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EXPERIMENTAL ARTICLES

Cell Wall Glycopolymers as a Diagnostic Trait of *Arthrobacter crystallopoietes*

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Abstract—The composition and structure of the cell wall glycopolymers from *Arthrobacter crystallopoietes* VKM Ac-1107^T (family *Micrococcaceae*, phylum *Actinobacteria*), previously assigned to the *"A. globiformis"* group based on the high similarity of 16S rRNA gene sequences and traditional chemotaxonomic markers were studied. Teichoic acid—1,3-poly(glycerol phosphate) substituted with β-glucose residues, and diglycosyl 1-phosphate polymer with -6)-α-D-Gal*p*NAc-(1→6)-α-D-Glc*p*NAc-(1-*P*-repeating unit were identified by chemical and NMR spectroscopy methods. The results of phylogenomic (taxogenomic) analysis, *viz.* determination of the average amino acid identity (AAI) and the similarity of conserved proteins (POCP), indicate that *A. crystallopoietes* belongs to a new genus and the composition of the cell wall glycopolymers may serve as a diagnostic characteristic of this genus, which will be described on the basis of *A. crystallopoietes.*

Keywords: *Arthrobacter crystallopoietes*, cell wall glycopolymers, teichoic acids, systematics, taxogenomics
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Investigation of cell wall polymers is important in fundamental and applied respects, in particular, for the taxonomy of microorganisms. Though the recent prokaryotic taxonomy is increasingly based on phylogenomic data, the phenotypic traits, in particular, chemotaxonomic ones reflecting the chemistry of cells and cell walls, are still important (Chun et al., 2018; Nouioui et al., 2018; Salam et al., 2020). Peptidoglycan, which is characterized by a high structural diversity, is known to be the main cell wall glycopolymer of gram-positive bacteria (Schleifer and Kandler, 1972; Schumann, 2011). The types and variations in peptidoglycan structure serve as important chemotaxonomic markers of bacterial taxa at different ranks (Schumann et al., 2009; Schumann, 2011). Apart from peptidoglycan, gram-positive bacteria usually have secondary cell wall glycopolymers (Kohler et al., 2009). These include polymers covalently bound to peptidoglycan such as teichoic acids, poly(glycosyl phosphates) and various phosphate-free glycopolymers, both neutral and acidic ones (among them teichuronic and teichulosonic acids), as well as the membrane-bound lipoteichoic acids (Kohler et al., 2009; Potekhina et al., 2011, 2021; Shashkov et al., 2020a, 2020b). Compared to peptidoglycan, secondary bacterial glycopolymers are less studied in the taxonomic aspect. However, the information available in the literature indicates that the composition, chemical structures, and some individual structural components of glycopolymers can be specific for species, genera, and higher taxa of actinobacteria (Takeuchi and Yokota, 1989; Schumann et al., 2009; Potekhina et al., 2011, 2021; Evtushenko and Ariskina, 2015; Goodfellow and Jones, 2015; Nouioiui et al., 2018; Shashkov et al., 2020b).

While the species *A. crystallopoietes* is a member of the revised genus *Arthrobacter* sensu lato, its position on 16S rRNA-based phylogenetic trees constructed using several methods is separate from *A. globiformis* and other species of the "*A. globiformis*" group (*A. humicola, A. oryzae*, and *A. pascens*) (Busse et al., 2012; Busse, 2016). It was, however, tentatively assigned to the "*A. globiformis*" group, taking into account its high similarity to *A. globiformis* in the 16S rRNA gene sequences (97.6%) and similarities to it and other species of the "*A. globiformis*" group in the key chemotaxonomic traits marker—A3α type pepti-

Accepted abbreviations: HSQC, proton-detected heteronuclear single-quantum correlation; COZY, correlation spectroscopy; TOCSY, total correlation spectroscopy; ROESY, rotating-frame nuclear overhauser effect correlation spectroscopy; HMBC, heteronuclear multiple bond correlation through several bonds; δ_C, δ_H, δ_P are the chemical shifts of the ^{13}C, ^1H, and ^{31}P atoms, respectively; AAI, average amino acid identity; POCP, percentage of conserved proteins.

doglycan, the main menaquinone MK-9(H_2) and polar lipid composition (Busse et al., 2012; Busse, 2016).

