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EXPERIMENTAL ARTICLES

# Changes in Cell Surface Properties and Biofilm Formation Efficiency in *Azospirillum brasilense* Sp245 Mutants in the Putative Genes of Lipid Metabolism *mmsB1* and *fabG1*

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**Abstract**—The previously obtained insertion mutants of *Azospirillum brasilense* Sp245 in the genes *mmsB1* and *fabG1* (strains SK039 and Sp245.1610, respectively) were characterized by impaired flagellation and motility. The putative products of expression of these genes are 3-hydroxyisobutyrate dehydrogenase and 3-oxoacyl-[acyl-carrier protein] reductase, respectively. In the present work, *A. brasilense* strains Sp245, SK039, and Sp245.1610 were found to have differences in the content of 3-hydroxyhexadecanoic, hexadecanoic, 3-hydroxytetradecanoic, hexadecenoic, octadecenoic, and nonadecanoic acids in their lipopolysaccharide preparations, as well as in cell hydrophobicity and hemagglutination activity and dynamics of cell aggregation, in biomass amount, and in the relative content of lipopolysaccharide antigens in mature biofilms formed on hydrophilic or hydrophobic surfaces.

*Keywords:* biofilms, fatty acids, lipid A, intercellular interaction, lipid metabolism genes, alphaproteobacteria
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Alphaproteobacteria *Azospirillum brasilense* inhabit diverse ecological niches, including the phytosphere. They actively move in liquids (the $Mot^+$ phenotype) due to the rotation of a single polar flagellum (Fla). They swarm on semisolid media and on moist surfaces (the $Swa^+$ phenotype) using numerous lateral flagella (Laf) and the polar flagellum (Tarrand et al., 1978; Hall and Krieg, 1983; Scheludko et al., 1998). Apart from motility, the polar flagellum is employed by azospirilla for adherence to the organs of plants, with which these bacteria form mutually beneficial associations (Croes et al., 1993).

Upon attaching to surfaces, bacteria form cell aggregates and biofilms (Bogino et al., 2013). Apart from a large amount of water, the extracellular matrix of biofilms contains various kinds of biopolymers (polysaccharides, proteins, nucleic acids, lipids, etc.) that secure their attachment to surfaces and structural and functional integrity. Bacterial flagella and pili are also incorporated into the matrix; they maintain the matrix architecture that depends on a large number of factors including hydrodynamic conditions, nutrient concentration, bacterial motility, and intercellular communication. The diversity of the structural elements of the biofilm matrix is comparable to the number of species that form these biofilms (Flemming and Wingender, 2010; López et al., 2010; Bogino et al., 2013).

Only scanty data are available on the role of exopolymers and extracellular organelles in the formation and stabilization of *Azospirillum* biofilms. Defects in the formation of Fla, the lipopolysaccharide (LPS), and surface Calcofluor-binding polysaccharides (CBPS) markedly affected the efficiency of biofilm formation by the relevant *A. brasilense* Sp245 mutants on hydrophilic and hydrophobic surfaces (Shelud'ko et al., 2008). Biofilm formation was suppressed upon inactivation of the Sp245 genes that are probably encoding the putative TAD pili (Wisniewski-Dyé et al., 2011). The biofilms of the $Fla^-$ $Laf^-$ mutant with an inactivated chromosomal copy of the flagellar gene *flhB* (*flhB1*) that were formed on hydrophobic and hydrophilic surfaces contained less biomass and were less stable than the biofilms of the parent strain Sp245. Retention of the Fla on *A. brasilense* Sp245 cells incorporated in a mature biofilm presumably contributed to the maintenance of its integrity under the influence of hydrodynamic shear force (Shelud'ko et al., 2015).

Impaired flagellation and motility was unexpectedly revealed in *A. brasilense* Sp245 insertion mutants with disrupted putative lipid metabolism genes coding for 3-hydroxyisobutyrate dehydrogenase MmsB1 (accession no. ADT80774) and 3-oxoacyl-[acyl-carrier protein (ACP)] reductase FabG1 (accession no. CCD01190) (Kovtunov et al., 2013). No significant differences between strain Sp245 and its *mmsB1*

and *fabG1* mutants were found in terms of viability, growth rate, average cell size, and the content of detectable fatty acids (FAs) in the total cell lipid fraction (Kovtunov et al., 2013). Retention of the normal viability of these mutants was apparently due to the existence of several genes encoding the aforementioned enzymes (accession nos. HE577327–HE577333; HM776586) in the *A. brasilense* Sp245 genome. It is not inconceivable that mutations in the *mmsB1* and *fabG1* loci resulted in changing the *Azospirillum* cell envelope structure, which considerably influenced flagellum assembly, intercellular interaction, and the social behavior of the bacteria.

