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EXPERIMENTAL ARTICLES

Role of the Agglutinating Proteins of Bacilli and Rhizobia in Bacterial Interactions

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Abstract—The surface agglutinating proteins of the soil nitrogen-fixing bacteria *Bacillus polymyxa* 1460 and *Rhizobium leguminosarum* 252 were found to be able to interact with the polysaccharide complexes of certain azospirilla and rhizobia. Such interactions are most likely involved in the formation of nitrogen-fixing plant-bacterial associations in the rhizosphere.

Key words: agglutinating proteins, polysaccharides, nitrogen-fixing bacteria, interaction.

Data available in the literature on bacterial interactions are scarce and primarily deal with intrageneric interactions, such as the flocculation of rhizobia [1] and the aggregation of agrobacteria and bradyrhizobia on the root surface [2, 3]. At the same time, such data are of interest, especially as concerned with the nitrogen-fixing rhizosphere bacteria and with the molecular mechanisms underlying bacterial cell recognition. Little is known about the interaction of microorganisms in mixed cultures, including nitrogen-fixing associations, although it would be reasonable to suggest that the surface components of bacterial cells, particularly lectins and polysaccharides, are involved in such interaction.

The aim of the present work was to study the role of the surface agglutinating proteins of soil nitrogen-fixing bacilli and rhizobia in bacterial interactions.

MATERIALS AND METHODS

Experiments were performed with the lectins LI and LII of *Bacillus polymyxa* 1460 and the agglutinins R_1 and R_2 *Rhizobium leguminosarum* 252 of *Rhizobium leguminosarum* 252, which were stripped from the surface of bacterial cells as described earlier [4, 5]. The polysaccharide–lipid complex (PSLC) and lipopolysaccharide–protein complex (LPPC) of *Azospirillum brasilense* strains Sp7, Sp245, SR75, and S17 and *Azospirillum lipoferum* strains 59b and RG20a were isolated from the capsular material of these strains using chromatographic methods [6]. The exopolysaccharide (EPS) and lipopolysaccharide (LPS) of *R. leguminosarum* 252 were obtained from L.V. Kosenko, Zabolotnyi Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine in Kiev.

The monosugar composition of the polysaccharide-containing complexes of azospirilla was analyzed by thin-layer chromatography (TLC) on DC-Alufolien cellulose plates in a pyridine–ethylacetate–acetic acid–water (5 : 5 : 1 : 3) solvent system. Separated monosugars were visualized by spraying the developed plates with a solution of anisidine phthalate in butanol. Samples for TLC were prepared by hydrolyzing polysaccharide complexes in 4 N trifluoroacetic acid (TFA) at 100°C for 4 h in sealed ampules; the TFA was then removed with a rotary vacuum pump [7].

The interaction of agglutinating proteins with the polysaccharide complexes was studied by the blotting technique [8], detecting immune complexes on nitrocellulose membranes with the protein A–gold colloid conjugate manufactured at the Institute of Biochemistry and Physiology of Plants and Microorganisms. The concentration of bacillary lectins and rhizobial agglutinins was 40 μg/ml and that of the polysaccharide complexes was 1 mg/ml. Positive reaction manifested itself as a pink spot on a nitrocellulose membrane.

The specificity of interaction was studied by incubating bacillary lectins (40 μg/ml; 28°C; 30 min) with one of the specific haptens (such as 0.3 M glucuronic acid) and by incubating rhizobial agglutinins (40 μg/ml) with their antibodies. The blocking of hemagglutinating activity was evaluated by assaying this activity with trypsinized rabbit erythrocytes.

Antibodies to the lectins LI and LII of *B. polymyxa* 1460 and the agglutinins R_1 and R_2 of *R. leguminosarum* 252 were prepared as described earlier [4, 5].

RESULTS AND DISCUSSION

As was shown in our previous works [4, 5, 9–11], the lectins of *B. polymyxa* 1460 and *R. leguminosarum* 252 are involved in the attachment of bacterial cells to host plants, binding to plant receptors of a protein or carbohydrate nature. The role of bacterial lectins in