Phylogenetic separation of *A. crystallopoietes*, as well as its differences from the "*A. globiformis*" group in terms of the structure of the peptidoglycan interpeptide bridge (the number of alanine residues), support the suggestion that it belongs to a genus-rank group different from "*A. globiformis*" (Busse, 2016).

Moreover, early works (Sadikov et al., 1983; Takeuchi and Yokota, 1989) reported that *A. crystallopoietes* differed from members of the *"A. globiformis"* group (*A. globiformis* and *A. pascens*) in the composition of cell wall glycopolymers. *A. crystallopoietes* cell wall contains teichoic acid, while *A. globiformis* and *A. pascens* are characterized by the presence of phosphate-free glycopolymers. However, the chemical structures of the aforementioned polymers in *A. crystallopoietes* have not been established. Neither is there any information on the presence or absence in the cell wall of *A. crystallopoietes* of neutral polysaccharides characteristic of *A. globiformis, A. pascens, A. citreus, A. ramosus,* and some other *Arthrobacter* species (Sadikov et al., 1983; Takuchi and Yokota, 1989; Potekhina et al., 2021; Zhou et al., 2009; Busse, 2016; Busse and Moore, 2018). Moreover, conclusions about the presence or absence of glycopolymers of one type or another in the cell wall based on the results of destructive methods alone (analysis of acid degradation products of whole cell walls and of carbohydrate-containing polymer preparations isolated from cell walls) may be erroneous. NMR spectroscopy is required to confirm the polymer types and structures (Fiedler and Schäffler, 1987; Takeuchi and Yokota, 1989; Shashkov et al., 2020a, 2020b).

It is also worth noting that with the accumulation of data on whole genomes and the development of phylogenomics (taxogenomics), it became obvious that the results of a comparative study of 16S rRNA genes are often not sufficient for discrimination between closely related genera (Konstantinidis and Tiedje, 2005; Qin et al., 2014). Methods of comparative genomics, including determination of average amino acid identity (AAI) (Konstantinidis and Tiedje, 2005; Kim et al., 2021) and the percentage of conserved proteins (POCP) (Qin et al., 2014), provide better resolution for determination of the genus position of prokaryotic microorganisms and elucidating the structure of taxa above the species rank.

The goal of the present work was to determine the composition and structures of secondary cell wall glycopolymers of the *A. crystallopoietes* type strain, as well as to clarify the taxonomic status of this species based on taxogenomic analysis.

MATERIALS AND METHODS

The studied strain *Arthrobacter crystallopoietes* VKM Ac-1107^T (=DSM 20117^T) was obtained from the All-Russian Collection of Microorganisms (VKM) (https:// www.vkm.ru).

The culture was grown aerobically at 28°C in flasks on a shaker until the mid-exponential growth phase in a peptone-yeast medium (Potekhina et al., 2011). Cell walls were obtained by differential centrifugation after cell disruption on an UP100H ultrasonic disintegrator (Hielscher, Germany). The isolation of glycopolymers from cell walls was carried out with trichloroacetic acid, as described previously (Potekhina et al., 2011). To study the qualitative composition of the cell wall and glycopolymer preparations, acid hydrolysis was carried out with 2 M HCl, 3 h, 100°C; hydrolysis products were analyzed by electrophoresis and paper chromatography as described previously (Potekhina et al., 2011).

