**Bacillus pycnus sp. nov. and Bacillus neidei sp. nov., round-spored bacteria from soil**

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*Bacillus sphaericus sensu lato* currently consists of seven or more groups of unrelated taxa, one of which is *B. sphaericus sensu stricto* and another of which is *Bacillus fusiformis*. Members of two groups (groups 6 and 7), in common with all other *B. sphaericus*-like organisms, are unable to grow anaerobically or to use common hexoses, pentoses and hexitols as sources of carbon, have G+C contents of 34–36 mol% and form round spores. Groups 6 and 7 can be differentiated from other *B. sphaericus*-like organisms by low DNA relatedness and by variations in whole-cell fatty acid composition. Unique characteristics of group 6 include the ability to oxidize β-hydroxybutyrate, the non-requirement for biotin and thiamin and failure to grow in 5% NaCl. Distinctive traits of group 7 include the inability to oxidize pyruvate and a requirement for biotin, thiamin and cystine for growth. These data show that groups 6 and 7 represent two novel species, for which the names *Bacillus pycnus* sp. nov. and *Bacillus neidei* sp. nov., respectively, are proposed; the corresponding type strains are NRRL NRS-1691T (= JCM 11075T) and NRRL BD-87T (= JCM 11077T).

**Keywords:** *Bacillus pycnus, Bacillus neidei*, novel species, 16S rDNA

The round-spored, mesophilic species *Bacillus sphaericus* was first described by Neide (1904). Because early differentiation tests were ineffective, all round-spored mesophiles were classified as *B. sphaericus*; the lack of adequate differentiating tools deterred taxonomic study of mesophilic, round-spored bacteria. However, the discovery of mosquitoicidal activity among some of these organisms (Kellen et al., 1965) and the development of molecular-biological techniques have rekindled taxonomic and phylogenetic examination of the round-spored organisms. Taxonomic heterogeneity of *B. sphaericus* had been suggested by variability of insecticidal activity among strains and was confirmed by comparative studies using DNA similarity assessment (Krych et al., 1980), numerical taxonomy (Alexander & Priest, 1990; Priest et al., 1988), randomly amplified polymorphic DNA fingerprinting (Woodburn et al., 1995) and rRNA gene restriction (Aquino de Muro et al., 1992). These developments led to the discovery of two more mesophilic species, *Bacillus fusiformis* (Priest et al., 1988) and *Bacillus silvestris* (Rheims et al., 1999). In a phylogenetic study based on 16S rDNA sequences (Nakamura, 2000), *B. sphaericus*-like species segregated into seven groups wherein group 3 represented *B. sphaericus* and group 2 comprised *B. fusiformis*; group 1 appeared to be a subgroup of 2. Groups 4–7 were unknown taxa.

In the present study, DNA relatedness evaluation and phenotypic characterization were used to differentiate groups 6 and 7 from each other, from representative strains of groups 4 and 5 and from type strains of recognized round-spore-forming relatives.

DNA was extracted from 24-h cultures from groups 1 (NRRL B-23269), 4 (NRRL NRS-593), 5 (NRRL B-1876), 6 (NRRL NRS-1691T, NRRL NRS-1693, NRRL NRS-1694, NRRL NRS-1695) and 7 (NRRL BD-87T, NRRL BD-101, NRRL BD-103) and the following type cultures: *Bacillus aminovorans*’ DSM 4337, *B. fusiformis* DSM 2898T, *Sporosarcina globispora* NRRL NRS-1533T, *Bacillus insolitus* NRRL NRS-1531T, *Marinibacillus marinus* DSM 1297T, *Sporosarcina psychrophila* IFO 15381T, *B. silvestris* DSM 12223T, *B. sphaericus* JCM 2502T and *Ureibacillus thermosphaericus* DSM 10633T. DNA reassociation was carried out using the method of Ezaki et al. (1989). Probes were prepared with DNA from *B. sphaericus* JCM 2502T, *B. fusiformis* DSM 2898T, NRRL NRS-1691T, NRRL NRS-1694, NRRL BD-87T and NRRL BD-103.
Fig. 1. Neighbour-joining tree showing the phylogenetic position of groups 6 and 7 among the mesophilic and psychrophilic round-spored *Bacillus* species and selected oval-spored species. The tree is based on 1321-nt sequences. Confidence limits estimated from bootstrap analyses (500 replicates) appear at the nodes. This is a modification of the tree generated by Nakamura (2000). A maximum-parsimony tree generated from the sequence data showed a similar topology. *Alicyclobacillus cycloheptanicus* was designated the outgroup species for the analysis. B. *thermosphaericus* has been reclassified as *Ureibacillus thermosphaericus* (Fortina et al., 2001); *B. globisporus*, *B. psychrophilus* and *B. pasteurii* have been reclassified as *Sporosarcina globispora*, *Sporosarcina psychrophila* and *Sporosarcina pasteurii*, respectively (Yoon et al., 2001a); *B. marinus* has been reclassified as *Marinibacillus marinus* (Yoon et al., 2001b); and *B. stearothermophilus* has been reclassified as *Geobacillus stearothermophilus* (Nazina et al., 2001). Bar, 1 nt substitution per 10 nt.

