Bacillus hisashii sp. nov., isolated from the caeca of gnotobiotic mice fed with thermophile-fermented compost

Ayaka Nishida,† Hirokuni Miyamoto,† Sankichi Horiuchi,‡ Ryo Watanabe,† Hidetoshi Morita,‡ Shinji Fukuda,§ Hiroshi Ohno,§§ Shizuko Ichinose,∥ Hisashi Miyamoto∥ and Hiroaki Kodama†

†Graduate School of Advanced Integration Science, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan
‡Japan Eco-science (Nikkan Kagaku) Co. Ltd, Shiomiigaoka-cho 11-1-2F, Chuuou-ku, Chiba-city, Chiba 260-0034, Japan
§Miroku Co. Ltd, Iwaya 706-27, Kitsuki-city, Oita 873-0021, Japan
¶Department of Biochemistry and Integrative Medical Biology, Keio School of Medicine, Shinnomachi 35, Shinjuku-ku, Tokyo 160-8582 Japan
∥Department of Molecular Virology, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-8519, Japan
¶Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan
∥Institute for Advanced Biosciences, Keio University, 264-2 Mizukami, Kakuganji, Tsuruoka, Yamagata 997-0052, Japan
§Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
∥Graduate School of Nanobioscience, Yokohama City University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
§§Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
∥∥Research Center for Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

A taxonomic study was performed on 15 bacterial isolates from the caeca of gnotobiotic mice that had been fed with thermophile-fermented compost. The 15 isolates were thermophilic, Gram-stain-positive, facultatively anaerobic, endospore-forming bacteria, and were most closely related to Bacillus thermoamylovorans CNCM I-1378T. The 16S rRNA gene sequence of strain N-11T, selected as representative of this new group, showed a similarity of 99.4 % with Bacillus thermoamylovorans CNCM I-1378T, 94.7 % with Bacillus thermolactis R-6488T, and 94.4 % with Bacillus kokeshiiformis MO-04. The isolates were then classified into two distinct groups based on a (GTG)5-fingerprint analysis. Two isolates, N-11T and N-21, were the representatives of these two groups, respectively. The N-11T and N-21 isolates showed 66–71 % DNA–DNA relatedness with one other, but had less than 37 % DNA–DNA relatedness with B. thermoamylovorans LMG 18084T. The other 13 isolates showed DNA–DNA relatedness values above 74 % with the N-11T isolate. All 15 isolates grew at 25–60 °C (optimum 50 °C), pH 6–8 (optimum pH 7) and were capable of growing on a medium containing 6 % (w/v) NaCl (optimum 0.5 %). The 15 isolates could be distinguished from B. thermoamylovorans LMG 18084T because they showed Tween 80 hydrolysis activity and did not produce acid from melibiose. The major fatty acids were anteiso-C15 : 0, C16 : 0, iso-C15 : 0, iso-C14 : 0 and iso-C16 : 0. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and several unidentified phospholipids. The diagnostic diamino acid in the cell-wall peptidoglycan was meso-diaminopimelic acid. The menaquinone was MK-7.
The genus *Bacillus* is a significantly heterogeneous group of Gram-staining-positive, aerobic, or facultatively anaerobic, endospore-forming bacteria. Most species of the genus *Bacillus* have been isolated from soil, water, food and clinical specimens. The vast majority of species of the genus *Bacillus* are mesophiles, with an optimal growth temperature of approximately 30 °C. However, several species of the genus *Bacillus* have higher optimal growth temperatures (40 to 55 °C). *meso*-Diaminopimelic acid is most common cell-wall peptidoglycan type in species of the genus *Bacillus*. The DNA G+C contents of members of the genus *Bacillus* range from 32 to 66 mol%. The type species of the genus is *Bacillus subtilis* (Logan & de Vos, 2009).