The complete structure of polymers, including the composition of monomers, the position of phosphodiester bonds in the chain, as well as the position and configuration of glycoside bonds, was determined by NMR spectroscopy. NMR spectra of the preparations were recorded in solutions of 99.96% deuterated water at temperatures that ensured minimal overlap of the residual signal of deuterated water with the signals of polymers using an Avance 600 spectrometer (Bruker, Germany). Chemical shifts were measured using sodium salt of 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid as an internal standard, TSP (δ_H 0.0 and δ_C −1.6), and an external standard, 80% phosphoric acid (δ_P 0.0) at 313 K. Two-dimensional NMR experiments were performed using the standard software (Bruker Optik GmbH, Germany). The spin-lock time in the experiments ^{1}H,^{1}H TOCSY was 250 ms, and the mixing time in the experiment ^{1}H,^{1}H ROESY was 150 ms. Two-dimensional ^{1}H,^{13}C HSQC, HMBC and ^{1}H,^{31}P HMBC experiments were optimized for a spin-spin coupling constant $J_{H,C}$ of 8 Hz.

Phylogenomic (taxogenomic) analysis was performed on the basis of 23 relevant genomic sequences available from the GenBank and GOLD databases: *Acaricomes phytoseiuli* DSM 14247^T AQXM00000000, *Arthrobacter agilis* DSM 20550^T VHIM00000000, *A. citreus* DSM 20133^T VTFV00000000, *A. crystallopoietes* DSM 20117^T CP018863, *A. globiformis* NBRC 12137^T, BAEG00000000, *A. koreensis* DSM 16760^T WACG00000000, *A. luteolus* DSM 13067^T WBJQ00000000, *A. oryzae* DSM 25586^T RBIR00000000, *A. pascens* DSM 545^T JAFHKT000000000, *A. pigmenti* DSM 16403^T JAATJL000000000, *A. psychrolactophilus* B7^T QJVC00000000, *A. woluwensis* DSM 10495^T FNSN00000000, *Citricoccus muralis* DSM 14442^T QREH00000000, *Glutamicibacter protophormiae* DSM 20168^T JAGIOJ000000000, *Haematomicrobium*

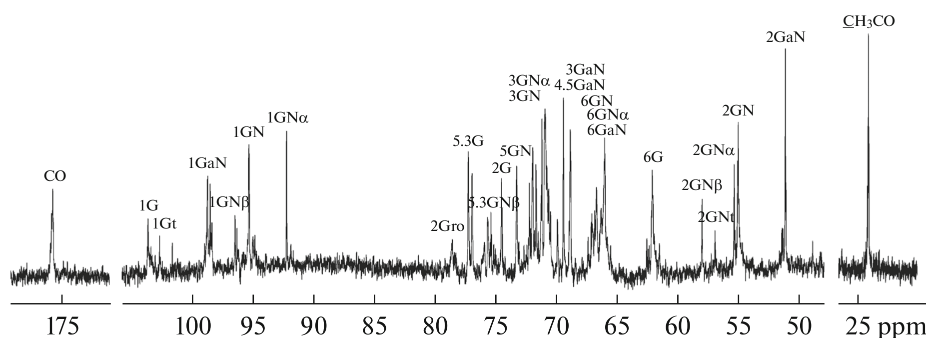

Fig. 1. The ^{13}C NMR spectrum of *A. crystallopoietes* VKM Ac-1107^T cell wall glycopolymers. Arabic numerals refer to the numbers of atoms in residues according to Table 1. The index t (terminal) refers to residues at the ends of the chain.

sanguinis DSM 21259^T JIAG00000000, *Micrococcus luteus* NCTC 2665^T LS483396, *Paenarthrobacter aurescens* NBRC 12136^T BJMD00000000, *Paeniglutamicibacter sulfureus* DSM 20167^T Gold Id:Ga0480495, *Pseudarthrobacter polychromogenes* CGMCC 1.1927^T BMKU00000000, *Pseudoglutamicibacter cumminsii* DSM 10493^T JAFBCO000000000, *Psychromicrobium silvestre* DSM 102047^T JACBYQ000000000, *Sinomonas atrocyanea* KCTC 3377^T CP014518, and *Specibacter cremeus* C1-50^T RWKQ00000000.

AAI values were calculated from the amino acid sequences of the annotated whole genomes using the AAI calculator at http://enve-omics.ce.gatech.edu/ (Rodriguez-R and Konstantinidis, 2016). POCP values were calculated using the runPOCP.sh script (Pantiukh and Grouzdev, 2017) as described previously (Qin et al., 2014).

