**Limisphaera ngatamarikiensis** gen. nov., sp. nov., a thermophilic, pink-pigmented coccus isolated from subaqueous mud of a geothermal hotspring

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A novel bacterial strain, NGM72.4T, was isolated from a hot spring in the Ngatamariki geothermal field, New Zealand. Phylogenetic analysis based on 16S rRNA gene sequences grouped it into the phylum **Verrucomicrobia** and class level group 3 (also known as OPB35 soil group). NGM72.4T stained Gram-negative, and was catalase- and oxidase-positive. Cells were small cocci, 0.5–0.8 μm in diameter, which were motile by means of single flagella. Transmission electron micrograph (TEM) imaging showed an unusual pirellulosome-like intracytoplasmic membrane. The peptidoglycan content was very small with only trace levels of diaminopimelic acid detected. No peptidoglycan structure was visible in TEM imaging. The predominant isoprenoid quinone was MK-7 (92%). The major fatty acids (>15%) were C16 : 0, anteiso-C15 : 0, iso-C16 : 0 and anteiso-C17 : 0. Major phospholipids were phosphatidylethanolamine (PE), phosphatidylmonomethyl ethanolamine (PMME) and cardiolipin (CL), and a novel analogous series of phospholipids where diacylglycerol was replaced with diacetylglycerol (sPE, sPMME, sCL). The DNA G+C content was 65.6 mol%. Cells displayed an oxidative chemoheterotrophic metabolism. NGM72.4T is a strictly aerobic thermophile (growth optimum 60–65 °C), has a slightly alkaliphilic pH growth optimum (optimum pH 8.1–8.4) and has a NaCl tolerance of up to 8 g l⁻¹. Colonies were small, circular and pigmented pale pink. The distinct phylogenetic position and phenotypic traits of strain NGM72.4T distinguish it from all other described species of the phylum **Verrucomicrobia** and, therefore, it is considered to represent a novel species in a new genus for which we propose the name **Limisphaera ngatamarikiensis** gen. nov., sp. nov. The type strain is NGM72.4T (ICMP 20182T = DSM 27329T).

The phylum **Verrucomicrobia** is highly divergent, globally distributed and can be found in many different aquatic and terrestrial habitats. These include various aquatic systems such as drinking water, freshwater lakes (Lindström et al., 2005), and marine waters and sediments (Polymenakou et al., 2005). In addition, members of the phylum have been detected in man-made ecosystems such as acid rock drainage, rice paddies and leachate from a municipal solid-waste landfill (Huang et al., 2005), as endo- or ectosymbionts of nematodes or ciliates (Petroni et al., 2000), and in the human intestine (Wang et al., 2005) and the digestive systems of termites and sea cucumbers (Wertz et al., 2012). Bacteria belonging to the phylum **Verrucomicrobia** have also been detected in extreme environments such as soda lakes (Humayoun et al., 2003), submarine hydrothermal systems (Hirayama et al., 2007), acidic hot springs (Dunfield et al., 2007), and mud volcanoes (Alain et al., 2006). Members of the phylum **Verrucomicrobia** are also nearly ubiquitous in soil environments, making up approximately 23% of all bacterial phylotypes detected (Bergmann et al., 2011). Their ubiquitous abundance implies a considerable ecological impact. Nevertheless, the phylum **Verrucomicrobia** is
