**Thermorudis pharmacophila** sp. nov., a novel member of the class *Thermomicrobia* isolated from geothermal soil, and emended descriptions of *Thermomicrobium roseum*, *Thermomicrobium carboxidum*, *Thermorudis peleae* and *Sphaerobacter thermophilus*

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An aerobic, thermophilic and cellulolytic bacterium, designated strain WKT50.2^T, was isolated from geothermal soil at Waikite, New Zealand. Strain WKT50.2^T grew at 53–76 °C and at pH 5.9–8.2. The DNA G+C content was 58.4 mol%. The major fatty acids were 12-methyl C_{18:0} and C_{18:0}. Polar lipids were all linked to long-chain 1,2-diols, and comprised 2-acylalkyldiol-1-O-phosphoinositol (diolPI), 2-acylalkyldiol-1-O-phosphoacylmannoside (diolP-acylMan), 2-acylalkyldiol-1-O-phosphoinositol acylmannoside (diolPI-acylMan) and 2-acylalkyldiol-1-O-phosphoinositol mannoside (diolPI-Man). Strain WKT50.2^T utilized a range of cellulolic substrates, alcohols and organic acids for growth, but was unable to utilize monosaccharides. Robust growth of WKT50.2^T was observed on protein derivatives. WKT50.2^T was sensitive to ampicillin, chloramphenicol, kanamycin, neomycin, polymyxin B, streptomycin and vancomycin. Metronidazole, lasalocid A and trimethoprim stimulated growth. Phylogenetic analysis of 16S rRNA gene sequences showed that WKT50.2^T belonged to the class *Thermomicrobia* within the phylum *Chloroflexi*, and was most closely related to *Thermorudis peleae* KI4^T (99.6% similarity). DNA–DNA hybridization between WKT50.2^T and *Thermorudis peleae* DSM 27169^T was 18.0%. Physiological and biochemical tests confirmed the phenotypic and genotypic differentiation of strain WKT50.2^T from *Thermorudis peleae* KI4^T and other members of the *Thermomicrobia*. On the basis of its phylogenetic position and phenotypic characteristics, we propose that strain WKT50.2^T represents a novel species, for which the name *Thermorudis pharmacophila* sp. nov. is proposed, with the type strain WKT50.2^T (=DSM 26011^T=ICMP 20042^T). Emended descriptions of *Thermomicrobium roseum*, *Thermomicrobium carboxidum*, *Thermorudis peleae* and *Sphaerobacter thermophilus* are also proposed, and include the description of a novel respiratory quinone, MK-8 2,3-epoxide (23%), in *Thermomicrobium roseum*.

**Abbreviations**: diolPI, 2-acylalkyldiol-1-O-phosphoinositol; diolP-acylMan, 2-acylalkyldiol-1-O-phosphoacylmannoside; diolPI-acylMan, 2-acylalkyldiol-1-O-phosphoinositol acylmannoside; diolPI-Man, 2-acylalkyldiol-1-O-phosphoinositol mannoside.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WKT50.2^T is HE794997.

Supplementary methods, six supplementary tables and six supplementary figures are available with the online Supplementary Material.
Members of the class *Thermomicrobia* are broadly distributed across a wide range of both aquatic and terrestrial habitats including geothermally heated soils, sediments and hot springs (Costa et al., 2009; Engel et al., 2013), mesophilic soils (Joynt et al., 2006; Lesaulnier et al., 2008) and rock (de la Torre et al., 2003). In addition, they have been detected in compost (Partanen et al., 2010; Gladden et al., 2011) and sewage sludge (Demharter et al., 1989) and on human skin (Grice et al., 2009). The class *Thermomicrobia* comprises five characterized isolates assigned to species with validly published names: *Thermomicrobiunm roseum* (Jackson et al., 1973), isolated from a hot spring, *Sphaerobacter thermophilus* (Hugenholtz & Stackebrandt, 2004), isolated from sewage sludge, *Nitrolancea hollandica* (Sorokin et al., 2014), isolated from a nitrifying bioreactor, and *Thermorudis peleae* and *Thermomicrobiunm carboxidum* (King & King, 2014b), isolated from a volcanic soil biofilm. Although the genome sequences of the type strains of both *Thermomicrobiunm carboxidum* and *S. thermophilus* are available (Wu et al., 2009; Pati et al., 2010), they have only been partially characterized. It has further been suggested that ‘*Candidatus Thermobaculum terrenum’* (Botero et al., 2004) may also be incorporated into the *Thermomicrobia*, although its phylogenetic position is uncertain and remains contentious (Kunisawa, 2011; Gupta et al., 2013). All members of the *Thermomicrobia* are neutrophilic, either thermotolerant or thermophilic, and have a broad chemo-organotrophic substrate specificity.