The goal of the present work was to investigate the cell surface and biofilm formation characteristics of *A. brasilense* Sp245 in comparison to those of the strains with mutant putative lipid metabolism genes *mmsB1* and *fabG1*.

## MATERIALS AND METHODS

**Bacterial strains and nutrient media.** Strain *A. brasilense* Sp245 isolated in Brazil from wheat roots (Baldani et al., 1983) and its mutants leaky Fla$^-$ Mot$^-$ Swa$^-$ SK039 (*mmsB1*::Omegon-Km) and leaky Fla$^-$/Mot$^-$ Laf$^-$ Swa$^-$ Sp245.1610 (*fabG1*::Omegon-Km) (Scheludko et al., 1998; Kovtunov et al., 2013) were used in this work. The mutants SK039 and Sp245.1610 contain a single insertion of the artificial transposon Omegon-Km (Km$^R$) in the central part of the putative lipid metabolism genes that are located in the chromosome (*mmsB1*) and in the AZOBR_p1 megaplasmid (*fabG1*) (accession nos. HM776586 and HE577328, respectively) (Kovtunov et al., 2013). In the cultures of the parent strain *A. brasilense* Sp245, 85.8 ± 3.8% of cells are motile and contain a long polar flagellum, whereas ~5% of the cells carry a short polar organelle. In cultures of the mutant SK039, ~21% of the cells have a long Fla, while ~1.6% of the cells contain a shortened polar organelle; most bacteria are nonmotile. In cultures of the mutant Sp245.1610, ~9 and ~29% of the cells synthesize the long and the drastically shortened polar flagellum, respectively; 2.7 ± 0.4% of the cells retain motility in liquid media. Unlike *A. brasilense* Sp245 and SK039 cells, Sp245.1610 cells fail to synthesize Laf on solid media; both mutants (SK039 and Sp245.1610) lost swarming capacity (Scheludko et al., 1998; Kovtunov et al., 2013).

The malate–salt medium (MSM) (Döbereiner and Day, 1976) or the LB medium (Sambrook et al., 1989) was used to cultivate azospirilla. When necessary, the media were supplemented with kanamycin (Km) (at a concentration of 50 μg/mL) and the vital fluorescent dye Calcofluor (Fluorescent Brightener 28; Sigma–Aldrich, United States) (at a concentration of 1 mg/mL).

**Determination of the relative hydrophobicity of bacteria.** Salt fractionation was used to determine the relative hydrophobicity of azospirilla cells (Lindahl et al., 1981). Bacterial cells from 18-h broth cultures were washed with 2 mM phosphate buffer (PB, pH 7.0) to remove the medium and resuspended in the same buffer. Aliquots of the suspension (25 μL, $A_{590}$ = 1.2) were applied to the wells of a polystyrene plate that contained 25 μL of serial dilutions of ammonium sulfate solution in PB. The minimum salt concentration was determined that caused bacterial aggregation (more hydrophobic cells aggregate at lower ammonium sulfate concentrations) (Lindahl et al., 1981).

**Examination of bacterial motility in liquid media.** The hanging drop technique was applied to cell suspensions ($A_{590}$ = 0.5). Slides were examined under a transmission microscope; the data were recorded for 1–3 s using a DCR-TRV900E video camera (SONY, Japan). The motility of all cells in one microscope field was estimated. Video images were analyzed using the software developed at the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (IBPPM RAS). The software enables monitoring of the position of a cell in the Cartesian coordinate system in the frame mode (Schelud'ko et al., 2009).

**Analyses of biofilm formation and biofilm microstructure in azospirilla.** After incubation for 18 h in liquid LB or MSM, the cultures were diluted with fresh medium to an $A_{590}$ value of 0.05–0.10, and samples were transferred to glass test tubes (2 mL), polystyrene plates with 96 flat-bottom wells (200 μL), and polystyrene petri plates with glass cover slips placed on their bottom (3 mL); these samples were incubated at 30°C under stationary conditions.