defined primarily through 16S rRNA gene sequences, and is represented by less than 100 described isolates in microbiological repositories (Bergmann et al., 2011). The phylum Verrucomicrobia (Hugenholtz et al., 1998a) is part of a monophyletic group referred to as the PVC (Planctomycetes-Verrucomicrobia-Chlamydiae) superphylum (Wagner & Horn, 2006). This deeply rooted taxonomic group is formed by the phyla Planctomycetes, Chlamydiae and Lentisphaerae, together with the candidate phyla Poribacteria and OP3. To date, there are seven defined subdivisions of the phylum Verrucomicrobia, including the classes Verrucomicrobia, ‘Spartobacteria’, Opitutae and the mostly uncultivated subphyla groups 3, 5, 6 and 7 (Hedlund, 2010). Representatives of subphylum groups 3 and 6 have been cultivated (Op den Camp et al., 2009; Sangwan et al., 2005), although there currently are no representatives with validly published names in these groups. Sangwan et al. (2005) in particular, isolated a range of mesophilic verrucomicrobial representatives of subphylum group 3 from pasture soil, including ‘Candidatus Pedosphaera parvula’ Ellin514. A phenotypic characterization of this strain has not been completed although a number of studies including analyses of its genome and membrane structure have been published (Kant et al., 2011; Lee et al., 2009). Only four moderately thermophilic verrucomicrobial strains have been described: Alterococcus agarolyticus (T_{opt} 54 °C) of the family Opitutaceae (Shieh & Jean, 1998) and three strains belonging to subphylum 6; the acidophilic methanotrophic strains ‘Candidatus Methylococcoides infernorum’ V4 (T_{opt} 60 °C) (Dunfield et al., 2007), ‘Candidatus Methylococcoides kamchtkensis’ Kam1 (T_{opt} 55 °C) (Islam et al., 2008) and ‘Candidatus Methylococcoides fumarophilum’ SoLV (T_{opt} 55 °C) (Pol et al., 2007). Despite the paucity of thermophilic isolates, verrucomicrobial phytophotes have been readily detected in various hot springs in Yellowstone National Park (Hugenholtz et al., 1998b) and Thailand (Kanokratana et al., 2004) extending the ecological range of this phylum. NGM72.4T is the first truly thermophilic strain (T_{opt} >60 °C) described within the phylum Verrucomicrobia. Here we describe the phenotypic and phylogenetic characteristics of this isolate. NGM72.4T represents the first strain from the class-level group 3 in the phylum Verrucomicrobia to be proposed as a representative of a novel genus and species. The name Limisphaera ngatamarikienis gen. nov., sp. nov. is proposed to accommodate this strain.