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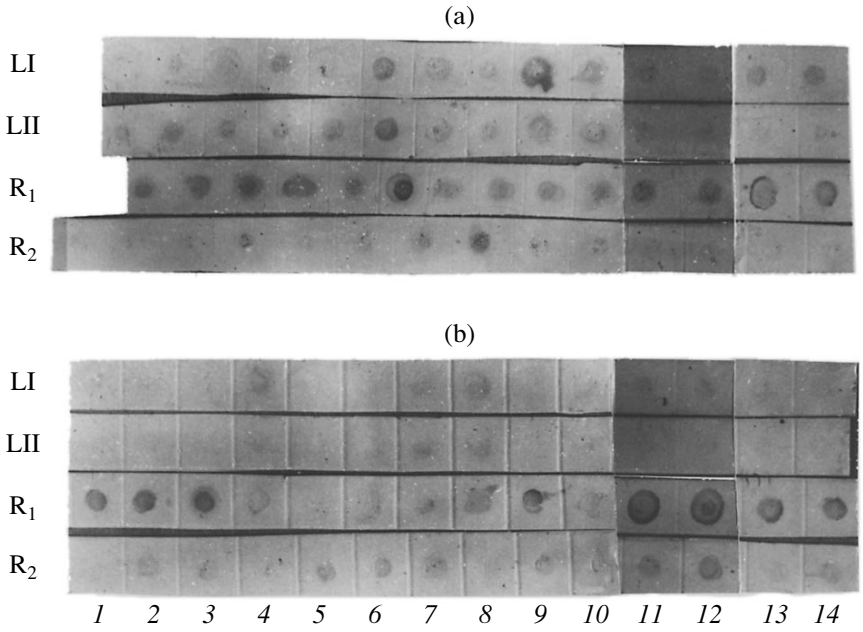

Fig. 1. Dot immunoblotting analysis of the interaction of the *B. polymyxa* 1460 lectins and *R. leguminosarum* 252 agglutinins with the polysaccharide-containing complexes of soil bacteria: (a) native lectins and agglutinins; (b) bacillary lectins blocked with glucuronic acid and rhizobial agglutinins blocked with antibodies; (*1*, *2*, *3*, and *4*) PSLC; (*5*, *6*, *7*, and *8*) the LPPC of *A. brasilense* Sp7, SR75, Sp245, and S17; (*9* and *10*) the EPS and LPS of *R. leguminosarum* 252; (*11* and *12*) the PSLC and LPPC of *A. lipoferum* 59b; and (*13* and *14*) the PSLC and LPPC of *A. lipoferum* RG20a.

intercellular interactions was not studied in depth, although there is evidence that they may be involved in microbial flocculation [12]. In the present work, we found that the bacillary lectins and rhizobial agglutinins are able to recognize receptors in some bacterial polysaccharide complexes: lectin LI, which is specific for glucuronic acid and fructose-1,6-diphosphate, and lectin LII, which is also specific for galacto- and glucosamine [4], interacted with all of the studied azospirillary PSLC and LPPC and rhizobial EPS and LPS.

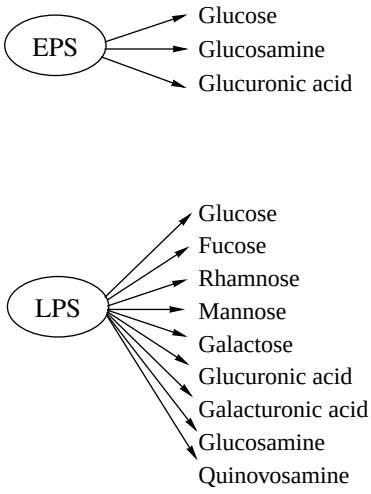

Fig. 2. Monosugar composition of the surface polysaccharides of *R. leguminosarum* 252.

Similarly, the rhizobial agglutinins interacted with both azospirillary and rhizobial polysaccharide complexes; in this case, the agglutinin R_2 was much more active than the agglutinin R_1 (Fig. 1).

The blocking of the agglutinating activity of lectin LI with glucuronic acid partially inhibited its interaction with the PSLC and LPPC of *A. brasilense* Sp7, SR75, and Sp245 and *A. lipoferum* 59b and RG20a and with the EPS and LPS of *R. leguminosarum* 252, as follows from the decrease in the intensity of the pink color of the spots on the nitrocellulose filters (Fig. 1). The incomplete inhibition of the interaction is indicative of the involvement of specific and nonspecific mechanisms. The specific interaction of lectin LI with the rhizobial EPS and LPS can be due to the presence of glucuronic acid in these complexes [13] (Fig. 2). The specific interaction of lectin LI with the azospirillary polysaccharide complexes, which lack glucuronic acid and fructose-1,6-diphosphate (see table), can be explained by its interaction with 2-keto-3-deoxyoctonate (KDO) present in these complexes [6]. A similar effect was observed by Yagoda-Shagam *et al.* [14], who found that the sialic acid–specific lectin is able to interact with the capsular material of azospirilla, although this material lacks sialic acid. On the other hand, lectin LI was unable to interact with the polysaccharides of *A. brasilense* S17, presumably due to the fact that these polysaccharides lack carbohydrates specific for this lectin (see table). It should be noted that the polysaccharides of this strain differ from the polysaccharides

