**Bacillus methanolicus** sp. nov., a New Species of Thermotolerant, Methanol-Utilizing, Endospore-Forming Bacteria

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The generic position of 14 strains of gram-positive bacteria able to use methanol as a growth substrate was determined. All are obligately aerobic, thermotolerant organisms that are able to grow at temperatures of 35 to 60°C. Nine of the strains produce oval spores at a subterminal-to-central position in slightly swollen rod-shaped cells. DNA-DNA hybridization studies, SS rRNA sequence analysis, and physiological characteristics revealed that all 14 strains cluster as a well-defined group and form a distinct new genus. Analysis of the 16S and SS rRNA sequences indicated that this new species is distinct from *Bacillus brevis* but closely related to *B. firmus* and *B. azotoformans*. The name proposed for this new species is *B. methanolicus*. The type strain, PB1, has been deposited in the National Collection of Industrial and Marine Bacteria as NCIMB 13113.

Bacteria able to grow on methanol at elevated temperatures are of technological interest for single-cell protein production, solvent degradation in aerobic thermophilic biotreatment processes, and fermentative production of amino acids (1, 25, 42). Following an earlier report on growth of a mixed culture with spore-forming bacteria on methanol (45), a claim appeared in the patent literature (31) concerning *Bacillus* strains that grow on methanol with optimum growth temperatures of ca. 55°C and maximum growth temperatures of ca. 65°C. In recent years, several other groups have successfully employed continuous culture techniques for isolation of pure cultures of *Bacillus* strains that grow rapidly on methanol at 55°C (5, 15, 24, 29, 42). Metabolism of methanol, ethanol, and glucose (7, 9, 23, 47) and environmental control of metabolic fluxes (2-4, 14, 15, 30) have been studied in detail in these organisms, grown in batch and continuous cultures under a variety of nutrient limitations and steady-state as well as transient-state conditions. The methylotrophic *Bacillus* strains display a strong resistance to high methanol concentrations, and the molar growth yields on methanol at the optimum growth temperatures in methanol-limited chemostats are among the highest reported for methylotrophic bacteria (6, 24). Enzyme analysis revealed that all isolates employ a novel NAD-dependent methanol dehydrogenase for methanol oxidation and the ribulose monophosphate pathway for formaldehyde assimilation (7-9).

On the basis of a number of phenotypic tests, Al-Awadhi et al. (5) concluded that seven of their isolates were *Bacillus brevis* strains. A further isolate was a sheathed, filamentous, gram-positive, endospore-forming, obligately aerobic bacterium that could not be allocated to any previously described genus. In the present investigation, 14 organisms, including most of Al-Awadhi's isolates, were further characterized, and a classification of these strains based on various properties, including DNA-DNA hybridization, is proposed.

**MATERIALS AND METHODS**

Test strains and cultivation conditions. The thermotolerant methanol-utilizing *Bacillus* strains PB1 (NCIMB 13113), CI (NCIMB 13114), AR2, TS1, TS2, and TS4, isolated by Dijkhuizen et al. (24); 4(55) (NCIMB 12523), S1 (NCIMB 12524), WMS.2 (NCIMB 12525), TFB (NCIMB 12526), WM5.1 (NCIMB 12527), S2 (NCIMB 12528), and KA (NCIMB 12529), isolated by Al-Awadhi et al. (5); and 40M, isolated by Govorukhina and Trotsenko (29), were the type strains in batch cultures and carbon-, oxygen- or nitrogen-limited continuous cultures. The nonmethylotrophs *B. firmus* DSM 12 and *B. brevis* DSM 30 were used as reference strains in some of the physiological tests. All strains were stored without supplements as frozen stocks at ~80°C and routinely grown in Trypentine soya broth (TSB; Oxoid CM131; 30 g/1) adjusted to pH 7.5. For plates, TSB was solidified with 1.5% (wt/vol) agar (TSBA). Growth of the methylotrophic *Bacillus* strains in batch cultures and carbon-, oxygen- or nitrogen-limited continuous cultures has been described previously (7, 9, 14). DNA, 16S rRNA, 5S rRNA, cell wall fractions, fatty acids, and lipids were isolated from cells grown in batch cultures in trimethylamine (25 mM; strain S2A1 only) or methanol (100 mM; all other strains) mineral medium supplied with vitamins (24).

