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FTIR spectroscopic study of biofilms formed by the rhizobacterium *Azospirillum brasilense* Sp245 and its mutant *Azospirillum brasilense* Sp245.1610

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ABSTRACT

Biofilms are spatially and metabolically structured communities of microorganisms, representing a mode of their existence which is ubiquitous in nature, with cells localised within an extracellular biopolymeric matrix, attached to each other, at an interface. For plant-growth-promoting rhizobacteria (PGPR), the formation of biofilms is of special importance due to their primary localisation at the surface of plant root systems. In this work, FTIR spectroscopy was used, for the first time for bacteria of the genus *Azospirillum*, to comparatively study 6-day-mature biofilms formed on the surface of ZnSe discs by the rhizobacterium *Azospirillum brasilense* Sp245 and its mutant *A. brasilense* Sp245.1610. The mutant strain, having an Omegon Km insertion in the gene of lipid metabolism *fabG1* on the plasmid AZOBR_p1, as compared to the wild-type strain Sp245 (see http://dx.doi.org/10.1134/S1022795413110112), had previously been shown to possess alterations in the synthesis of fatty acids, as well as in the amount of biomass and relative content of lipopolysaccharide antigens in mature biofilms formed at a hydrophilic or hydrophobic surface (see http://dx.doi.org/10.1134/S002626171602017X). The 6-day biofilm of the wild-type strain *A. brasilense* Sp245 was found to contain moderate amounts of poly-3-hydroxybutyrate (PHB, a reserve biopolyester) featured by its typical bands (e.g., the ester carbonyl stretching mode at 1732 cm^{-1}), while its mutant strain showed a diminished PHB content in the biofilm (the observed differences were also confirmed by analysing the second derivatives of the FTIR spectra). Thus it may be assumed that in this mutant strain, PHB synthesis is also affected which, in turn, can affect the formation and stability of biofilms.

1. Introduction

The formation of biofilms is a natural process in microbial ecology which plays diverse important roles in the environment (see, e.g. Refs. [1–3] and references reported therein). For plant-growth-promoting rhizobacteria (PGPR), this feature is yet more important in view of their primary localisation at the surface of plant root systems. Among PGPR, bacteria of the genus *Azospirillum*, and particularly the ubiquitous species *A. brasilense*, are widely studied because of their phytostimulating potential [4,5]. They can form the associations with plant roots and fix atmospheric nitrogen [5,6]. Our previous studies using Fourier transform infrared (FTIR) spectroscopy in various modes (see, e.g. Refs. [7–10]) showed this technique to be a sensitive and informative tool for monitoring molecular-level changes in bacteria (particularly on the examples of *A. brasilense*) induced by various environmental factors, including different stresses.

In natural conditions, the bacteria of the genus *Azospirillum* (as well as many other PGPR) exist mainly in the form of biofilms and are often under various stress conditions. Biofilms are spatially and metabolically structured communities of microorganisms, representing a mode of their existence which is ubiquitous in nature,

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with cells localised within an extracellular biopolymeric matrix, attached to each other, at an interface. Biofilms of various bacteria are currently under thorough investigation [1–3], as information on their structure, composition and modes of formation is of topical interest and importance. However, information on PGPR biofilms, including those of azospirilla [11–15], is scarce, and the application of FTIR spectroscopy may be helpful in elucidating possible molecular structural and compositional changes which accompany the transformation of planktonic cultures (i.e. cells in a cultural solution) to a biofilm.

In this communication, FTIR spectroscopy was used, for the first time for bacteria of the genus *Azospirillum*, to comparatively characterise 6-day-mature biofilms of *A. brasilense* wild-type strain Sp245 and its mutant strain Sp245.1610 (defective in mobility and capability of swimming [16]).

2. Materials and methods

2.1. Bacterial strains and growth conditions

Wild-type *A. brasilense* strain Sp245 [17] (from The Collection of Rhizosphere Microorganisms, [WDCM 1021], Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia) and its mutant leaky Fla^-/Mot^- Laf^- Swa^- Sp245.1610 (*fabG1*::Omegon-Km) [16], which forms biofilms different from those of the wild-type strain [12], were used in this work.

Bacteria were cultivated in a liquid modified malate salt medium (MSM) [18] which contained the following salts ($g \cdot l^{-1}$): K_2HPO_4, 3.0; KH_2PO_4, 2.0; NH_4Cl, 1.0; NaCl, 0.1; $FeSO_4 \cdot 7H_2O$, 0.02 (added as chelate with nitrilotriacetic acid); $CaCl_2$, 0.02; $MgSO_4 \cdot 7H_2O$, 0.2; $Na_2MoO_4 \cdot 2H_2O$, 0.002; sodium malate, 5.0 (obtained by mixing 3.76 g of malic acid with 2.24 g NaOH per litre), pH 6.8–7.0. Kanamycin (Km) was added to the medium at a final concentration of 50 μg/ml in all cases when the mutant strain was cultivated.

