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ORIGINAL ARTICLE

Morphogenesis of wheat calluses treated with *Azospirillum* lipopolysaccharides

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Abstract

Callus tissue is a popular tool in modern plant breeding and biotechnology. Macromolecules of plant-growth-promoting rhizobacteria can be beneficial for callus morphogenesis. We compared the effects of the lipopolysaccharides (LPSs) of three *Azospirillum* strains (*A. brasilense* SR55, *A. brasilense* SR75, and *A. lipoferum* SR65) on the calluses of two wheat (*Triticum aestivum* L. cv. Saratovskaya 29) lines (LRht-B1c and LRht-B1a) that differ in their morphogenic activity. The LPSs differed in the chemical structure of their O polysaccharides and in their physicochemical and serological properties. The LPS of *A. lipoferum* SR65 significantly promoted callus morphogenesis and regenerant development in both wheat lines. The yield of regenerated plants in terms of the total number of explants was significantly increased—2.15-fold in the highly morphogenic line LRht-B1c and 3.75-fold in the weakly morphogenic line LRht-B1a. In both lines, the LPSs of *A. brasilense* SR55 and SR75 increased either only the yield of morphogenic calluses or only the yield of regenerated plants, respectively. Overall, the *Azospirillum* LPSs affected the weakly morphogenic line LRht-B1a stronger than they did the highly morphogenic line LRht-B1c, and this resulted in a leveling of differences between the activities of the LRht-B1c and LRht-B1a morphogenic calluses. The LPSs of some *Azospirillum* strains are promising promoters of plant morphogenesis and may, in the future, find frequent use in plant breeding and genetic engineering experiments with callus tissue.

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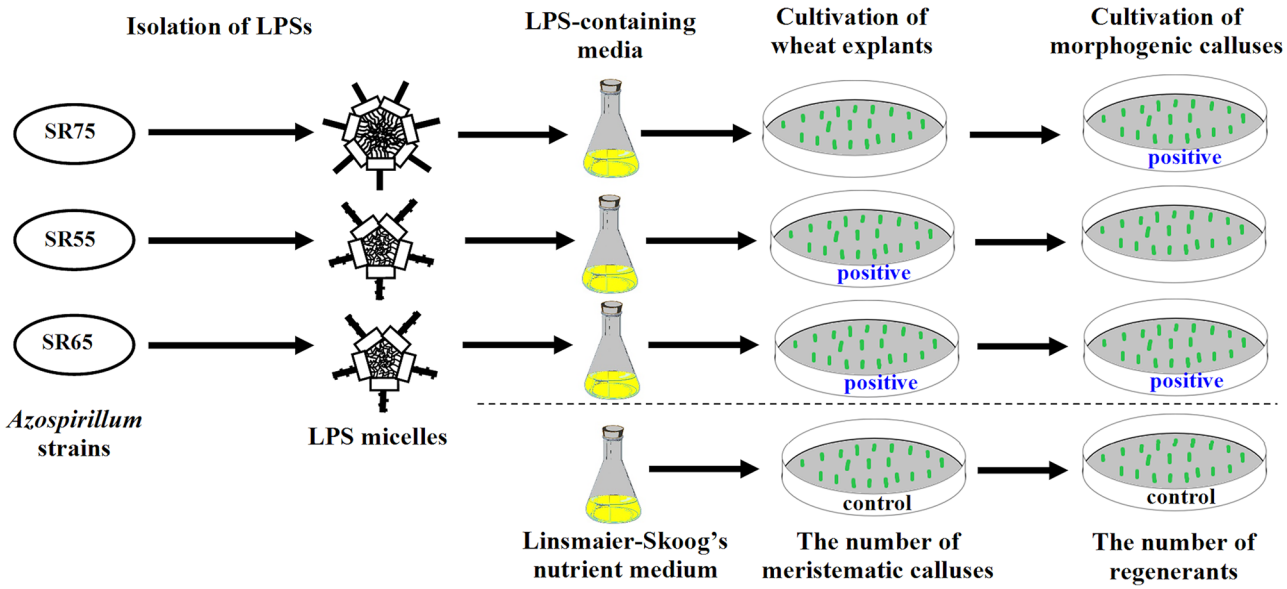
Graphic abstract



Key message

Lipopolysaccharides isolated from the outer membranes of various strains of plant-growth-promoting rhizobacteria of the genus *Azospirillum* increase the morphogenic activity of soft wheat calluses with different efficiency.

