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Effect of exopolysaccharides of *Paenibacillus polymyxa* rhizobacteria on physiological and morphological variables of wheat seedlings

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***Paenibacillus polymyxa* is a promising plant-growth-promoting rhizobacterium that associates with a wide range of host plants, including agronomically important ones. Inoculation of wheat seedlings with *P. polymyxa* strains CCM 1465 and 92 was found to increase the mitotic index of the root cells 1.2- and 1.6-fold, respectively. Treatment of seedlings with the exopolysaccharides (EPSs) of these strains increased the mitotic index 1.9-fold (*P. polymyxa* CCM 1465) and 2.8-fold (*P. polymyxa* 92). These increases indicate activation of cell division in the root meristems. Analysis of the morphometric variables of the seedlings showed that *P. polymyxa* CCM 1465, *P. polymyxa* 92, and their EPSs promoted wheat growth, increasing root and shoot length up to 22% and root and shoot dry weight up to 28%, as compared with the control. In addition, both strains were found to intensely colonize the seedling root surface. Thus, *P. polymyxa* EPSs are active metabolites that, along with whole cells, are responsible for the contact interactions of the bacteria with wheat roots and are implicated in the induction of plant responses to these interactions. The strains used in this work are of interest for further study to broaden the existing understanding of the mechanisms of plant–bacterial interactions and to develop effective biofertilizers for agricultural purposes.**

***Keywords*:** *Paenibacillus polymyxa,* exopolysaccharides, mitotic index, plant-growth-promoting activity, root colonization, *Triticum aestivum* L.

Introduction

Biological, or organic, farming systems are becoming more and more popular worldwide. Such systems, based on the substantial reduction in the use of mineral fertilizers and pesticides, are advantageous in being able to maintain soil fertility, improve environmental health, and produce high-quality agricultural products. One method used in biological farming is the application of inoculants based on rhizosphere bacteria and their metabolites. These bacteria, used with a wide range of cultivated plants, include those of the genus *Paenibacillus*, which currently comprises more than 200 species of facultative anaerobes, with *P. polymyxa* as the type species (Grady *et al.*, 2016; Lee *et al.*, 2020). Although *P. polymyxa* occurs naturally in soil and marine sediments, it prefers plant-associated habitats such as the rhizosphere and roots of crop plants (McSpadden Gardener, 2004). In addition, *P. polymyxa* can be endophytic. Endophytic *Paenibacillus* spp. have been found associated with various plants, including *Arabidopsis thaliana* (Timmusk *et al.*, 2005), *Pinus contorta* (Anand *et al.*, 2013; Puri *et al.*, 2016), hybrid spruce (*Picea glauca* × *P. engelmannii*; Shishido *et al.*, 1999), *Curcuma longa* (Aswathy *et al.*, 2013), and *Lilium lancifolium* (Khan *et al.*, 2020). Beneficial *Paenibacillus* spp. can overgrow other endophytes in plant cell cultures (Ulrich *et al.*, 2008).

Paenibacillus polymyxa is a polyfunctional Gram-positive bacterium used in agriculture, medicine, and industry (Lal and Tabacchioni, 2009; Hao and Chen, 2017; Yu *et al.*, 2017). It is considered safe and is commercially available. The use of paenibacilli in agriculture includes two main aspects: biocontrol of plant diseases and promotion of plant growth. *Paenibacillus polymyxa* is a promising plant-growth-promoting rhizobacterium that can fix nitrogen (Puri *et al.*, 2016), mobilize phosphorus from poorly accessible compounds (Wang *et al.*, 2012), and synthesize a broad range of physiologically active substances: phytohormones, antibiotics, enzymes (Grady *et al.*, 2016; Rybakova *et al.*, 2016; Zhang *et al.*, 2018; Yuan *et al.*, 2020); volatile organic components (Raza *et al.*, 2015); and exopolysaccharides (EPSs) (Haggag, 2007; Liang and Wang, 2015). *Paenibacillus polymyxa* is a relatively new species and is the basis for several commercial formulations against plant pathogens (Rybakova *et al.*, 2016).