Monosugar composition of the polysaccharide complexes of azospirilla

Strain		Complex	Monosugar								
			Rhamnose	Galactose	Fucose	Xylose	Mannose	Glucosamine	Galactosamine	Galacturonic acid	Glucose
A. brasilense	Sp7	LPPC	+	+	+			+	+	+	
		PSLC	+	+	+			+		+	
	Sp245	LPPC	+	+		+		+	+	+	
		PSLC	+	+		+		+	+	+	
	SR75	LPPC	+	+				+	+	+	
		PSLC	+	+				+	+	+	
	S17	LPPC	+				+	+	+	+	
		PSLC	+	+			+	+		+	
A. lipoferum	RG20a	LPPC	+	+				+	+		
		PSLC		+	+			+			
	59b	LPPC	+					+	+		+
		PSLC	+						+		+

Note: The "+" sign indicates the presence of the particular monosugar.

of the other *A. brasilense* strains studied in the high mannose content (see table).

The absence of the formation of pink spots on nitrocellulose membranes as a result of the incubation of the glucuronic acid–treated lectin LII with the PSLC of *A. brasilense* Sp245 and the LPPC of *A. brasilense* Sp7 is indicative of the specificity of the interaction of lectin LII with these polysaccharide complexes. This specific interaction may be due to glucosamine and galactosamine present in the PSLC (see table). On the other hand, lectin LII was unable to interact with the PSLC and LPPC of *A. brasilense* S17, although these complexes contain galacto- and glucosamine; this could be related to the steric inaccessibility of carbohydrate-binding sites in these complexes. The incubation of lectin LII with the PSLC of *A. brasilense* Sp7, the LPPC and PSLC of *A. brasilense* SR75, the LPPC of *A. brasilense* Sp245, the PSLC and LPPC of *A. lipoferum* 59b and RG20a, and the EPS and LPS of *R. leguminosarum* 252 produced only faint pink spots on nitrocellulose filters; this suggests that the interaction of lectin LII with these polysaccharide complexes is due to both the specific haptens of these complexes and nonspecific binding (Figs. 1 and 2).

Experiments on the blocking of the agglutinating activity of the rhizobial agglutinin R_1 with the respective antibodies showed that this lectin interacts, both specifically and nonspecifically, with the LPPC of *A. brasilense* Sp7, SR75, and Sp245; the PSLC of *A. brasilense* S17; and rhizobial LPS. The interaction of agglutinin R_1 with the PSLC of *A. brasilense* Sp7, SR75, and Sp245; the EPS of *R. leguminosarum* 252; the LPPC of *A. brasilense* S17; and the PSLC and LPPC of *A. lipoferum* 59b and RG20a was only nonspecific. Data on the interaction of agglutinin R_1 with LPS are in good agreement with our earlier findings that haptens for this agglutinin may represent complex carbohydrates [13].

As for agglutinin R_2, it was able to interact, both specifically and nonspecifically, with the PSLC and LPPC of *A. brasilense* S17 and the LPS of *R. leguminosarum* 252, whereas its interaction with the other polysaccharide complexes was only nonspecific (Fig. 1).

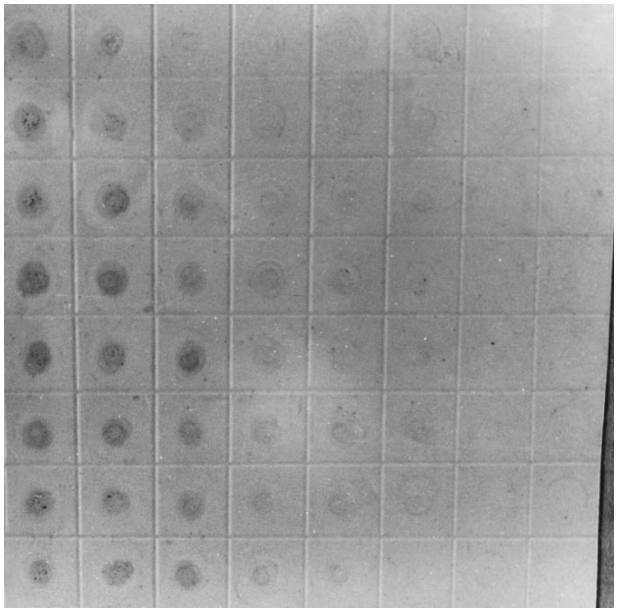

Fig. 3. Interaction of the agglutinin R_1 of *R. leguminosarum* 252 with the LPPC of *A. brasilense* Sp7. The twofold agglutinin dilutions are in the downward direction and the twofold LPPC dilutions are in the right direction.