The inoculation cultures (100 ml in 250-ml Erlenmeyer flasks) were grown under aerobic conditions on a shaker (140 rpm) for up to 18 h. Cell growth was monitored by optical density A_{590} at $\lambda = 590$ nm (Spekol 221, Germany, optical path length 0.5 cm).

Biofilms of the cultures under study were obtained as follows (all procedures were carried out under sterile conditions). A clean ZnSe disc (CVD-ZnSe, "R'AIN Optics", Dzerzhinsk, Russia; diameter 2.5 cm, thickness 0.2 cm) was placed on the bottom of a Petri dish (diameter 4 cm), in which 3 ml of the MSM medium with bacteria (*A. brasilense* strains inoculated up to the final optical density $A_{590} = 0.05$ to 0.1) were added. Then the Petri dishes were placed into a thermostat at 28 °C and incubated for 6 d.

2.2. Sample preparation and Fourier transform infrared (FTIR) spectroscopy

For FTIR spectroscopic measurements, the 6-day-mature biofilms that had formed on the surface of the ZnSe glass discs (see above) were carefully separated from the culture medium with the remaining planktonic culture and dried at 45 °C up to a constant weight. (It should be noted that this sample preparation procedure, without active washing, was necessary to obtain an intact biofilm. It was experimentally found that, under active washing in order to fully remove the culture medium components, the formed biofilm could be partly damaged or even removed by the water flow.) As a cell-free control, a minimal volume of the MSM medium was placed as a thin film on a clean flat ZnSe disc and dried.

Infrared spectroscopic measurements were performed on a Nicolet 6700 FTIR spectrometer (Thermo Electron Corporation, USA; DTGS detector; KBr beamsplitter). Spectra were collected in the transmission mode with a total of 64 scans (resolution 2 cm^{-1}) against the ZnSe disc background and manipulated using the OMNIC (version 8.2.0.387) software supplied by the manufacturer of the spectrometer. All spectra were smoothed using the standard "automatic smooth" function of the software which uses the Savitsky–Golay algorithm (95-point moving second-degree polynomial), and then the baseline was corrected using the "automatic baseline correct" function. All the FTIR spectroscopic measurements were done in triplicate and were reproducible. The second derivatives of the FTIR spectra of the biofilms were calculated using the OMNIC software (the Savitsky–Golay method; number of points: 29; third-degree polynomial).

3. Results and discussion

For a successful functioning of a plant-bacterial association, the ability of rhizobacteria, including azospirilla, to form biofilms may be essential [13,19]. In the recent years, biofilms formed by agro-biotechnologically promising strains *A. brasilense* Sp7 and *A. brasilense* Sp245 have been under investigation; particularly, metabolic processes important for biofilm formation were studied using their mutant strains defective in flagellation, motility, etc. [11–15].

In 1985, a report was published on the use of FTIR spectroscopy in microbial ecology for the analysis of bacteria, bacteria-polymer mixtures and biofilms [20]. Currently, FTIR spectroscopy is a well-established tool in microbiology [21–23], including a few reported studies of biofilms formed by various bacteria [24–27]. This technique can be used to obtain information on the physicochemical state of biofilms and on the relative composition of their macrocomponents (using an appropriate sample preparation procedure) virtually *in situ* without isolation and characterisation of the matrix constituents. This information complements that obtained by other (mainly biochemical) methods on the formation and structure of microbial biofilms.

Fig. 1(A–C) shows FTIR spectra (registered in the transmission mode) of the samples (as dried 6-day-mature biofilms formed on the surface of ZnSe discs) of *A. brasilense* Sp245 (Fig. 1A) and its mutant Sp245.1610 (Fig. 1B), as well as of the control sample of the MSM (Fig. 1C). Wavenumbers of the maxima for the main bands in the FTIR spectra are summarised in Table 1 together with their tentative assignment [8,9,21–31].