Current research has been intensely interested in *P. polymyxa* EPSs, which have highly diverse physiological and biotechnological functions and little or no toxicity (Raza *et al.*, 2011; Liu *et al.*, 2012; Rafigh *et al.*, 2014; Liang and Wang, 2015). Owing to their surface localization, EPSs can shield the underlying cellular structures, thereby determining the immunological properties of the bacteria, and can also mediate the interaction of *P. polymyxa* with other microorganisms and with macroorganisms (Vasilyev *et al.*, 1984). During batch cultivation of *P. polymyxa* CCM 1465 and 92, we isolated and characterized their EPSs and prepared polyclonal rabbit antibodies to the total preparations of EPS_{1465} (Yegorenkova *et al.*, 2008) and EPS_{92} (Yegorenkova *et al.*, 2011). Recently, we have shown that when grown in a liquid mineral medium with sucrose, *P. polymyxa* 92 produces an EPS

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that is a linear β-(2→6)-linked fructan (levan). This EPS is of potential biotechnological interest (Grinev *et al*., 2020).

EPS is an important metabolite in plant–bacterial interactions. According to Timmusk *et al.* (2005), the EPSs of *P. polymyxa* are implicated in biofilm formation on the roots of *Arabidopsis thaliana*. The EPSs of *P. polymyxa* A26 biofilms can antagonize *Fusarium graminearum*, and their uronate content is of critical importance in the antagonism (Timmusk *et al*., 2019). *Paenibacillus polymyxa* EPSs play a part in biofilm formation on abiotic surfaces (Yegorenkova *et al*., 2011), colonization of wheat seedling roots, and induction of changes in root hair morphology (Yegorenkova *et al*., 2013).

In associative symbiosis, plants do not form any structures similar to nodules or tumors; instead, root hair deformations are observed. Root hair deformation usually correlates with the ability of bacteria to colonize plant roots (Jain and Patriquin, 1984; Yegorenkova *et al*., 2013). Root hair deformation is one of the earliest plant responses to bacteria and can be a quantitative indicator of plant responsiveness to inoculation (Gaskins and Hubbell, 1979; Baldani *et al*., 1983).

During intense division, the wheat meristem cells express a specific antigen called the proliferative antigen of initials (PAI; Sumaroka *et al*., 2000). Analysis of the treatment of wheat with *Azospirillum* bacteria and with their lipopolysaccharides (LPSs) suggested that the degree of the mitotic activity of the root meristem cells and the content of PAI may characterize the effectiveness of associative plant–bacterium interactions (Evseeva *et al*., 2011). However, the search for criteria to predict the effectiveness of associations remains topical.

We investigated the effect of *P. polymyxa* CCM 1465, *P. polymyxa* 92, and their EPSs on the functional activity of the root meristem cells and on the physiological and morphological variables of wheat seedlings.

Materials and Methods

Strains and growth conditions

Paenibacillus polymyxa CCM 1465 was from the Czech Collection of Microorganisms (Brno, Czech Republic). *Paenibacillus polymyxa* 92 (IBPPM 109, VNIISHM 92) was from the IBPPM RAS Collection of Rhizosphere Microorganisms (http://collection.ibppm.ru/). This strain had been isolated from wheat roots by Dr. Y.M. Voznyakovskaya (All-Russia Research Institute for Agricultural Microbiology, Russian Academy of Agricultural Sciences, Pushkin-8, St. Petersburg, Russia). Cells were grown in a liquid nutrient medium that contained: yeast extract, 4 g/L; Na_2HPO_4, 1.1 g/L; KH_2PO_4, 0.5 g/L; $MgSO_4 \times 7H_2O$, 0.2 g/L; $(NH_4)_2SO_4$, 0.1 g/L; $CaCO_3$, 0.2 g/L; glucose, 30 g/L; DW, up to 1 L (pH 7.2–7.5). After the cells had been grown with shaking (220 rpm) at 30°C for 2 days, the viscosity of the culture liquid was decreased by twofold dilution with DW. The cells were separated by centrifugation at 15,000 × *g* for 30 min and were resuspended in phosphate-buffered saline (PBS; KH_2PO_4, 0.43 g/L; Na_2HPO_4, 1.68 g/L; NaCl, 7.2 g/L; pH 7.2) to 1.8×10^8 cells/ml. Cells were counted by counting the colony-forming units (CFU). The bacterial suspension was used to inoculate wheat seedlings.