Here we describe the phenotypic and phylogenetic characteristics of a novel strain of the *Thermomicrobia*, WKT50.2T, that was isolated from geothermally heated soil in New Zealand. We propose that it represents a novel species within the genus *Thermorudis*. In addition, we performed supplementary characterization of the type strains of *Thermomicrobiunm roseum* (Jackson et al., 1973), *Thermomicrobiunm carboxidum* and *Thermorudis peleae* (King & King, 2014b) and *S. thermophilus* (Demharter et al., 1989) and propose the emendation of their formal descriptions.

Strain WKT50.2T was isolated from geothermally heated soil at Waikite, New Zealand. Soil gas fluxes at Waikite are low and are composed principally of CO₂ (>95 mol%) with minor concentrations of NH₃ and CH₄ (Giggenbach et al., 1994). The soil was sampled from a pink-coloured soil profile directly above the hot spring. The soil sample had a pH of 4.5 (25 °C) and a temperature *in situ* of 64.8 °C. Soil crumbs were spread on AOM1 plates (Stott et al., 2008) and incubated at 60 °C in an aerobic atmosphere. Individual bacterial colonies were picked and purified with the streak-plate method until an axenic culture was obtained (Stott et al., 2008). Unless otherwise stated, all physiological and metabolic characteristics were determined by growing WKT50.2T at 65 °C in liquid CPS medium (see the online Supplementary Material), and all materials and methodologies for phenotypic characterization are listed in the Supplementary Material.

Genomic DNA was isolated from strain WKT50.2T using a NucleoSpin Tissue kit (Macherey Nagel) according to the manufacturer’s instructions. The 16S rRNA gene was amplified using the universal bacterial primers 9F and 1492R (Weisburg et al., 1991). The near-complete 16S rRNA gene sequence was 1357 bp, and was checked manually for quality. Strains closely related to WKT50.2T were determined by subjecting the 16S rRNA gene sequence to BLASTN discontinuous megablast search (Altschul et al., 1997). The 16S rRNA gene sequences from WKT50.2T and closely related strains and phylotypes were aligned (all retrieved sequences were >1212 bp), and phylogenetic distances were calculated using the Jukes–Cantor correction within the ARB software environment SILVA SSU NR (non-redundant) Release 119 database (Jukes & Cantor, 1969; Ludwig et al., 2004). WKT50.2T was most closely related to *Thermorudis peleae* KI4T (99.6% similarity), *Thermomicrobiunm carboxidum* KI3T (91.6%) and *Thermomicrobiunm roseum* DSM 5159T (91.2%). 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 2 000 000 cycles, four chains (temperature parameter of 0.5) and a sampling frequency of 500. The phylogenetic placement of strain WKT50.2T shows that it groups within the class *Thermomicrobia* and is a member of the genus *Thermorudis* (Fig. 1).

Strain WKT50.2T formed pale-pink, convex and entire colonies, 2–3 mm in diameter after 2 days of incubation on CPS medium at 70 °C. Gram staining was negative. No cell lysis was observed when cells were treated with a 3% (v/v) solution of KOH (Halebain et al., 1981). Neither motility nor spore formation were observed under any growth conditions. Cells of strain WKT50.2T had a characteristic dumb-bell or short-rod morphology (Fig. 2). As the cultures aged, pairs or chains of cells were frequently observed. Cells were 2.1–2.8 μm long and 0.9–1.1 μm wide (Fig. 2a). Cryo-transmission electron microscopy of cells of strain WKT50.2T showed a classical Gram-negative structure of a cytoplasmic membrane, a peptidoglycan layer, outer membrane and putative thin proteinaceous and/or polysaccharide-rich S-layer (Fig. 2b). The widths of the two cited lipid membranes were approximately 6 nm (data not shown), which is consistent with a typical membrane thickness of 4–7 nm. The peptidoglycan composition (Schumann, 2011) and cell-wall sugars (Staneck & Roberts, 1974) of WKT50.2T were analysed by the Identification Service of the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The analysis found substantial amounts of protein present in the cell membrane, which could not be fully removed from the cell hydrolysate and therefore prevented full analysis of the peptidoglycan content. No diaminopimelic acid was detected in the cell wall of strain WKT50.2T, but ornithine, l-alanine and d-glutamic acid were identified and are normally associated with Gram-positive-type cell membranes (Schleifer & Kandler,
1972). The same amino acids were detected in the cell wall of *N. hollandica* (Sorokin et al., 2014). The cell envelopes of *Thermomicrobium roseum* and *S. thermophilus* were also composed largely of protein, and only minor amounts of peptidoglycan could be detected (Merkel et al., 1980; Demharter et al., 1989). The cell-wall sugars of WKT50.2T were determined by TLC and included xylose, mannose, glucose, galactose, rhamnose and ribose. These sugars are not reported routinely in bacterial cell walls but, with the exception of ribose, they reflect the composition of the large polysaccharide complex bound to the peptidoglycan observed in the monoderm *Chloroflexus aurantiacus* (Jürgens et al., 1987; Meissner et al., 1988). These data suggest that the cell envelope of WKT50.2T (Fig. 2b) includes substantial polysaccharide and protein components.