The presented FTIR spectra of the 6-day-mature biofilms formed by both *A. brasilense* Sp245 and its mutant *A. brasilense* Sp245.1610 show all the typical bands corresponding to bacterial macrocomponents (proteins, polysaccharides, lipids, etc.; see Fig. 1A and B and Table 1). The differences between the biofilms are small but clearly visible. First of all, in the spectrum of the wild-type strain (Fig. 1A) there is a well-resolved band at 1732 cm^{-1} typical of the ν(C=O) mode in poly-3-hydroxybutyrate (PHB; reserve biopolyester that can be synthesised and accumulated by azospirilla as intracellular granules [8]). It should be noted that in *A. brasilense* the accumulation of noticeable amounts of PHB (detectable by FTIR spectroscopy) is induced mainly by nitrogen deficiency [7,8]. In this case, however, the MSM contained 1 g l^{-1} of NH_4Cl, which is commonly sufficient for this nitrogen-fixing bacterium to avoid trophic stress caused by lack of bound nitrogen (see, e.g. the data reported in Ref. [32]). The accumulation of PHB up to about 10% of dry cell biomass (estimated from the FTIR spectrum in Fig. 1A as reported in Ref. [7]) in this case is evidently related to the prolonged cultivation (6 d) of the biofilm when most of the nitrogen might have been consumed causing its deficiency and, consequently, PHB accumulation (note that carbon-containing nutrients in the culture medium available for this moderate PHB biosynthesis in the biofilm might well have been represented by bacterial exopolysaccharides

