ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 3, pp. 432–435. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © M.R. Sharipova, A.A. Toymentseva, A.R. Sabirova, A.D. Mukhametzyanova, A.I. Akhmetova, A.M. Mardanova, N.P. Balaban, 2011, published in Mikrobiologiya, 2011, Vol. 80, No. 3, pp. 424–426.

SHORT COMMUNICATIONS

A New Phylogenetic Position of Strain *Bacillus intermedius* 3-19

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Received July 1, 2010

DOI: 10.1134/S0026261711030192

Members of the *Bacillus* genus are phenotypically heterogeneous aerobic gram-positive rod-shaped spore-forming bacteria [1]. Their heterogeneity is the result of a wide range of the G + C content (32–69 mol %), as well as the diversity of metabolic pathways (nitrogen-fixing, thermophilic, psychrophilic, acidophilic, alkaliphilic, and halophilic bacilli) [2]. Due to this diversity, *Bacillus* species are of great practical interest [3]. New data and modern methods of molecular and genetic analysis used in taxonomy suggest updating previously obtained data on the phylogenetic position of bacteria.

Sequencing of the 16S ribosomal RNA (16S rRNA) gene is the most commonly used method in bacterial taxonomy. Comparison of the primary structure of the 16S rRNA gene with the sequences deposited in the GenBank database makes it possible to assign the studied bacterial species to specific taxonomic groups. Analysis of the 16S rRNA genes, which contain both highly conserved and variable sites, is a source of important evolutionary information [4].

In 1978, a bacterium capable of producing RNase was isolated from the samples of Tatarstan soils by scientists from Kazan State University; the obtained strain was identified as *Bacillus intermedius* 7R using the Krasil'nikov manual [5] (patent no. 587156, 1978). In 1988, a streptomycin-resistant strain *B. intermedius* 7R/3-19 that exhibited high RNase productivity was obtained from the above-mentioned strain (patent no. 151354, 1988). Extracellular alkaline ribonuclease (binase) exhibits antiviral [6, 7] and antiphage activities (patent no. 1781295, 1992).

It was established that, in addition to RNase production, strain *B. intermedius* 3-19 was able to synthesize extracellular serine proteinases and metalloendopeptidase [8–10]. These enzymes are used as models in the studies of the gene structures and functions of enzymes [11] and can potentially be used for thrombolytic therapy [12]. However, the species *B. intermedius* is not listed in the modern edition of *Bergey's Manual of Systematic Bacteriology* and international databases (GenBank, EMBL). In this study, we examined the taxonomic position of this species in terms of the results of genetic analyses.

Genetic analysis was performed to specify the phylogenetic position of strain *B. intermedius* 3-19. PCR amplification of the 16S rRNA gene fragments (~1500 bp) was carried out [13] using direct (GAGTTTGATCCTGGCTCAG) and reverse (ACGGTTACCTTGTTACGACTT) primers. The primary structure of the amplified DNA fragment was determined by Syntol Co. (Russia). Comparison of the 16S rRNA gene sequences determined in this work with the gene sequences from the GenBank database showed high homology to bacteria from the family *Bacillaceae*. *B. pumilus* strains were found to be closest to the studied strain (Fig. 1). The level of 16S rRNA similarity between strain *B. intermedius* 3-19 and representatives of the species *B. pumilus* is 99%.

Since modern molecular taxonomy does not employ criteria for taxonomic significance, the sequences of the functional genes are actively used as additional characteristics for determination of identity between the organisms. Therefore, in order to confirm the species relation of *B. intermedius* and *B. pumilus*, comparative analysis of the nucleotide sequences of proteolytic enzymes genes from these strains was carried out.

According to the results of comparative analysis of the proteinase genes *aprBi* (AY754946.1), *gseBi* (Y15136.1), and *mprBi* (EU678894.2), as well as the RNase gene (*binBi*) (X53697.1), strain *B. intermedius* 3-19 is closest to the species *B. pumilus* (table). The level of homology between the above-listed genes of these species is 94–97%. Hence, this level corresponds to the level of similarity between their 16S rRNA gene sequences.

An additional comparative analysis of the two previously sequenced fragments of the *B. intermedius* 3-19 chromosomal DNA (EU678894.2 GenBank) was car-

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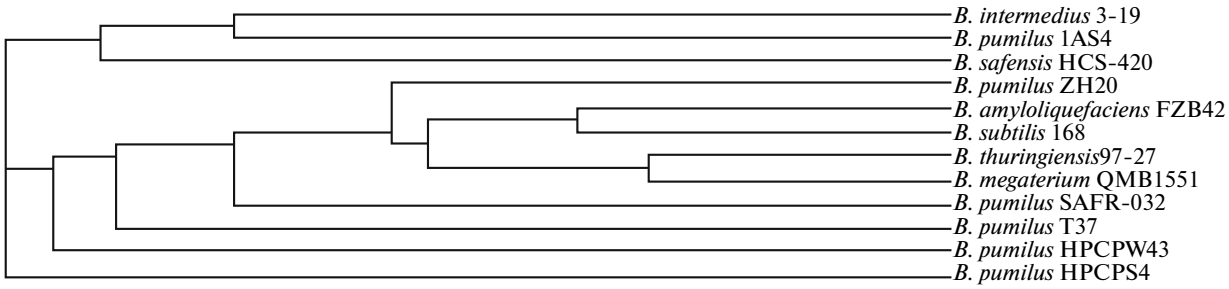