Keywords *Azospirillum* · Bacterial lipopolysaccharide · Callus culture · Somatic embryogenesis · *Triticum aestivum* L. · Plant-growth-promoting rhizobacteria

Introduction

Biotechnologies based on in vitro cell and tissue culture are used widely in modern basic and applied research (Bednarek and Orłowska 2020). Embryogenesis and organogenesis in a somatic cell culture, which are based on the property of totipotency, are induced by various growth stress factors, among which a large part is played by endogenous and exogenous hormonal signals and by nonhormonal inducers (Fehér 2003; Kim and Moon 2007; Lee and Huang 2013; Miroshnichenko et al. 2016; Zang et al. 2001). The influence of bacteria and their metabolites on plant cells has been understudied. There have been sporadic reports describing positive effects of cell suspensions of plant-associated *Bacillus* spp. on the morphogenic activity of geranium calluses (Visser-Tenyenhuis et al. 1994), positive effects of the diazotrophic bacterium *Herbaspirillum seropedicae* Z78 on the cells of oil palm (Lim et al. 2016), and positive effects of methylotrophic bacteria on the cells of barley (Shirokikh et al. 2010) and wheat (Kalyaeva et al. 2003). However, in vitro bacterization of plant somatic cells is not always successful, because it may cause culture contamination and/or toxicity (Ilchukov 2012).

Lipopolysaccharide (LPS) is the principal component of the outer layer of the outer membrane of gram-negative bacteria (Serrato 2014). It forms the surface layer of the cell wall and is involved in the interaction of bacteria with biotic and abiotic environmental constituents (Makin and Beveridge 1996). LPS plays a part in bacterial attachment to host plant cells (Lee et al. 2014) and in induction of plant responses to the presence of symbiotic bacteria (Leeman et al. 1995; Sumayo et al. 2013). The LPSs of some rhizosphere strains promote the growth of plant seedlings (Evseeva et al. 2011; Sigida et al. 2020) and improve yields in wheat (Chávez-Herrera et al. 2018). Previously, we have found that by contrast to the LPS of *Escherichia coli* K-12, the LPS of the plant-growth-promoting bacterium *Azospirillum brasilense* Sp245 increases the morphogenic activity of wheat calluses, improving the efficacy of culturing of genotypes with low morphogenic potential (Evseeva et al. 2018).

In recent years, much information has been accumulated to show that the O-polysaccharide (OPS) structure and the LPS physicochemical characteristics can be very different in different *Azospirillum* strains (Burygin et al. 2016; Fedonenko et al. 2015). However, no comparative studies have

been made of LPS effects on plant calluses. We examined the effects of *Azospirillum* LPSs with different chemical structures and properties on the morphogenic activity of soft wheat calluses.

Materials and methods

Plant material

Two near-isogenic lines of soft spring wheat (*Triticum aestivum* L. cv. Saratovskaya 29) were used—LRht-B1c and LRht-B1a. These have been described in detail elsewhere (Evseeva et al. 2018; Lobachev 2000). The LRht-B1c line has greater morphogenic potential in a somatic tissue culture than does its sister line LRht-B1a.

LPS characteristics

We used LPSs from *Azospirillum* spp. of three serogroups: *A. brasilense* SR75, *A. brasilense* SR55, and *A. lipoferum* SR65 (Fedonenko et al. 2015). The LPSs were isolated by the method of Westphal and Jann (1965). Proteins and nucleic acids were precipitated with CCl_3CO_2H (Kul'shin et al. 1987) and were centrifuged away. The LPSs were dialyzed and freeze dried (Boyko et al. 2011; Fedonenko et al. 2005, 2008).