NGM72.4T was isolated from a hot spring in the Ngatamariki geothermal field, New Zealand. It was enriched from subaqueous clay sediments (67 °C) immediately adjacent to the spring edge. Sediment samples were collected in a sterile 50 ml plastic centrifuge tube. The soil pH was determined at room temperature by suspending 1 g of the sample in deionized H$_2$O and was measured as pH 7.6. Soil crumbs were transferred to NRP plates (Supplementary Methods, available in the online Supplementary Material) with the addition of 0.01 % (w/v) glucosemann. The plates were incubated at 70 °C in Oxoid anaerobic jars with an aerobic atmosphere. Bacterial colonies that formed within 4 weeks were examined by means of a stereo microscope and subcultured until pure cultures were obtained. Unless otherwise stated, all physiological and metabolic characteristics were determined by growing NGM72.4T at 65 °C in liquid NRP-V medium (Supplementary Methods) with mannose (0.5 g l$^{-1}$) as an energy source. Unless otherwise stated, all methodologies for phenotypic characterization were as listed in the Supplementary Methods.

Genomic DNA was extracted from single colonies using a Nucleospin Tissue kit (Macherey-Nagel) as per the manufacturer’s instructions. The 16S rRNA gene was amplified using PCR with universal bacterial primers 9f and 1492r (Weisburg et al., 1991) and purified using a Nucleospin Gel and PCR clean-up kit (Macherey-Nagel) and sequenced at the University of Waikato Sequencing Service (Hamilton, New Zealand). The almost complete 16S rRNA gene sequence was 1410 bp and was checked manually for quality. Strains closely related to NGM72.4T were determined by subjecting the 16S rRNA gene sequence to BLASTn discontiguous megablast search (Altschul et al., 1997). The 16S rRNA gene sequences from NGM72.4T and closely related strains and phylotypes were aligned (all retrieved sequences were >1395 bp) and phylogenetic distances calculated using the Jukes–Cantor correction within the ARB software environment (Jukes & Cantor, 1969; Ludwig et al., 2004). NGM 72.4T was most closely related (89.4 % sequence similarity) to three clonal phylotypes from geothermal hotsprings in Yellowstone National Park, USA (clone OPB35, GenBank accession no. AF027005), Velingrad, Bulgaria (clone VS-21, FM994912) and Bor Klueng, Thailand (clone PK291, AY555799) (Hugenholtz et al., 1998b; Kanokratana et al., 2004). The closest cultivated relatives were members of the Ellinbank pasture soils study (Sangwan et al., 2005), Ellin5102 (AY234519; 86.5 % similarity), Ellin515 (AY960778; 86.0 %) and ‘Candidatus Pedosphaera parvula’ Ellin514 (AY960777; 86.5 %). A phylogenetic tree (Fig. 1) was reconstructed using MrBayes, which uses a Bayesian inference model to calculate phylogeny (Ronquist et al., 2012). The phylogenetic tree in Fig. 1 was calculated using a 0.25 burn, and Markov chain Monte Carlo estimation of 100 000 cycles, four chains (temperature parameter of 0.5), and a sampling frequency of 500. The phylogenetic placement of strain NGM72.4T clearly shows it groups within the class-level group 3 (OPB35 soil group).

The respiratory quinone and DNA base composition of strain NGM 72.4T were determined externally by the Identification Service at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The primary respiratory quinones were determined by HPLC according to the method of Tindall (1990) and were MK-6 (8 %) and MK-7 (92 %). For DNA base composition, approximately 0.5 g of freeze-dried material was washed in sterile water and lyzed by French press. Genomic DNA was then purified on a hydroxyapatite column (Cashion et al., 1977) and composition determined by HPLC (Mesbah et al., 1989). The genomic DNA G + C content was 65.6 mol%. Fatty acid methyl esters

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were prepared (Ferreira et al., 1999) and analysed without prior extraction as described previously (Lee et al., 2011) and detailed in the Supplementary Methods. The cell membrane of NGM72.4\textsuperscript{T} was composed primarily of long, branched fatty acids (Table S1). The major fatty acids were C\textsubscript{16}:0 (15.0%), anteiso-C\textsubscript{15}:0 (16.7%), iso-C\textsubscript{16}:0 (15.5%) and anteiso-C\textsubscript{17}:0 (18.5%), with lesser amounts of C\textsubscript{18}:0 (11.7%) and C\textsubscript{18}:0 3-OH (4.5%). Total lipid analysis performed using two-dimensional TLC (Fig. S1) showed six major phospholipids which were quantified using \textsuperscript{31}P NMR (MacKenzie et al., 2009) (Fig. S2). Common phospholipids, phosphatidylethanolamine (19 mol%), phosphatidylmonomethylethanolamine (19 mol%) and cardiolipin (8.2 mol%), were present alongside a novel series of analogous lipids which had a serinol unit (2-amino-1,3-propanediol) replacing glycerol (see Supplementary Methods). These serinol phospholipids were 1-O-acyl-2-N-acyl-serinophosphoethanolamine (13 mol%), 1-O-acyl-2-N-acyl-serinophosphomonomethylethanolamine (19 mol%) and cardiolipin with one of the two diacylglycerol units replaced by diacyselserinol (4.4 mol%). Phosphatidylglycerol and 1-O-acyl-2-N-acyl-serinophosphoglycerol were also detected as minor lipids by mass spectrometry (Fig. S3). The total phospholipid content was 68% (w/w) of total lipid.