EPS isolation

The total EPSs of *P. polymyxa* CCM 1465 and 92 were isolated as follows. After the cells were separated by centrifugation, the supernatant liquid was concentrated to the original volume of the culture liquid by rotary vacuum evaporation (40°C), and the EPS was precipitated with 3 volumes of acetone. The precipitate was separated by centrifugation at 3,000 × *g* for 20 min, washed repeatedly with acetone, and lyophilized in a BENCHTOP 2K freeze dryer (VirTis; Yegorenkova *et al*., 2010). The resultant EPS samples were used to treat wheat seedlings.

Plant material and inoculation

Seeds of common spring wheat (*Triticum aestivum* L. cv. Saratovskaya 29; Federal Center of Agriculture Research of the South-East Region, Saratov) were prepared for inoculation as follows. Healthy, visibly undamaged seeds were washed with water, surface sterilized in 70% (v/v) ethanol for 30–40 sec, washed in sterile DW, treated with aqueous diacide (ethanol mercury chloride, 33 mg; cetylpyridine chloride, 66 mg; DW, 100 ml) for 1–2 min, and washed again several times in sterile DW. For germination, sterilized seeds were placed on glass rods in plastic cuvettes containing sterile DW and were germinated in a thermostat at 25°C for 3 days without lighting. The *P. polymyxa* strains were grown and centrifuged as described above. Further, 3-day-old seedlings were inoculated within 24 h with living cells of both strains. The strains were inoculated separately, each at 1.8×10^8 cells/ml. Alternatively, the seedlings were treated with aqueous EPS solutions (EPS concentration, 0.2 mg/ml). After inoculation, the plants were grown for another 2 days in aquatic culture under controlled conditions (temperature, 24°C; air humidity, 60%; light intensity, 60 $\mu mol/m^2 \cdot sec$). Untreated plants grown in sterile DW were used as controls.

Analysis of physiological and morphological variables of wheat seedlings

The following variables of 6-day-old seedlings were analyzed: root and shoot length and root and shoot dry weight. The roots were also used to measure the mitotic index of the meristem cells. For dry weight measurements, samples were placed in capped aluminum cups and dried in a desiccator at 105°C until weight attained constancy. The presented data were calculated per one seedling. Comparison of the physiological and morphological variables of the experimental and control plants was used as an indicator of the biological effect of the bacteria and their EPSs on seedling growth and development.

Examination of *P. polymyxa*'s colonizing activity

This was done with excised roots of 3-day-old seedlings, as described by Yegorenkova *et al.* (2001). From the seedling apices, 2-cm-long root pieces were cut off, and each piece was transferred aseptically to an individual test tube containing 4.5 ml of PBS and was inoculated with 0.5 ml of a bacterial suspension (cell density, 1.8×10^8 cells/ml). The experiments used 2-day-old *P. polymyxa* cultures grown in the liquid nutrient medium with glucose. The living bacteria in the suspensions used for inoculation were counted by plating on the nutrient medium solidified with agar. The inoculated roots were shaken at 30°C for 0.25–48 h. After that the roots were gently agitated three times in PBS and homogenized. Root-attached cells were counted by counting the CFU, and the number of bacteria adsorbed per centimeter of root was determined (Yegorenkova *et al*., 2001).

Light microscopy

Roots were observed with a Biolar PI polarizing interference microscope (objective lenses, 20× and 40×; ocular lens, 12×). Images were recorded with a SCOPETEK DCM35 digital camera and were processed with the MiniSee 1.0 program.

Measurement of mitotic index of root meristem cells

This was done by a method modified from that described in Elkonin *et al.* (1993). For mitotic index measurements, samples were taken in a room with a temperature of not less than 25°C. Root tips (2–3 mm) of 6-day-old seedlings were fixed in acetic acid–96% ethanol (Carnoy's fixative; 1:3), stained with acetohematoxylin (Diaem Co.), and macerated in cytase (a mixture of enzymes isolated from the stomach of the vineyard snail, *Helix pomatia*; courtesy of M.I. Tsvetova, Federal Center of Agriculture Research of the South-East Region, Saratov). The dividing and resting cells were counted with a Leica DM 2500 microscope at 600× magnification. The microscopy was done at the Simbioz Center for the Collective Use of Research Equipment, IBPPM RAS. Each experiment was replicated three times, and in each replicate the root tips of five seedlings were used. About 1,000 cells were analyzed for each root tip.