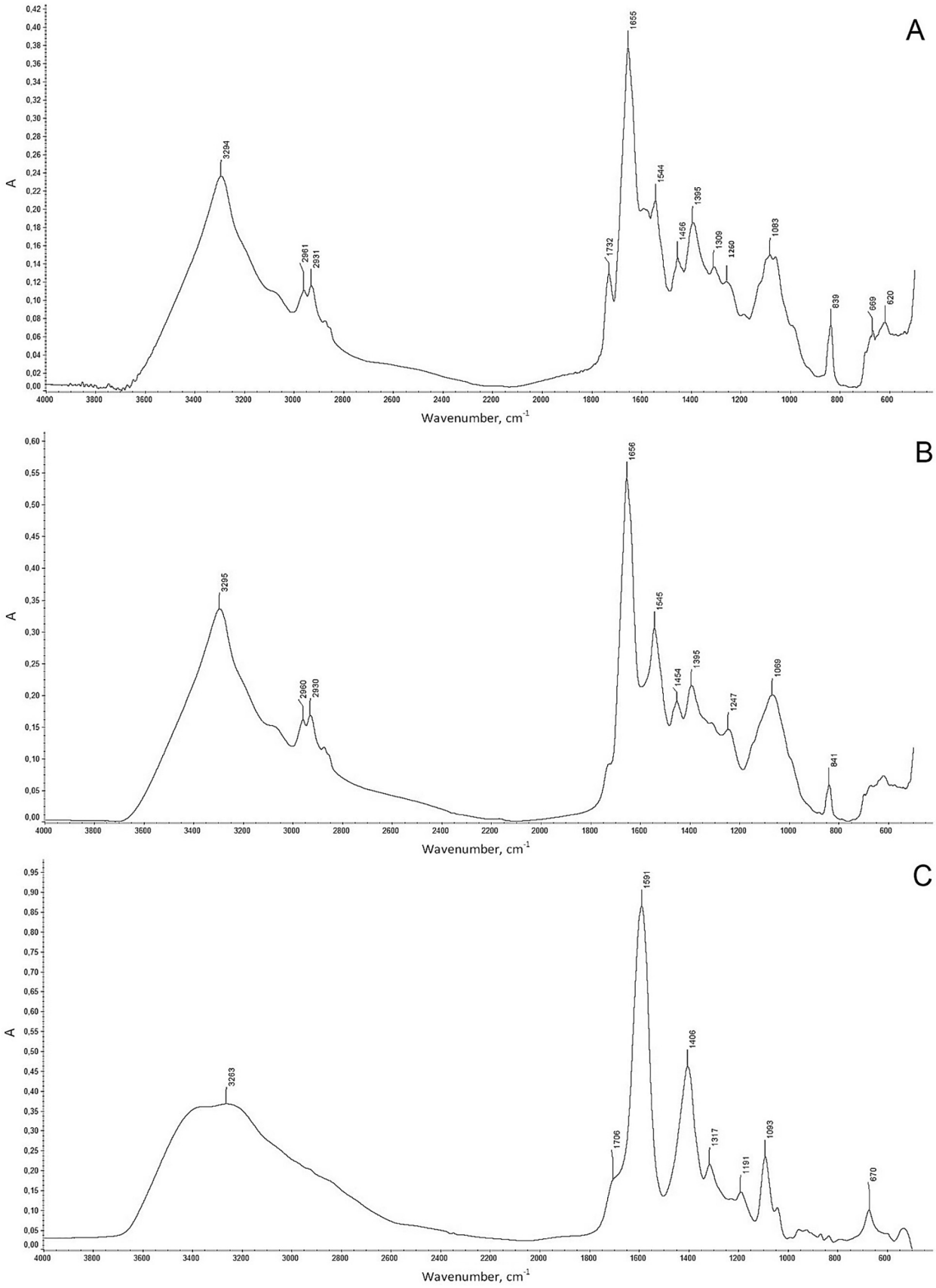

Fig. 1. Fourier transform infrared spectra (in the transmission mode, measured as dried films) of the biofilms of *Azospirillum brasilense* Sp245 (A) and its mutant *Azospirillum brasilense* Sp245.1610 (B) grown on the ZnSe disc surfaces, as well as the cell-free control (dried MSM) on a flat ZnSe disc (C).

Table 1
Wavenumbers of the main bands in the FTIR spectra (see Fig. 1) and their tentative assignment [8,9,21–31].

Sample	Tentative assignment of main bands to the relevant functional groups (wavenumbers, $\gtrsim m^{-1}$)													
	O–H; N–H (amide A in proteins, etc.), ν	C–H in $-CH_3$, ν_{as} in $>CH_2$, ν_{as}	C–H	C=O, ν (ester moiety)	C=O, ν (in carboxyl)	Amide I (in proteins)[a]	Carboxyl COO^-, ν_{as}	Amide II (in proteins)	$-CH_3$, δ (in proteins, lipids, polyesters, etc.)	COO^-, ν_s	C–O ν (in malate)	C–O–C/C–C–O ν (in ester moieties)	Amide III/O–P=O ν_{as}	C–O, C–C, "Fingerprint region" C–OH ν; C–O–H, C–O–C δ		
6-day-mature biofilm of *A. brasilense* Sp245	3294	2961	2931	1732	–[b]	1655	–[c]	1544	1456	1395	–	1309	1260	1083	839	669
																620
6-day-mature biofilm of *A. brasilense* Sp245.1610	3295	2960	2930	–[d]	–[b]	1656	–[c]	1545	1454	1395	–	–	1247	1069	841	620
Control (MSM)	3200–3400[g]	–	–[e]	–	1706	–	1591	–	–	1406	1317	–	–	1191[f] 1093[f]	–	670

Designations: ν – stretching vibrations; ν_s – symmetric stretching vibrations; ν_{as} – antisymmetric stretching vibrations; δ – bending vibrations; dash (–) means the absence of a band or that a possible similar weak band is evidently overlapped by a neighbouring stronger one.

[a] The registered wavenumbers of amide I (in proteins) correspond to a dominating α-helix in bacterial biofilms in both cases.
[b] Evidently weak (overlapped by ν(C=O) of ester carbonyl).
[c] Evidently overlapped by amide II (in proteins).
[d] A weaker shoulder is registered in this region.
[e] Evidently weak ν(CH_2) modes in malate (overlapped by the right-hand wing of the broad ν(O–H) envelope).
[f] May include overlapped bands related to phosphates contained in the culture medium (see Section 2.1).
[g] Flat broad ν(O–H) envelope.

and/or lysed cell components [32]). It may also be mentioned that the position of the maximum of this ν(C=O) band (1732 cm^{-1}), which is sensitive to the degree of PHB crystallinity [33,34] (commonly appearing at even higher wavenumbers, up to ~1750 cm^{-1}, in a fully amorphous biopolyester, including that in bacterial cells, or down to 1725 cm^{-1} in its more crystalline form [32]), corresponds to PHB of a relatively low (intermediate) degree of crystallinity, typical of intracellular PHB granules.

In contrast to the wild-type strain, the mutant strain *A. brasilense* Sp245.1610 shows only a relatively weak shoulder in this region (see Fig. 1B), reflecting a significantly lower PHB accumulation under the same conditions (the shoulder may also be assigned to other common cellular esters, such as lipopolysaccharides and phospholipids [8–10,23,32]). This mutant strain, having an Omegon Km insertion in the gene of lipid metabolism *fabG1* on the plasmid AZOBR_p1 [16], as compared to the wild-type strain *A. brasilense* Sp245, is known to possess alterations in the synthesis of fatty acids, as well as in the amount of biomass and relative content of lipopolysaccharide antigens in mature biofilms formed at a hydrophilic or hydrophobic surface [12]. Thus it may be assumed that in this mutant strain, PHB synthesis is also affected which, in turn, can affect the formation and stability of biofilms.