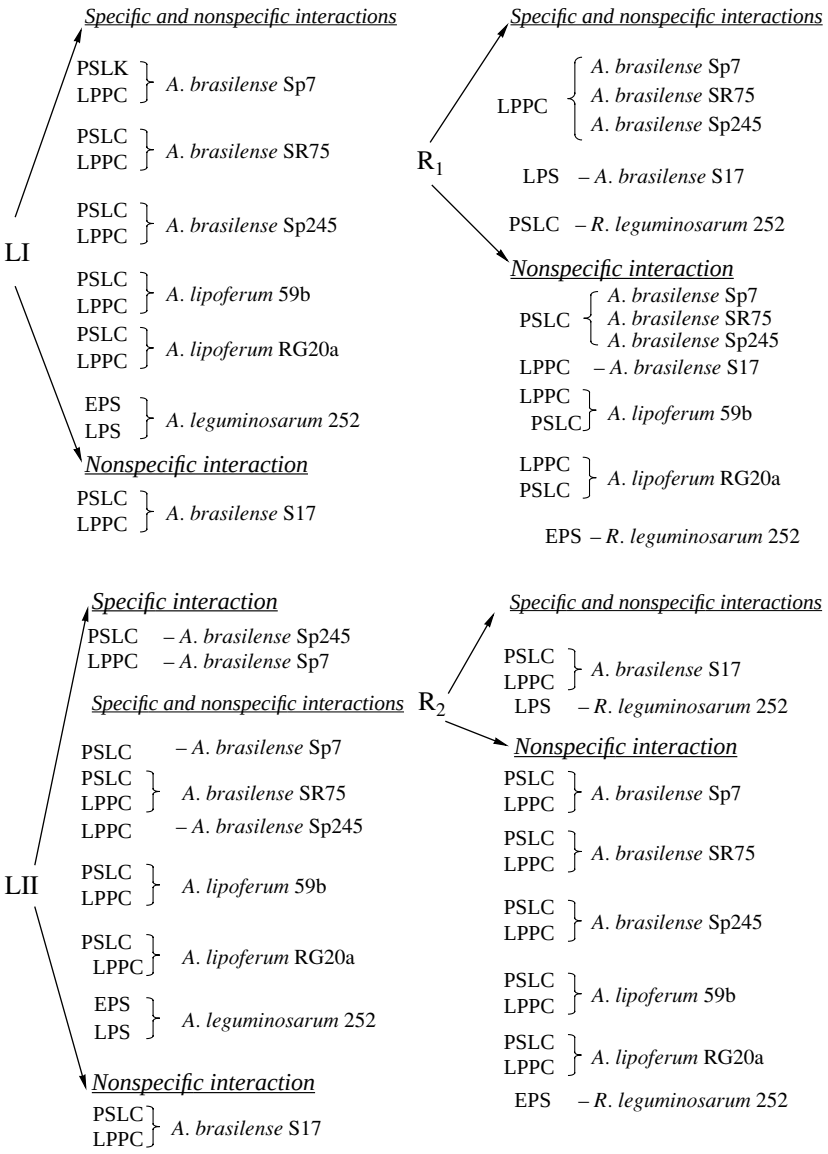

Fig. 4. Schematic representation of the interactions of the *B. polymyxa* 1460 and *R. leguminosarum* 252 lectins with the polysaccharide-containing complexes of azospirilla and rhizobia.

Dot immunoblotting analysis showed (Fig. 3) that the minimum concentration of agglutinin R_1 necessary for its interaction with the LPPC of *A. brasilense* Sp7 is 0.3 μg/ml (the LPPC concentration in this experiment was 67.5 μg/ml).

Figure 4 schematically shows the specific and nonspecific interactions of the *B. polymyxa* 1460 and *R. leguminosarum* 252 lectins with the polysaccharide-containing complexes of rhizobia and azospirilla. The specific interaction of the galactose-specific lectin with polysaccharides was also described by Behhary *et al.* [12] with respect to its involvement in bacterial cell aggregation.

Thus, the surface lectins of soil bacteria can interact with the polysaccharide complexes of other soil bacteria liberated into the medium. Such interactions may be responsible for the formation of nitrogen-fixing bacterial associations in the plant rhizosphere. The blocking of the binding sites of bacterial lectins by the polysaccharide complexes of rhizobia and azospirilla may contribute to their competition for binding sites on the host plant roots.