Cells of strain NGM72.4\textsuperscript{T} were small single cocci, 0.5–0.8 \textmu m in diameter (Fig. 2), with diplococci morphologies commonly observed. No spores were detected at any stage of growth for NGM72.4\textsuperscript{T}. The cells were highly motile in liquid culture, although ‘swarming’-type colony morphology on solid medium was not observed. Flagella could be detected by Ryu staining (Heimbrook et al., 1989) (Fig. S4). Cells were montrichously flagellated and flagella were up to 10 \textmu m in length. NGM72.4\textsuperscript{T} cells stained Gram-negative. However, transmission electron micrograph (TEM) imaging did not show the typical Gram-negative tri-layered composition of the cell wall (Fig. 2). Only the outer cytoplasmic membrane was easily detectable with what appears to be a pirellulosome-like intracytoplasmic membrane also visible. This intracellular organization was characterized by the separation of an inner compartment (pirellulosome)
NGM72.4T formed pink-pigmented colonies on solid medium. Colonies were smooth, irregular-shaped, slightly translucent and were fully developed 48 h after inoculation. Colony pigmentation deepened marginally as the colonies aged. On NRP plates, NGM72.4T colonies formed indentations into Phytagel (Sigma)-containing solid medium, indicating that NGM72.4T possesses glycoside hydrolase activity (Fig. S5). In liquid culture, NGM72.4T grew as a pink turbid solution (Fig. S5). It required gentle shaking (120 r.p.m.) in an orbital incubator, whereas no growth was observed with static or rapid (>120 r.p.m.) incubation. The pink pigmentation of NGM72.4T did not change colour under any growth conditions tested, neither did it change after treatment with 20 % (w/v) aqueous KOH solution, which would indicate flexirubin-like pigments (Fautz & Reichenbach, 1980). The pigments were isolated via a crude solvent extraction using methanol or acetone and photospectrometrically scanned (330–900 nm). They had an absorbance maximum at 500 nm (acetone) and 495 nm (ethanol) and a second smaller peak/plateau was observed at 527 nm (acetone) and 513–523 nm (ethanol), respectively. We were unable to link these absorbance maxima with known pink or red carotenoids described in the literature. NGM72.4T had a growth temperature optimum between 60 and 65 °C and a growth temperature range of 45–71 °C. It was able to grow at between pH 5.6 and 8.9 (optimum pH 8.1–8.4), but no growth was observed at pH 5.4 or below or at pH 9.5 or above. NGM72.4T did not show enhanced growth by the addition of NaCl but tolerated up to 8 g NaCl l⁻¹. Cells also tolerated up to 3 % (v/v) ethanol but were sensitive to ethanol concentrations >4 % (v/v). NGM72.4T was an obligate aerobe. It required a minimum of 1 % (v/v) O₂ for growth and exhibited no growth at lesser O₂ concentrations. No growth was observed anaerobically using nitrate or sulphate as terminal electron acceptors.

Substrate utilization tests were conducted in triplicate in 125 ml serum bottles (NRP-V medium) and were incubated at 60 °C under aerobic conditions at 120 r.p.m. for 7 days. A list of substrates tested, focusing mainly on the utilization of various carbon sources is presented in Table S2 and summarized in the species description. In addition, a selection of carbohydrates and their derivatives were also tested using API 50 CH 300 strips (bioMérieux; Table S3). NGM72.4T has an oxidative heterotrophic metabolism, utilizing primarily low concentrations of organic substrates. Oxidation of inorganic energy sources such as hydrogen or nitrite as energy sources was not detected. No growth was observed for alcohols or sugar alcohols, and hydrogen or nitrite as energy sources was not detected. No growth was observed for any standard complex media tested such as nutrient broth (NB - Sigma), R2A (Difco) and Luria–Bertani broth (LB - Merck), even when diluted to 10 % or 1 % (w/v) strength. NGM72.4T was capable of growing on all simple sugars and their derivatives tested, but it was not able to degrade crystalline polysaccharides or cellulose such as Avicel (Fluka) or CM-cellulose (Hercules).
Similarly, starch (Sigma), xanthan (CP Kelco) and glycogen were not utilized, indicating the absence of endoglucanases and/or alpha-acting exoglucanases. Interestingly, however, starch breakdown products such as pullulan (Sigma) and dextrin (Sigma) were growth-promoting. Furthermore, NGM72.4\textsuperscript{T} was able to metabolize simpler polymers with mannose or mannose/glucose backbones such as galactomannan and glucomannan, and bacterial exopolysaccharides such as gellan gum (Sigma). As a general observation, NGM72.4\textsuperscript{T} seems to be adapted to very low substrate concentrations of simple oligosaccharides and appears to be inhibited by concentrated, nutrient-rich media.