The FTIR spectrum of the freshly prepared MSM used for culturing the strains (dried as a thin film on a clean flat ZnSe disc; see Fig. 1C) shows no bands coinciding with those of the biofilms (see Fig. 1A and B). This shows that the minimal non-invasive sample preparation procedure used in this work (careful removal of the medium with the remaining planktonic culture from the biofilm formed on the ZnSe disc with subsequent drying at 45 °C) is sufficient, so that possible remainders of the culture medium components do not contribute appreciably to the FTIR spectra of the biofilms.

The differences in the spectra of the biofilms (see Fig. 1, spectra A and B) mentioned above are corroborated and more clearly illustrated by their second derivatives (Fig. 2, spectra A and B, respectively) calculated and presented in the most informative spectroscopic region (under 2000 cm^{-1}; note that peaks in the second derivatives, directed downwards, correspond both to peaks and to poorly resolved shoulders (i.e., spectral components overlapped by stronger neighbouring peaks) in the initial spectrum, allowing them to be better resolved). In particular, note the significantly more relatively intensive peak at 1732 cm^{-1} of the ν(C=O) mode in PHB in Fig. 2A than that at 1733 cm^{-1} in Fig. 2B (as compared to the strong amide I protein-related peaks at 1655 cm^{-1} in Fig. 2A and B, respectively), with correspondingly more pronounced peaks in the region at and under 1309 cm^{-1} in Fig. 2A. It may also be noticed that, along with the strong amide I peaks at 1655–1656 cm^{-1} related to the dominating α-helix in cellular proteins in both bacterial biofilms (see Fig. 1A and B and Table 1), in Fig. 2A and B they both are accompanied by weaker peaks at 1633 cm^{-1} revealing the presence of a minor contribution of β-structured secondary structure components (β-sheets) in cellular proteins [8,9].

4. Conclusions

FTIR spectroscopy was used, for the first time for bacteria of the genus *Azospirillum*, to comparatively study 6-day-mature biofilms formed on the surface of ZnSe discs by the rhizobacterium *A. brasilense* Sp245 and its mutant starin *A. brasilense* Sp245.1610. It has been found that the biofilm of the wild-type strain *A. brasilense* Sp245 contains moderate amounts of poly-3-hydroxybutyrate (PHB), while the mutant strain showed its diminished content in the biofilm. This may be related to alterations in the synthesis of fatty acids, as well as in the amount of biomass and relative content