The LPS carbohydrate content was measured as recommended by Dubois et al. (1956). The measurements were made on a Specord 250 spectrophotometer (Analytik, Jena, Germany) at 490 nm, by using glucose solutions for calibration. The LPS fatty acids were analyzed as methyl esters by gas–liquid chromatography (GLC) on a GC-2010 chromatograph (Shimadzu, Japan). The acids had been methylated as described by Mayer et al. (1985), with a bacterial acid methyl ester mix (Sigma–Aldrich, USA) as the standard. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of the LPSs and gel staining were done as recommended by others (Hitchcock and Brown 1983; Tsai and Frasch 1982).

The dynamic light scattering of aqueous LPS solutions (LPS concentration, 2 mg ml^{-1}) was measured with a Malvern Nano-ZS system (Malvern, UK; Burygin et al. 2016). We determined the intensity of light scattering at 173 ° and the correction function of scattering intensity fluctuations in time. Using these data, we estimated the number distribution over the particle size and the most probable modal hydrodynamic diameter (d_m). Measurements were made at 37°C. The laser was focused on the cuvette center (4.65 mm), and the diaphragm diameter was constant. Four-sided plastic cuvettes (10 mm; SARSTED, Germany) were used, and the solutions were incubated for 3 min before use. The zeta-potential of micelles in the aqueous LPS solutions (LPS concentration, 2 mg ml^{-1}) was measured with a Malvern Nano-ZS system at 25 °C by using DTS 1060 folded capillary cells (Malvern) and standard settings for zeta-potential measurements.

The serological reactions of antibodies against the O-antigens of *Azospirillum* serogroups I, II, and III (Fedonenko et al. 2015) with the LPSs were detected by enzyme-linked immunosorbent assay (ELISA) in 96-well polystyrene plates (Yegorenkova et al. 2010). The absorbance at 492 nm was read on a Multiskan Ascent analyzer (Thermo, Finland).

Determination of the morphogenic activity of wheat calluses

This was done as described earlier (Evseeva et al. 2018). Donor plants were grown under field conditions, and immature (14-day-old) embryos were used as explants to produce somatic calluses. For callus formation, sterile embryos were removed and placed scutellum up in petri dishes containing Linsmaier and Skoog's nutrient medium (Linsmaier and Skoog 1965) with the inclusion of 2 mg l^{-1} of 2,4-dichlorophenoxyacetic acid and 10 µg ml^{-1} of the LPS of *A. brasilense* SR75, *A. brasilense* SR55, or *A. lipoferum* SR65. The 10 µg ml^{-1} concentration was chosen as the most effective on the basis of the earlier work with the LPS of *A. brasilense* Sp245 (Tkachenko et al. 2010). Because bacterial LPSs are heat stable (Ramos-Sanchez et al. 1991), they were added to the growth medium before autoclaving (pressure, 0.8 atm; time, 20 min). The nativity of the structure and the concentration of each LPS in the autoclaved medium and during the experiment were confirmed by ELISA. All experiments were done in triplicate. In the control treatment, calluses were initiated on an LPS-free medium. No fewer than 50 embryos were used in each treatment. On day 30, the total number of formed calluses and the number of calluses with meristematic centers were counted by eye (He et al. 1986). Morphogenic calluses were transferred to test tubes containing a regeneration medium of the same composition that lacked 2,4-dichlorophenoxyacetic acid but included 0.5 mg l^{-1} of indole-3-acetic acid and 0.5 mg l^{-1} of kinetin. Regenerated plants were counted 30 days later.