Many strains of species of the genus *Bacillus* have been identified during composting processes (e.g. Ryckeboer et al., 2003), but in general, these *Bacillus* bacteria disappeared upon maturation of the compost product (Ishii et al., 2000; Partanen et al., 2010). In our previous study (Niisawa et al., 2008) we characterized the microbiota of thermophile-fermented compost. This compost was produced by the fermentation of small marine animals, such as fish, crabs and shrimp that are not suitable for human consumption. The temperature during the composting process reaches approximately 75 °C or higher by fermentation-associated self-heating. One distinct characteristic of this thermophile-fermented compost is that it includes many thermophilic members of the family *Bacillaceae* (Niisawa et al., 2008). Recently, a thermotolerant bacterium, *Bacillus kokeshiformis*, was obtained during the isolation of l-lactic acid-producing bacteria from this compost (Poudel et al., 2014). The oral administration of thermophile-fermented compost confers health benefits to fish and pigs (Tanaka et al., 2010; Miyamoto et al., 2012). To delineate the probiotic function of thermophile-fermented compost, potentially probiotic thermophilic bacteria were isolated from the caeca of gnotobiotic mice that had ingested this compost. The predominant isolates were closely related to *Bacillus thermoamylovorans* (Miyamoto et al., 2013). *B. thermoamylovorans* was first isolated in 1995 from the fermented sap of *Palmae* trees (Combet-Blanc et al., 1995); later, an emended description of *B. thermoamylovorans* was reported (Coorevits et al., 2011). Following the characterization of 15 novel isolates from the caeca of the compost extract-fed mice using a polyphasic taxonomic approach (Logan et al., 2009), a novel species of the genus *Bacillus* is proposed.

The DNA G+C content was 37.9 mol%. Based on the phenotypic properties, the 15 strains represent a novel species of the genus *Bacillus*, for which the name *Bacillus hisashii* sp. nov. is proposed. The type strain is N-11T (=NRBC 110226T=LMG 28201T).

Thermophile-fermented compost was produced using an aerobic repeated fed-batch fermentation system (Niisawa et al., 2008). The suspension containing this compost at a final concentration of 1 % (v/v) was incubated under aerobic conditions at 60 °C for at least 10 h. The compost extract was prepared by filtering this suspension through a nylon mesh (100 μm pore size). BALB/c germ-free male mice were fed with water containing 0.5 % (v/v) compost extract for 21 days (Miyamoto et al., 2013). The predominant isolates from the caeca of these mice were closely related to *B. thermoamylovorans* (Miyamoto et al., 2013). After subculture on heart infusion agar (Nissui Pharmaceutical) at 37 °C, 15 well-grown isolates were further characterized.

The 16S rRNA gene sequence of the 15 isolates was amplified with a colony PCR method using a universal bacterial primer pair (27F and 1525R). Sequence similarity was calculated by the FASTA method (Pearson & Lipman, 1988). Multiple alignments of the sequences were made using the software package GENETYX version 15 (GENETYX Co.). A phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony algorithm (http://mobyle.pasteur.fr/cgi-bin/portal.py#forms:dnapers). The tree topology was evaluated using a bootstrap analysis based on 1000 replicates. In the following analyses, *Bacillus thermoamylovorans* LMG 18084T was included as a reference strain. The diversity of the isolates was evaluated using their (GTG)₃-PCR fingerprints (Gevers et al., 2001). A DNA–DNA hybridization (DDH) analysis was performed using the microplate method of Ezaki et al. (1989) with a hybridization temperature of 42 °C in a DIG Easy Hyb buffer (Roche). Six to eight replicates of each hybridization were obtained. Three independently prepared DNA samples from each strain were used, and the DDH values reflect the mean ± SD of at least three independent hybridization experiments. Hybridization with reciprocal probes was also conducted.

Gram staining and endospore staining were performed according to standard procedures (Bartholomew & Mittwer, 1950). The cellular morphology of strain N-11T was observed using scanning electron microscopy and transmission electron microscopy (Ichinose et al., 2013). In brief, the specimens were placed on MAS-coated glass slides (Matsunami Glass Ind.) at room temperature for 30 min and were then fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer saline (PBS) for 2 h. The specimens were washed with 0.1 M PBS, post-fixed for 2 h in 1 % OsO₄ buffered with 0.1 M PBS, and dehydrated in a graded series of ethanol solutions. The specimens were mixed with isoamyl acetate for 15 min and dried in a critical-point drying apparatus (HCP-2; Hitachi) with liquid CO₂; they were then sputter-coated with platinum and examined with scanning electron microscopy (S-4500; Hitachi). For transmission electron microscopy observations, the ethanol-dehydrated specimens were embedded in Epon 812.