Fig. 1. Phylogenetic position of strain *B. intermedius* 3-19 among bacteria of the genus *Bacillus* according to the 16S rRNA gene analysis. The dendrogram was constructed using the online CLUSTALW2 software package.

ried out. Alignment of the two genome fragments (6000 bp) and matching of the obtained sequences with the sequences in the NCBI database showed a high level of similarity between these sequences and, especially, to strain *B. pumilus* SAFR-032 (Figs. 2a and 2b). The homology level was 96%.

The level of the protease and RNase genes similarity of *B. intermedius* 3–19 according to the results of screening in the GenBank database

Bacillus intermedius 3–19 genes	Homologous genes of other *Bacillus* species from the GenBank database	Gene homology, %
aprBi (subtilisin-like proteinase)	*Bacillus pumilus* SAFR-032, *aprE1* gene (CP000813.1)	95
	Bacillus pumilus TMS55, gene of the subtilisin-like proteinase (FJ5 84420.1)	93
	Bacillus pumilus, *sapB* gene (AM748727.1)	93
	Bacillus pumilus NJM4, serine proteinase gene (FJ869878.1)	90
	Bacillus pumilus, serine proteinase gene (AB029082.1)	89
	Bacillus pumilus, *bppA* gene (AB211527.1)	89
gseBi (metalloproteinase)	*Bacillus pumilus* SAFR-032, gene encoding glutamyl endopeptidase of the SI family (CP000813.1)	94
	Bacillus pumilus, *bppB* gene (AB 174896.1)	88
mprBi (metalloproteinase)	*Bacillus pumilus* SAFR-032, hypothetical protein (CP 000813.1)	97
Extracellular RNase (binase)	*Bacillus pumilus* SAFR-032, guanine-specific RNase (CP 000813.1)	95
	Bacillus pumilus, RNase gene (U06867.1)	96
	Bacillus pumilus, RNase gene (DQ339154.1)	91

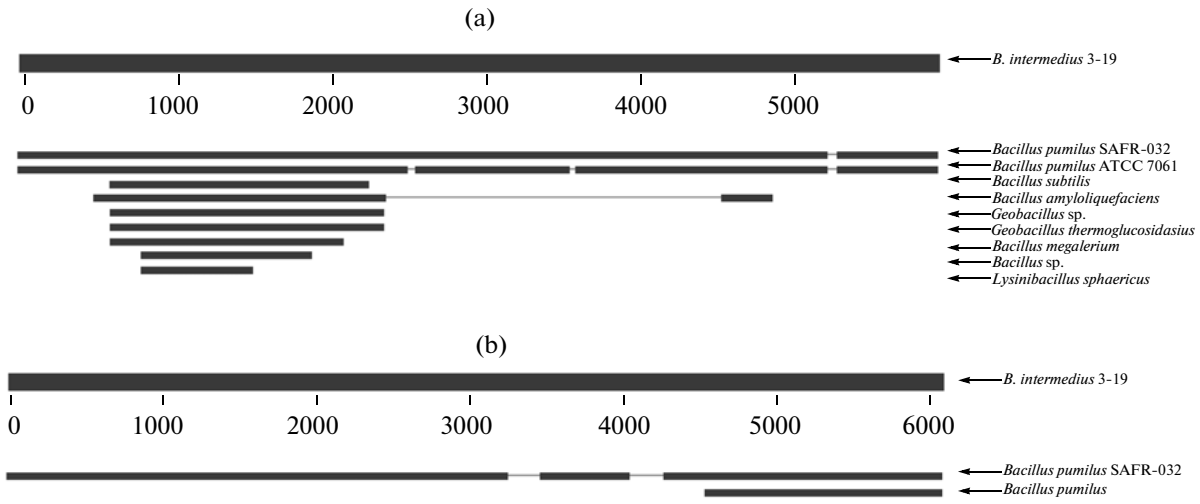

Fig. 2. Schematic diagram of the results of alignment of two gene fragments (6000 bp) with corresponding sequences retrieved from the NCBL database. The sequences of the *mprBi* (EU678894.2) (a) and *aprBi* (during uploading to the database server) (b) genes were compared using the online NCBI/BLAST software package (homologous species are depicted with arrows).