**Morphology.** Colony morphology was examined in isolated colonies grown on TSBA for 2 days at 55°C. Cellular
Table 1. 16S rRNA sequence similarity values for B. methanolicus C1 and other bacilli

<table>
<thead>
<tr>
<th>Organism</th>
<th>B. methanolicus</th>
<th>B. azotoformans</th>
<th>B. firmus</th>
<th>B. coagulans</th>
<th>B. megaterium</th>
<th>B. succitale</th>
<th>B. cereus</th>
<th>B. globisporus</th>
<th>B. stearothermophilus</th>
<th>B. aneurinolyticus</th>
<th>B. luteusporus</th>
<th>B. brevis</th>
<th>B. pumilus</th>
<th>B. cycloheptanicus</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Similarity (%)</td>
<td>96.3</td>
<td>96.1</td>
<td>95.7</td>
<td>95.4</td>
<td>95.4</td>
<td>95.4</td>
<td>95.4</td>
<td>94.1</td>
<td>92.8</td>
<td>93.7</td>
<td>91.5</td>
<td>90.1</td>
<td>90.2</td>
<td>89.0</td>
<td>85.9</td>
</tr>
<tr>
<td>Similarity (%)</td>
<td>95.8</td>
<td>94.9</td>
<td>94.5</td>
<td>93.5</td>
<td>92.0</td>
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<td>84.9</td>
<td>84.7</td>
</tr>
<tr>
<td>Similarity (%)</td>
<td>94.2</td>
<td>93.4</td>
<td>94.7</td>
<td>93.2</td>
<td>92.3</td>
<td>92.2</td>
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<td>85.0</td>
<td>84.6</td>
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<td>91.5</td>
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<tr>
<td>Similarity (%)</td>
<td>92.5</td>
<td>92.8</td>
<td>93.2</td>
<td>91.8</td>
<td>91.6</td>
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<td>85.2</td>
<td>84.4</td>
<td>84.6</td>
<td>84.9</td>
</tr>
</tbody>
</table>

* Mean values are given for the groups of related bacilli defined in Fig. 2. The values on the lower left are overall similarity values; the values on the upper right are based on a set of data reduced to those positions which are invariant in at least 40% of the entire set of sequences.
results were recorded after 1, 3, and 5 days. Poly-β-hydroxy- 
butyric acid accumulation was determined as described by 
Braunegg et al. (12) and Suzuki et al. (46).

**Antibiotic sensitivity.** Cultures were incubated overnight in 
peptone water broth. Disposable bioassay plates were pre-
pared with 200 ml of Oxoid Sensitest agar (CM409) with 3% 
(wt/vol) agar. The plates were seeded with a 3-ml culture 
volume and allowed to dry. Oxoid antibiotic sensitivity discs 
were then placed on these plates, which were incubated 
overnight. The zone width from the edge of the disc to the 
edge of the bacterial growth was then measured.

**Computer analysis.** Physiological and morphological data 
were analyzed by using the simple matching, Jaccard, and 
pattern difference coefficients (44). Clustering was achieved 
by using the unweighted pair group method with arithmetic 
averages algorithm (44).

**Determination of G+C contents of DNAs and DNA homol-
gy studies.** Purification of DNA was performed as described 
by Meyer and Schleifer (37). The melting point of the 
purified DNA was determined by using the method of 
Marmur and Doty (36), and the G+C content was calculated 
by using the method of De Ley et al. (22). DNA from 
*Escherichia coli* B (Sigma Chemical Co., St. Louis, Mo.), 
with a G+C content of 51.7 mol%, was used as the refer-
ence. DNA-DNA hybridization studies were performed by 
using the DNA filter method described by Kilpper et al. and 
Kilpper-Bälz et al. (33, 34).