> The type strain is N-11T (=NRBC 110226T=LMG 28201T).

†These authors contributed equally to this paper.

**Abbreviation:** DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N-11T is AB618491.

Three supplementary figures and two supplementary tables are available with the online Supplementary Material.

http://ijs.microbiologyresearch.org
Ultrathin sections (90 nm) were double-stained with uranyl acetate and lead citrate, and observed with transmission electron microscopy (H-7100; Hitachi). Anaerobic growth was determined after a two-day-incubation at 37 °C on a general anaerobic medium (GAM) agar (Nissui Pharmaceutical) using an AnaeroPack jar (Mitsubishi Gas Chemical). Motility was examined using the semisolid Sulphide-Indol-Motility medium. Growth at various temperatures (20, 25, 30, 37, 45, 50, 55, 60 and 65 °C) and NaCl concentrations (0.5, 1, 2, 3, 4, 5, 6 and 7 %, w/v) was monitored on heart infusion agar medium at 45 °C for up to 3 days. Growth at different pH (pH 5, 6, 7, 8, 9 and 9.5) was monitored in heart infusion broth at 45 °C; the pH was adjusted to acidic, neutral or alkaline levels with sodium acetate, Tris or sodium borate, respectively. Biochemical characteristics were assessed using API 20E and API 50 CHB kits with incubation at 50 °C. The methyl red test was performed according to the protocol described by Ventosa et al. (1982). The nitrite reduction test was performed in a medium composed of 2 % (w/v) Bacto Tryptone (BD), 0.2 % (w/v) disodium phosphate, 0.1 % (w/v) glucose and 0.1 % (w/v) potassium nitrate. The levels of nitrite and nitrate were determined using ion chromatography. The Tween 80 hydrolysis test was performed according to the procedure described by Slikkin (2000). Sole carbon source utilization tests were performed using C medium (Dye, 1962) with 0.5 % (w/v) substrate and 1.5 % (w/v) bromocresol purple. The minimal standards described by Logan et al. (2009) were considered for characterization of the novel isolates.

Chemotaxonomic characterization was conducted using cells cultured in tryptic soy broth for 2 days at 45 °C. The fatty acid composition was identified using the standard Microbial Identification System version 6 and the TSBA40 database (MIDI). Polar lipids were extracted and identified after undergoing two-dimensional silica-gel TLC as described by Coorevits et al. (2011). The peptido-glycan type was determined after purification of the bacterial cell walls as described by Schleifer & Kandler (1972), and the hydrolyses of the purified cell-wall fraction were analysed by TLC using the methods of Harper & Davis (1979). The extraction and analysis of menaquinones was performed according to Komagata & Suzuki (1987). The G+C content of the DNA was determined using HPLC after digestion with nuclease (Katayama-Fujimura et al., 1984).