Statistics

The experimental and control treatments each used not less than 30 plants. Data were analyzed by one-way ANOVA. We used the AGROS program package for statistical and biometrical–genetic analysis in plant breeding and selection (Version 2.09; Department of Statistical Analysis, Russian Academy of Agricultural Sciences). Least significant differences ($LSD_{0.05}$) were calculated at a significance level p of 0.05. The table and histograms report the means for the analyzed variables and the $LSD_{0.05}$ values.

Results and Discussion

Functional activity of root tip meristem cells

In studies of plant response to inoculation with plant-growth-promoting rhizobacteria, special attention should be paid to the functioning of root apical meristems, which are the formative and regulatory centers of the plant partner (Ivanov, 2004). Interaction with the associative microflora activates cell division in root meristems (Levanony and Bashan, 1989; Evseeva *et al*., 2011). In particular, Levanony and Bashan (1989) showed that inoculation of germinating wheat seeds with *Azospirillum brasilense* Cd enhances cell division in the root meristem and enlarges the elongation zone of the roots. The authors speculated that these changes may be responsible for the larger root system of the inoculated plants. Subsequently, we found that the LPS of *Azospirillum* bacteria increases cellular mitotic activity in the root meristems of wheat seedlings—an effect comparable to that of living bacteria (Evseeva *et al*., 2011). In addition, our preliminary work has shown that the LPS of the associative rhizobacterium *A. brasilense* Sp245 promotes the formation of meristematic centers and the formation of embryoids in the callus tissues of plants growing *in vitro* (Evseeva *et al*., 2018). All this indicates that LPS can be considered an active component of the *Azospirillum* cell surface. It not only determines the interactions of the bacteria with wheat roots (Fedonenko *et al*., 2001) but also participates in the induction of plant response to these interactions.

Here we used two *P. polymyxa* strains, CCM 1465 and 92, and their produced EPSs. These bacteria and their EPSs have valuable properties that may be important for the establishment of plant–bacterial associations (Yegorenkova *et al*., 2011, 2013, 2016). The functional activity of the meristem cells of the root tip was evaluated by their mitotic index. Treatment of wheat seedlings with strain CCM 1465 or with strain 92 (or with EPS of either strain) led to a 1.2- to 2.8-fold increase in the mitotic index of root cells, as compared with the control, depending on the strain and treatment method (Fig. 1). This increase indicates intensified cell division in the root meristems and the formation of many new cells, which intensify the development of the plant roots. The EPS concentration and the inoculation density were chosen by us on the basis of our earlier work (Yegorenkova *et al*., 2001, 2013). After the seedlings were inoculated with *P. polymyxa* CCM 1465 and 92 (cell density, 1.8×10^8 cells/ml), the mitotic index increased about 1.2- and 1.6-fold, respectively, and treatment with bacterial EPSs (concentration, 0.2 mg/ml) increased the mitotic index 1.9- and 2.8-fold, respectively (Fig. 1). These results are consistent with the previous data showing that in response to inoculation with the associative bacterium *A. brasilense* Sp245, the mitotic index of the wheat root mer-

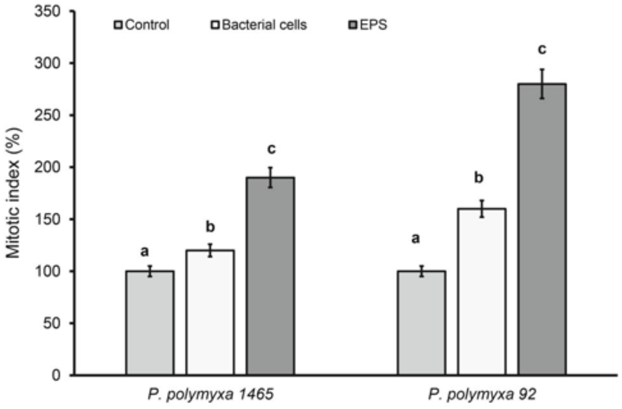

Fig. 1. Change in the mitotic index of the root meristem cells of 6-day-old wheat seedlings inoculated separately with *P. polymyxa* strains CCM 1465 and 92 or treated with their EPSs. The control (untreated plants) was taken as 100%. The histograms show the mean values for the analyzed measures and the least significant differences ($LSD_{0.05}$) at $p \leq 0.05$. Different letters (a, b, and c) above bars indicate values that differ significantly at $p \leq 0.05$, according to Duncan's multiple range test.