Statistics

Data on the physicochemical and antigenic properties of the LPSs (quantitative indicators) came from at least three independent experiments done in three replicates. The measured data were processed by one-way ANOVA ($p \leq 0.05$) and then by Duncan's multiple range tests for independent samples. The yields of calluses, morphogenic calluses, and regenerated plants (qualitative indicators) were evaluated with the alternative variability formulas given in Evseeva et al. (2018). The significances of differences between control and

Table 1 Structures of the OPS repeating units for *A. brasilense* SR75, *A. brasilense* SR55, and *A. lipoferum* SR65

Azospirillum brasilense SR75
→2)-β-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→

Azospirillum brasilense SR55
→3)-β-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→3)-α-D-Gal*p*-(1→3)-β-L-Rha*p*-(1→4)-β-L-Rha*p*3OMe-(1→4)-α-L-Rha*p*-(1→3)-α-D-Gal*p*-(1→
α-D-Glc*p*A-(1$^{]2}$

Azospirillum lipoferum SR65
→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→
β-D-Glc -(1$^{]3}$

experimental treatments were tested by Student's *t* test for $P = 0.10$, 0.05, 0.01, and 0.001.

Results

LPS isolation and characterization

Wheat calluses were treated with the LPSs of *A. brasilense* SR75, *A. brasilense* SR55, and *A. lipoferum* SR65, which differ in OPS structure (Table 1). Earlier, these strains had been assigned to three *Azospirillum* serogroups owing to the substantial differences in OPS structure (Fedonenko et al. 2015). These differences include a linear D-rhamnan in *A. brasilense* SR75; a branched acidic heteropolysaccharide formed from residues of L-rhamnose, D-galactose, and 3-O-methyl-L-rhamnose in its main chain and a residue of D-glucuronic acid in its side chain in *A. brasilense* SR55; and a branched heteropolysaccharide formed from L-rhamnose and D-glucose residues in *A. lipoferum* SR65 (Boyko et al. 2011; Fedonenko et al. 2005, 2008).

LPSs were isolated from dry bacterial mass with hot phenol–water. Proteins were precipitated with trichloroacetic acid, and the extracts were dialyzed and freeze dried. The LPS yields were about 5–7% of the bacterial mass. Fatty acid composition analysis by GLC showed the predominance of 3-hydroxytetradecanoic, 3-hydroxyhexadecanoic, octadecenoic, and hexadecenoic acids. As is known and was observed in this study, the fatty acid profile and ratio are often similar in the LPSs from bacteria of the same genus. SDS–PAGE followed by silver nitrate staining showed the predominance of S-form molecules containing OPSs (Fig. 1).

Determination of the LPS carbohydrate content and measurement of the size and zeta-potential of the micelles formed from LPS molecules in aqueous media revealed the individual characteristics of each of the three LPSs examined (Table 2). The LPS of *A. brasilense* SR75 had a relatively high carbohydrate content and formed micelles with a diameter of 31 nm and an average zeta-potential. The carbohydrate mass fractions and the micelle sizes for the LPSs of *A. brasilense* SR55 and *A. lipoferum* SR65 were small, but the zeta-potentials of the micelles differed greatly. The LPS