Strain N-11T shared 99.7 % 16S rRNA gene sequence similarity with B. thermoamylovorans CNCM I-1378T, 94.7 % similarity with Bacillus thermodenitrificans DSM 4241T and 94.4 % similarity with Bacillus koreensis DSM 411T. Other isolates have the same 16S rRNA sequence as that of the type strain. The phylogenetic tree showed that the closest relative to these isolates was B. thermoamylovorans CNCM I-1378T (Fig. 1). We then considered whether these isolates consisted of a uniform bacterial species. As a genotypic tool for the classification and identification of these isolates, the (GTG)3-PCR fingerprinting test was performed. Thirteen of the 15 tested isolates produced an identical fingerprinting pattern. These 13 isolates were classified into one group, and one strain (N-21) was chosen as their representative isolate. The fingerprinting patterns of the other two isolates (N-11T and H-11) were identical, and these two isolates were grouped together. The fingerprints of both groups were different from the fingerprint of B. thermoamylovorans LMG 18084T (Fig. S1, available in the online Supplementary Material). To delineate bacterial species, DDH should be determined between these strains. Strains with a high DNA–DNA relatedness (70–100 %) were considered to be conspecific (Wang et al., 2007). When DNA isolated from strain N-11T was used as a probe, DDH values were 94 ± 12 % (strain H-11), 66 ± 4 % (strain N-21), 37 ± 11 % (B. thermoamylovorans LMG 18084T) and 16 ± 7 % (B. thermolactis LMG 25569T). The DDH values of the other 12 isolates with probe-DNA from strain N-11T were 74–94 %. In a reciprocal experiment, DNA isolated from B. thermoamylovorans LMG 18084T was used as a probe. The resulting DDH values were 33 ± 22 % (strain N-11T) and 33 ± 19 % (strain N-21). The DDH values obtained with an N-11T probe were 66 ± 4 % (strain N-21), while the reciprocal DDH analysis showed a DDH value of 71 % between the N-11T and N-21 strains (these data were obtained in a single hybridization experiment with eight replicates). These DDH values suggested that all 15 strains represented a species distinct from B. thermoamylovorans. The DNA G+C content of strain N-11T was 37.9 mol%, which was within the range of the DNA G+C content of the genus Bacillus (32 to 66 mol%, Logan & de Vos, 2009) but was higher than the 37.0 mol% found in B. thermoamylovorans LMG 18084T (Coorevits et al., 2011).

The biochemical characteristics of the 15 isolates and B. thermoamylovorans LMG 18084T are summarized in Table S1. None of the 15 strains produced acid from melibiose, whereas B. thermoamylovorans LMG 18084T was positive for this characteristic. Acid production from rhamnose was negative in strain N-11T, whereas the remaining 14 strains and the type strain of B. thermoamylovorans LMG 18084T were positive in our laboratory conditions; acid production from rhamnose was negative in the emended description of B. thermoamylovorans (Coorevits et al., 2011). Acid production from D-xylose was observed in strains N-11T and H-11, which displayed a similar (GTG)3-PCR fingerprinting pattern (see Fig. S1); the other 13 strains showed a negative reaction for acid production from D-xylose. None of the other biochemical characteristics investigated showed a link with the genotypic pattern produced by the (GTG)3-PCR fingerprinting.

Based on the biochemical and genotypic data described above, the 15 isolated strains represented a novel species that was distinct from B. thermoamylovorans LMG 18084T. All 15 strains showed a positive reaction for Tween 80 hydrolysis, whereas B. thermoamylovorans LMG 18084T did not.

Colonies of the 15 strains grown on heart infusion agar plates were beige, round, smooth and/or glossy at the surface, and had a flat morphology with an entire margin. Cells were facultatively anaerobic, Gram-stain-positive, endospore-forming, and rod-shaped. The cells of strain N-11T were usually single.
but often combined to form diplobacilli (Fig. S2). The cells produced ellipsoidal endospores that lay subterminally in a swollen sporangium. These endospores were encased in a striated endospore coat (Fig. S2), as observed in

\[ \text{Bacillus subtilis} \]

(Henriques & Moran, 2007). Strain N-11T showed a better sporulation ability than

\[ \text{Bacillus thermoamylovorans} \text{LMG 18084}^T \].

Sporulation ability was determined by growing cells of strain N-11T and

\[ \text{Bacillus thermoamylovorans} \text{LMG 18084}^T \]

in half-strength heart infusion medium for 2 days at 45 \( ^\circ C \). The number of endospores were found from heat-resistant (75 \( ^\circ C \) for 30 min) colony-forming units on the heart infusion agar plates. Viable cells were measured as the total number of colony forming units, and the percentages of sporulation were calculated. No detectable

\[ \text{Bacillus thermoamylovorans} \text{LMG 18084}^T \]

endospores were formed. In contrast, approximately \( 5 \times 10^9 \) out of the \( 1.9 \times 10^{10} \) total viable N-11T cells were sporulated after 24 h culture (sporulation rate, ca. 0.004 %). Generally, these endospores can tolerate low pH gastric conditions, and the endospores of several species of the genus

\[ \text{Bacillus} \]

have been used as probiotics (Colenutt & Cutting, 2014; Jeong & Kim, 2014). When endospores of strain N-11T were orally administered to conventionally maintained mice, a slight increase in body weight, an increased tendency towards feeding efficiency and faecal IgA production were observed (Miyamoto et al., 2013). These results suggested that the administration of strain N-11T improved the health of its host animals.