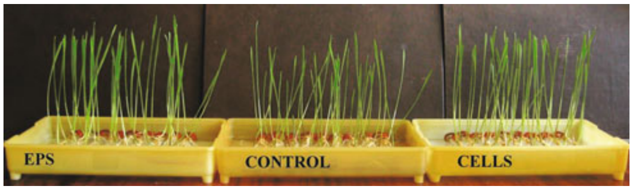

Fig. 2. **Promotion of wheat seedling growth by *P. polymyxa* 92 (cell density, 1.8×10^8 cells/ml) and by its EPS (EPS concentration, 0.2 mg/ml).**

istem cells increases 2-fold and that root treatment with LPS isolated from the outer membrane of Sp245 increases the mitotic index 1.8-fold. By contrast, interaction of seedling roots with cells or LPS of *E. coli*, a non-plant-growth-promoting bacterium, does not significantly alter the mitotic index (Evseeva *et al*., 2011).

The mechanism by which bacteria and their exoglycans activate cell division in the plant root meristems is not quite clear. Probably, EPSs mediate the interaction of *P. polymyxa* with host organisms and with other microorganisms. EPSs may be implicated in bacterial attachment to plants and in plant infection, may protect the bacteria against plant defense responses, and may function as signal molecules. In addition, their role in the establishment of symbiosis may be similar to that of flavonoids and lipochitooligosaccharides (York *et al*., 1996). For example, our previous experiments have shown that the pretreatment of wheat seeds with the EPSs of *P. polymyxa* CCM 1465 increases the root content of *o*-phenylene- and guaiacol-dependent peroxidases 2- and 1.5-fold, respectively; the protein concentration in the samples increases 4-fold, as compared with the control (Yegorenkova *et al*., 2016). Analysis of the data generated in this study suggests that EPS_{1465} induces plant protective responses, the "switch-on" of which may be related to the interaction of the EPS with the protein receptors in the plant cell plasmalemma. Bacterial polysaccharides can regulate metabolic processes and immunomodulate both plant and animal cells (Liu *et al*., 2012; Liang and Wang, 2015). In particular, EPS_{1465} stimulates the phagocytosis of bacterial cells by macrophages, intensifies metabolism in human and animal leukocytes, and modestly influences the production of proinflammatory cytokines (interleukin-1 [IL-1] and tumor necrosis factor α [TNF-α]) by human mononuclear cells (Yegorenkova *et al*., 2018).

Measurement of physiological and morphological variables of wheat seedlings

A promising direction in basic and applied research is the study of the regulation of plant growth and development by physiologically active natural substances, particularly during the first stages of plant development. In the initial stage of ontogenesis (when seedlings form from seeds), there occur the most noticeable, substantial, and fundamental physiological changes. Therefore, plant seedlings are a convenient and promising model in the search for environmentally safe natural regulators of plant growth and development.

The promotion of wheat growth by *P. polymyxa* CCM 1465, *P. polymyxa* 92, and their EPSs was examined by measuring the physiological and morphological variables of wheat seedlings. Treatment of 3-day-old seedlings with an aqueous EPS solution (EPS concentration, 0.2 mg/ml), followed by their further growth in the greenhouse under controlled conditions for 3 days, increased the length and weight of roots and shoots (Fig. 2). Specifically, treatment with EPS_{1465} promoted average increases in root and shoot length of 22% and 12%, respectively, and average increases in root and shoot dry weight of 20% and 17%, respectively. Inoculation with living *P. polymyxa* cells (cell density, 1.8×10^8 cells/ml) increased shoot length and root and shoot weight while changing root length only slightly. In particular, inoculation with *P. polymyxa* CCM 1465 increased shoot length by 12% and root and shoot weight by 11% and 22%, respectively (Table 1).

Paenibacillus polymyxa 92, isolated from wheat roots, and its EPS promoted wheat seedling growth (Table 1). Root and shoot length increased by 13% and 20%, respectively, and root and shoot dry weight increased by 22% and 18%, respectively (treatment with EPS_{92}; Table 1). Inoculation with living bacteria increased shoot length by 21% and root and shoot weight by 28% and 21%, respectively (Table 1). In a previous study of the biological activity of *P. polymyxa* EPS in plant cells, we showed that EPS_{1465} and EPS_{92} significantly increase the number of root hair deformations in wheat seedlings (6.5- and 5.7-fold, respectively), as compared to the control, and are more active than other *P. polymyxa* strains analyzed by us (Yegorenkova *et al*., 2013).