To estimate relative biomass amounts in bacterial biofilms, they were stained with crystal violet (O'Toole and Kolter, 1998). Prior to analyzing biofilms, planktonic cultures surrounding them were removed by aspiration. Upon carefully washing with distilled water, biofilms were supplemented with an equal volume (2 mL or 200 μL) of 1% aqueous solution of crystal violet, incubated at room temperature for 10 min, and washed with water again upon removing the solution. The biomass-bound dye was dissolved in 2 mL or 200 μL of ethanol; optical density of the solution obtained was measured at a wavelength of 590 nm with a KFK-2 photocolorimeter (Zagorsk Optico-Mechanical Plant, Russia) or a Multiskan Ascent microplate photometer (ThermoLabsystems, Finland). The photometer is located at Symbiosis, the Center for the Collective Use of Research Equipment (CCURE) in the Field of Physical-Chemical Biology and Nanobiotechnology of IBPPM RAS (Saratov, Russia).

In some experiments, water-washed biofilms were carefully stained for 30 min with 0.02% water solution of Calcofluor (Fluorescent Brightener 28; Sigma–

Aldrich, United States) and thereupon washed with distilled water again.

Planktonic cultures sampled from glass test tubes were used for determining the aggregation degree of the cells in the medium around the biofilms; a modified method of Madi and Henis (1989) was employed. Liquid cultures of azospirilla were allowed to settle for 30 min, the supernatant was carefully decanted, and the precipitated cell aggregates were suspended in 50 mM PB (pH 7.0). After the suspension was allowed to settle for 2 h, the $A_{590}$ of the supernatant was measured. Thereupon, the bacteria were dispersed for 2 min using a magnetic stirrer; the optical density of the suspension was determined again. The cell aggregation percentage was calculated using the formula $A = [((A_{590})_2 - (A_{590})_1)/(A_{590})_2] \times 100\%$, where $(A_{590})_1$ is the optical density of the supernatant after aggregate precipitation and $(A_{590})_2$ is the optical density of the suspension obtained by dispersing the precipitated bacterial aggregates.

Phase contrast, fluorescence, transmission electron, and atomic force microscopy of biofilms and individual cells was performed with the equipment located at the Symbiosis CCURE of IBPPM RAS (Saratov, Russia), including the Leica DM6000 B (Leica-Microsystems, Germany), Libra 120 (Carl Zeiss, Germany), and SolverBio (NT-MDT, Russia) devices. Detailed information concerning the preparation of samples for microscopy and analysis is available in Shelud'ko et al. (2015).

**Determination of the hemagglutinating activity of biofilm bacteria.** Planktonic bacteria were removed from glass test tubes after 6 days of cultivation. The biofilms were washed with 50 mM phosphate–salt buffer (PSB; pH 7.0) and detached by pipetting them with the buffer. 50 μL of cell suspensions obtained ($A_{590} = 0.5$) were applied to 96-well immunochemical plates, and a series of their 1 : 1 dilutions in PSB was prepared. The resulting dilutions were supplemented with 50 μL of 2% suspension of trypsinized rabbit erythrocytes in PSB; the hemagglutination titer was determined after 18 h of incubation at 4°C.

**LPS isolation and determination of their fatty acid composition.** LPS preparations were obtained by extraction with hot aqueous phenol of bacterial cells from 18-h cultures in liquid MSM; the cells were washed to separate the capsules and dried with acetone. The carbohydrate, protein, and nucleic acid contents were determined as was described earlier (Fedonenko et al., 2004). The FA composition of lipid A was determined by gas–liquid chromatography (GLC) of FA methyl ethers on a GC-2010 chromatographer (Shimadzu, Japan) at the Symbiosis CCURE of IBPPM RAS (Saratov, Russia).

**Solid-phase indirect enzyme-linked immunosorbent assay (ELISA).** ELISA was performed with cell suspensions from 6-day biofilms that were formed on 96-well polystyrene plates. The planktonic culture was removed from the wells. To block the vacant polystyrene sites, 100 μL of 0.05% aqueous solution of polyethylene glycol 2000 was applied to the wells for 40 min. The subsequent stages of the procedure were performed as was described earlier (Sheludko et al., 2008). Polysaccharide antigens were detected using rabbit antibodies (Ab) against the LPS of *A. brasilense* strain Sp245 at a concentration of 50 μg/mL. Horseradish peroxidase-conjugated goat antirabbit Ab (Sigma, United States) at a concentration of 1 μg/mL served as secondary antibodies. Peroxidase activity was determined using the solution of 0.03% *o*-phenylene diamine and 0.02% hydrogen peroxide in 0.1 M sodium citrate buffer (pH 4.5). Optical density ($A_{490}$) of the tested samples was measured with a Multiskan Ascent photometer (ThermoLabsystems, Finland) at the Symbiosis CCURE of IBPPM RAS (Saratov, Russia).