The phylogenetic tree was constructed by applying the Maximum Likelihood (ML) method based on 90 core genes (the length of protein sequences was 31 358 amino acids) using software packages MICR0B1AL1Z3R (https://microbializer.tau.ac.il/index.html; Avram et al., 2019). The strain *Microbacterium lacticum* DSM 20427 served as an outgroup.

RESULTS AND DISCUSSION

Acid hydrolysates (2 M HCl, 3 h, 100°C) of the cell wall of *A. crystallopoietes* VKM Ac-1107^T and glycopolymer preparations isolated from them by extraction with trichloroacetic acid were found to contain the degradtion products characteristic of teichoic acids (namely, glycerol mono- and bisphosphates), as well as glucose, glucosamine, and galactosamine. Analysis of the glycopolymer preparation by electrophoresis revealed two fractions with a mobility of m_{GroP} 0.68 and m_{GroP} 0.9, which indicated the presence of at least two phosphate-containing polymers.

The ^{13}C NMR spectrum (Fig. 1) of the glycopolymers preparation contained signals of different intensity, which is typical for a polymer with an irregular structure or for a mixture of polymers. Part of the signals (Table 1), judging by the magnitude of chemical shifts δ_C 92.1 ppm (GNα residue) and 96.4 ppm (GNβ residue), belonged to sugar residues with a free hydroxyl group in the C-1 position. Four signals with δ_C 98.7 ppm (GaN residue), 98.4 ppm (GaN' residue), 98.2 ppm (GaN'' residue), and 103.6 ppm (G residue), were characteristic for anomeric carbon atoms at glycosidic bonds, while one signal with δ_C 95.1 ppm belonged to an anomeric carbon atom in the phosphodiester bond (Table 1).

Signals resolution in one-dimensional spectra and conclusions about the structure of polymers were made on the basis of two-dimensional homonuclear $^1H,^1H$ COZY, TOCSY, and ROESY, as well as heteronuclear $^1H,^{13}C$ HSQC, and HMBC experiments (spectra not shown).

Analysis of the $^1H,^1H$ COZY, TOCSY, and ROESY spectra showed the presence in the polymer of substituted at C-6 hydroxyl residues of 2-acetamido-2-deoxy-α-galactopyranose (α-Gal*p*NAc, GaN), 2-acetamido-2-deoxy-α-glucopyranose (α-Glc*p*NAc, GNα), and 2-acetamido-2-deoxy-β-glucopyranose (β-Glc*p*NAc, GNβ), as well as unsubstituted β-glucopyranose residues (β-Glc*p*, G) and trisubstituted glycerol residues (Gro).

The $^1H,^{31}P$ HMBC spectrum showed that phosphoric acid residues were localized on the hydroxyl at C-1 (GNα residues) and C-6 (GaN residues), which are characteristic of the polymer chain of a diglycosyl phosphate polymer (Polymer I), as well as on hydroxyls at C-1,3 of glycerol residues constituting the teichoic acid chain (Polymer II).

Analysis of the two-dimensional $^1H,^{13}C$ HSQC spectrum made it possible to identify all signals in the one-dimensional ^{13}C NMR spectrum (Table 1). As follows from the analysis of chemical shifts, GNα and GNβ residues are substituted at the hydroxyl in C-6

Table 1. Chemical shifts in ¹H (δ_H TSP 0.0) and ¹³C NMR (δ_C TSP −1.6) spectra of *A. crystallopoietes* VKM Ac-1107ᵀ cell wall polymers and Polymer I fragments