Nitrogen fixation from air was not detectable. The preferred nitrogen source was determined by providing different nitrogen sources in otherwise nitrogen-free NRP-V medium (Supplementary Methods). Nitrogen sources tested were KNO\textsubscript{3}, KNO\textsubscript{2}, (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, urea, Casamino acids (Difco) and peptone. Ammonium was the preferred nitrogen source; Casamino acids provided weak growth. No increased biomass production was observed when additional CO\textsubscript{2} was supplemented to the oxic headspace, indicating a lack of anaerobic mechanisms that employ pyruvate carboxylase or phosphoenolpyruvate carboxylase.

NGM72.4\textsuperscript{T} was catalase- and oxidase-positive. Further enzymic activities were tested using bioMérieux API ZYM strips (Table S4); the results are also included in the species description. A broad range of antibiotics was tested at different concentrations for resistance or sensitivity of NGM72.4\textsuperscript{T}. This organism was not inhibited by up to 250 \mu g ampicillin ml\textsuperscript{-1}, 250 \mu g trimethoprim ml\textsuperscript{-1} or 250 \mu g vancomycin ml\textsuperscript{-1}, or up to 100 \mu g chloramphenicol ml\textsuperscript{-1} or 100 \mu g metronidazole ml\textsuperscript{-1}, or by 10 \mu g lasalocid A ml\textsuperscript{-1}. In contrast, it was susceptible to erythromycin, kanamycin, monensin, neomycin, polymyxin B, rifampicin, streptomycin and tetracycline at all concentrations tested.

The phylum Verrucomicrobia is defined primarily by 16S rRNA gene sequences as few representatives are in pure culture. Table 1 lists the phenotypic characteristics of NGM72.4\textsuperscript{T} with other characterized verrucomicrobial strains. Alterococcus agarolyticus shares some similar characteristics including cocci morphology, motility by a single flagella, a slightly alkaliphilic growth range and an almost identical DNA G+C content. However, it only shares 88.2 % 16S rRNA gene sequence similarity with NGM72.4\textsuperscript{T}, and has a lower growth temperature optimum, an obligate salt requirement, an ability to hydrolyse agar and grows anaerobically via fermentation, all of which clearly differentiates it from NGM72.4\textsuperscript{T}. The ‘Methylacidiphilum’ strains are equally dissimilar to NGM72.4\textsuperscript{T}, particularly as these strains are obligately acidophilic and methanotrophic. The most closely related cultivated strains were enriched and isolated from pasture soil at Ellinbank, a diary research farm in Australia (Sangwan et al., 2005). A number of Ellinbank isolates (Ellin5102, Ellin 515 and Ellin514) position phylogenetically in subphylum group 3 (also known as OPB35 soil group) of the phylum Verrucomicrobia. However, almost no phenotypic characterization of these isolates has been published and, therefore, these strains were not included in Table 1. Ellinbank strains 514, 515, 5101 and 516 were all cocci, approximately 0.6 to 0.8 \mu m in diameter, and stained Gram-negative. However, considering what is known of the Ellinbank isolates, NGM72.4\textsuperscript{T} differs notably by its pigmentation, temperature optimum and DNA G+C content. The Ellinbank isolates formed cream-coloured, flat, smooth and entire colonies when grown on plates and had a temperature optimum of ~30 °C (Sangwan et al., 2005). In addition, the genome sequence of ‘Candidatus Pedosphaera parvula’ Ellin 514 was published in 2011 (Kant et al., 2011) but included very little in the way of insights into its physiological or metabolic capabilities. Given the phylogenetic dissimilarity and significant physiological differences with known and characterized verrucomicrobial strains, we consider NGM72.4\textsuperscript{T} to represent a distinctive genus and a novel species within the class-level group 3. We propose the name Limisphaera ngatamarikiensis gen. nov., sp. nov., to accommodate this isolate.

