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EXPERIMENTAL ARTICLES

Larkinella arboricola sp. nov., a New Spiral-Shaped Bacterium of the Phylum *Bacteroidetes* Isolated from the Microbial Community of Decomposing Wood

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Abstract—An aerobic gram-negative bacterial strain Z-0532 with ring-shaped cells forming spirals in the course of growth was isolated from the humified solution produced by spruce wood decomposition. The new isolate was a chemoorganotrophic, mesophilic, moderately acidophilic organism with the temperature range of 6–32°C (optimum at 25–28°C) and pH range from 4.7 to 7.2 (optimum at pH 5.5–6.5). A broad range of substrates was used as carbon and energy sources, including sugars, some organic acids and polyalcohols, and soluble polymeric compounds (gelatin, esculin, starch, xylan, laminarin, dextrin, casein hydrolysate, and Tween-40). According to its physiological and biochemical characteristics, strain Z-0532 is a typical member of the trophic group of oligotrophic bacteria, which utilize the products of wood hydrolysis dissipated by xylotrophic microorganisms. The G+C base content of strain Z-0532 was 52.1 mol %. Sequencing of the 16S rRNA gene of the new isolate revealed 98% similarity to *Larkinella insperata* LMG 22510^T, which is a recently described species of the family *Spirosomaceae* of the phylum *Bacteroidetes*. The level of DNA : DNA homology between this species and strain Z-0532 was only 40%. The differences in the phenotypic and genotypic characteristics suggested classification of the isolate obtained from decomposing wood as a new species of the genus *Larkinella, Larkinella arboricola* sp. nov., with the type strain Z-0532^T (=VKM B-2528^T = DSM 21851^T).

Key words: phylum *Bacteroidetes*, family *Spirosomaceae*, genus *Larkinella*, microbial decomposition of wood.

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Aerobic gram-negative bacteria of the phylum *Bacteroidetes* play the key role in the transformation of biopolymers in terrestrial, marine, and freshwater ecosystems [1, 2]. According to the results of in situ hybridization with rRNA-specific fluorescent probes (FISH), the members of this heterogeneous phylogenetic group predominate in aquatic ecosystems; they are second in numbers only to the *Proteobacteria* [3]. One of the phylogenetic subgroups within the *Bacteroidetes* is represented by the family *Spirosomaceae*, comprising gram-negative aerobic chemoorganotrophic bacteria of the genera *Spirosoma, Flectobacillus, Runella* [4, 5], *Dyadobacter* [6], *Arcicella* [7], *Larkinella* [8], and *Rudanella* [9]. A morphological trait shared by many members of this family is the possession of highly characteristic, ring- or horseshoe-shaped cells, sometimes forming spirals and S-shaped aggregates [2, 4, 5].

A bacterial strain, Z-0532, with a similar annular shape of the cells arranged in spirals and S-like structures, was isolated from the humified solution formed in the course of microbial decomposition of spruce wood. Partial sequencing of the 16S rRNA gene of the new isolate revealed its phylogenetic relatedness to the only known member of the recently described genus and species *Larkinella insperata* [8], of the family *Spirosomaceae*. The goal of the present work was investigation of the phenotypic, chemotaxonomic, and genotypic characteristics of strain Z-0532 in comparison to *Larkinella insperata* LMG 22510^T and determination of the taxonomic position of the new bacterium.

MATERIALS AND METHODS

Isolation and cultivation. The isolate was obtained from the xylotrophic microbial community formed in the course of spruce wood decomposition in a laboratory experiment [10]. Strain Z-0532 was isolated by plating an aliquot of the dark-colored solution formed in the course of wood decomposition on the M1 complex agarized medium containing the following (g/l): KH_2PO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.05; $(NH_4)_2SO_4$, 0.1; $CaCl_2 \cdot 2H_2O$, 0.01; glucose, 0.5; peptone, 0.1; yeast extract, 0.1; and agar, 15; pH 5.8.

