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EXPERIMENTAL ARTICLES

The Bactericidal Activity of Lectins from Nitrogen-Fixing Bacilli

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Abstract—Lectins I and II isolated from the nitrogen-fixing soil bacterium *Paenibacillus polymyxa* 1460 were found to be able to suppress the growth of *Rhizobium leguminosarum* 252 and *Bacillus subtilis* 36 at nearly all the concentrations tested (from 1 to 10 μg/ml). Lectin I was also inhibitory to *Azospirillum brasilense* 245 and *Erwinia carotovora* subsp. *citrulis* 603, while lectin II exerted bactericidal activity against *Xanthomonas campestris* B-610 and B-611 and *A. brasilense* 245. The bacillar lectins incubated with *Rhizobium* and *Azospirillum* cells caused leakage of low-molecular-weight substances from the cells, presumably resulting from impairment of the membrane barrier function. We believe that one of the possible mechanisms of the bacterial growth inhibition by lectins is mediated by the lectin-specific receptors occurring on the bacterial membrane, whose interaction with the lectin molecules induces conformational alterations in the membrane and concurrent malfunction of the metabolism of bacterial cells.

Key words: bacterial lectins, *Paenibacillus polymyxa*, bactericidal activity.

There is currently increasing research interest in the lectins and agglutinins of soil bacteria, which play an important role in the formation of symbiotic associations. There is evidence that the lectins of nitrogen-fixing bacteria are involved in the attachment of bacterial cells to plants and are able to affect enzymatic processes occurring in plant roots [1–4]. Interactions between various microorganisms in the rhizosphere of higher plants are not clearly understood, although it has been recognized that the antagonistic activity of rhizosphere microorganisms may be associated with the production of antibiotics [5], toxins [6], and, in the case of nitrogen-fixing soil pseudomonads, azospirilla and rhizobia, which are bacteriocins of a lectin nature [7, 8].

The aim of this work is to study the bactericidal activity of lectins isolated from *Paenibacillus polymyxa* 1460, which have not yet been studied in this respect.

MATERIALS AND METHODS

Lectins I and II were isolated, as described by Karpunina *et al.* [1], from the surface of cells of the nitrogen-fixing bacterium *Paenibacillus* (*Bacillus*) *polymyxa* 1460, obtained from the Czech Collection of Microorganisms (CCM) at the Masaryk University, Brno, Czech Republic.

The bactericidal activity of the lectins was assayed using test microorganisms from different taxonomic groups, which were obtained from the collection at the Institute of Biochemistry and Physiology of Plants and Microorganisms. These microorganisms included *Agrobacterium radiobacter* B-1218; *Agrobacterium rhizogenes* B-1219; *Azospirillum brasilense* Sp7 and Sp245; *P. polymyxa* 1460; *Bacillus subtilis* 36; *Erwinia carotovora* subsp. *citrulis* MI, 21, 603, and 215; *Escherichia coli* M65-1, C600, and QD12/25; *Rhizobium leguminosarum* 252; *Pseudomonas putida* B-1458; and *Xanthomonas campestris* B-610 and B-611. Bactericidal activity was assayed by the method of diffusion into agar [9]. Solid agar (20 ml) in petri dishes was overlaid with 1.8 ml of soft 0.8% agar (Difco, United States) mixed with 0.2 ml of a bacterial suspension containing 10^6 cells/ml. After the agar had hardened, 0.01-ml aliquots of bacillar lectin solutions containing from 1 to 10 μg lectin/ml were poured into wells made in the agar and the plates were then incubated at the optimal growth temperature of the particular test culture.

The lectin-induced leakage of low-molecular-weight substances was studied as follows: One-day-old cells of a test culture were washed thrice with 0.05 M phosphate buffer (pH 7.4) by suspending the cells in the buffer and centrifuging them at 6000 *g* for 5 min. The final bacterial suspension containing 5 mg cells per ml buffer was supplemented with a lectin at a concentration of 10 μg/ml and incubated either at 37°C (azospirilla) or 28°C (rhizobia and bacilli). After incubation, cells were removed from the suspension by centrifugation at 6000 *g* and the concentration of low-molecular-weight substances in the supernatant was determined spectrophotometrically at 260 nm.