**Description of Limisphaera gen. nov.**


Cells stain Gram-negative. Strictly aerobic and catalase- and oxidase-positive. The peptido glycan content is below detectable limits, but contains diaminopimelic acid. Cells are small coccoid, non-spore-forming and motile by means of monotrichous flagella. Cell division is by binary fission. Chemoheterotrophic. Typical substrates include monosaccharides and simple oligosaccharides. The major respiratory quinone is MK-7. The primary fatty acids are C\textsubscript{16:0}, anteiso-C\textsubscript{15:0}, iso-C\textsubscript{16:0} and anteiso-C\textsubscript{17:0}. The type species is *Limisphaera ngatamarikiensis*.

**Description of Limisphaera ngatamarikiensis sp. nov.**

*Limisphaera ngatamarikiensis* (nga.ta.ma.ri.ki.en.sis. N.L. fem. adj. *ngatamarikiensis* pertaining to the Ngatamariki geothermal features where the type strain was isolated).

In addition to the characteristics of the genus, cells are very small single cocci or diplococci and are 0.5–0.8 \mu m in diameter. They are motile by means of monotrichous flagella (up to 10 \mu m in length). Colonies are smooth, irregular-shaped, slightly translucent and pale pink-pigmented. Pigments have an absorbance maximum at 500 nm (acetone) and 495 nm (ethanol). Thermophilic. Growth occurs at 45–71 °C (optimum 60–65 °C), at pH 5.6–8.9 (optimum pH 8.1–8.4) and with 0–0.8 % NaCl (w/v; optimum 0 %) and up to 3 % (v/v) ethanol (optimum 0 %). Exhibits an oxidative heterotrophic metabolism. Grows on simple sugars [D-L-arabinose, (-)-D-ribose, (+)-D-xylene, L-rhamnose, (+)-D-galactose,
Table 1. Differential characteristics of NGM72.4<sup>T</sup> and thermophilic and moderately thermophilic representatives of the phylum Verrucomicrobia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecosystem</td>
<td>Water/clay hot spring, New Zealand</td>
<td>Coastal hot spring, Lutoa, Taiwan</td>
<td>Thermal soil, hot spring, New Zealand</td>
<td>Outflow hot spring, Kamchatka, Russia</td>
<td>Thermal mudpot, Solfatara, Italy</td>
</tr>
<tr>
<td>Temp. range for growth (&lt;T&lt;sub&gt;opt&lt;/sub&gt;) (°C)</td>
<td>45–71 (60–65)</td>
<td>38–58 (48)</td>
<td>40–60 (60)</td>
<td>37–60 (55)</td>
<td>40–65 (55)</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>5.6–8.9 (8.1–8.4)</td>
<td>7.0–8.5 (8.0)</td>
<td>1.0–6.0 (2.0–2.5)</td>
<td>2–5 (3.5)</td>
<td>0.8–5.8 (2.0)</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Pink</td>
<td>White/opaque</td>
<td>White</td>
<td>n.r.</td>
<td>White</td>
</tr>
<tr>
<td>Primary fatty acids</td>
<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15 : 0&lt;/sub&gt;, iso-C&lt;sub&gt;16 : 0&lt;/sub&gt;, anteiso-C&lt;sub&gt;17 : 0&lt;/sub&gt; (≥15%)</td>
<td>C&lt;sub&gt;18 : 0&lt;/sub&gt;</td>
<td>iso-C&lt;sub&gt;14 : 0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
<td>n.r.</td>
<td>iso-C&lt;sub&gt;14 : 0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15 : 0&lt;/sub&gt; C&lt;sub&gt;18 : 0&lt;/sub&gt;</td>
</tr>
<tr>
<td>NaCl tolerance (w/v)</td>
<td>0–0.8%</td>
<td>1.0–3.5%</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td>Motility</td>
<td>Single flagellum</td>
<td>Single flagellum</td>
<td>Non-motile</td>
<td>Non-motile</td>
<td>Non-motile</td>
</tr>
<tr>
<td>Morphology</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Rods</td>
<td>Oval rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>0.5–0.8</td>
<td>0.8–0.9</td>
<td>0.3–0.5 × 1.0–4.0</td>
<td>0.8–1.0 × 0.45–0.65</td>
<td>0.4–0.6 × 0.8–2.0</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>65.6</td>
<td>65.5–67.0</td>
<td>45.5</td>
<td>n.r.</td>
<td>40.9</td>
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<tr>
<td>Primary metabolic lifestyle</td>
<td>Heterotrophic, aerobic</td>
<td>Heterotrophic, aerobic and anaerobic fermentation</td>
<td>Autotrophic, obligate methanotroph</td>
<td>Autotrophic, obligate methanotroph</td>
<td>Autotrophic, obligate methanotroph</td>
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<tr>
<td>Nitrogen sources</td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;+, Casamino acids</td>
<td>n.r.</td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;+, N&lt;sub&gt;2&lt;/sub&gt; fixation</td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;+, N&lt;sub&gt;2&lt;/sub&gt; fixation</td>
<td>n.r.</td>
</tr>
<tr>
<td>Major quinones</td>
<td>MK-7</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
</tbody>
</table>