Residue	Chemical shifts NMR ¹³C (δ_C TSP −1.6) and ¹H (δ_H TSP 0.0)					
	C-1 *H-1*	C-2 *H-2*	C-3 *H-3* (*H-3e,3a*)	C-4 *H-4*	C-5 *H-5*	C-6 *H-6* (*H-6a,6b*)
Polymer I						
-6)-α-D-Gal*p*NAc-(1→ (GaN)	98.7 *4.90*	51.1** *4.21*	68.8 *3.96*	69.4 *4.05*	70.9 *4.14*	65.8* *4.04, 4.00*
→6)-α-D-Glc*p*NAc-(1-*P*- (GN)	95.1* *5.47*	55.0** *3.96*	72.0 *3.79*	70.6 *3.93*	73.2 *3.99*	66.7 *4.08, 3.69*
Polymer I disaccharides						
α-Gal*p*NAc-(1→ (GaN')	98.4 *4.95*	51.1 *4.18*	68.9 *3.96*	69.7 *4.01*	72.2 *4.00*	62.4 *3.77, 3.75*
→6)-α-D-Glc*p*NAc (GNα)	92.1 *5.20*	55.3 *3.88*	72.0 *3.76*	71.0 *3.60*	71.6 *3.98*	66.8 *4.02, 3.67*
α-D-Gal*p*NAc-(1→ (GaN")	98.2 *4.95*	51.0 *4.21*	69.0 *3.96*	69.7 *4.01*	72.2 *4.00*	62.4 *3.77, 3.75*
→6)-β-D-Glc*p*NAc (GNβ)	96.4 *4.72*	57.9 *3.68*	75.2 *3.53*	70.7 *3.59*	75.6 *3.59*	66.7 *3.97, 3.74*
Polymer II						
-1)-snGro-(3-*P*- (Gro) 2) ↑	65.9*** *4.21, 4.18*	78.2 *4.21*	66.2*** *4.12, 4.11*			
β-D-Glc*p*-(1 (G)	103.6 *4.63*	74.4 *3.32*	76.9 *3.52*	70.9 *3.40*	77.2 *3.47*	61.5 *3.92, 3.77*

* ³¹P at δ_P −1.4 ppm.
** CH_3CON at δ_C 23.3, 23.4 ppm and 176.8, 175.9 and δ_H 2.07 ppm.
*** ³¹P at δ_P +0.5 ppm.

(downfield shift 66.7 and 66.8 ppm, 61–62 ppm in the corresponding unsubstituted residues), and the glycerol residue is additionally substituted at the hydroxyl in the C-2 position (78.2 ppm).

The final conclusion about the structure of polymer chains followed from the analysis of the ¹H,¹H ROESY, and ¹H,¹³C HMBC spectra (Table 1). In the ¹H,¹H ROESY spectrum, a correlation was observed between the anomeric protons of the GaN residues and the protons of the GN residues at C-6 position (4.90/4.08; 3.69), which indicated the spatial proximity of these atoms and the presence of a 1→6 bond between the residues. The H-1 (G)/H-2 (Gro) correlation peak (4.63/4.21) is typical of a 1→2 bond between these residues. The ¹H, ¹³C HMBC spectrum had correlation peaks for the atoms of residues connected by a glycosidic bond: H-6,6' (GNα)/C-1 (GaN') (4.02; 3.67/98.4) and (GNβ)/C-1 (GaN") (3.97; 3.74/98.2) and H-2 (Gro)/C-1(G) (4.21/103.6). These peaks correspond to the structure of the repeating units of two polymers: Polymer I -6)-α-Gal*p*NAc-(1→6)-α-Glc*p*NAc-(1-*P*- and Polymer II -1)-[β-Glc*p*-(1→2)]-snGro-(3-*P*-.

Thus, using NMR spectroscopic methods, we established the complete structures of carbohydrate-containing polymers of the *A. crystallopoietes* cell wall for the first time. The polymers identified are a teichoic acid, 1,3-poly(glycerol phosphate) substituted with β-glucose residues and a diglycosyl 1-phosphate polymer with the repeating unit structure -6)-α-D-Gal*p*NAc-(1→6)-α-D-Glc*p*NAc-(1-*P*-. Phosphate-free glycopolymers characteristic of other studied species of the "*A. globiformis*" group and *Arthrobacter* sensu lato (Sadikov et al., 1983; Takeuchi and Yokota, 1989) were not found in *A. crystallopoietes*.