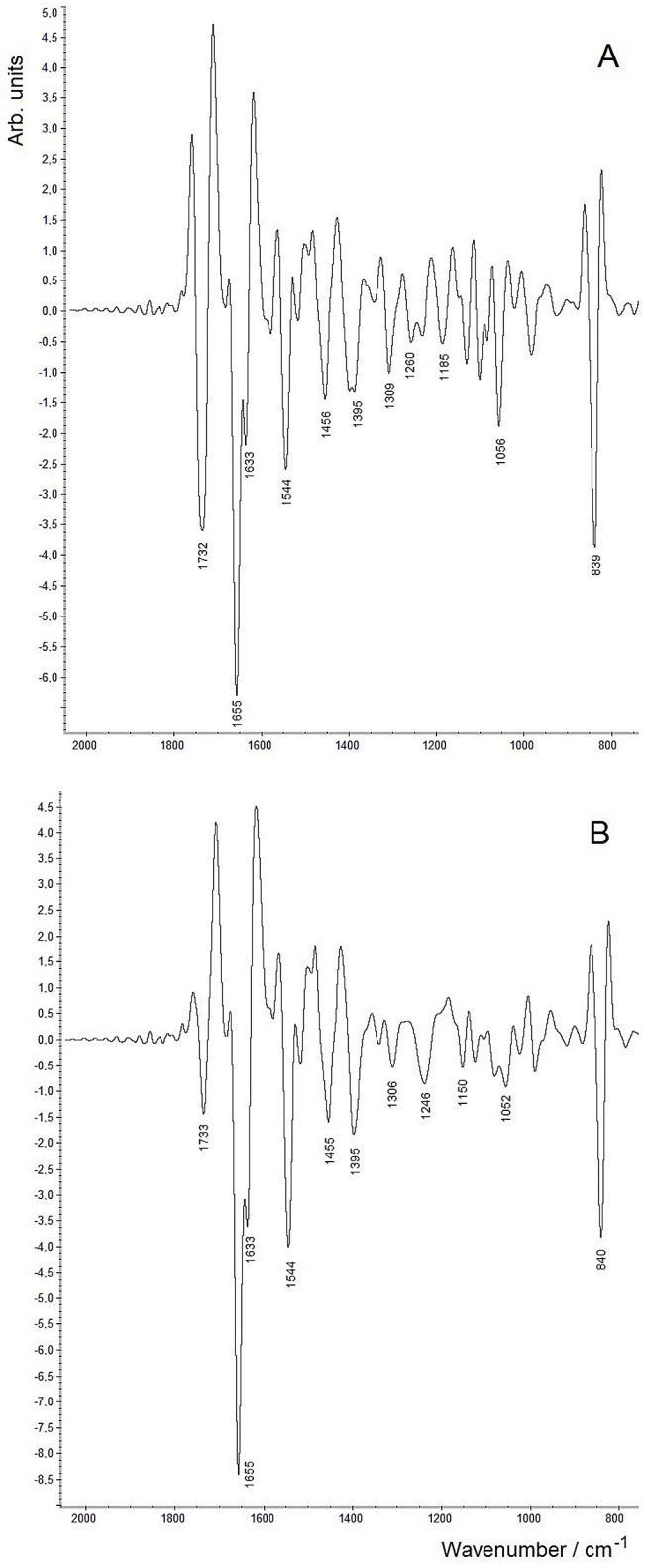

Fig. 2. Second derivatives of the Fourier transform infrared spectra (in the transmission mode, measured as dried films) of the biofilms of *Azospirillum brasilense* Sp245 (A) and its mutant *Azospirillum brasilense* Sp245.1610 (B) grown on the ZnSe disc surfaces (see Fig. 1A and B, respectively).

of lipopolysaccharide antigens in mature biofilms, characteristic of the mutant strain. It may therefore be assumed that in this mutant strain, PHB synthesis is also affected which, in its turn, can affect the formation and stability of biofilms.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

[1] K. Vickery, H. Hu, A.S. Jacombs, D.A. Bradshaw, A.K. Deva, A review of bacterial biofilms and their role in device-associated infection, Healthc. Infect. 18 (2) (2013) 61–66, http://dx.doi.org/10.1071/HI12059.
[2] D. Kusić, B. Kampe, A. Ramoji, U. Neugebauer, P. Rösch, J. Popp, Raman spectroscopic differentiation of planktonic bacteria and biofilms, Anal. Bioanal. Chem. 407 (22) (2015) 6803–6813, http://dx.doi.org/10.1007/s00216-015-8851-7.
[3] K.N. Kragh, J.B. Hutchison, G. Melaugh, C. Rodesney, A.E.L. Roberts, Y. Irie, P.Ø. Jensen, S.P. Diggle, R.J. Allen, V. Gordon, T. Bjarnsholt, Role of multicellular aggregates in biofilm formation, mBio 7 (2) (2016), http://dx.doi.org/10.1128/mBio.00237-16 e00237–16.
[4] F. Cassán, M. Diaz-Zorita, *Azospirillum* sp. in current agriculture: from the laboratory to the field, Soil Biol. Biochem. 103 (2016) 117–130, http://dx.doi.org/10.1016/j.soilbio.2016.08.020.
[5] Y. Bashan, L.E. de-Bashan, How the plant growth-promoting bacterium *Azospirillum* promotes plant growth – a critical assessment, Adv. Agron. 108 (2010) 77–136, http://dx.doi.org/10.1016/S0065-2113(10)08002-8.
[6] L. Pereg, L.E. de-Bashan, Y. Bashan, Assessment of affinity and specificity of *Azospirillum* for plants, Plant Soil 399 (1–2) (2016) 389–414, http://dx.doi.org/10.1007/s11104-015-2778-9.