Hence, comparative analysis of the 16S rRNA, *aprBi*, *gseBi*, *mprBi*, and *binBi* gene sequences, as well as the 6000-bp genome fragments, revealed the phylogenetic position of strain *B. intermedius* 3-19 as a representative of *B. pumilus*. Therefore, strain 3-19, previously described as *B. intermedius*, was renamed as *B. pumilus* 3-19.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 09-04-99044) and by the federal targeted program "Scientific and Pedagogical Personnel of Innovative Russia in 2009–2013," state contract no. P406 (12.05.2010).

REFERENCES

1. Sneath, P.H.A., Endospore-Forming Gram-Positive Rods and Cocci, in *Bergey'S Manual of Systematic Bacteriology*, Kreig, N.R. and Holt, J.G., Eds., Baltimore: Williams & Wilkins, 1984, vol. 2, pp. 1104–1207.
2. Asoodeh, A., Chamani, J., and Lagzian, M., A Novel Thermostable, Acidophilic Alpha-Amylase from a New Thermophilic "*Bacillus* sp. Ferdowsicous" Isolated from Ferdows Hot Mineral Spring in Iran: Purification and Biochemical Characterization, *Int. J. Biol. Macromol.*, 2010, vol. 46, no. 3, pp. 289–297.
3. Mala, B.R., Aparna, M.T., Mohini, S.G., and Vasanti, V.D., Molecular and Biotechnological Aspects of Microbial Proteases, *Microbiol. Mol. Biol. Rev.*, 1998, vol. 62, no. 3, pp. 597–635.
4. Amann, R.I., Ludwig, W., and Schleifer, K.-H., Phylogenetic Identification and in situ Detection of Individual Microbial Cells Without Cultivation, *Microbiol. Rev.*, 1995, vol. 59, no. 1, pp. 143–169.
5. Krasil'nikov, N.A., Opredelitel' bakterii i aktinomitsetov, in (Guide for Identification of Bacteria and Actinomycetes), Moscow, 1949.
6. Kluge, S., Rockstroh, A., Stenz, E., Leschinskaja, I.B., Scharipova, M.R., Atabekov, I.G., Taljanski, M.E., Kaplan, I.B., Katkov, V.S., and Karpejski, M.Ja., Biogene mittel zur Chemotherapie pflanzlicher Virosen, *Deutsches Patentamt*, 1991, DD A 01 N/340 707 1.
7. Gribencha, S.V., Potselueva, L.A., Barinskii, I.F., Balandin, T.G., Deev, S.M., and Leshchinskaya, I.B., Antiviral Activity of *Bacillus iniremedius* RNase in Experiments on CBA Linear Mice Infected with the Rabies Virus, *Vopr. Virusol.*, 2004, no. 6, pp. 38–41.
8. Balaban, N.P., Sharipova, M.R., Gabdrakhmanova, L.A., Mardanova, A.M., Tokmakova, Yu.S., Sokolova, E.A., Rudenskaya, T.N., and Leshchinskaya, I.B., Synthesis and Secretion of Proteinases by *Bacillus intermedius* in the Late Stages of Sporulation, *Mikrobiologiya*, 2003, vol. 72, no. 3, pp. 338–342 [*Microbiology* (Engl. Transl.), vol. 72, no. 3, pp. 295–299].
9. Leshchinskaya, I.B., Shakirov, E.V., Itskovitch, E.L., Balaban, N.P., Mardanova, A.M., Sharipova, M.R., Viryasov, M.B., Rudenskaya, G.N., and Stepanov, V.M., Glutamyl Endopeptidase of *Bacillus intermedius*, Strain 3-19, *FEBS Lett.*, 1997, pp. 241–244.
10. Mikhailova, E.O., Balaban, N.P., Mardanova, A.M., Rudakova, N.L., Ilynskaya, O.N., Rudenskaya, G.N., Rizvanov, A.A., and Sharipova, M.R., Purification of a Subtilisin-Like Serine Proteinase from Recombinant

Bacillus subtilis during Different Phases of Growth, *Ann. Microbiol.*, 2009, vol. 59, no. 2, pp. 1–8.

11. Meijers, R., Blagova, E.V., Levdikov, V.M., Rudenskaya, G.N., Chestukhina, G.G., Akimkina, T.V., Kostrov, S.V., Lamzin, V.S., and Kuranova, I.P., The Crystal Structure of Glutamyl Endopeptidase from *Bacillus intermedius* Reveals a Structural Link between Zymogen Activation and Charge Compensation, *Biochemistry*, 2004, vol. 43, no. 10, pp. 2784–2791.
12. Itskovich, E.L., Lyutova, L.I., Balaban, N.P., Mardanova, A.M., Shakirov, E.V., Sharipova, M.R., Leshchinskaya, I.B., and Rudenskaya, G.N., Thrombolytic and Anticoagulant Properties of the Thiol-Dependent Serine Proteinase from *Bacillus intermedius* 3-19*, Vopr. Med. Khim.*, 1999, no. 5, pp. 288–291.
13. Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning: a Laboratory Manual, 2nd ed.*, New York: Cold Spring Harbor Laboratory Press, 1989.