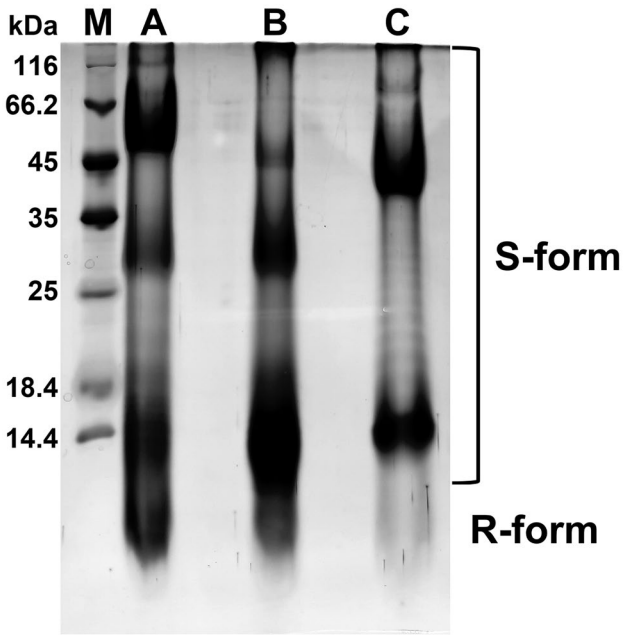


Fig. 1 Silver-stained 12.5% Tris–glycine SDS–PAGE. Lane A, *A. brasilense* SR75 LPS; lane B, *A. brasilense* SR55 LPS; lane C, *A. lipoferum* SR65 LPS; lane M, protein molecular weight marker (β-galactosidase, 116 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45 kDa; lactate dehydrogenase, 35 kDa; REase Bsp98I, 25 kDa; β-lactoglobulin, 18.4 kDa; lysozyme, 14.4 kDa)

Table 2 Carbohydrate contents, micelle diameters, and micelle zeta-potentials for the LPSs of four *Azospirillum* strains

LPS	Carbohydrate mass ratio (%)	Micelle diameter (nm)	Micelle zeta-potential (mV)
A. brasilense SR75 LPS	64.9b	30.5bc	− 20.5c
A. brasilense SR55 LPS	37.2a	22.9a	− 22.0d
A. lipoferum SR65 LPS	35.0a	21.1a	− 17.5a
A. brasilense Sp245 LPS	80.3c	34.5c	− 18.9b

Different letters (a, b, c, d) show that values differ significantly at $p \leq 0.05$, according to Duncan's multiple range test

of *A. brasilense* SR55 had the most negative zeta-potential, which is due to the presence of glucuronic acid residues in the OPS. By contrast, the zeta-potential of the LPS micelles for *A. lipoferum* SR65 was the lowest in modulus.

ELISA with rabbit polyclonal monospecific antibodies against the O-antigens of *A. brasilense* Sp245 (serogroup I), *A. brasilense* Sp7 (serogroup II), and *A. lipoferum* Sp59b (serogroup III) showed that the LPSs of *A. brasilense* SR75, *A. brasilense* SR55, and *A. lipoferum* SR65 belonged to serogroups I, II, and III, respectively (Fig. 2). Thus, of the variety of described *Azospirillum* LPSs, we had chosen those that differed in OPS structure, physicochemical characteristics, and serological properties.

LPS effects on callus formation and on yield of morphogenic calluses

Explants of the LRht-B1c and LRht-B1a lines were cultured on LPS-containing media to form calluses. On day 30 of culturing, the callus yield from explants (Table 3) of both lines treated with the LPSs from *A. brasilense* SR55 and *A. lipoferum* SR65 increased significantly (at $P=0.001$), as compared to the controls (calluses obtained on LPS-free media). No significant differences between the genotypes were detected in any experimental treatment. For LRht-B1c, wthe yield of morphogenic calluses increased 1.7- and 1.6-fold with the LPSs of *A. brasilense* SR55 ($P=0.05$) and *A. lipoferum* SR65 ($P=0.01$), respectively; for LRht-B1a, it increased 2.4- and 2.0-fold ($P=0.001$), respectively (Fig. 3). When the explants were cultured with the LPS of *A. brasilense* SR75, the formation of calluses and the yield of morphogenic calluses did not differ from the control in either line.

Yield of regenerated plants

After the morphogenic calluses were transferred to Linsmaier and Skoog's regeneration medium (no LPS), the yield