Cell size and morphology were analyzed under a Zeiss Axioplan 2 epifluorescence microscope using the Axiovision 4.2 software package (Jena, Germany). The type strain of *Larkinella insperata* LMG 22510^T iso-

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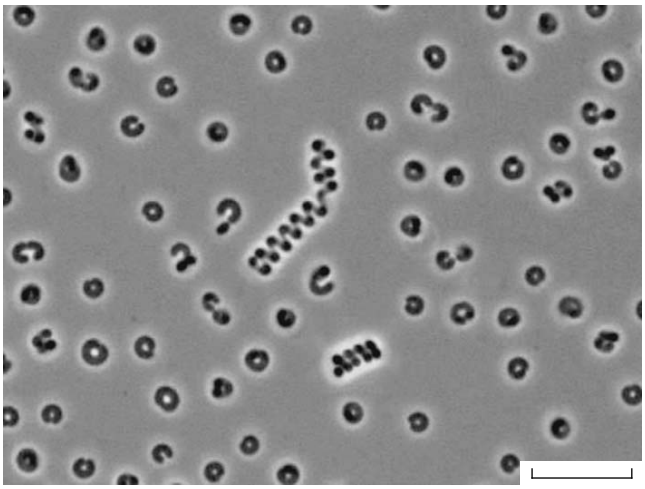

Fig. 1. Cell morphology of a 3-day-old culture of strain Z-0532; phase contrast. Scale bar, 10 μm.

lated from water of a steam generator [8] was used for comparison. The type strain was maintained on MS agarized medium recommended by the authors and containing the following (g/l): glucose, 1.0; peptone, 1.0; yeast extract, 1.0; and agar, 15 [5].

The fatty acid composition of strain Z-0532 was determined in the cells grown on M1 medium and collected at the late exponential growth phase. Analysis was carried out by the DSMZ service (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany).

Physiological and biochemical characteristics of isolate Z-0532 were determined according to the generally accepted procedures [11] and with the standard API 20 NE kit (BioMérieux, France). The spectrum of the substrates utilized as carbon sources was determined in M1 liquid medium without peptone and glucose; the content of yeast extract was decreased to 0.05 g/l. The concentration of the tested substrates was 0.5 g/l. Cultivation was carried out in 120-ml vials with 20 ml of the medium. Bacterial growth was monitored by measuring OD_{600} on an Eppendorf Bio-Photometer spectrophotometer. Specific growth rates μ were calculated from OD_{600} changes per unit time during the exponential growth phase and related to the current pH. The enzymatic activity was determined with the standard API ZYM kit (BioMérieux, France). Capacity for anaerobic growth was determined in a desiccator with AnaeroGen packages (Oxoid). Antibiotic sensitivity of strain Z-0532 was determined on agarized M1 medium using test disks containing the following antibiotics (Oxoid): ampicillin (10 mg), gentamycin (10 mg), kanamycin (30 mg), neomycin (10 mg), novobiocin (30 mg), streptomycin (10 mg), chloramphenicol (30 mg), and lincomycin (10 mg).

DNA G+C base content was determined as described earlier [12]. DNA was isolated by the Marmur method [13].

Phylogenetic analysis. PCR amplification of the 16S rRNA gene was carried out with universal eubacterial primers [14] on a PE GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, United States). The amplified fragments were sequenced on an ABI 377A sequencer (Perkin-Elmer Applied Biosystems, United States). Analysis of the nucleotide sequences and construction of phylogenetic trees were carried out with the ARB software package (http://www.arb-home.de). Statistical validity of the dendrograms was calculated with the Phylip software package by bootstrap analysis by constructing 1000 alternative trees. The 16S rRNA gene sequence of strain Z-0532 is deposited to GenBank (accession no. FN391025).

RESULTS AND DISCUSSION

Isolation of the new bacterium. Strain Z-0532 was isolated on M1 medium from the acidic (pH 4.3), dark-colored solution resulting from the transformation of the products of spruce wood decomposition in a laboratory experiment [10]. Primary identification of the isolate by in situ hybridization with an equimolar mixture of Cy3-labeled probes (CF319a + CFB560) specific for the *Bacteroidetes* [3] confirmed its affiliation to this phylogenetic group.

Cultural characteristics. On agarized M1 medium strain Z-0532 formed circular, pink-pigmented colonies of liquid consistency, up to 1–3 mm in diameter after 3–4 days of incubation at 25°C. In liquid culture, this isolate grew homogeneously, forming a pale pink suspension.

Morphology. Gram-negative cells of strain Z-0532 were horseshoe-shaped or formed almost closed rings (Fig. 1). The outer diameter of the ring varied from 1.8 to 2.5 μm. In the exponential growth phase, spirals of varying length were formed, which later broke into short S-shaped aggregates and horseshoe-shaped cells. Spores were not formed in the cells of Z-0532. Unlike the phylogenetically related species *Larkinella insperata* LMG 22510^T, the cells of strain Z-0532 did not exhibit gliding motility.