**Statistical treatment of the results.** All quantitative studies were based on at least three independent experiments; at least three repeats of each experiment were done. The biomass of the biofilm of each strain was estimated at least six times. For each measurement, biofilms formed in five glass test tubes or ten wells of polystyrene plates were stained. The results were processed using the Microsoft Office Excel 2007 software package; the 95% confidence intervals were determined.

## RESULTS AND DISCUSSION

**Characterization of the biofilms of *A. brasilense* strain Sp245 and its *fabG1* (Sp245.1610) and *mmsB1* (SK039) mutants.** During biofilm formation, the attachment and adherence of the cells of all the three tested *A. brasilense* strains to the solid surface below the liquid medium occurred on day 2–3 of cultivation, and the maximum growth and stabilization of biofilm biomass were observed by day 5–6. At the adherence stage, bacteria formed thin biofilms, and their microscopy revealed scattered cell aggregates. Starting from day 3 (day 4 for strain SK039) of incubation, the microcolonies merged into a biofilm with a flattened surface. Starting from day 2 of incubation, all strains also formed a biofilm on the liquid medium surface, which subsequently precipitated to the test tube bottom.

Differences between relative biomass amounts in the biofilms of strain Sp245 and its mutants became visible by day 6 of incubation (Figs. 1a, 1b). On the hydrophilic glass surface under liquid LB medium, approximately equal biomass amounts were contained in the biofilms of strains Sp245 and Sp245.1610, whereas the biomass amount in the biofilms of strain SK039 was significantly lower. Decreased biomass amounts were detected in the biofilms of both mutants under the layer of liquid MSM (Fig. 1a). On the hydrophobic polystyrene surface under LB or MSM, both mutants formed less prominent biofilms than the