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The effect of the *P. polymyxa* 1460 lectins on the growth of some bacteria

Bacterial strains	Growth inhibition zone in the presence of							
	Lectin I (μg/ml)				Lectin II (μg/ml)			
	10	5	2.5	1	10	5	2.5	1
E. coli M65-1	–	–	–	–	–	–	–	–
E. coli C600	–	–	–	–	–	–	–	–
E. coli QD 12/25	–	–	–	–	–	–	–	–
E. carotovora subsp. *citrullis* MI	–	–	–	–	–	–	–	–
E. carotovora subsp. *citrullis* 21	–	–	–	–	–	–	–	–
E. carotovora subsp. *citrullis* 603	+	–	–	–	–	–	–	–
E. carotovora subsp. *citrullis* 215	–	–	–	–	–	–	–	–
A. brasilense Sp7	–	–	–	–	–	–	–	–
A. brasilense Sp245	–	–	–	+	–	+	–	–
B. subtilis 36	–	+	+	+	+	+	+	+
P. polymyxa 1460	–	–	–	–	–	–	–	–
R. leguminosarum 252	+	+	+	+	+	+	+	+
A. radiobacter B-1218	–	–	–	–	–	–	–	–
A. rhizogens B-1219	–	–	–	–	–	–	–	–
X. campestris B-610	–	–	–	–	–	+	–	–
X. campestris B-611	–	–	–	–	+	+	–	–
P. putida B-1458	–	–	–	–	–	–	–	–

Note: "+" and "–" indicate, respectively, the presence and absence of the growth inhibition zone.

RESULTS AND DISCUSSION

It is known that there are bacilli that are able to lyse some bacteria and yeasts [10]. The culture liquid filtrates of these bacilli degrade the live cells of gram-negative microorganisms [11], the nature of substances involved in this process being unknown in depth.

As was shown in our previous works, lectins I and II isolated from the surface of *P. polymyxa* 1460 cells are glycoproteins specific for glucuronic acid and fructose-1,6-diphosphate. In addition, lectin II is also specific for D-galactosamine and D-glucosamine, whose residues occur on the surface of *P. polymyxa* 1460 cells [1]. The lectins are able to inhibit the growth of some microorganisms (table). In particular, both lectins inhibited the growth of *R. leguminosarum* 252 cells at all of the concentrations tested (1, 2.5, 5, and 10 μg/ml). Similarly, the growth of *B. subtilis* 36 was inhibited by lectin II at all of the concentrations tested, although lectin I at the concentration 10 μg/ml turned out to be noninhibitory. The latter finding can be explained by the fact that lectins possess regulatory properties as well. At the concentration 5 μg/ml, lectin II exerted bactericidal activity against *X. campestris* B-610 and B-611 and *A. brasilense* Sp245. Lectin I inhibited the growth of *A. brasilense* Sp245 at the concentration 1 μg/ml and *E. carotovora* subsp. *citrulis* 603 at the concentration 10 μg/ml. The wider range of bactericidal activity of lectin II as compared with that of lectin I may be due to the fact that lectin II is specific for four carbohydrates, whereas lectin I is specific for only two of them. It can be presumed that lectin II recognizes its specific carbohydrate haptens on the cell surface more easily than lectin I does. Correspondingly, lectin II may induce more drastic conformational alterations in the cell membrane and, hence, more severely inhibit bacterial growth than lectin I. This suggestion requires a serious experimental underpinning.

To the best of our knowledge, there is no evidence that the bacterium *P. polymyxa* can coexist with the bacterium *B. subtilis* or *R. leguminosarum* in the plant rhizosphere [12, 13]. This fact may be due to the bactericidal activity of lectins occurring on the surface of *P. polymyxa* cells. On the other hand, the lectins of *P. polymyxa* 1460 do not affect the growth of the agrobacteria, pseudomonads, and azospirilla that were found, together with *P. polymyxa*, in the rhizosphere of winter rye [12]. Nor did these lectins inhibit the growth of *Erwinia* bacteria, one representative of which (*E. herbicola*) was detected in the sugarcane rhizosphere together with *P. polymyxa* [13].