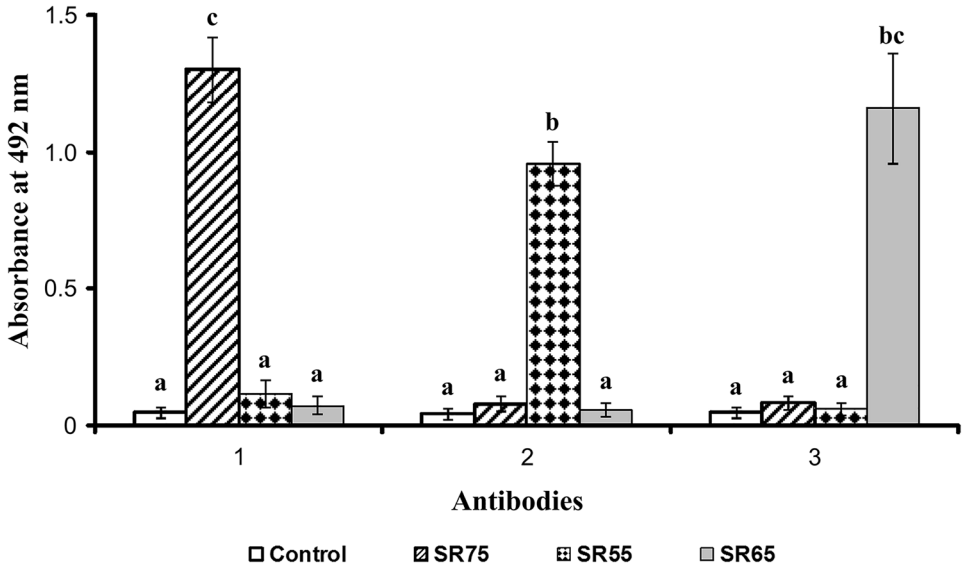


Fig. 2 ELISA of solutions of the LPSs (10 μg ml^{-1}) from *A. brasilense* SR75, *A. brasilense* SR55, and *A. lipoferum* SR65 by using antibodies against the O-antigens of *A. brasilense* Sp245 (serogroup I; **1**), *A. brasilense* Sp7 (serogroup II; **2**), and *A. lipoferum* Sp59b (serogroup III; **3**). Bars indicate standard deviation of the mean. Different letters (a, b, c) show that values differ significantly at $p \leq 0.05$, according to Duncan's multiple range test

Table 3 Effect of LPSs on total callus yield in LRht-B1c and LRht-B1a

Genotype	LRht-B1c			LRht-B1a		
Experiment	*n*	*q* (%)	*t*	*n*	*q* (%)	*t*
Control	1025	98.4–0.76	–	1022	97.7–0.93	–
A. brasilense SR75 LPS	200	99.5–0.98	1.68*	200	99.0–1.39	1.59
A. brasilense SR55 LPS	153	100–0.00	4.03**	171	100–0.00	4.96**
A. lipoferum SR65 LPS	345	100–0.00	4.03**	351	99.7–0.56	3.73**

n, the number of observations for each treatment; *q* (%), the percent of the sample proportion with a confidence interval at $P=0.05$; *t*, the experimental value of Student's coefficient. When the control treatments for the two lines were compared, $t=1.29<t_{0.10}=1.65$, i.e., it was nonsignificant at $P=0.10$ (significance level, 90%)

*Statistically significant differences at $P=0.10$ ($t \geq t_{0.10}$)

**Statistically significant differences at $P=0.001$ ($t \geq t_{0.001}$)

Fig. 3 Yield of morphogenic calluses on LPS-containing media (sample proportion q, %). Bars indicate confidence intervals at $P=0.05$ (significance level, 95%). Differences between controls (no LPS) for the two callus genotypes are significant at $P=0.05$; $t=1.98>t_{0.05}=1.97$. The asterisks show the significance of the differences between the LPS-treated calluses and the respective controls: one asterisk, at $P=0.05$; two asterisks, at $P=0.01$; and three asterisks, at $P=0.001$

of LRht-B1a regenerants increased significantly—2.6-fold ($P=0.05$) with the LPS of *A. brasilense* SR75 in the initial medium and 1.8-fold ($P=0.05$) with the LPS of *A. lipoferum* SR65 (Table S1). The LPS of *A. brasilense* SR55 did not significantly affect regeneration from LRht-B1a morphogenic calluses. The yield of LRht-B1c regenerants did not significantly change relative to the control in any of the LPS treatments.