Fatty acid methyl esters were prepared and analysed as described previously (Lagutin et al., 2015). The fatty acid composition of WKT50.2T was presented in Table S1, available in the online Supplementary Material. The cell membrane of WKT50.2T was composed primarily of the fatty acids 12-methyl C<sub>18:0</sub> (54.0%) and C<sub>18:0</sub> (30.6%). Polar lipids were analysed using TLC and phospholipids were quantified by <sup>31</sup>P-NMR (MacKenzie et al., 2009) (Supplementary Material). TLC analysis of polar lipids indicated seven glycolipids and four unknown phospholipids (Fig. S1). Phospholipids comprised 25.2% of the total lipids and included four unusual 1,2-diol-linked lipids (Lagutin et al., 2015), 2-acylalkyldiol-1-O-phosphoinositol (diolPI) (64.0 mol%), 2-acylalkyldiol-1-O-phospho-acylmannoside (diolP-acylMan) (17.9 mol%), 2-acylalkyldiol-1-O-phosphoinositol acylmannoside (diolPI-acylMan) (11.5 mol%) and 2-acylalkyldiol-1-O-phospho-acylmannoside (diolP-Man) (6.7 mol%) (Table S2 and Figs S2 and S3). Diol-linked lipids were previously reported in *Thermomicrobium roseum* (Pond et al., 1986) and *N. hollandica* (Sorokin et al., 2012), but were not fully characterized.

The DNA base composition and DNA–DNA hybridization were determined by the Identification Service of the DSMZ. The G + C base content was determined by disrupting cells via a French pressure cell and purifying the DNA extract using a hydroxyapatite column (Cashion et al., 1977). The DNA was hydrolysed with P1 nuclease and dephosphorylated with bovine alkaline phosphatase and
then quantified using HPLC (Mesbah 

et al., 1989). The DNA G + C content was 58.4 mol%. DNA–DNA hybridization between WKT50.2 T and Thermorudis peleae DSM 27169 T was performed as described previously (Ziemke et al., 1998). Strain WKT50.2 T and Thermorudis peleae DSM 27169 T exhibited a mean DNA–DNA relatedness of 18.0%, which is below the threshold value of 70% recommended for bacterial species delineation (Wayne et al., 1987). The extraction and determination of respiratory quinones was undertaken using a modified LC-MS method (Supplementary Material) described by Nishijima et al. (1997), and the quinone profile was determined to contain MK-8 (95.3%) and MK-9 (4.7%).

Strain WKT50.2 T was unable to grow anaerobically using sulphur, sulphate or nitrate as terminal electron acceptors (Supplementary Material). It grew at 53–76 °C (optimum 68–73 °C), and the pH range for growth was pH 5.9–8.2 (optimum pH 6.8–7.0). No growth was observed at or below pH 5.5 or at or above pH 8.9. Growth was observed with 0–1% (w/v) NaCl, but optimum growth occurred in the absence of NaCl. All substrate utilization experiments were conducted in triplicate, with shaking incubation at 125 r.p.m. at 68 °C, using Castenholz salts solution (Supplementary Material) with 50% (v/v) air headspace. Table S3 contains a list of substrates tested (0.2% w/v unless otherwise stated). WKT50.2 T grew chemo-organotrophically using some di- and trisaccharide substrates, but no growth was observed when using monosaccharides or sugar derivatives as sole energy sources. WKT50.2 T was able to hydrolyse soluble starch (Sigma-Aldrich), crystalline cellulose (Avicel; Sigma-Aldrich) and sodium CM-cellulose (Hercules). Tested organic acids, with the exception of sodium citrate, were used as sole carbon sources. Growth was also observed in media containing peptone (Oxoid), Bacto tryptone (BD), Casamino acids (Difco) or BBL yeast extract (BD), as well as in standard complex media such as R2A and nutrient broth. Ethanol and methanol were utilized as sole carbon sources, but not 1-propanol or 2-propanol (all 0.05%, v/v). Oxidation of carbon monoxide was tested as described previously (Hardy & King, 2001), but was not observed. No homologues of the gene of the large-subunit of carbon monoxide dehydrogenase (coxL) were found using PCR primers (Table S4) based on the gene sequences from Thermomicrobium roseum and S. thermophilus, or through analysis of the draft genome sequence of WKT50.2 T. The preferred nitrogen source was determined by providing different nitrogen sources in otherwise nitrogen-free Castenholz salts solution with sucrose as a carbon and energy source (Supplementary Material). Nitrogen sources tested were N2, KNO3, KNO2, (NH4)2SO4, yeast extract, urea, alanine and serine. Strain WKT50.2 T was able to use nitrate, ammonia and alanine as sole nitrogen sources.