It can be suggested that bacillar lectins inhibit bacterial growth because of binding to specific receptors on the surface of bacterial cells. In the case of gram-positive bacteria, the role of such receptors may be played by glucosamine, a constituent of peptidoglycan (a major component of the bacterial cell wall). In the

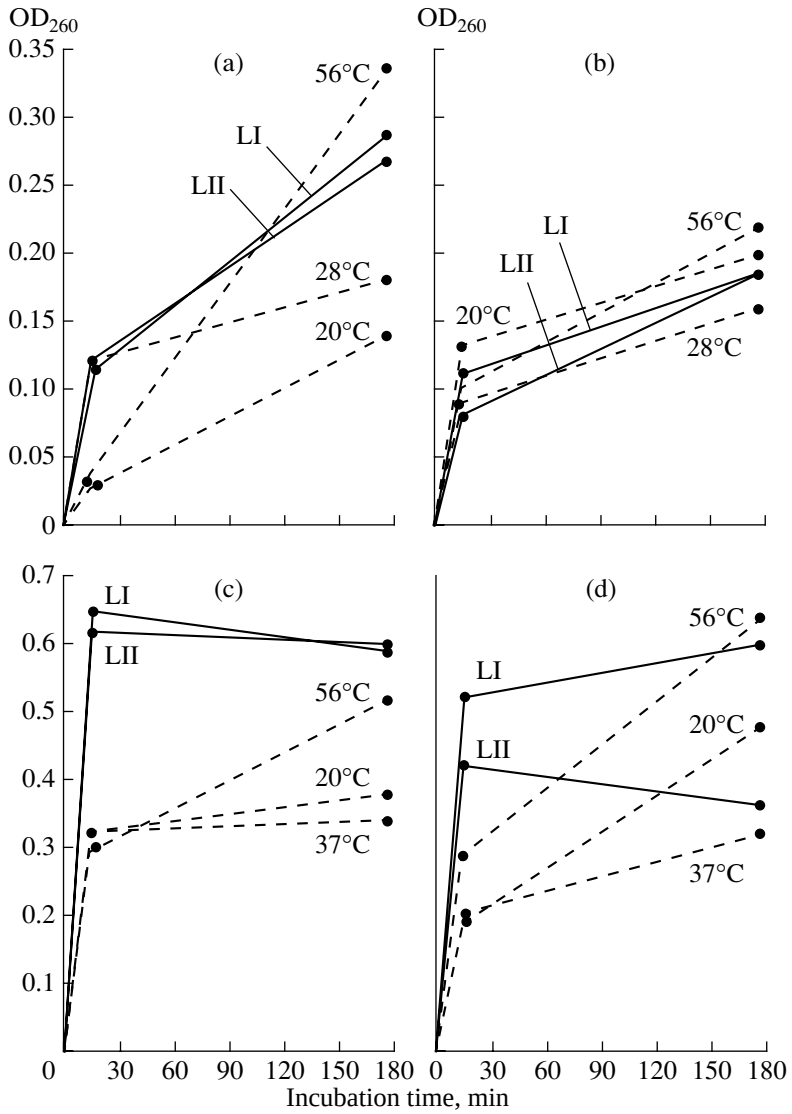

Fig. 1. The leakage of low-molecular-weight substances from cells of the nitrogen-fixing soil bacteria (a) *P. polymyxa* 1460, (b) *R. leguminosarum* 252, (c) *A. brasilense* Sp7, and (d) *A. brasilense* Sp245 in response to the action of lectin I (LI) and lectin II (LII) and incubation at different extreme temperatures (20 and 56°C). The optimum growth temperature for bacilli and rhizobia is 28°C, while it is 37°C for azospirilla.

case of gram-negative bacteria, bacillar lectins may directly interact with receptors occurring on the outer bacterial membrane.