Table 1. **Morphological measurements of 6-day-old wheat seedlings after root inoculation with *P. polymyxa* (cell density, 10^8 cells/ml) or after treatment with *P. polymyxa* EPSs (EPS concentration, 0.2 mg/ml)**

Treatment with *P. polymyxa* cells or EPS	Root length (mm)	Shoot length (mm)	Root dry weight (mg)	Shoot dry weight (mg)
CCM 1465				
Control	263.9 *a*	108.8 *a*	3.6 *a*	7.9 *a*
Cells	269.2 *a*	121.5 *b*	4.0 *ab*	9.6 *c*
EPS	322.2 *b*	121.7 *b*	4.3 *b*	9.2 *b*
$\text{LSD}_{0.05}$	11.0	1.3	0.5	0.3
92				
Control	323.8 *a*	109.7 *a*	3.2 *a*	7.3 *a*
Cells	319.7 *a*	132.6 *b*	4.1 *b*	8.8 *b*
EPS	366.1 *b*	131.9 *b*	3.9 *b*	8.6 *b*
$\text{LSD}_{0.05}$	9.8	2.1	0.6	0.3

Values followed by different letters differ significantly at $p \leq 0.05$, according to Duncan's multiple range test; $\text{LSD}_{0.05}$, least significant differences calculated at a significance level of $p = 0.05$.

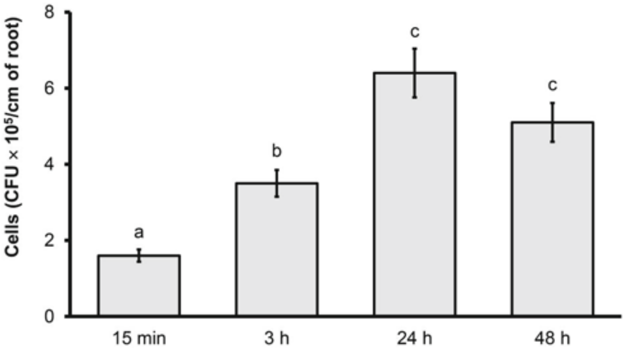

Fig. 3. **Time course of *P. polymyxa* adsorption to wheat seedling roots.** The cell density was 1.8×10^8 cells/ml. The histograms show the mean values for the analyzed measures and the least significant differences ($LSD_{0.05}$) at $p \leq 0.05$. Different letters (a, b, and c) above bars indicate values that differ significantly at $p \leq 0.05$, according to Duncan's multiple range test.

Extensive results have been generated on the effect of *P. polymyxa* inoculation on the yield of major cereal crops such as wheat, barley, rice, sorghum, millet, and maize (Maes and Baeyen, 2003; Lal and Tabacchioni, 2009; Grady *et al.*, 2016). Several authors have reported a large positive effect from the introduction of *P. polymyxa* into the plant rhizosphere, considering such variables as the viability and weight of the plants, the concentration of chlorophyll in the leaf mesophyll, the state of the root, and the formation of root hairs. *P. polymyxa* treatment of seeds improves seed germinability and seedling growth (Maes and Baeyen, 2003). Inoculation with the endophyte *P. polymyxa* P2b-2R, originally isolated form a lodgepole pine seedling, and with its green fluorescent protein (GFP) derivative, P2b-2Rgfp, promoted maize growth through the enhancement of seedling length and biomass (by 52% and 53% and by 68% and 67%, respectively; Puri *et al.*, 2016).

Our present results show a pronounced response of the plant partner to *P. polymyxa* CCM 1465 and 92 and to their glycopolymers. This response was manifested as a change in the plant's physiological and morphological variables. Thus, the changes in the functional activity of the root tip meristem cells under the influence of *P. polymyxa* or their EPSs correlate with the changes in the physiological and morphological variables of the wheat seedlings inoculated with the bacteria or treated with their EPSs.