**16S rRNA analysis.** Determination of the 16S rRNA pri-
mary structure was done by direct sequencing of the RNA 
and sequencing of cloned in vitro-amplified rDNA. RNA was 
isolated from strain C1 as described by Embley et al. (26). 
Sequencing of 16S rRNA was performed by using reverse 
transcriptase as described by Lane et al. (35). Sequence 
ambiguities were resolved by using terminal transferase (21). 
A DNA fragment containing the greater part of a 16S rRNA 
genome (homologous to positions 54 to 1542 of *E. coli* 16S 
rRNA), the intragenic spacer, and a small 5′-terminal part 
of a 23S rRNA gene (homologous to positions 1 to 130 of *E. 
coli* 23S rRNA) was amplified in vitro by applying the 
polymerase chain reaction technique (41) in combination 
with site-specific primers (5′-CATGCAAGTCGARCG-3′ 
[16S rRNA specific] and 5′-GGGTTYCCCCATTGC-3′ 
[23S rRNA specific]). The amplified rDNA fragment was 
cloned as PCI-611-118 in the vector pBluescript (Stratagene, 
La Jolla, Calif.). DNA sequencing was done as described by 
Chen and Seeberg (17). Oligonucleotide primers were syn-
thesized by standard methods by using a Biosearch Cyclone 
DNA synthesizer. The sequences of these primers are com-
plementary to highly conserved regions of 16S rRNA. The 
new sequence was added to an alignment of about 500 almost 
complete 16S rRNA primary structures of (eubacteria). 
Alignment of sequences was performed with respect to 
conserved primary structures, as well as secondary struc-
tures. Phylogenetic distance values (K_Nuc; 32) were calcu-
lated, including those positions which had been determined 
for both of the particular sequence pairs. Phylogenetic trees 
were reconstructed by applying the distance, parsimony, 
and bootstrapped parsimony methods by using the programs 
NEIGHBOR, FITCH, DNAPARS, and DNABOOT as im-
pemented in Felsenstein’s PHYLIP program package (27).

**5S rRNA analysis.** Isolation, sequencing, and phylogenetic 
analysis of 5S ribosomal RNA were performed by published 
procedures (16, 18).
TABLE 2. Physiological characteristics of thermotolerant methylotrophic *Bacillus* strains,$^a$ *B. firmus*, and *B. brevis$^b$

<table>
<thead>
<tr>
<th>Test</th>
<th>Result obtained with <em>Bacillus</em> sp. strain:</th>
<th><em>B. firmus</em> result</th>
<th><em>B. brevis</em> result</th>
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<tbody>
<tr>
<td></td>
<td>C1</td>
<td>PB1</td>
<td>AR2</td>
</tr>
<tr>
<td>Fermentation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fructose</td>
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<td>0</td>
</tr>
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</tr>
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<tr>
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<tr>
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<tr>
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<td>0</td>
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<tr>
<td>Colony morphology</td>
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<td>Transparent</td>
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<tr>
<td>Rough</td>
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<td>0</td>
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</tr>
<tr>
<td>Raised$^c$</td>
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<td>0</td>
</tr>
<tr>
<td>Crenated$^d$</td>
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</tr>
<tr>
<td>Length</td>
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<td>2</td>
</tr>
<tr>
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<td>0</td>
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</tr>
<tr>
<td>Chains$^e$</td>
<td>0</td>
<td>2</td>
<td>0</td>
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</table>

$^a$ All strains produced acid from glucose but not from i-erythritol, inositol, xylose, galactose, or adonitol. All strains grew at pH 6.5 and displayed oxidase activity. All methanol-utilizing bacilli showed catalase activity. None of the strains hydrolyzed hippurate or were able to reduce nitrate or nitrite. No growth was observed in the presence of 5% NaCl. The methylotrophic bacilli grew on mannitol but failed to grow on succinate or cellobiose (*B. brevis* and *B. firmus* were not tested). All colonies contained nonmotile, rod-shaped cells that stained positive in the Gram test. The methylotrophic bacilli grew at temperatures between 35 and 60°C.

$^b$ Scores: 2, positive; 1, weakly positive; 0, negative; —, test not done or results unsatisfactory. Scores for cell lengths are described in Materials and Methods.

$^c$ Score 0, colony is flat.

$^d$ Score 0, colony is convex.