Physiological and biochemical characteristics. Strain Z-0532 is an aerobic chemoorganotrophic organism with cytochrome oxidase and catalase activity. A broad range of sugars was used as carbon and energy sources. In contrast to *L. insperata* LMG 22510^T, growth of strain Z-0532 on carbohydrates resulted in acidification of the medium (Table 1). The isolate grew well on glucose, fructose, sucrose, galactose, maltose, cellobiose, xylose, arabinose, mannose, and lactose. Unlike the related *Larkinella* species, the new isolate utilized some organic acids (succinate, fumarate, gluconate, and pyruvate), as well as salicin and polyalco-

Table 1. Differences in the phenotypic characteristics of strain Z-0532 and *Larkinella insperata* LMG 25510^T

Characteristics	Strain Z-0532	*Larkinella insperata* LMG 25510^T
Cell morphology	Ring- or horseshoe-shaped cells forming spirals in the course of growth	Ring- or horseshoe-shaped cells
Gliding	–	+*
NaCl range for growth, %	0–0.8	0–2*
Growth on MS medium	–	+
pH range (optimum) for growth	4.5–7.2 (5.5–6.5)	5.5–8.2 (6.5–7.5)
Temperature growth range	10–32°C	10–40°C*
Starch hydrolysis	+	–*
Indole formation	+	–*
α-Chymotrypsin activity	–	+**
Assimilation:		
succinate	+	–
fumarate	+	–
gluconate	+	–
pyruvate	+	–
sorbitol	+	–
mannitol	+	–
salicin	+	–
Medium acidification during growth on:		
cellobiose	+	–*
fructose	+	–*
galactose	+	–*
sucrose	+	–*

Notes: * data from [8].
** data from [9].

hols (sorbitol, dulcitol, and mannitol). Raffinose, oxalate, citrate, malate, acetate, ethanol, methanol, formate, benzoate, and phenylacetic acid were not utilized as carbon sources. Ammonium, nitrate, and *N*-acetylglucosamine were used as nitrogen sources; no growth occurred in the presence of nitrite or in the medium without nitrogen sources. The new isolate obtained from the microbial community of decomposing wood hydrolyzed a broad range of soluble biopolymers: gelatin, esculin, starch, xylan, laminarin, dextrin, casein hydrolysate, and Tween-40. Cellulose, agar, chitin, and pectin, as well as Tween-20 and Tween-80, were not hydrolyzed.

Analysis of the enzymatic characteristics of strain Z-0532 with the standard API ZYM kit (BioMérieux, France) revealed activity of β-galactosidase, alkaline phosphatase, esterase–lipase (C8), leucine arylami-

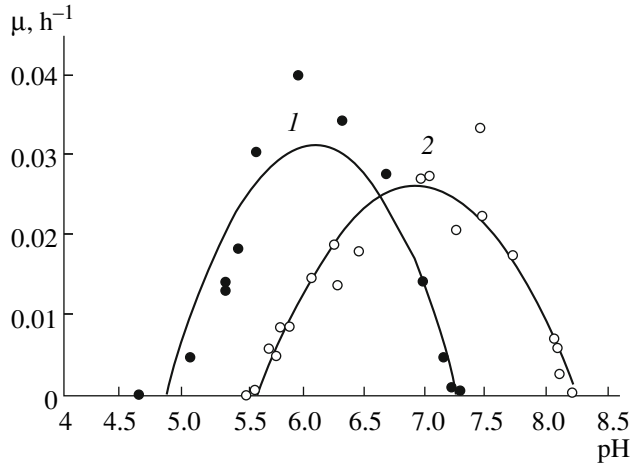

Fig. 2. Specific growth rate (μ) of strain Z-0532 (*1*) and *Larkinella insperata* LMG 25510^T (*2*) depending on pH of the medium.

dase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol phosphohydrolase, β-glucosidase, *N*-acetyl-β-glucosaminidase, β-mannosidase, and indole formation. For urease, arginine hydrolase, lipase (C14), β-glucuronidase, and α-fucosidase, the results were negative. Similar to phylogenetically related *L. insperata* LMG 22510^T, strain Z-0532 did not ferment glucose and was not capable of nitrate reduction. The strain was sensitive to ampicillin and streptomycin; it was resistant to gentamycin, kanamycin, neomycin, novobiocin, chloramphenicol, and lincomycin.

