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Biological Role of Lectins from the Nitrogen-Fixing *Paenibacillus polymyxa* Strain 1460 During Bacterial–Plant-Root Interactions

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Abstract. Enzyme-lectins LI and LII from *Paenibacillus polymyxa* 1460, when incubated with the carbohydrate moiety of the wheat-root exocomponent fraction, showed an increase in their proteolytic activity. This increase may be associated with the presence of lectin-specific carbohydrates in the root fraction. The lectins of the nitrogen-fixing paenibacilli enhance cellulose degradation in the plant cell, thus increasing the activity of β-glucosidase in the wheat-root cell wall.

Problems associated with the detection of possible natural receptors for bacterial lectins at the initial stages of bacterial–plant-cell attachment are still little known, as are problems in the study of the role of lectins during such an interaction. In this connection, we were interested in examining the biological role of lectins from *Paenibacillus polymyxa* 1460 during interaction with the carbohydrate components of wheat roots.

Materials and Methods

Bacterial strains and agglutinins. *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) 1460 was obtained from a culture collection held in Brno (Czech Republic). The culture was grown in Moore's synthetic medium [11] at 28°C for 72 h. Lectins LI and LII were isolated from the cell surface of strain 1460 as described earlier [4].

Obtainment of the plant fraction. To obtain the exocomponent fraction (EF) of wheat-seedling roots, we used 4-day-old roots of the cultivar Saratovskaya-29. The roots were grown aseptically in petri dishes containing sterile distilled water. The EF was mildly washed off the plant-seedling roots with phosphate-buffered saline (pH 7.2) [14].

Hemagglutinating activity. The hemagglutinating activity of LI and LII was determined by hemagglutination tests with spontaneous erythrocyte sedimentation, by using a 2% suspension of trypsinized rabbit erythrocytes [6, 8].

Enzyme assay. Proteolytic enzyme activity was determined according to Preston and Kruger [13]. β-glucosidase (EC 3.2.1.2.1) was determined according to Kwon et al. [5].

Carbohydrate determination. When proteins were removed, the EF was hydrolyzed in 4 N trifluoroacetic acid for 4 h at 100°C. The monosaccharide constituents of the EF were determined by thin-layer chromatography on a 3 × 10-cm DC-Alufolien Cellulose plate (Fluka Chemie AG, Buchs, Switzerland). Pyridine–ethyl acetate–acetic acid–water (5:5:1:3, vol/vol) was used as the solvent system [16]. The amino sugars of the EF were determined with an AAA339 amino acid analyzer (Czech Republic).

Statistics. Results of the study were analyzed for significance by Student's *t*-test [12].

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Results and Discussion

Lectins LI and LII are located on the surface of *P. polymyxa* 1460. Both LI and LII are specific for glucuronic acid and fructose-1,6-diphosphate, with LII being also specific for D-galactosamine · HCl and D-glucosamine · HCl. As we showed previously [4], proteins that are part of the protein–carbohydrate EF of wheat-seedling roots function as plant receptors for these lectins. The question whether the carbohydrates of the wheat-root EF could also be receptors for the *P. polymyxa* lectins remained open. When LI and LII were incubated with the carbohydrate moiety of the wheat-root EF at a 1:1 ratio for 30 min at 28°C (hemagglutination titer, 1:4), their hemagglutinating activity was abolished. This fact indicated that the wheat-seedling-root exocomponents contain specific carbohydrate receptors that block the lectin center of the bacteria.

Determination of the qualitative monosaccharide composition of the carbohydrate moiety of the wheat-root EF confirmed our assumption. The EF had the following lectin-specific carbohydrates: glucose, glucu-

ronic acid, glucosamine, and galactosamine. These carbohydrates could be readily available to the bacterial-surface lectins and could function as receptors for them. Many workers believe that the specific contacts between plant carbohydrate components and microbial receptors may occur in root-hair-surface mucilage [1, 7, 10, 15]. Much of it is a mixture of high-molecular-weight plant slime whose components are formed on root caps and are secreted by the Golgi apparatus. The monosaccharide composition of mucilage is similar to that of the wheat-seedling-root fraction. Literature data suggest that besides various monosaccharidic residues, the mucilage from the wheat-root surface contains glucuronic acid, galactosamine, and glucosamine, i.e., sugars that are found as part of the wheat-root fraction and may well serve as specific carbohydrate haptens for the lectins of paenibacilli in the wheat-root zone.