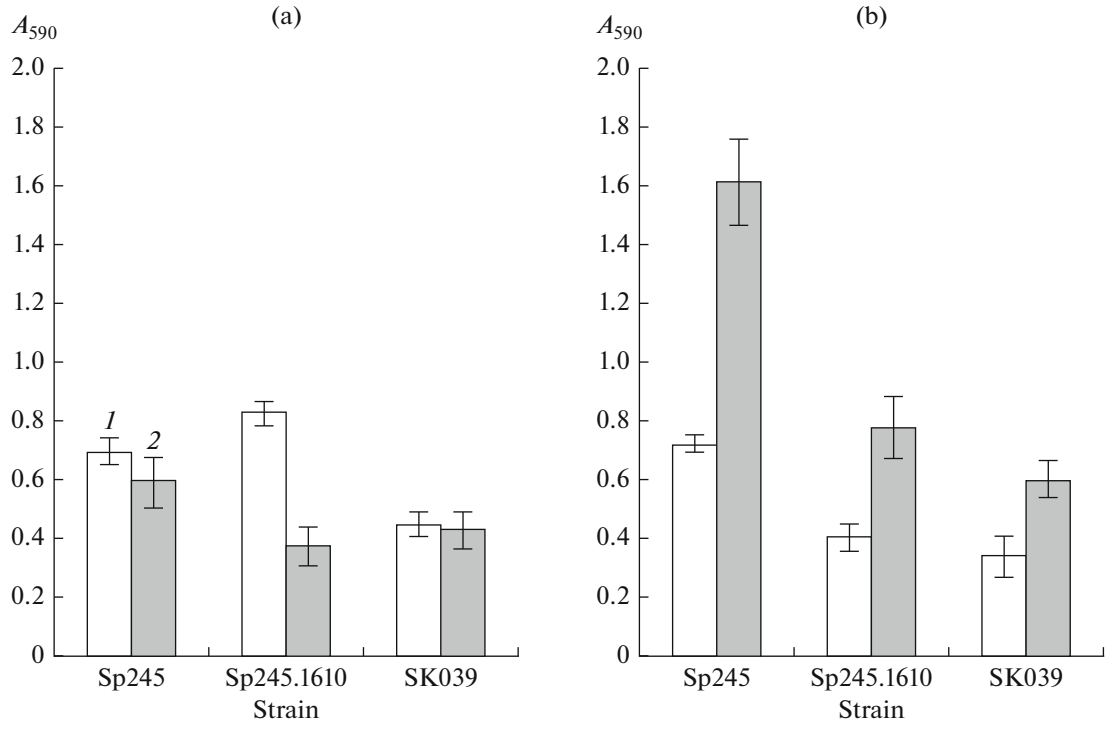


**Fig. 1.** Relative biomass amount in the biofilms of strain *A. brasilense* Sp245 and its mutants, leaky Fla$^-$/Mot$^-$ Laf$^-$ Swa$^-$ Sp245.1610 (*fabG1*::Omegon-Km) and leaky Fla$^-$ Mot$^-$ Swa$^-$ SK039 (*mmsB1*::Omegon-Km), that were formed after 6 days on glass (a) or polystyrene (b) under liquid LB (*1*) or MSM (*2*). $A_{590}$, optical density of crystal violet extracted from stained biofilms. The 95% confidence intervals were determined.

wild type strain (Fig. 1b). The relative biomass amount in the biofilms of all strains on polystyrene under MSM was considerably higher than that in the biofilms under the LB medium (Fig. 1b).

In the case of *A. brasilense* Sp245, numerous cells with long flagella, which intertwined to form networks, were revealed at all biofilm formation stages (Shelud'ko et al., 2015). The biofilms of the leaky Fla$^-$ Mot$^-$ Swa$^-$ strain SK039 contained a very low number of cells with a long Fla, and a drastically shortened Fla was present on some cells of the leaky Fla$^-$/Mot$^-$ Laf$^-$ Swa$^-$ mutant strain Sp245.1610 (Fig. 2). Lateral flagella were not detected in the biofilms of any of the strains. Mature biofilms of all *A. brasilense* strains contained vesicles of variable size (Fig. 2) that were more frequently revealed by atomic force microscopy in the samples from the liquid/air interphase than in those from the solid surface (native air-dried biofilm samples were used for atomic force microscopy). As a rule, such vesicles are formed from the components of the outer membrane of gram-negative bacteria. They contain LPSs, phospholipids, enzymes, and nucleic acids (Flemming and Wingender, 2010).

On day 2 of cultivation in LB medium, the aggregation degree of the planktonic cultures of Sp245.1610 and SK039 exceeded that of Sp245. On day 6, the aggregation degree of planktonic cells remained unchanged in the mutants and increased approximately twofold in strain Sp245, matching that in the mutant Sp245.1610. It seems likely that active aggregation of bacteria that already proceeded in the planktonic culture promoted their attachment to the solid surface, particularly in the mutants that lack the normal Fla, which is involved in cell adherence (Figs. 3b, 3c).

During cultivation in MSM, cells of all strains aggregated to an approximately equal extent, regardless of the incubation time; this process was more intense than in LB medium (Figs. 2b, 2c). Presumably, this factor promoted accumulation of a larger biomass amount in biofilms on the hydrophobic surface under MSM, in contrast to that under LB medium (Fig. 1b). Nonetheless, the differences between biofilm thickness in the mutants and the parent strain remained in both MSM and LB media.

The relative hydrophobicity of planktonic cells was almost the same in strains Sp245.1610 and Sp245 from ~20 h cultures in LB medium. However, it drastically increased in strain Sp245.1610 cultivated in MSM; the mutant SK039 cells were distinguished by elevated hydrophobicity, irrespective of the cultivation medium (Fig. 3a). We did not monitor the possible changes in relative hydrophobicity of *Azospirillum* cells during biofilm formation and maturation.