Growth of the isolates could occur between pH 6 and pH 8 but did not occur at pH 5 or pH 9.5. Growth at pH 9.0 was

---

**Fig. 1.** Neighbour-joining phylogenetic tree of strain N-11T with its closest relatives in the genus

\[ \text{Bacillus} \]

and other members of the family

\[ \text{Bacillaceae} \]

based on nearly complete 16S rRNA gene sequences (1342 bp). Bootstrap values (percentages of 1000 replications) >50 % are shown at the nodes. The sequence of

\[ \text{Paenibacillus polymyxa} \text{DSM 36}^T \]

was used as the outgroup. GenBank accession numbers are shown in parentheses. Filled circles indicate branches that were consistent in both the maximum-likelihood and maximum-parsimony trees. Bar, 0.01 nt substitutions per site.

---
Acid production from methylD-mannoside, melezitose and raffinose

Temperatures between 25°C and 45°C could tolerate up to 6% (w/v) NaCl. All 15 strains grew at 25–60°C (optimum 25°C), but did not grow at 20°C or 65°C, whereas growth of _B. thermoamylovorans_ LMG 18084^T_ occurred throughout the temperature range of 30°C to 55°C in our laboratory conditions. The physiological and biochemical characteristics that differentiate the novel isolates and _B. thermoamylovorans_ LMG 18084^T_ are listed in Table 1. The major fatty acids detected in strain N-11^T_ were anteiso-C15 : 0 (32.5%), C16 : 0 (15.7%), iso-C15 : 0 (13.3), iso-C14 : 0 (10.7%) and iso-C16 : 0 (10.0%). A large amount of anteiso-C15 : 0 and iso-C15 : 0 and low levels of unsaturated fatty acids were observed in the genus _Bacillus_ (Kämpfer, 1994). A detailed fatty acid composition is displayed in Table S2. The polar lipid pattern of strain N-11^T_ was similar to that of _B. thermoamylovorans_ LMG 18084^T_, showing diphostatidylglycerol, phosphatidylglycerol, phosphatidyl-

Table 1. Characteristics for distinguishing _Bacillus hisashii_ sp. nov. from _Bacillus thermoamylovorans_.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility</td>
<td>+</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>−</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>+</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Arbutin</td>
<td>+</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Methyl d-mannoside*</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Melezitose*</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Melibiose</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Raffinose*</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>V</td>
<td>−</td>
</tr>
<tr>
<td>Growth at/in:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>60°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6 % NaCl</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*Acid production from methyl d-mannoside, melezitose and raffinose was reported to be variable between strains for _B. thermoamylovorans_ (Coorevits et al., 2011).

variable between strains. _B. thermoamylovorans_ LMG 18084^T_ grew at pH 9. Cells of _B. thermoamylovorans_ LMG 18084^T_ did not tolerate 5% (w/v) NaCl, but all 15 novel strains could tolerate up to 6% (w/v) NaCl. All 15 strains grew at temperatures between 25°C and 60°C, but did not grow at 20°C or 65°C, whereas growth of _B. thermoamylovorans_ LMG 18084^T_ occurred throughout the temperature range of 30°C to 55°C in our laboratory conditions. The physiological and biochemical characteristics that differentiate the novel isolates and _B. thermoamylovorans_ LMG 18084^T_ are listed in Table 1. The major fatty acids detected in strain N-11^T_ were anteiso-C15 : 0 (32.5%), C16 : 0 (15.7%), iso-C15 : 0 (13.3), iso-C14 : 0 (10.7%) and iso-C16 : 0 (10.0%). A large amount of anteiso-C15 : 0 and iso-C15 : 0 and low levels of unsaturated fatty acids were observed in the genus _Bacillus_ (Kämpfer, 1994). A detailed fatty acid composition is displayed in Table S2. The polar lipid pattern of strain N-11^T_ was similar to that of _B. thermoamylovorans_ LMG 18084^T_, showing diphostatidylglycerol, phosphatidylglycerol, phosphatidyl-