![Fig. 2. (a) Negative-stain transmission electron micrograph of cells of strain WKT50.2 T. Bar, 500 nm. (b) Cryo-transmission electron micrograph of cells of strain WKT50.2 T. The insert shows an enlarged view of the cell-envelope structure. CM, Cytoplasmic membrane; OM *, outer membrane; P *, peptidoglycan; Sr *, S-layer; C, Formvar-carbon support mesh. Bar, 200 nm. Asterisks (*) denote that, while the cryo-transmission electron micrograph shows a classical Gram-negative/diderm structure, chemotaxonomic and genomic data presented in the text suggest that WKT50.2 T has an atypical Gram-positive monoderm-type cell envelope.](image-url)
Antibiotic sensitivity was assessed by growing strain WKT50.2\textsuperscript{T} on Castenholz salts solution with 5 g peptone l\textsuperscript{-1} and 2.5 g sucrose l\textsuperscript{-1}, with either 3 or 30 \textmu g antibiotic ml\textsuperscript{-1}. No growth was observed with the addition of ampicillin, which possibly supports a peptidoglycan assembly structure in the cell, chloramphenicol, kanamycin, neomycin, polymyxin B, streptomycin or vancomycin at 3 \textmu g ml\textsuperscript{-1}. The addition of 3 \textmu g lasalocid A ml\textsuperscript{-1} or 3 or 30 \textmu g metronidazole or trimethoprim ml\textsuperscript{-1} resulted in a higher OD\textsubscript{600} as well as a faster growth rate than the control with no antibiotic added (Table S5). Further experiments showed a dosage-dependent increase in growth of WKT50.2\textsuperscript{T} following the addition of up to 150 \textmu g metronidazole or trimethoprim ml\textsuperscript{-1} (data not shown). None of metronidazole, lasalocid A and trimethoprim was able to support growth as a sole carbon or nitrogen source.

Strain WKT50.2\textsuperscript{T} demonstrated negative reactions for both catalase and oxidase tests. Other enzyme activities were assessed using the API ZYM kit (bioMérieux), prepared according to the manufacturer’s instructions. Esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase and trypsin activities were observed (Table S6).