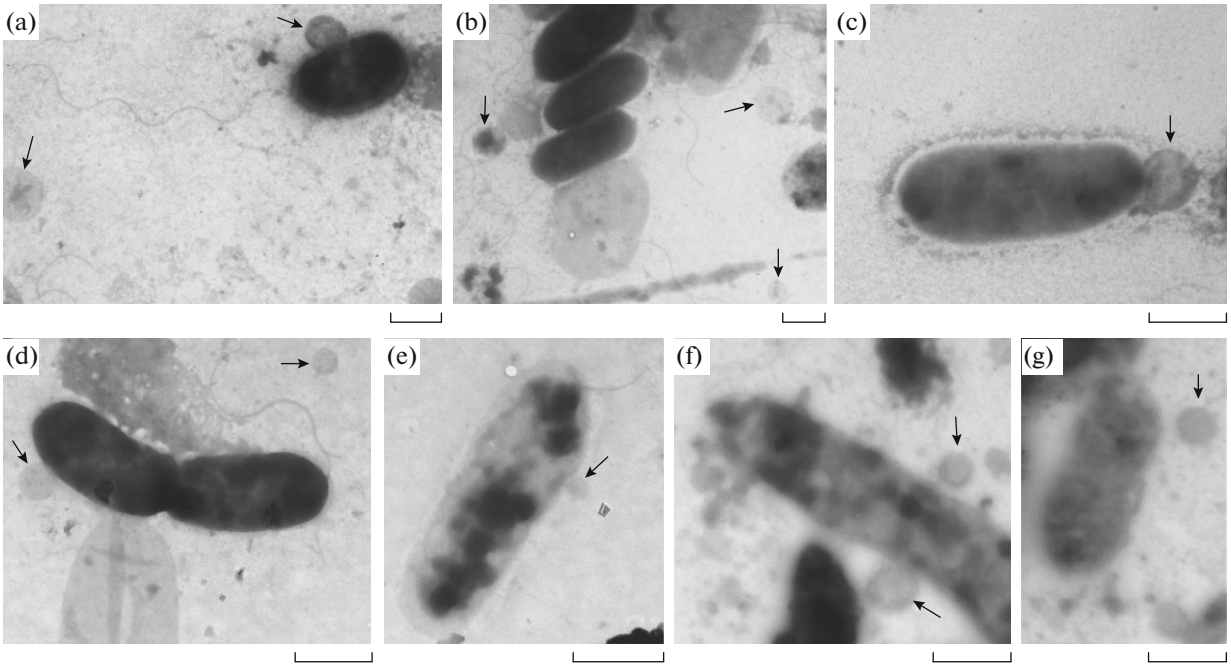


**Fig. 2.** Transmission electron microscopy of the material from the biofilms of strains *A. brasilense* Sp245 (a–c), Sp245.1610 (d, e), and SK039 (f, g) that were detached from the glass surface after 6 days of cultivation under liquid LB (a, b, d, f) or MSM (c, e, g) medium. Arrows indicate vesicles. Scale bar, 1 μm.

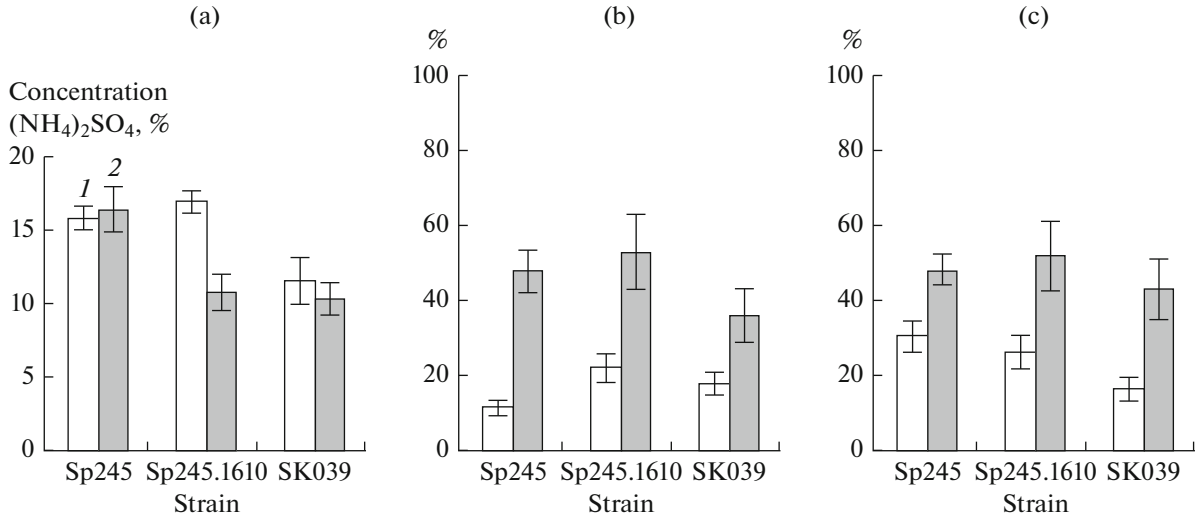


**Fig. 3.** Characteristics of planktonic cells of *A. brasilense* strain Sp245 and its *fabG1* (Sp245.1610) and *mmsB1* (SK039) mutants cultivated in LB (*1*) or MSM (*2*): relative hydrophobicity of the cells from ~20-h cultures determined by salt fractionation (a); aggregation degree of planktonic cells after 2 (b) and 6 (c) days of cultivation without stirring. The 95% confidence intervals were determined.