Strain Z-0532 was a mesophilic and moderately acidophilic organism. Its optimal growth temperature was 25–28°C (growth range from 6 to 32°C), and the pH range was 4.7–7.2 with the optimum at pH 5.5–6.5 (Fig. 2). The strain did not require sodium chloride for growth. At NaCl concentrations above 0.8%, its growth was inhibited.

Fatty acid composition. In strain Z-0532, $C_{16:1}\omega5c$, *iso*-$C_{17:0}$ 3OH, and *iso*-$C_{15:0}$ acids were predominant, similar to the type species *L. insperata* (Table 2).

Phylogenetic position. Sequencing of the 16S rRNA gene of strain Z-0532 confirmed its affiliation to the *Bacteroidetes. Larkinella insperata* LMG 22510^T, the only known strain of this species, was the closest phylogenetic relative of the new isolate (98% similarity) (Fig. 3). According to the previously accepted taxonomic hierarchy of the phylum, the genus *Larkinella* belonged to the family *Spirosomaceae.* Presently, however, the taxonomic structure of the *Bacteroidetes* is being reconsidered, so that in the outlines for the Bergey's Manual currently in preparation, classification of the genus *Larkinella*, as well as of other genera comprising gram-negative aerobic bacteria with characteristic ring-shaped cells, within the family *Cytophagaceae* was suggested [15].

DNA G+C content of strain Z-0532 was 52.1 mol %. DNA : DNA hybridization of the new isolate with the type strain *L. insperata* LMG 22510^T revealed only 40% homology.

Comparative analysis of the morphological, physiological, biochemical, and genetic characteristics of the new isolate Z-0532 and the type strain *L. insperata* LMG 22510^T revealed significant differences (Table 1). Since the two strains belonged to the same genus, their morphological and cultural characteristics were similar, as well as their type of metabolism and fatty acid composition. Strain Z-0532, however, had a growth optimum at lower pH, was more sensitive to NaCl, and grew on a number of organic acids and polyalcohols

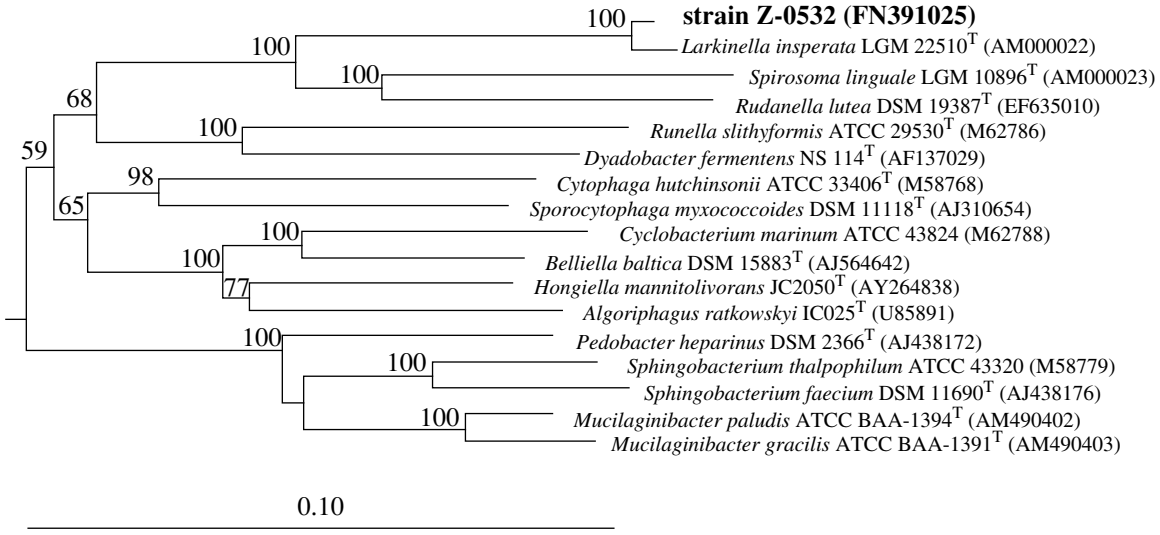

Fig. 3. Phylogenetic tree constructed based on comparative analysis of 16S rRNA gene sequences of strain Z-0532, *Larkinella insperata* LMG 25510^T, and some other members of the phylum *Bacteroidetes*. Scale bar, 0.1 substitutions per nucleotide position.