[7] A.A. Kamnev, A.V. Tugarova, P.A. Tarantilis, P.H.E. Gardiner, M.G. Polissiou, Comparing poly-3-hydroxybutyrate accumulation in *Azospirillum brasilense* strains Sp7 and Sp245: the effects of copper (II), Appl. Soil Ecol. 61 (2012) 213–216, http://dx.doi.org/10.1016/j.apsoil.2011.10.020.
[8] A.A. Kamnev, J.N. Sadovnikova, P.A. Tarantilis, M.G. Polissiou, L.P. Antonyuk, Responses of *Azospirillum brasilense* to nitrogen deficiency and to wheat lectin: a diffuse reflectance infrared Fourier transform (DRIFT) spectroscopic study, Microb. Ecol. 56 (4) (2008) 615–624, http://dx.doi.org/10.1007/s00248-008-9381-z.
[9] A.A. Kamnev, FTIR spectroscopic studies of bacterial cellular responses to environmental factors, plant-bacterial interactions and signalling, Spectrosc. Int. J. 22 (2–3) (2008) 83–95, http://dx.doi.org/10.3233/SPE-2008-0329.
[10] A.A. Kamnev, A.V. Tugarova, L.P. Antonyuk, P.A. Tarantilis, L.A. Kulikov, Yu.D. Perfiliev, M.G. Polissiou, P.H.E. Gardiner, Instrumental analysis of bacterial cells using vibrational and emission Mössbauer spectroscopic techniques, Anal. Chim. Acta 573–574 (2006) 445–452, http://dx.doi.org/10.1016/j.aca.2006.04.041.
[11] A. Ramírez-Mata, L.I. López-Lara, M.L. Xiqui-Vázquez, S. Jijón-Moreno, A. Romero-Osorio, B.E. Baca, The cyclic-di-GMP diguanylate cyclase CdgA has a role in biofilm formation and exopolysaccharide production in *Azospirillum brasilense*, Res. Microbiol. 167 (3) (2016) 190–201, http://dx.doi.org/10.1016/j.resmic.2015.12.004.
[12] E.M. Shumilova, A.V. Shelud'ko, Yu.A. Filip'echeva, S.S. Evstigneeva, E.G. Ponomareva, L.P. Petrova, E.I. Katsy, Changes in cell surface properties and biofilm formation efficiency in *Azospirillum brasilense* Sp245 mutants in the putative genes of lipid metabolism *mmsB1* and *fabG1*, Microbiol. Moscow 85 (2) (2016) 172–179, http://dx.doi.org/10.1134/S002626171602017X.
[13] A.V. Shelud'ko, Yu.A. Filip'echeva, E.M. Shumilova, B.N. Khlebtsov, A.M. Burov,