To clarify the taxonomic status of *A. crystallopoietes,* we determined the AAI and POCP values (Table 2). The POCP similarity of the studied strains (90 core genes with the length of protein sequences was 31 358 amino acids) is presented in the dendrogram (Fig. 2). As can be seen, *A. crystallopoietes* forms a common group with *A. citreus* (Fig. 2). These two

Table 2. Pairwize values (%) of the average amino acid identity (AAI) and percentage of conserved protein (POCP) of 23 species of actinobacteria

No.	Species	AAI, to the right of the diagonal; POCP, to the left of the diagonal																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	*Arthrobacter crystallopoietes*	*	61.5	61.8	58.4	59.4	60.1	60.2	56.3	57.3	52.5	50.8	46.8	54.3	52.2	47.9	46.2	59.0	62.9	60.0	39.9	49.4	57.3	53.8
2	*Arthrobacter globiformis*	64.6	*	79.9	70.0	55.5	55.6	55.3	56.9	56.2	56.9	56.5	48.6	47.9	48.0	48.9	43.9	69.4	54.7	72.1	37.8	53.4	61.0	56.6
3	*Arthrobacter pascens*	65.0	84.1	*	68.4	55.8	55.5	55.5	56.8	56.9	56.6	56.3	49.3	48.2	48.5	49.1	44.8	69.1	55.5	72.2	38.4	53.1	60.7	56.2
4	*Arthrobacter oryzae*	64.2	77.3	77.4	*	59.0	58.9	58.0	59.1	55.9	58.1	57.5	52.0	47.6	48.9	51.4	46.7	65.8	55.2	68.4	39.5	56.7	64.8	59.3
5	*Arthrobacter citreus*	65.6	62.9	63.0	63.2	*	89.4	83.4	63.7	59.5	57.1	55.7	54.8	55.1	54.2	55.2	52.3	60.2	57.5	58.6	45.0	55.5	55.9	55.5
6	*Arthrobacter koreensis*	65.8	63.0	63.1	63.1	91.9	*	83.2	64.3	59.8	57.6	55.3	55.4	55.6	54.4	55.9	53.3	59.2	58.1	58.9	46.4	55.5	55.8	54.6
7	*Arthrobacter luteolus*	65.7	62.4	62.9	63.0	83.2	82.9	*	61.1	58.5	56.2	54.8	52.4	55.1	53.7	53.2	50.4	59.2	57.2	58.1	44.1	53.8	55.1	55.0
8	*Arthrobacter agilis*	65.1	63.7	63.7	63.7	66.3	66.1	65.7	*	65.9	58.2	53.2	55.2	52.6	50.0	56.7	52.2	59.7	52.8	61.1	43.9	55.3	54.1	52.5
9	*Arthrobacter pigmenti*	65.1	62.9	63.2	62.8	65.0	65.0	64.9	68.9	*	55.0	51.3	50.4	48.9	47.7	53.4	46.9	58.8	52.0	58.5	39.5	53.5	51.7	53.6