Substrate oxidation profiles were obtained using the BIOLOG GP system under conditions prescribed by the manufacturer. Other physiological traits were examined using the methods of Gordon et al. (1973). Antibiotic sensitivity was assessed by placing commercially prepared antibiotic discs on TGY agar plates spread-inoculated with the test organisms. The test plates were incubated at 28 °C and the extent of inhibition was determined at 48 h. Decomposition of Tweens 40 and 80 was determined by the method...
of Breuil & Gounot (1972). Vitamin and amino acid requirements were determined using the method of Poom et al. (1955).

The extent of similarity among strains based on fatty acid composition data (Nakamura, 2000) was estimated using the simple matching coefficient and clustering was based on arithmetic averages (Sneath & Sokal, 1973). All cultures were grown on trypticase soy agar for 24 h at 30 °C. Cell-wall peptidoglycan composition was analysed using the method described by Suzuki et al. (1993).

In a phylogenetic tree based on 16S rDNA sequences, strains of groups 4–7 were positioned within a clade that consisted generally of mesophilic, round-spored species, namely B. sphaericus, B. fusiformis and B. silvestris; the thermophile, U. thermosphaericus, is situated outside the clade [Fig. 1; this tree is a modification of one generated by Nakamura (2000)]. High bootstrap values validate the uniqueness of groups 4–7 within the clade of round-spored organisms. Groups 6 and 7 were more closely related to each other than to the other mesophilic groups.

High levels of DNA relatedness (97–100%) were observed between NRRL NRS-1531 and other group 6 strains, namely NRRL NRS-1693, NRRL NRS-1694 and NRRL NRS-1695; 100% relatedness was also observed between NRRL NRS-1694 and two other group 6 strains (NRRL NRS-1693 and NRRL NRS-1695). Low DNA relatedness values, of 27 and 19%, respectively, were determined between NRRL NRS-1694 and two group 7 strains, NRRL BD-87 T and NRRL BD-103. Similarly, low levels of DNA relatedness were observed between NRRL NRS-1694 and representative strains from groups 1 (NRRL B-23269), 4 (NRRL NRS-593) and 5 (NRRL B-1876); the respective values were respectively 23, 27 and 15%. Finally, the DNA relatedness between NRRL NRS-1691 and other group 7 strains, NRRL BD-87 T and NRRL BD-103 were also 100%. Low DNA relatedness values, of 27 and 15%, were respectively observed between NRRL BD-87 T and NRRL BD-103. The levels of DNA relatedness between NRRL NRS-1694 and the type strains of existing named mesophilic species. Numerical analysis of substrate-oxidation patterns segregated the existing named mesophilic species. Numerical analysis of substrate-oxidation patterns segregated the existing named mesophilic species. Numerical analysis of substrate-oxidation patterns segregated the existing named mesophilic species. Numerical analysis of substrate-oxidation patterns segregated the existing named mesophilic species. Numerical analysis of substrate-oxidation patterns segregated the existing named mesophilic species. Numerical analysis of substrate-oxidation patterns segregated the existing named mesophilic species. Numerical analysis of substrate-oxidation patterns segregated the existing named mesophilic species.
Description of *Bacillus pycnus* sp. nov.