D-glucose, (+)-D-mannose, (-)-D-fructose, (+)-cellobiose, lactose, (+)-maltose, sucrose, (+)-trehalose, (+)-rafafinose and sugar derivatives (D-N-acetylglucosamine, galacturonic acid, D-glucuronic acid). Dextrin, pullulan, galactomannan (Locust bean gum), glucomannan, gellan and Phytalgel are hydrolysed, but Avicel, CM-cellulose, starch, agarose, alginic acid sodium salt, pectin, chitin, glycopen and xanthan are not. Growth is observed on sodium gluconate and sodium pyruvate, but no growth on alcohols (methanol, ethanol, 1-propanol, 2-propanol, butanol, pentanol), sorbitol, mannitol, sodium acetate, sodium citrate, sodium fumarate, sodium lactate, sodium succinate, benzoic acid, malic acid, oxalic acid, yeast extract, Casamino acids, peptone, tryptone, gelatin, B-vitamins, glycerol, methane gas, vanillin, ammonium sulphate, hydrogen gas, sodium nitrate, sulphur (elemental), NB, tryptic soy broth, R2A, LB or Todd–Hewitt Broth. In API 50 CH 300 strips, oxidation of D-arabinose, L-arabinose, D-ribose, D-xylene, L-xylene, D-glucose, D-fructose, D-mannose, L-sorbose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, lactose (bovine origin), melibiose, sucrose, trehalose, melezitose, raffinose, gentiobiose, fructose, D-lyxose, D-tagatose, fructose and Fucose. No oxidation detected for glycerol, erithritol, D-adonitol, methyl β-D-xylopyranoside, L-ramnosse, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, starch, glycopen, xylitol, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketoglucolate or potassium 5-ketoglucolate. Ammonia (as ammonium salt) is the preferred nitrogen source. In API ZYM strips (bioMérieux) enzymic activity for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, β-galactosidase, β-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fructosidase, but weak or no activity for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phospho-hydrolase and β-glucoronidase. Resistant to ampicillin, chloramphenicol, lasalocid A, metronidazole, trimethoprim and vancomycin, but susceptible to erythromycin, kanamycin, monensin, neomycin, polymyxin B, rifampicin, streptomycin and tetracycline. The predominant isoprenoid quinone is MK-7. The major fatty acids (≥15%) are C<sub>16 : 0</sub>, anteiso-C<sub>15 : 0</sub>, iso-C<sub>16 : 0</sub> and anteiso-C<sub>17 : 0</sub>. The primary lipids are phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PMME), cardiolipin (CL) and an analogous series of phospholipids where diacylglycerol is replaced with diacylserinol (sPE, sPMME, sCL).

The type strain, NGM72.4<sup>T</sup> (ICMP 20182<sup>T</sup> =DSM 27329<sup>T</sup>), was isolated from geothermally heated sediment at the periphery of a hot spring at Ngatamariki, New Zealand. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Limisphaera ngatamarikiensis is HF947551. The DNA G+C content of the type strain is 65.6 mol%.
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References