Thus, the use of the LPS from *A. lipoferum* SR65 significantly increased the yield of regenerants from the LRht-B1c and LRht-B1a calluses obtained at the first stage. The respective increases were 2.15- and 3.75-fold ($P=0.01$), as compared to the controls (Table 4). A positive effect of the *A. brasilense* SR55 and SR75 LPSs on the final yield of regenerants was detected only for the weakly morphogenic LRht-B1a line ($P=0.1$). However, the final yields of LRht-B1a regenerants in all LPS treatments did not differ significantly from the corresponding treatments or from the controls for the LRht-B1c line. This is in contrast to the control for LRht-B1a, for which the yield of regenerants was 2.4-fold lower ($P=0.01$) than it was for the LRht-B1c control.

Discussion

LPS is one of the most conserved structures within all gram-negative bacteria. This property makes LPS an important pathogen-associated molecular pattern (PAMP) to be recognized by mammals and plants (Kutschera and Ranf 2019). In plants, LPSs activate the genes of the salicylate pathway of plant immunity (*pr* genes) (Iizasa et al. 2016) and initiate systemic resistance to plant pathogens (Leeman et al. 1995; Sumayo et al. 2013). The great progress in the study of *Azospirillum* OPSs that has been made in the past two decades (Fedonenko et al. 2005) has made it possible here to choose LPSs that differ in their physicochemical and serological properties. The OPSs of many *Azospirillum* strains contain two or more types of repeating oligosaccharide units (Fedonenko et al. 2015; Matora et al. 2008). The OPS of each LPS used in this study contained only one type of repeating unit with a unique chemical structure.

As shown by Tkachenko et al. (2010), the LPS of *A. brasilense* Sp245 promotes morphogenesis in wheat. The 10 μg ml^{-1} LPS concentration was found optimal for the promotion of morphogenesis in a wheat somatic callus culture. Here, however, we have found that when used at 10 μg ml^{-1}, the LPSs differed considerably in the magnitude of their effect. The most active LPS was that isolated from *A. lipoferum* SR65 (serogroup III). Its micelles in water had

Table 4 Effect of LPSs on regenerant yield in LRht-B1c and LRht-B1a

Genotype	LRht-B1c			LRht-B1a		
Experiment	n	q (%)	t	n	q (%)	t
Control	1009	3.77–1.18	–	998	1.60–0.78	–
A. brasilense SR75 LPS	199	2.51–2.19	0.99	198	4.55–2.92	1.94*
A. brasilense SR55 LPS	153	6.54–3.95	1.33	171	4.68–3.19	1.85*
A. lipoferum SR65 LPS	345	8.12–2.89	2.74**	350	6.00–2.50	3.31**

n, the number of observations for each treatment; q (%), the percent of the sample proportion with a confidence interval at $P=0.05$; t, the experimental value of Student's coefficient. When the control treatments for the two lines were compared, $t=3.01>t_{0.01}=2.58$, i.e., it was significant at $P=0.01$ (significance level, 99%)

*Statistically significant differences at $P=0.10$ ($t\geq t_{0.10}$)

**Statistically significant differences at $P=0.01$ ($t\geq t_{0.01}$)

a diameter of 21 nm and a weak zeta-potential. This LPS, like that from *A. brasilense* Sp245 (serotype I), promoted morphogenesis at all stages of culturing in vitro. By contrast, the LPS of *A. brasilense* SR75, in which the OPS repeating unit is structurally identical to that in *A. brasilense* Sp245 (Fedonenko et al. 2005), increased only the yield of regenerants from morphogenic calluses in the weakly embryogenic line LRht-1Ba, but it did not affect the yield of morphogenic calluses in either line, as compared to the control.