Changes in hemagglutination activity testified to alterations in cell surface structure in mature biofilms of the three azospirilla strains. Cell suspensions of strains Sp245, Sp245.1610, and SK039 from 6-day biofilms under LB/MSM that were detached (by washing) from the glass surface caused agglutination of trypsinized erythrocytes with titers of (1 : 16)/(1 : 16), (1 : 4)/(1 : 4), and (1 : 1)/(1 : 4), respectively. The hemagglutinin protein that interacts with the O-polysaccharide (OPS) of strain *A. brasilense* Sp245 (apparently promoting the establishment of intercellular contacts) was isolated from the surface of its cells ear-

**Table 1.** Characterization of the lipopolysaccharides (LPS) of *A. brasilense* strain Sp245 and its *fabG1* (Sp245.1610) and *mmsB1* (SK039) mutants

| Components | | | LPS of *A. brasilense* strain | | |
|---|---|---|---|---|---|
| | | | Sp245 | Sp245.1610 | SK039 |
| Content, wt % | | Carbohydrates | 65.8 ± 2.1 | 75.9 ± 5.5 | 44.1 ± 2.5 |
| | | Proteins | 0.7 ± 0.1 | 0.5 ± 0.2 | 0.5 ± 0.1 |
| | | DMOA* | 0.6 ± 0.02 | 1.8 ± 0.2 | 1.8 ± 0.1 |
| | | Phosphorus | 2.7 ± 0.1 | 4.6 ± 0.2 | 2.7 ± 0.4 |
| | wt % of FAME** | 3-OH-$C_{14:0}$ | 42.0 ± 0.6 | 35.4 ± 0.2 | 44.0 ± 0.2 |
| | | $C_{16:1}$ | Tr.*** | 3.1 ± 0.1 | Tr. |
| | | $C_{16:0}$ | 29.2 ± 1.3 | 3.1 ± 0.1 | 3.0 ± 0.4 |
| | | 3-OH-$C_{16:0}$ | 22.0 ± 0.9 | 11.2 ± 0.4 | 14.2 ± 0.8 |
| | | $C_{18:1}$ | 6.8 ± 0.2 | 43.1 ± 1.1 | 33.4 ± 1.2 |
| | | $C_{19:0}$ | Tr. | 5.1 ± 0.6 | 4.8 ± 0.5 |

* DMOA, 3-deoxy-D-manno-2-octulosonic acid.
** FAME, fatty acid methyl ethers.
*** Tr., trace amounts.
The 95% confidence intervals were determined.

lier (Shelud'ko et al., 2009). Since hemagglutinin activity in mutant cells was decreased 4- to 16-fold, hemagglutinin-mediated intercellular interaction in the cultures of the relevant strains was presumably weakened. Importantly, alterations in the structure or arrangement on the cell surface of not only hemagglutinins per se but also of other biomolecules could account for decreased hemagglutinin activity of the mutant cells.

ELISA with polyclonal Ab against the LPS of strain *A. brasilense* Sp245 was also used for analyzing *Azospirillum* biofilms on polystyrene. The ratios between the index of LPS antigen content in biofilms under LB/MSM (Table 2) and that of biofilm biomass (Fig. 1b) were 0.5/0.3, 1.0/0.8, and 0.8/0.6, respectively, in strains Sp245, SK039, and Sp245.1610. Apparently, the relative LPS antigen content in mature biofilms of flagellation-deficient mutants Sp245.1610 and SK039 was higher than that of parent strain Sp245.

**Fatty acid profile of the LPSs of *A. brasilense* strain Sp245 and its *fabG1* and *mmsB1* mutants.** We earlier attempted to elucidate the differences in FA composition of the total cell lipids of strains *A. brasilense* Sp245, SK039, and Sp245.1610 grown on liquid and solid LB and MSM. The *trans*-9-octadecanoic, *cis*-9-hexadecenoic, and hexadecanoic acids prevailed in all tested extracts; their total content exceeded 84%. Octadecanoic and *cis*-9-octadecanoic acids accounted for another ~6.5–8.0%. Insignificant interstrain fluctuations were revealed in the relative content of virtually all detectable FAs in the extracts of total cell lipids (Kovtunov et al., 2013).