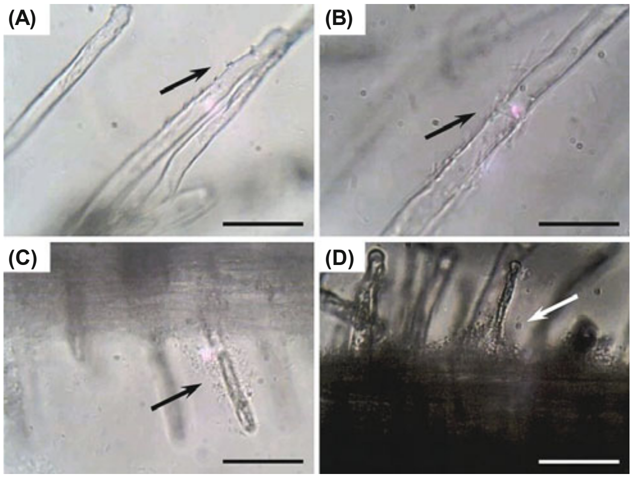

Fig. 4. **Microphotographs of *P. polymyxa* 92 on wheat seedling roots.** Bar marker: (A) 50 µm; (B) 35 µm; (C) 100 µm; (D) 100 µm. The arrows show bacterial cells on the root surface.

Examination of *P. polymyxa*'s ability to colonize wheat seedling roots

Effective attachment of associative bacteria to plant roots and the maintenance of bacterial population size at an ecologically friendly level are important for plant growth promotion, regardless of the operating mechanism (synthesis of metabolites and antibiotics against phytopathogens, stimulation with nutrients, or induction of plant resistance) (Timmusk *et al.*, 2005; Yi *et al.*, 2019). The exoglycans, produced by *P. polymyxa* in substantial quantities, are implicated in bacterial colonization of and biofilm formation on plant roots (Timmusk *et al.*, 2005, 2019; Haggag, 2007).

We investigated the ability of *P. polymyxa* to colonize the roots of wheat seedlings. Plating of root homogenate dilutions on solid media showed that after 15 min of incubation, the root adsorption of strain 92 was 1.6×10^5 cells/cm root (inoculum density, 1.8×10^8 cells/ml) and increased with time, reaching 6.4×10^5 cells/cm root after 24 h. Further extending the incubation time barely affected the number of attached cells, which at 48 h was 5.1×10^5 cells/cm root (Fig. 3). The adsorption of *P. polymyxa* CCM 1465 to wheat roots had been studied by us earlier (Yegorenkova *et al.*, 2013). The adsorption time courses for strains 92 and CCM 1465 were similar: in both strains, the number of adsorbed cells increased with increasing incubation time, and the cell number on the roots stabilized after 24 h of contact (Fig. 3). Strain 92 adsorbed to wheat roots slightly worse than did strain CCM 1465, for which the number of adsorbed cells had reached 1.7×10^6 cells/cm root by 24 h of contact. Nonetheless, the colonization ability of strain 92 was quite high, a finding confirmed by light microscopy. The bacteria intensely colonized the roots from the first minutes of contact (Fig. 4A and B), and at prolonged coincubation, they formed multilayered cellular clumps on the root surface that were embedded in a slimy material (Fig. 4C and D).

Previous studies led us to assume that *P. polymyxa* EPSs play a large part in root colonization and in biofilm formation. Enzyme-linked immunosorbent assay with rabbit polyclonal antibodies against isolated EPSs of *P. polymyxa* CCM 1465 and 92 permitted the detection of *P. polymyxa*'s EPS determinants in the biofilm materials (Yegorenkova *et al.*, 2011). Biofilms, which are formed from cells and a matrix consisting of polysaccharides and proteins, prevent pathogens from accessing the roots (Timmusk *et al.*, 2005; Haggag, 2007; Yi *et al.*, 2019) and help bacteria to survive in natural systems (Redmile-Gordon *et al.*, 2014; Timmusk *et al.*, 2019).

Conclusion

We have shown that either inoculation with *P. polymyxa* strains CCM 1465 and 92 or treatment with their EPSs increases the mitotic activity of the root meristem cells. The mitotic activity positively correlates with the morphological variables of wheat seedlings. Both strains can intensely colonize seedling roots, which is essential for the formation of effective plant–bacterial associations when *P. polymyxa* is introduced into farm ecosystems. Bacterial polysaccharides have diverse biological functions, primarily receptor functions, which ensure the interaction of cells with each other and with cells of other partners. *P. polymyxa* is an active producer of EPS, whose valuable properties have been described by us in earlier reports. Further studies of *P. polymyxa* and its metabolites are necessary for broadening the existing knowledge of the mechanisms of plant–bacterial interactions and for developing potent microbial inoculants for agricultural purposes.

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Conflict of Interest

The authors have no conflicts of interest to report.

Research involving human participants and/or animals

Not applicable.

Informed consent

Not applicable.

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