The considerable differences in the effect of the LPSs of *A. brasilense* SR75 and Sp245 on wheat calluses are difficult to explain. These LPSs contain structurally identical OPSs (Fedonenko et al. 2005) but differ slightly in the mass ratio between carbohydrates and in the physicochemical characteristics of their micelles (Table 2). The structural identity of the OPS units of *A. brasilense* SR75 and Sp245 suggests that neither OPSs nor their fragments are essential for the enhancement of the morphogenetic activity in wheat calluses. The LPS of *A. brasilense* Sp245 greatly promoted morphogenesis in both lines (Evseeva et al. 2018), as distinct from the LPS of *A. brasilense* SR75 (this study). This difference may be due to the multifactorial nature of the interaction between LPS molecules and callus cells. The combined effect of the small differences between the sizes of the LPS molecules and between the zeta-potentials of their micelles, together with the influence of other parameters, may be important for the formation of meristematic centers in dedifferentiated plant tissue. Also, one should not overlook the specificity of the action of lipids A and core oligosaccharides. These LPS portions are highly conserved (Caroff and Novikov 2019; Casillo et al. 2017); therefore, we did not account for their effects in this work. Further, one cannot rule out the differences in the structure of lipid A or the core between bacterial strains of the same species. These differences are important for interaction with plant cells (Caroff and Novikov 2019; Raetz and Whitfield 2002). Desaki et al. (2018) showed that the lipid A of the plant pathogen *Xanthomonas oryzae* pv. *oryzae* is implicated in the activation of plant responses through the specific interaction with the multifunctional co-receptor kinase OsCERK1 in suspension-cultured rice cells. It should be assumed that there are other plant receptors that trigger different biochemical and physiological responses of plants to LPS.

The LPS of *A. brasilense* SR55 ($d = 23$ nm, high negative zeta-potential, serogroup II) significantly promoted the yield of morphogenic calluses in both lines but not the yield of regenerants from morphogenic calluses. Nonetheless, for calluses of the weakly embryogenic line LRht-1Ba grown with *A. brasilense* SR55 LPS, we experimentally observed a 2.9-fold ($P = 0.1$) increase in regenerant yield (from explants to regenerants), as compared to the control group. This increase was due to the substantial activation of morphogenesis during the formation of morphogenic calluses.

In summary, the observed effects of the three *Azospirillum* LPSs tested, together with their physicochemical and serological properties, have enabled us to establish a (weak) relationship between the ability to increase morphogenic activity in wheat calluses and the low value of the zeta-potential of micelles. Yet, it may be that the LPS promotion of callus growth is not related to the chemical structure of the OPS repeating unit or to the size of the micelles formed. The entire mechanism of LPS action on plants is still poorly understood. Of note also is that the LPSs of different *Azospirillum* strains may differ in the concentration at which they are most effective as activators of wheat callus morphogenesis. The data generated in this study could be a step to elucidating the mechanisms by which the LPSs of plant-growth-promoting rhizobacteria affect plant cell differentiation.

Conclusion

The production of regenerants and the regulation of culture development in biotechnological approaches that use plant tissue culture (calluses) should be improved. The use of substances elaborated by bacteria may assist in this process. The study of plant tissue culture responses to bacterial components may both contribute to the understanding of basic issues in plant–microbe interactions and help researchers choose bacterial molecules able to regulate plant development.

Specifically, soft wheat calluses have weak morphogenic activity, which makes the production of regenerated plants less effective. The study of the effects of the LPSs from three *Azospirillum* strains has found significant promotion by *A. lipoferum* SR65 LPS of the morphogenic activity of wheat calluses—an effect comparable to that of *A. brasilense* Sp245 LPS (Evseeva et al. 2018). Not all *Azospirillum* strains are effective in activating plant tissue culture morphogenesis through their LPSs. Presumably, neither the structural differences between OPS repeating units nor the physicochemical characteristics of micelles determine the nature of LPS action on callus cells. Although LPS effects on plant objects (cells, tissues, organs) is a very promising area of plant biotechnology, the nature of the differences in efficacy and the mechanisms of LPS action on plant calluses remain unknown and call for further research.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11240-021-02114-2.

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Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval This article does not contain any human or animal studies performed by any of the authors.

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