The mutants *A. brasilense* SK039 or Sp245.1610 retained the ability to synthesize CBPS, and their LPS immunochemically interacted with polyclonal antibodies against the LPS of strain Sp245. MmsB enzymes are characterized by a broad substrate spectrum, and may catalyze reversible oxidation of 3-hydroxyisobutyrate, other 3-hydroxy acids, and a number of amino acids (Chowdhury et al., 1996). FabG enzymes catalyze reduction of 3-oxoacyl-[ACP] to 3-hydroxy-[ACP] at the first stage of FA de novo synthesis (accession no. CCD01190). Lipid A is one of the main components of bacterial LPS, along with the carbohydrate moiety that is composed of OPS and the core polysaccharide. Analysis of the FA profiles of lipid A of the wild type strain and its mutants was performed to test the suggestion that the FA composition of the LPS could change upon inactivation of the *mmsB1* and *fabG1* genes.

As mentioned above, the hydrophobicity of *A. brasilense* Sp245 cells during cultivation in LB medium or MSM did not change, and the differences between the biomass amount in the biofilms of strain Sp245 and those of its mutants were especially prominent in MSM (see Figs. 1a, 1b). Therefore, bacteria grown in MSM were used to obtain LPS preparations for chemical analysis.

Analysis of the LPS FA profiles by GLC of FA methyl ethers revealed that the content of 3-hydroxyhexadecanoic (3-OH-$C_{16:0}$) and hexadecanoic ($C_{16:0}$) acids was decreased in the LPS of the mutants Sp245.1610 and SK039, compared to those of strain *A. brasilense* Sp245 (Table 1). An insignificant decrease in 3-hydroxytetradecanoic acid (3-OH-

**Table 2.** Results of the enzyme-linked immunosorbent assay (ELISA) of the content of lipopolysaccharide (LPS) antigens in the biofilms of strain *A. brasilense* Sp245 and its mutants *fabG1* (Sp245.1610) and *mmsB1* (SK039) that were formed after 6 days on polystyrene

| *A. brasilense* strain | Results of ELISA with anti-LPS antibodies ($A_{490}$) of biofilms formed under liquid medium | |
|---|---|---|
| | LB | MSM |
| Sp245 | 0.38 ± 0.03 | 0.50 ± 0.09 |
| Sp245.1610 | 0.24 ± 0.02 | 0.40 ± 0.08 |
| SK039 | 0.27 ± 0.04 | 0.38 ± 0.08 |

The 95% confidence intervals were determined.

$C_{14:0}$) content and a slight increase in hexadecenoic acid ($C_{16:1}$) content occurred in the LPS of strain SK039. The octadecenoic acid ($C_{18:1}$) and nonadecanoic acid ($C_{19:0}$) content in the LPS of both mutants was increased (Table 1).

A change in FA ratio in the LPSs may account for the aforementioned differences in the physical and chemical properties of the cell surface and biofilm formation efficiency in azospirilla; the effect could be direct or indirect (e.g., caused by altering the exposure degree of the biomolecules involved in interactivity among azospirilla cells and between them and inert surfaces).

Hence, apart from disrupting flagellation and cell motility (as described earlier, Kovtunov et al., 2013), inactivation of the putative lipid metabolism genes *fabG1* and *mmsB1* in *A. brasilense* Sp245 resulted in changes in FA ratio in the LPS and in relative hydrophobicity, hemagglutination activity, cell aggregation dynamics, and biofilm formation efficiency in the mutants on abiotic surfaces. Similar multiple phenotype changes caused by a different modification of lipid A were described in alphaproteobacteria of the order *Rhizobiales* (see, e.g., Vanderlinde et al., 2009; Vanderlinde and Yost, 2012). Interestingly, FA can perform the function of intercellular communication signals and are components of biosurfactants that facilitate the motility of some bacteria on the surfaces they colonize. A relationship between the FA profile of some bacterial cells and their swarming capacity was revealed (Lai et al., 2005). Motility is not only a prerequisite for bacterial biofilm formation or dispersal; it is also involved in molding the biofilm architecture (Guttenplan and Kearns, 2013). Of considerable interest seems to be a subsequent comparative analysis of changes in the lipidome of the biofilms of *A. brasilense* strain Sp245 and its *fabG1* and *mmsB1* mutants during the course of their formation and degradation.

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