10	*Arthrobacter psychrolactophilus*	61.2	63.9	63.6	63.4	61.5	61.3	61.0	61.1	60.0	*	57.2	53.6	48.1	52.3	53.4	47.6	63.1	53.6	60.1	40.4	57.8	52.9	60.7
11	*Arthrobacter woluwensis*	61.2	66.0	66.1	65.9	61.3	60.9	60.4	61.2	60.2	61.5	*	54.5	48.0	50.0	54.3	47.6	61.7	51.0	57.2	42.8	58.5	55.1	55.0
12	*Acaricomes phytoseiuli*	61.3	63.2	62.6	63.2	61.6	61.6	61.2	61.6	60.8	62.0	61.4	*	47.4	46.7	58.2	52.0	52.4	45.8	52.5	47.9	63.5	47.9	50.3
13	*Citricoccus muralis*	58.0	56.7	56.2	56.3	57.7	57.9	57.4	57.4	56.0	55.2	55.6	55.7	*	52.1	47.8	62.8	50.0	54.3	49.3	46.3	45.9	48.6	46.4
14	*Glutamicibacter protophormiae*	58.1	56.6	56.7	56.9	57.8	57.5	57.6	57.0	55.9	56.7	56.6	56.2	55.4	*	47.2	50.1	52.3	63.0	49.8	45.1	46.8	48.6	49.8
15	*Haematomicrobium sanguinis*	59.4	58.8	58.9	58.7	59.7	59.6	59.3	59.3	58.8	57.3	58.0	59.9	54.8	55.0	*	51.4	53.1	47.5	51.5	44.8	57.2	47.4	49.7
16	*Micrococcus luteus*	57.1	56.5	56.6	56.8	57.3	57.6	57.4	57.3	56.1	55.3	56.1	56.4	63.3	55.8	55.9	*	46.2	48.7	46.3	51.1	48.0	44.6	44.9
17	*Paenarthrobacter aurescens*	64.2	74.6	75.4	73.5	63.2	63.1	62.9	63.7	62.9	63.9	66.2	62.5	56.1	57.0	58.7	56.0	*	55.6	71.2	39.8	57.6	60.3	58.4
18	*Paeniglutamicibacter sulfureus*	62.2	59.5	60.1	60.0	60.5	60.6	60.5	59.7	58.9	59.1	58.8	58.2	57.3	63.4	56.7	56.6	60.4	*	54.8	43.7	48.1	53.7	53.4
19	*Pseudarthrobacter polychromogenes*	64.9	77.4	77.8	76.0	63.8	64.3	63.3	64.2	63.3	64.1	65.9	62.6	56.9	56.7	58.7	56.4	74.6	60.4	*	39.3	54.5	60.5	55.0
20	*Pseudoglutamicibacter cumminsii*	55.2	54.1	54.2	54.3	55.1	55.4	55.0	54.2	53.5	53.4	54.2	54.2	54.8	55.1	53.2	55.0	54.3	55.9	54.2	*	42.9	39.2	39.7
21	*Psychromicrobium silvestre*	62.0	64.5	64.3	64.3	61.9	61.9	61.3	62.4	61.6	63.6	62.2	68.7	55.2	56.0	59.3	55.9	64.1	58.1	64.0	54.1	*	52.3	56.4
22	*Sinomonas atrocyanea*	62.0	65.3	64.9	65.7	61.7	61.7	61.2	61.7	60.6	60.8	63.9	61.7	57.0	56.6	58.2	57.1	64.7	59.4	65.2	55.0	62.5	*	56.6
23	*Specibacter cremeus*	63.2	65.5	65.1	65.3	62.3	62.3	61.9	62.4	61.8	70.4	62.8	63.1	56.2	57.3	58.6	56.8	64.7	60.1	64.8	54.5	65.1	63.4	*