*Bacillus pycnus* (pyc’nus. Gr. adj. *pyknos* thick; N.L. adj. *pycnus* thick, referring to thick cells).

Rods are 1×1-1.5×3.0–5.0 μm (determined from photomicrograph). Gram-positive. Motile. Round spores form in swollen sporangia. Agar colonies are non-pigmented, translucent, thin, smooth, circular, entire and the mean diameter is about 1 mm after 24 h of incubation on TGY agar at 28°C. Catalase is produced. Strictly aerobic. Nitrate is not reduced to nitrite. Acetylmethylnitrocin, dihydroxyacetone, indole and H₂S are not produced. The pH in Voges–Proskauer broth ranges from 7.2 to 7.6. Starch, casein, tyrosine, urea, Tweens 40 and 80 and egg-yolk lecithin are not decomposed. Common hexoses, pentoses, hexitols, disaccharides and trisaccharides are not fermented. Grows at pH 5.7, but not in the presence of 0.001% lysozyme and 5% NaCl. Sensitive to chloramphenicol, tobramycin, streptomycin, erythromycin and tetracycline. Biotin, thiamin and cystine are not required for growth. Based on the BIOLOG GP method, pyruvate and β-hydroxybutyrate are oxidized. Citrate, propionate, l-alanine, glycyl l-glutamate, 2-deoxyadenosine, inosine, AMP and UMP are not oxidized. Optimum growth temperature is 28–30°C (minimum, 5–10°C; maximum, 40–45°C). Whole-cell fatty acids detected are 15:0iso (70.3%), 15:0anteiso (8.1%), 16:1ω7cis alcohol (6.0%), 16:0iso (3.1%) and 16:1ω11cis (1.4%). Cell wall peptidoglycan type is L-Lys–d-Glu. Isolated from soil. G+C content is 35 mol%. Type strain is NRRL NRS-1691T (= JCM 11075T).

Description of *Bacillus neidei* sp. nov.

*Bacillus neidei* (nei’de.i. N.L. gen. n. *neidei* of Neide, in recognition of the early microbiologist E. Neide).

Rods are about 1×0.3–0.5 μm (determined by photomicrograph). Gram-positive. Motile. Round spores form in swollen sporangia. Agar colonies are non-pigmented, translucent, thin, smooth, circular, entire and the mean diameter is about 1 mm after 24 h of incubation on TGY agar at 28°C. Catalase is produced. Strictly aerobic. Nitrate is not reduced to nitrite. Acetylmethylnitrocin, dihydroxyacetone, indole and H₂S are not produced. The pH in Voges–Proskauer broth ranges from 7.2 to 7.6. Starch, casein, tyrosine, urea, Tweens 40 and 80 and egg-yolk lecithin are not decomposed. Common hexoses, pentoses, hexitols, disaccharides and trisaccharides are not fermented. Grows at pH 5.7 and in 5% NaCl, but not in the presence of 0.001% lysozyme. Sensitive to chloramphenicol, tobramycin, streptomycin, erythromycin and tetracycline. Biotin, thiamin and cystine are required for growth. Based on the BIOLOG GP method, pyruvate and β-hydroxybutyrate are oxidized. Citrate, propionate, l-alanine, glycyl l-glutamate, 2-deoxyadenosine, inosine, AMP and UMP are not oxidized. Optimum growth temperature is 28–30°C (minimum, 5–10°C; maximum, 40–45°C). Whole-cell fatty acids detected are 14:0iso (5.0%), 15:0 (1.7%), 16:1ω7cis alcohol (4.7%) and 16:0iso (8.1%). L-Lysine and d-glutamic acid were the key amino acids found in the cell wall peptidoglycans of NRRL NRS-1691T and NRRL BD-87T. Like many of the round-spored species (Stackebrandt et al., 1987), the peptidoglycan type for groups 6 and 7 was L-Lys–d-Glu.

Based on these results, groups 6 and 7 merit recognition as two novel species, for which the respective names *Bacillus pycnus* sp. nov. and *Bacillus neidei* sp. nov. are proposed. The respective type strains are NRRL NRS-1691T (= JCM 11075T) and NRRL BD-87T (= JCM 11077T).

References


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