Earlier [9], we showed that both LI and LII possess proteolytic as well as hemagglutinating activity. We found that the proteolytic activity of the lectins is dependent on their hemagglutinating activity, and we showed that blocking of hemagglutinating activity with specific carbohydrate haptens decreases (with glucuronic acid and fructose-1,6-diphosphate) or increases (with galactosamine and glucosamine) the enzyme activity of both lectins. The detection of such an interrelationship suggested that the binding of the *Paenibacillus* lectins to the EF carbohydrates of wheat-seedling roots can also be attended by changes in enzyme activity. The determination of proteolytic activity after incubation of the lectins with the carbohydrate moiety of the wheat-root EF confirmed our assumption. When the EF carbohydrate moiety was incubated with LI, the proteolytic activity of the lectin increased slightly (0.006 μg alanine/min/μg protein), as compared with control (0.005 μg alanine/min/μg protein). Incubation of the EF carbohydrate moiety with LII augmented enzyme activity about twofold, as compared with control.

These results are in good agreement with the earlier data [9] for the proteolytic activity of the lectins after blocking of hemagglutinating activity with pure galactosamine and glucosamine preparations.

In the lectins of soil paenibacilli, the combination of such activities as hemagglutinating and enzymatic apparently has a significant biological meaning during the establishment of nitrogen-fixing associations. We believe that when the bacteria make contact with a plant, the lectins find carbohydrate receptors in the root-hair-covering mucilage, thus blocking the lectin center, and begin to show their enzymatic activity to a larger extent. The increase in enzyme activity possibly facilitates the bacterial penetration of the plant-cell interior.

Our further work showed that the paenibacillar colonization of wheat roots was followed by the action of the bacterial lectins on certain hydrolytic enzymes, in particular β-glucosidase. When choosing the experimental conditions, we found that the optimum time of lectin incubation of wheat-seedling roots was 1 h, and the lectin concentration that best stimulated wheat-root β-glucosidase activity was 0.1 μg/ml. The determination of β-glucosidase activity in non-lectin-incubated roots (control) showed that the enzymatic activity of these plant cells was 0.04 mmol/min/mg protein (Table 2). After seedling-root incubation with LI, the cellular enzymatic activity increased to as high as 0.006 mmol/min/mg protein; after incubation with LII, to as high as 0.02 mmol/min/mg protein.

The experimental data suggest that the paenibacillar lectins enhance cellulose degradation, thus increasing the activity of β-glucosidase in the wheat-root cell wall. Earlier [3], we found that proteolytic, β-glucosidase, and succinate-dehydrogenase activities increase after incubation of *Rhizobium leguminosarum* 252 agglutinins with pea roots.

Evidence accrued to date does not give an accurate account of the mechanisms underlying the effect of bacterial lectins on various enzymatic processes. Our data support the views [2] that the biological action of many

Table 1. Proteolytic activity of the *P. polymyxa* 1460 lectins after their interaction with the carbohydrate moiety of the seedling-root EF[a]

Lectins	Proteolytic activity[b]
LI + EF	0.0059 ± 0.0009
LI	0.0050 ± 0.0003
LII + EF	0.0215 ± 0.0018
LII	0.0108 ± 0.0004

[a] Results are expressed as mean ± SEM of 10 determinations. All differences significant ($p < 0.001$) when compared with control (proteolytic activity of lectins that did not interact with the carbohydrate moiety of the seedling-root EF).
[b] Activity is expressed as μg substrate (alanine)/min/μg protein.

Table 2. Effect of the *P. polymyxa* 1460 lectins on the β-glucosidase activity in wheat-seedling roots[a]

Lectins	β-glucosidase[b]
LI	0.006 ± 0.001
LII	0.020 ± 0.003
Control	0.004 ± 0.0005

[a] Results are expressed as mean ± SEM of 10 determinations. All differences significant ($p < 0.001$) when compared with control (β-glucosidase activity of non-lectin-incubated seedling roots).
[b] Activity is expressed as mmol *p*-nitrophenol/min/mg protein.

bacterial lectins is determined by their co-functioning with lytic enzymes.

Thus, the results of this study suggest that: (i) bacterial lectins are involved in the complex process of establishment of an association; (ii) plant-root carbohydrates, alongside plant proteins [15], can act as receptors for the lectins; and (iii) the surface lectins of the nitrogen-fixing soil bacteria can increase β-glucosidase activity in wheat roots.

Taken together, our data do not rule out the possibility that the paenibacillar lectins facilitate the plant-tissue penetration by *Paenibacillus* cells during the establishment of a plant–bacterial nitrogen-fixing association.

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