$^e$ Score 0, single cells.

**Other analytical methods.** Preparation of cell wall fractions and determination of the diamino acid were carried out as described by Schleifer and Kandler (43). Fatty acid, lipid, and hopenoid extraction and analysis were carried out as described by Bringer et al. (13).

**Nucleotide sequence accession numbers.** The 16S rRNA sequence has been deposited in the EMBL data library under accession numbers Z11816 to Z11827.

**RESULTS AND DISCUSSION**

**16S rRNA sequence.** The 16S rRNA primary structure of strain C1 is shown in Fig. 1. The sequence was compared with all published homologous sequences of bacilli (10, 11,
40, 48). A matrix of sequence similarity values is shown in Table 1. Mean values are given for groups of more closely related bacilli. The phylogenetic tree shown in Fig. 2 is based on a matrix of phylogenetic distances which had been calculated by including those alignment positions which are invariant in at least 40% of the entire set of sequences. The combined 16s rRNA data show that Bacillus strain C1 is more closely related to B. azotoformans and B. firmus than to B. brevis, as originally thought (5).

**DNA-DNA hybridization studies.** DNA-DNA hybridization studies showed that the thermotolerant methanol-utilizing Bacillus strains are closely related and form a distinct taxon. The DNA similarity values obtained at optimal hybridization conditions (25°C below the Tm of the homologous hybridization) were 60% and higher.

**5S rRNA sequences.** 5S rRNA sequences of the methylophilic bacteria determined in this investigation are shown in Fig. 3. These sequences were compared with published data on nonmethylophilic mesophilic and thermophilic Bacillus species (49). The 5S rRNA data confirm that the thermotolerant methanol-utilizing Bacillus strains cluster as a well-defined group, separate from mesophilic trimethylamine-utilizing Bacillus strain S2A1 and nonmethylophilic thermophilic organisms such as B. steaerotherophilus. The 5S rRNA data also provide further evidence that the thermotolerant methylophilic bacilli are closely related to B. firmus and B. megaterium but not to B. brevis.

**Morphology and physiological characteristics.** All of the methanol-utilizing strains studied were obligately aerobic and able to grow in various media at temperatures of 35 to 60°C. Their physiological characteristics are listed in Tables 2 and 3. All strains formed circular colonies on TSBA after 2 days of incubation, in most cases with rough surfaces and crenated, undulating edges. Colonies of strains S2 and KA were different with respect to most of the properties tested (Table 2) (5). All colonies contained nonmotile, rod-shaped cells that stained gram positive. Strain KA forms filamentous cells during all growth stages. Some of the other isolates also formed filamentous cells but only towards the end of growth, e.g., in the centers of TSBA colonies. In methanol-limited continuous cultures of strains C1 and AR2, most of the cells were present as short chains of rod-shaped cells. Reduced growth rates caused formation of strongly helical filaments (data not shown) in both of these cultures. Similar cellular structures were detected in colonies of all of the methylophilic isolates grown on mannitol mineral agar for 2 days. Under these conditions, however, they constituted only a minority of the population. With the exception of strains C1, TS1, TS2, WM5.2, and TFB, cells of all of the strains sporulated on TSBA and/or on mannitol mineral agar. Sporulating cells were swollen and possessed oval spores at a subterminal-to-central position. The G+C content of the DNAs of strains C1, PB1, AR2, TS1, TS4, S2, (55), WM5.1, and KA was determined as 48 to 50%. The above data indicate that all endospore-forming isolates may be assigned to the genus Bacillus (39).