The agglutinins of nitrogen-fixing soil bacteria may affect the membrane of plant cells. For instance, the incubation of the agglutinins of *R. leguminosarum* 252 with pea seedling roots was accompanied by an increase in the activity of some membrane-bound enzymes of plant cells, succinate dehydrogenase in particular [4]. At the same time, there is no information in the literature concerning the influence of the lectins of soil bacteria on the membrane apparatus of bacterial cells, although the diverse bactericidal activity of bacillar lectins suggests that such an influence must exist. The occurrence of protein receptors for bacillar lectins in the membrane fraction of wheat roots [1] is also indicative of the ability of these lectins to affect the membrane apparatus of plant cells. All of these facts prompted us to study the effect of the *P. polymyxa* 1460 lectins on the membrane of nitrogen-fixing soil azospirilla, bacilli, and rhizobia, which are the most frequently encountered bacteria in the rhizosphere of various plants [12, 14].

The functional state of the membrane apparatus of *A. brasilense* Sp245 and Sp7, *P. polymyxa* 1460, and *R. leguminosarum* 252 cells was evaluated from the leakage of low-molecular-weight substances from these cells, since it is known that such leakage is indicative of membrane permeability impairment in microbial cells [15]. Experiments showed that, after 15 min

of incubation of bacillar lectins with *P. polymyxa* 1460 cells, the optical density (measured at 260 nm) of the incubation medium increased to 0.11 (in the case of lectin I) and to 0.12 (in the case of lectin II) (Fig. 1a). After 3 h of incubation at 28°C, the optical density of the medium rose to 0.29 and 0.27, respectively. The incubation of lectins I and II with *R. leguminosarum* 252 cells for 3 h was accompanied by an increase in the optical density of the incubation medium to 0.18 and 0.19, respectively (Fig. 1b). In contrast to the bacilli and rhizobia, the incubation of lectins I and II with *A. brasilense* Sp7 and Sp245 cells led to the drastic leakage of low-molecular-weight substances into the medium already within 15 min of incubation, so that during the next 2.5 h of incubation the further rise in the optical density of the medium was low, or even nonexistent. Specifically, the optical density of the incubation medium of *A. brasilense* Sp245 cells after 15 min of incubation with lectins I and II was 0.52 and 0.42, respectively, while 0.6 and 0.36 after 3 h (Fig. 1d). Correspondingly, the optical density of the incubation medium of *A. brasilense* Sp7 cells after 15 min of incubation with lectins I and II was 0.65 and 0.62, respectively, while 0.59 and 0.6 after 3 h (Fig. 1c).

The effect of lectins I and II on the permeability state of the membrane apparatus of bacilli, azospirilla, and rhizobia was compared with that of extreme temperatures, since it is known that the primary effect of extreme temperatures on bacterial cells manifests itself in the damage of their cytoplasmic membrane [16, 17]. Measurements showed that, indeed, the incubation of the four tested bacteria at 20°C and especially at 56°C caused leakage of low-molecular-weight substances from cells (Figs. 1a–1d). According to some authors [18, 19], the leakage of free nucleotides from cells in response to incubation at extreme temperatures is due to the induced conformational alterations of membranes.

Thus, the leakage of low-molecular-weight substances from bacterial cells under the action of bacillar lectins is most likely due to impairment of their membrane apparatus and may be responsible for the inhibition of the growth of one soil bacteria by others.

This suggestion is confirmed by the experimental data of Sharga *et al.* [20], who showed that, in a mixed culture of *B. subtilis* 1107 and *E. rhapontici* NCPPB 139, the former bacterium kills the latter through the impairment of systems responsible for the transport of potassium ions across the cytoplasmic membrane of the erwinia.

The lectin-enhanced permeability of the membrane of the nitrogen-fixing soil bacterium *P. polymyxa* 1460, which is accompanied by leakage of low-molecular-weight substances from cells, can be accounted for by the occurrence of lectin receptors on the membrane of susceptible bacteria and the concurrent conformational alterations in the membrane. The observation that lectins I and II impair the membrane of not only the susceptible bacteria but also the nonsusceptible bacteria *P. polymyxa* 1460 and *A. brasilense* Sp7 indicates that the bactericidal activity of these lectins, like that of some known antibiotics [5], may also be related to the inhibition of the synthesis of some enzymes occurring in the cell membrane or the cell wall.

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