Table 2. Fatty acid composition of strain Z-0532 and *Larkinella insperata* LMG 25510^T

Fatty acids	% of total content	
	Strain Z-0532	*Larkinella insperata* LMG 22510^T
C14:0	0.2	1.0
C15:0 *iso*	**11.6**	**13.9**
C15:0 *anteiso*	2.4	1.4
C15:0 *iso* 3-OH	2.5	2.1
C15:0	0.2	–
C15:0 2OH	0.2	–
C16:0 *iso*	1.1	–
C16:0 *iso* 3OH	0.9	1.8
C16:0	1.9	3.9
C16:1ω5c	**45.3**	**48.7**
C16:0 3OH	0.8	3.6
C17:0 *iso*	1.3	–
C17:0 *anteiso*	0.9	–
C17:0	0.3	–
C17:0 *iso* 3OH	**13.9**	**14.3**
C17:1ω6c	0.4	–
C17:0 2OH	0.6	–

Note: Predominant components are highlighted in bold.

which were not utilized by *L. insperata.* These substrate preferences mark the new isolate as a member of the trophic group of oligotrophic acidophilic bacteria, which utilize the products of wood decomposition by micromycetes. The above phenotypic differences, together with the low level of DNA : DNA homology to *L. insperata* LMG 22510^T, suggest classification of strain Z-0532 as a new species of the genus *Larkinella*, named *Larkinella arboricola* sp. nov.

Description of Larkinella arboricola sp. nov.

Larkinella arboricola (ar.bo.ri'cola L. fem, arbor, wood; colere, inhabit; arboricola, inhabiting wood).

Gram-negative, ring-shaped cells with the ring's outer diameter of 1.8–2.5 μm; form spirals, which break into short S-shaped aggregates and horseshoe-shaped cells in the course of growth. Do not exhibit gliding motility, and do not form spores. Round pink colonies with smooth edges are formed. Strictly aerobic and chemoorganotrophic. Glucose, fructose, sucrose, galactose, maltose, cellobiose, xylose, arabinose, mannose, lactose, succinate, fumarate, gluconate, pyruvate, salicin, sorbitol, dulcitol, and mannitol are utilized; raffinose, oxalate, citrate, malate, acetate, ethanol, methanol, formate, benzoate, and phenylacetic acid are not utilized. Ammonium, nitrate, and *N*-acetylglucosamine are used as nitrogen sources; nitrite is not utilized, and no growth occurs in the absence of available nitrogen sources. Cytochrome oxidase and catalase activities are present, as well as β-galactosidase, alkaline phosphatase, esterase–lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol phosphohydrolase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and β-mannosidase. Capable of indole formation. No activity of urease, arginine hydrolase, lipase (C14), β-glucuronidase, and α-fucosidase were revealed. Do not ferment glucose and are incapable of nitrate reduction. The following soluble biopolymers are hydrolyzed: gelatin, esculin, starch, xylan, laminarin, dextrin, casein hydrolysate, and Tween-40; cellulose, agar, chitin, pectin, Tween-20, and Tween-80 are not hydrolyzed. Sensitive to ampicillin and streptomycin; resistant to gentamycin, kanamycin, neomycin, novobiocin, chloramphenicol, and lincomycin. Growth temperature optimum at 25–28°C (range from 6 to 32°C); pH optimum at pH 5.5–6.5 (pH range 4.7–7.2). Among the fatty acids, $C_{16:1}$ω5c, *iso*-$C_{17:0}$ 3OH, and *iso*-$C_{15:0}$ are predominant. DNA G+C base content is 52.1 mol %. The type strain, Z-0532^T (VKM B-2528^T = DSM 21851^T), was isolated from the humified solution formed in the course of spruce wood microbial degradation.

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REFERENCES

1. Reichenbach, H., The Order *Cytophagales*, in *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*, Dworkin, M. et al, Eds.,

3rd Release 3.0, New York: Springer, 1999 (http://141.150.157.117:8080/prokRUB/index.htm).