Previously, we reported (24) that isolation of these methanol-utilizing strains in pure cultures was difficult when methanol agar plates were used. This may be due to accumulation of toxic formaldehyde from methanol. With various alternative techniques, six methanol-utilizing strains were isolated (strains PB1, C1, AR2, TS1, TS2, and TS4). All six strains lost this ability upon subcultivation, resulting in isolation of endospore-deficient mutants. All of these methylophilic Bacillus strains contained meso-diaminopimelic acid as a cell wall diamino acid. This murein type is also present in the cell walls of B. subtilis, B. pumilus, B. megaterium, B. cereus, B. firmus, B. fastidiosus, and B. brevis (43). Lipid analysis indicated the presence of squalene and phosphatidylethanolamine in strain C1. Hophonoids (38) were not detected. The fatty acid profile of strain C1 consists mainly of 13-methyltetradecanoic acid (iso-C15: 27% of total fatty acid composition), 12-methyltetradecanoic acid (anteiso-C15: 16%), 14-methylpentadecanoic acid (iso-C16: 12%), 13-methylpentadecanoic acid (anteiso-C16: 13%), 15-methylhexadecanoic acid (iso-C17: 4%), and 14-methylheptadecanoic acid (anteiso-C17: 14%). Cells of strains M40 and C1 grown on methanol in nitrogen-limited continuous cultures contained a storage polymer that was identified as poly-β-hydroxybutyric acid. Sudan black staining of cells of strains PB1 and AR2 grown on methanol in oxygen-limited

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**Table 1.** Mean values are given for groups of more closely related Bacillus strains that stained gram positive. Strain KA forms filamentous cells but only towards the end of growth, e.g., in the centers of TSBA colonies. In methanol-limited continuous cultures of strains C1 and AR2, most of the cells were present as short chains of rod-shaped cells. Reduced growth rates caused formation of strongly helical filaments (data not shown) in both of these cultures. Similar cellular structures were detected in colonies of all of the methylophilic isolates grown on mannitol mineral agar for 2 days. Under these conditions, however, they constituted only a minority of the population. With the exception of strains C1, TS1, TS2, WM5.2, and TFB, cells of all of the strains sporulated on TSBA and/or on mannitol mineral agar. Sporulating cells were swollen and possessed oval spores at a subterminal-to-central position. The G+C content of the DNAs of strains C1, PB1, AR2, TS1, TS4, S2, (55), WM5.1, and KA was determined as 48 to 50%. The above data indicate that all endospore-forming isolates may be assigned to the genus Bacillus (39).

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continuous cultures revealed the presence of intracellular lipophilic material, most likely poly-β-hydroxybutyric acid. These findings suggest that the ability to synthesize poly-β-hydroxybutyric acid is a feature common to the entire group of methylotrophic Bacillus strains.

Determination of the average linkage (simple matching coefficient/unweighted pair group clustering) of the methanol-utilizing Bacillus strains on the basis of 68 characteristics (Table 2 and 3) revealed that all of the isolates are linked at >80% similarity, which indicates that the strains are closely related.

The thermotolerant methylotrophic test strains can readily be separated from mesophilic B. firmus on the basis of growth temperature; growth at pH 6.0; methanol utilization; acid production from glycerol, inulin, mannitol, and sucrose; and hydrolysis of casein (Table 2). B. brevis differs with respect to methanol utilization; acid production from inulin, salicin, and sucrose; and hydrolysis of casein (Table 2).

Molecular systematic, chemotaxonomic, and phenotypic data confirm that all of the 14 strains examined in this study are closely related and belong to the genus Bacillus. Until the internal heterogeneity of this group of organisms is studied in more detail, we propose that all 14 isolates be considered as belonging to a new species, for which we propose the name B. methanolicus.

Description of B. methanolicus Arfman, Dijkhuizen, Kirchhof, Ludwig, Schleifer, Bulygina, Chumakov, Govorukhina, Trotsenko, White, and Sharp sp. nov. (me.tha.noli.cus. M. L. n. methanolicum, methanol; M. L. masc. adj. methanolicus, relating to methanol). Cells are rod shaped and stain gram positive. Older cultures may contain filaments. No motility has been observed. Sporulating cells are swollen and possess oval spores at a subterminal-to-central position, although the ability to sporulate has been lost in some cultures. Growth is obligately aerobic and occurs at temperatures between 35 and 60°C, with optimum growth at around 55°C. The G+C content of the DNA is 48 to 50 mol%. Phenotypic characteristics are shown in Tables 2 and 3. Organisms can be isolated from soil samples, aerobic (thermophilic) waste water treatment systems, and volcanic hot springs. Type strain: B. methanolicus PB1 NCIMB 13113.

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REFERENCES