L.P. Petrova, E.I. Katsy, Changes in biofilm formation in the nonflagellated flhB1 mutant of *Azospirillum brasilense* Sp245, Microbiol. Moscow 84 (2) (2015) 144–151, http://dx.doi.org/10.1134/S0026261715010129.
[14] L.P. Petrova, A.V. Shelud'ko, E.I. Katsy, Plasmid rearrangements and alterations in *Azospirillum brasilense* biofilm formation, Microbiol. Moscow 79 (1) (2010) 121–124, http://dx.doi.org/10.1134/S0026261710010169.
[15] A.V. Shelud'ko, O.V. Kulibyakina, A.A. Shirokov, L.P. Petrova, L.Yu. Matora, E.I. Katsy, The effect of mutations affecting synthesis of lipopolysaccharides and calcofluor-binding polysaccharides on biofilm formation by *Azospirillum brasilense*, Microbiol. Moscow 77 (3) (2008) 313–317, http://dx.doi.org/10.1134/S0026261708030107.
[16] E.A. Kovtunov, A.V. Shelud'ko, M.P. Chernyshova, L.P. Petrova, E.I. Katsy, Mutants of bacterium *Azospirillum brasilense* Sp245 with Omegon insertion in *mmsB* or *fabG* genes of lipid metabolism are defective in motility and flagellation, Russ. J. Genet. 49 (11) (2013) 1107–1111, http://dx.doi.org/10.1134/S1022795413110112.
[17] V.L.D. Baldani, J.I. Baldani, J. Döbereiner, Effects of *Azospirillum* inoculation on root infection and nitrogen incorporation in wheat, Can. J. Microbiol. 29 (8) (1983) 924–929, http://dx.doi.org/10.1139/m83-148.
[18] J.M. Day, J. Döbereiner, Physiological aspects of N_2-fixation by a *Spirillum* from *Digitaria* roots, Soil Biol. Biochem. 8 (1) (1976) 45–50, http://dx.doi.org/10.1016/0038-0717(76)90020-1.
[19] T. Danhorn, C. Fuqua, Biofilm formation by plant-associated bacteria, Annu. Rev. Microbiol. 61 (2007) 401–422, http://dx.doi.org/10.1146/annurev.micro.61.080706.093316.
[20] P.D. Nichols, J.M. Henson, J.B. Guckert, D.E. Nivens, D.C. White, Fourier transform-infrared spectroscopic methods for microbial ecology: analysis of bacteria, bacteria-polymer mixtures and biofilms, J. Microbiol. Methods 4 (2) (1985) 79–94, http://dx.doi.org/10.1016/0167-7012(85)90023-5.
[21] A. Alvarez-Ordonez, D.J.M. Mouwen, M. Lopez, M. Prieto, Fourier transform infrared spectroscopy as a tool to characterize molecular composition and stress response in foodborne pathogenic bacteria, J. Microbiol. Methods 84 (3) (2011) 369–378, http://dx.doi.org/10.1016/j.mimet.2011.01.009.
[22] J.J. Ojeda, M. Dittrich, Fourier transform infrared spectroscopy for molecular analysis of microbial cells, in: A. Navid (Ed.), Microbial Systems Biology: Methods and Protocols, Methods in Molecular Biology, vol. 881, Springer Science+Business Media, LLC, 2012, pp. 187–211, http://dx.doi.org/10.1007/978-1-61779-827-6_8 (Chapter 8).
[23] D. Naumann, Infrared spectroscopy in microbiology, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, UK, 2000, pp. 102–131, http://dx.doi.org/10.1002/9780470027318.a0117 (Updated version: P. Lasch, D. Naumann. Infrared spectroscopy in microbiology. In: R.A. Meyers (ed.). Encyclopedia of Analytical Chemistry. J. Wiley & Sons. Published online 12 March 2015. http://dx.doi.org/10.1002/9780470027318.a0117.pub2).
[24] S. Wu, M.M. Baum, J. Kerwin, D. Guerrero, S. Webster, C. Schaudinn, D. VanderVelde, P. Webster, Biofilm-specific extracellular matrix proteins of nontypeable *Haemophilus influenzae*, Pathog. Dis. 72 (3) (2014) 143–160, http://dx.doi.org/10.1111/2049-632X.12195.
[25] J.J. Ojeda, M.E. Romero-González, S.A. Banwart, Analysis of bacteria on steel surfaces using reflectance micro-Fourier transform infrared spectroscopy, Anal. Chem. 81 (15) (2009) 6467–6473, http://dx.doi.org/10.1021/ac900841c.
[26] J. Pink, T. Smith-Palmer, D. Chisholm, T.J. Beveridge, D.A. Pink, An FTIR study of *Pseudomonas aeruginosa* PAO1 biofilm development: interpretation of ATR–FTIR data in the 1500–1180 cm^{-1} region, Biofilms 2 (3) (2005) 165–175, http://dx.doi.org/10.1017/S1479050505001936.
[27] J. Pink, T. Smith-Palmer, T.J. Beveridge, D.A. Pink, An FTIR study of *Pseudomonas aeruginosa* PAO1 biofilm growth and dispersion. An improved ATR method for studying biofilms: the C–H stretch spectral region, Biofilms 1 (3) (2004) 157–163, http://dx.doi.org/10.1017/S1479050504001413.
[28] D. Naumann, FT-infrared and FT-Raman spectroscopy in biomedical research, Appl. Spectrosc. Rev. 36 (2–3) (2001) 239–298, http://dx.doi.org/10.1081/ASR-100106157.
[29] M. Beekes, P. Lasch, D. Naumann, Analytical applications of Fourier transform-infrared (FT-IR) spectroscopy in microbiology and prion research, Vet. Microbiol. 123 (4) (2007) 305–319, http://dx.doi.org/10.1016/j.vetmic.2007.04.010.
[30] A. Barth, Infrared spectroscopy of proteins, Biochim. Biophys. Acta 1767 (9) (2007) 1073–1101, http://dx.doi.org/10.1016/j.bbabio.2007.06.004.
[31] Z. Movasaghi, S. Rehman, I. ur Rehman, Fourier transform infrared (FTIR) spectroscopy of biological tissues, Appl. Spectrosc. Rev. 43 (2) (2008) 134–179, http://dx.doi.org/10.1080/05704920701829043.
[32] A.A. Kamnev, P.V. Mamchenkova, Yu.A. Dyatlova, A.V. Tugarova, FTIR spectroscopic studies of selenite reduction by cells of the rhizobacterium *Azospirillum brasilense* Sp7 and the formation of selenium nanoparticles, J. Mol. Struct. (2016), http://dx.doi.org/10.1016/j.molstruc.2016.12.003.
[33] M. Kansiz, A. Domínguez-Vidal, D. McNaughton, B. Lendl, Fourier-transform infrared (FTIR) spectroscopy for monitoring and determining the degree of crystallisation of polyhydroxyalkanoates (PHAs), Anal. Bioanal. Chem. 388 (5) (2007) 1207–1213, http://dx.doi.org/10.1007/s00216-007-1337-5.
[34] H. Sato, R. Murakami, K. Mori, Y. Ando, I. Takahashi, I. Noda, Y. Ozaki, Specific crystal structure of poly(3-hydroxybutyrate) thin films studied by infrared reflection–absorption spectroscopy, Vib. Spectrosc. 51 (1) (2009) 132–135, http://dx.doi.org/10.1016/j.vibspec.2008.11.010.