2. Raj, H.D. and Maloy, S.R., Family *Spirosomaceae*: Gram Negative Ring-Forming Aerobic Bacteria, *Critical Rev. Microbiol.*, 1990, vol. 17, no. 5, pp. 329–364.
3. Weller, R., Glöckner, F.O., and Amann, R., 16S rRNA-Targeted Oligonucleotide Probes for the in situ Detection of Members of the Phylum *Cytophaga-Flavobacterium-Bacteroides, Syst. Appl. Microbiol.*, 2000, vol. 23, pp. 107–114.
4. Larkin, J.M. and Borrall, R., *Spirosomaceae*, a New Family to Contain the Genera *Spirosoma* Migula 1894, *Flectobacillus* Larkin et al. 1977, and *Runella* Larkin and Williams 1978, *Int. J. Syst. Bacteriol.*, 1978, vol. 28, pp. 595–596
5. Larkin, J.M. and Borrall, R., Family I. *Spirosomaceae* Larkin and Borrall 1978, 595^{AL}, in *Bergey's Manual of Systematic Bacteriology.,* Eds. Krieg N.R., Holt J.G. 1984, Baltimore: Williams & Wilkins, vol. 1, pp. 125–126.
6. Chelius, M.K. and Triplett, E.W., *Dyadobacter fermentans* gen. nov., sp. nov., a Novel Gram-Negative Bacterium Isolated from Surface-Sterilized *Zea mays* Stems, *Int. J. Syst. Evol. Microbiol.,* 2000, vol. 50, p. 751.
7. Nikitin, D.I., Strompl, C., Oranskaya, M.S., and Abraham, W.-R., Phylogeny of the Ring-Forming Bacterium *Arcicella aquatica* gen. nov., sp. nov. (ex Nikitin et al. 1994), from a Freshwater Neuston Biofilm, *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 681 – 684.
8. Vancanneyt, M., Nedashkovskaya, O.I., Snauwaert, C., Mortier, S., Vandemeulebroecke, K., Hoste, B., Dawyndt, P., Frolova, G.M., Janssens, D., and Swings, J., *Larkinella insperata* gen. nov., sp. nov., a Bacterium of the Phylum *'Bacteroidetes'* Isolated from Water of a Steam Generator, *Int. J. Syst. Evol. Microbiol.*, 2006, vol. 56, p. 237.
9. Weon, H.-Y., Noh, H.-J., Son, J.-A., Jang, H.B., Kim, B.-Y., Kwon, S.-W., and Stackebrandt, E., *Rudanella lutea* gen. nov., sp. nov., Isolated from an Air Sample in Korea, *Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 474–478.
10. Zavarzin, G.A. and Zavarzina, A.G., Xylotrophic and Mycophilic Bacteria in Formation of Dystrophic Waters, *Mikrobiologiya*, 2009, vol. 78., no. 5, pp. 579–591 [*Microbiology* (Engl. Transl.), vol. 78, no. 5, pp. 523–534].
11. *Manual of Methods for General Bacteriology*, Gerhardt, P. et al., Eds., Washington: Amer. Soc. Microbiol., 1981 [Russ. Transl. Moscow: Mir, 1984].
12. Pankratov, T.A., Kulichevskaya, I.S., Liesack, W., and Dedysh, S.N., Isolation of Aerobic, Gliding, Xylanolytic and Laminarinolytic Bacteria from Acidic sphagnum Peatlands and Emended Description of *Chitinophaga arvensicola* Kampfer et al. 2006, *Int. J. Syst. Evol. Microbiol*, 2006, vol. 56, no. 12, pp. 2761–2764.
13. Marmur, J., A Procedure for the Isolation of Deoxyribonucleic Acid from Microorganisms, *J. Mol. Biol.*, 1961, vol. 3, pp. 208–218.
14. Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J., 16S Ribosomal DNA Amplification for Phylogenetic Study, *J. Bacteriol.*, 1991, vol. 173, pp. 697–703.
15. Ludwig, W., Euzéby, J., and Whitman, W.B., Draft Taxonomic Outline of the *Bacteroidetes, Planctomycetes, Chlamydiae, Spirochaetes, Fibrobacteres, Fusobacteria, Acidobacteria, Verrucomicrobia, Dictyoglomi*, and *Gemmatimonadetes*, 2008, http://www.bergeys.org/outlines/Bergeys_Vol_4_Outline.pdf