REFERENCES

1. Kijne, J.W., Schaal van der, A.M., Diaz, C.L., and Iron, F., Mannose-Specific Lectins and the Recognition of Pea Roots by *Rhizobium leguminosarum*, Lectins: Biology, Biochemistry, and *Clinical Biochemistry*, Berlin, 1983, vol. 3, pp. 521–529.
2. Loh, J.T., Ho, S.C., Wang, J.L., and Schindler, M., Carbohydrate Binding Activities of *Bradyrhizobium japonicum*. IV. Effect of Lactose and Flavones on the Expres-

sion of the Lectin BJ38, *Glycoconj.*, 1994, vol. 11, no. 4, pp. 363–370.

3. Depierreux, C., Kang, H.-C., Guerin, B., Monsigny, M., and Delmotte, F., Characterization of an *Agrobacterium tumefaciens* Lectin, *Glycobiology*, vol. 1, no. 6, pp. 643–649.
4. Karpunina, L.V., Mel'nikova, U.Yu., Vishnevetskaya, O.A., and Nikitina, V.E., *Bacillus polymyxa* Lectins: Location and Interaction with Wheat Roots, *Mikrobiologiya*, 1993, vol. 62, no. 2, pp. 307–313.
5. Karpunina, L.V., Savenkova, N.N., Vladimirova, M.V., Koltunova, E.F., and Nikitina, V.E., *Rhizobium leguminosarum* Agglutinins and Their Role in Bacterial Interaction with Plants, *Izv. Akad. Nauk, Ser. Biol.*, 1996, no. 6, pp. 698–704.
6. Konnova, S.A., Skvortsov, I.M., Makarov, O.E., and Ignatov, V.V., Properties of the *Azospirillum brasilense* Polysaccharide-containing Complexes and Their Polysaccharide Constituents, *Mikrobiologiya*, 1994, vol. 63, no. 6, pp. 1020–1030.
7. Konnova, S.A., Makarov, O.E., Skvortsov, I.M., and Ignatov, V.V., Exopolysaccharides of *Azospirillum brasilense* Sp245 and Sp107, *Mikrobiol. Zh.* (Kiev), 1992, vol. 54, no. 2, pp. 31–42.
8. Dykman, L.A. and Bogatyrev, V.A., Colloidal Gold in Solid-Phase Research Techniques, *Biokhimiya*, 1997, vol. 62, no. 4, pp. 411–418.
9. Karpunina, L.V., Savenkova, N.N., Vishnevetskaya, O.A., Mashkina, A.B., and Nikitina, V.E., Role of the Surface Lectins of *Rhizobium leguminosarum* Cells in Their Attachment to Pea Roots, *Mikrobiologiya*, 1996, vol. 65, no. 4, pp. 512–516.
10. Karpunina, L.V., Mel'nikova, U.Yu., Soboleva, E.F., Vishnevetskaya, O.A., and Nikitina, V.E., Investigation of the Adhesive Properties of *Bacillus polymyxa* Cells and the Role of Bacillary Lectins in Cell Adhesion, *Mikrobiologiya*, 1999, vol. 68, no. 4, pp. 445–448.
11. Karpunina, L.V., Mel'nikova, U.Yu., Konnova, S.A., and Stadnik, G.I., Investigation of the Role of *Bacillus polymyxa* Lectins in Bacterial Interaction with the Wheat Root Carbohydrates, *Izv. Akad. Nauk, Ser. Biol.* (in press).
12. Behhari, M.E., Gehin, G., Coulon, J., and Bonaly, R., Evidence for a Lectin of *Kluyveromyces* sp. That Is Involved in Co-Flocculation with *Schizosaccharomyces pombe*, *FEMS Microbiol. Lett.*, 2000, vol. 184, pp. 41–46.
13. Karpunina, L.V., Nikitina, V.E., Koltunova, E.F., Kosenko, L.V., and Patseva, M.A., Interaction of the *Rhizobium leguminosarum* 252 Agglutinin with Autogenous and Plant Receptors, *Mikrobiol. Zh.* (Kiev), 1995, vol. 57, no. 1, pp. 64–70.
14. Yagoda-Shagam, J., Barton, L.L., Reed, W.P., and Chiovetti, R., Fluorescein Isothiocyanate–labelled Lectin Analysis of the Surface of the Nitrogen-fixing Bacterium *Azospirillum brasilense* by Flow Cytometry, *Appl. Environ. Microbiol.*, 1988, vol. 54, no. 7, pp. 1831–1837.