+	±
Lactose	+	+	+	+	+	+	+	+	–	–	+	–
Mannitol	+	+	+	+	+	+	–	+	–	–	+	+
No growth	±	±	–	–	–	–	–	+	–	–	–	–

ificity probably take part not only in the formation of aggregated communities of luminescent bacteria, but also in the different stages of the colonization of the luminescent organs of marine animals by luminescent bacteria *P. phosphoreum*.

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