Based on the cell-wall composition, menaquinone, and the major fatty acids, the isolates from the caeca of gnotobiotic mice could be allocated to the genus _Bacillus_. These data, combined with the other phenotypic and genotypic data described above, indicate that the 15 isolates represent a novel species of the genus _Bacillus_, for which the name _Bacillus hisashii_ sp. nov. is proposed.

### Description of _Bacillus hisashii_ sp. nov.

_Bacillus hisashii_ (hi.sa’hi.i. N.L. gen. n. _hisashii_ of hisashi, named after Hisashi Miyamoto, a founder of thermophile-fermented compost production).

Cells are Gram-stain-positive, facultatively anaerobic rods (0.4–0.5 x 2–5 μm). Cells occur singly or in pairs. Cells are motile by peritrichous flagella, but some strains are non-motile. After 24 h of incubation at 45°C on heart infusion agar, colonies are beige, flat and circular with an entire margin, and the surfaces are smooth or glossy. Ellipsoidal endospores are formed in a sub-terminal position and cause cells to swell. Growth occurs at 25–60°C (optimum 50°C), in the presence of 0.5–6% (w/v) NaCl (optimum 0.5%) and at pH 6–8 (optimum pH 7) when tested in heart infusion medium. Starch is hydrolysed, but casein is not. Catalase- and oxidase-positive. Tests for the formation of acetoin (Voges–Proskauer test) and indole are negative. Hydrolysis of gelatin is positive. Cells are negative for nitrite reduction but positive for nitrate reduction. The reactions for ortho-nitrophenyl-β-D-galactopyranoside (ONPG) production, the methyl red test and Tween 80 hydrolysis are positive. The arginine dihydrolase, citrate utilization, hydrogen sulphide production, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, and urease reactions are negative. Acid is produced from aesculin, cellobiose, D-fructose, D-glucose, D-mannose, galactose, gentiobiose, glycogen, lactose, L-arabinose, maltose, methyl D-glucosamine, rhamnose, ribose, salicin and starch. Acid production is variable between strains for glycerol, amygdalin, arbutin, sucrose and trehalose. Acid is not produced from 2-keto-D-gluconate, 5-keto-D-gluconate, adonitol, D-arabinose, D-arabitol, D-fucose, D-lyxose, D-tagatose, D-xylose, dulcitol, erythritol, gluconate, inulin, L-arabitol, L-fucose, L-sorbose, L-xylose, mannitol, melezitose, methyl D-mannoside, methyl xyloside, myo-inositol, raffinose, sorbitol, turanose or xylitol. Utilizes L-arabinoce, amygdalin, D-fructose, D-mannose, glycogen, maltose and methyl D-glucoside as sole carbon sources, but not butyrate, fumarate, propionate, succinate or pyruvate. The major fatty acids (> 10%) are anteiso-C15 : 0, C16 : 0, iso-C15 : 0, iso-C14 : 0 and iso-C16 : 0. The predominant polar lipids are diphostatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. Only menaquinone MK-7 is detected. The cell-wall peptidoglycan contains meso-diaminopimelic acid.
The type strain N-11T (=NRBC 110226T=LMG 28201T) was isolated from the caeca of the gnotobiotic mice that were fed with thermophile-fermented compost in Japan. The genomic DNA G+C content of the type strain is 37.9 mol%. The type strain is motile, positive for growth at pH 9, and negative for hydrolysis of gelatin. In relation to the acid production data listed above, the type strain is positive for acid production from amygdalin, arbutin, trehalose, D-xyllose and sucrose, and negative for acid production from galactose, glycerol and rhamnose.

Acknowledgements
This work was supported in part by the Ministry of Economy, Trade and Industry. We are grateful to Dr Tadashi Kajita for reconstructing the phylogenetic tree using the maximum-parsimony method.

References