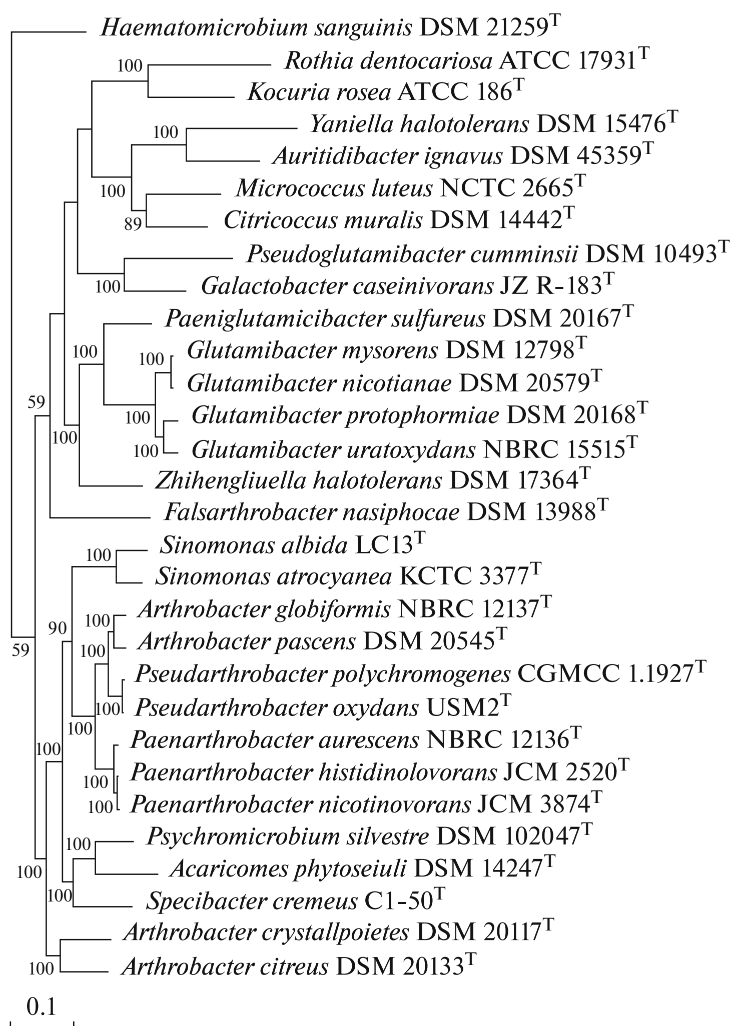

Fig. 2. Maximum-likelihood phylogenetic tree derived from concatenated sequences of 90 conserved proteins (31358 a.a.) constructed using the MICR0B1AL1Z3R server (https://microbializer.tau.ac.il/index.html; Avram et al., 2019). Outgroup—*Microbacterium lacticum* DSM 20427. Bootstrap values (>50%) are listed as percentages at the branching points. Scale bar, 1 amino acid substitution per 100 nucleotides.

species, however, are separated from each other and from *A. globiformis*, *A. pascens* (*"A. globiformis"* group), and also from other species of the *Micrococcaceae* at the genus level, as evidenced by the POCP and AAI values (Table 2).

The POCP values for *A. crystallopoietes* and *A. citreus* (59.4%), as well as for *A. crystallopoietes* in relation to the species of the "*A. globiformis*" group (*A. globiformis, A. pascens,* and *A. oryzae*) (61.5, 61.8, and 58.4%, respectively) are close to or lower than those between the type species of the *Micrococcaceae* genera (for example, *A. globiformis* and *Paenarthrobacter aurescens,* 69.4%; *Acaricomes phytoseiuli* and *Psychromicrobium silvestre,* 63.5%; *Glutamicibacter protophormiae* and *Paeniglutamicibacter sulfureus,* 63.0%; *Micrococcus luteus* and *Citricoccus muralis,* 62.9%). The AAI values for *A. crystallopoites* and each species of the "*A. globiformis*" group (*A. globiformis* (64.6%), *A. pascens* (65.0%), and *A. oryzae* (64.2%), as well as for *A. crystallopoietes* and *A. citreus* (65.6%)) were also close to or lower than the values determined for species of different genera of this group (Table 2). Thus, the AAI values were 77.4, 74.6, 65.5, and 65.3%, respectively, for *A. globiformis* with regard to *Pseudarthrobacter polychromogenes, Paenarthrobacter aurescens, Specibacter cremeus,* and *Sinomonas atrocyanea.* These AAI values correspond to intergeneric values for some other bacterial groups (Nicholson et al., 2020; Ramírez-Durán et al., 2021).

Thus, the results of taxogenomic analysis show that *A. crystallopoietes* is separated at the generic level from the species of the "*A. globiformis*" group and *Arthrobacter* sensu lato as a whole, and also from other members of the family *Micrococcaceae*. At the same time, *A. crystallopoietes,* unlike other *Arthrobacter* species which have A3α peptidoglycan and phosphate-free

(neutral) cell wall polysaccharides (Sadikov et al., 1983; Takeuchi and Yokota, 1989), is characterized by the presence of phosphate-containing polymers, *viz.*, teichoic acid 1,3-poly(glycerol phosphate) substituted with β-glucose residues, and a poly(diglycosyl 1-phosphate).

The data presented indicate that the composition and structure of cell wall glycopolymers can be considered as an important diagnostic feature of a new genus, which will be described in the future based on *A. crystallopoietes.*

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of the welfare of animals. This article does not contain any studies involving animals or human participants performed by any of the authors.

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