In addition to the characterization of WKT50.2\textsuperscript{T}, we undertook further chemotaxonomic or phenotypic analysis of the type strains of Thermomicrobium roseum (DSM 5159\textsuperscript{T}), Thermorudis peleae (DSM 27169\textsuperscript{T}), Thermomicrobium carboxidum (DSM 27067\textsuperscript{T}) and S. thermophilus (DSM 20745\textsuperscript{T}). The methods used for this characterization are described in full in the Supplementary Material but, where possible, reflect the methodologies used for the characterization of WKT50.2\textsuperscript{T}. The temperature and pH ranges and optima for growth, substrate utilization (Table S3), preferred nitrogen sources and salinity tolerance were determined for Thermomicrobium roseum DSM 5159\textsuperscript{T} and S. thermophilus DSM 20745\textsuperscript{T}. The growth responses to metronidazole, lasalocid A and trimethoprim were also determined (Table S5). Full results are found in the emended species descriptions for these species. The respiratory quinone content of Thermomicrobium roseum DSM 5159\textsuperscript{T} was determined by LC-MS (Supplementary Material) and was composed primarily of MK-8 (74\%) along with low levels of MK-9 (3\%). A third peak (23\%) observed in the LC-MS chromatograph (Fig. S4) corresponded to a novel quinone. We analysed this unknown peak (Supplementary Material) and determined that it is an MK-8 epoxide with the epoxy structure at the 2,3 position (see Supplementary Material and Figs S5 and S6). \textsuperscript{13}C-NMR analysis of the lipid extract from S. thermophilus DSM 20745\textsuperscript{T} identified the same four phospholipids as found in WKT50.2\textsuperscript{T}, diolPI (41.3 mol\%), diolP-acylMan (17.0 mol\%), diolP-acylMan (8.4 mol\%) and diolP-Man (33.3 mol\%), making up 53.5\% of the total lipid extract (Lagutin \textit{et al.}, 2015). The fatty acid methyl ester content of S. thermophilus DSM 20745\textsuperscript{T} was dominated by 12-methyl C\textsubscript{18:1} (63.2\%) and C\textsubscript{18:0} (17.1\%) and resembles the fatty acid profile of WKT50.2\textsuperscript{T} (54.0 and 30.6\%, respectively). The unusual fatty acid 12-methyl C\textsubscript{18:0} was previously determined to be the major fatty acid in Thermomicrobium roseum (68\%) (Pond \textit{et al.}, 1986) and \textit{N. hollandica} (Sorokin \textit{et al.}, 2012), and was found at low levels (1.6–7.6\%) in Thermogemmatispora strain T81 (Vyssotski \textit{et al.}, 2012). Outside the phylum Chloroflexi, 12-methyl C\textsubscript{18:0} fatty acid has only been detected in low concentrations (<5\%) in Rubrobacter xylanophilus (Carreto \textit{et al.}, 1996) and two strains of Rubrobacter taiwanensis (Chen \textit{et al.}, 2004). This fatty acid has not been found in the closely related species Rubrobacter radiotolerans (Suzuki \textit{et al.}, 1988), and thus could be considered as a useful taxonomic marker for the class Thermomicrobia.

Despite the classical diderm-type cell envelope observed in cryo-transmission electron microscope imaging of WKT50.2\textsuperscript{T} (Fig. 2b) and a negative Gram stain reaction, a number of chemotaxonomic observations reported here were more consistent with the characteristic monoderm envelope structure normally associated with the phylum Chloroflexi. The lack of detectible concentrations of diaminopimelic acid and the presence of ornithine, L-alanine and D-glutamic acid in the cell-wall lysose, a negative KOH reaction and susceptibility to vancomycin and ampicillin are all characteristic of Gram-positive, monoderm-type cell-envelope structures. Indeed, previous cell-envelope classifications of strains of the Chloroflexi have been reported inconsistently because of variable Gram-staining results for Chloroflexus aurantiacus and species of the genera Herpetosiphon and Roseflexus, despite the prevailing monoderm consensus in the Chloroflexi (Sutcliffe, 2010; references therein). Recent publications reviewing the cell envelopes of members of the Chloroflexi have argued that all genome sequences of members of the Chloroflexi (including Thermomicrobium roseum, ‘Candidatus Thermobaculum terrenum’ and \textit{S. thermophilus}) lack crucial genes required for lipopolysaccharide production and transport, and transporters specific to cell walls (Sutcliffe, 2010, 2011). We have reviewed the draft genome of WKT50.2\textsuperscript{T} (not shown) and were also unable to find evidence of crucial gene homologues required for the function and synthesis of diderm-type cells (Supplementary Material), nor secretory systems linked with the outer membrane. Based upon the lack of genomic evidence for a diderm-type cell envelope and chemotaxonomic observations consistent with monomers, we conclude that WKT50.2\textsuperscript{T} has an atypical monoderm cell-envelope rich in proteinaceous and polysaccharide materials. Previous reports cite multilayered outer-membrane structures for Thermogemmatispora onikobensis and Thermogemmatispora foliorum (Yabe \textit{et al.}, 2011), \textit{C. aurantiacus} (Hanada & Pearson, 2006) and other members of the phylum Chloroflexi (Jürgens \textit{et al.}, 1987), although the composition and purpose of these structures have yet to be investigated. We therefore caution that a focussed investigation of cell envelopes of isolates assigned to the Chloroflexi that includes electron microscopy, chemotaxonomic and genomic analyses is warranted and should be undertaken in the future to provide clarity in cell-envelope classification across this phylum.
Strain WKT50.2T could be differentiated from other members of the Thermomicrobia on the basis of both phenotypic and chemotaxonomic features, including differences in temperature and pH range, substrate utilization, oxidase and catalase reactions, Gram-staining and G+C content of the genomic DNA (Table 1). It was placed phylogenetically within the class Thermomicrobia and had the greatest 16S rRNA gene sequence similarity to the type strains of Thermorudis peleae (99.6%), Thermomicrobium carboxidum (91.6%) and Thermomicrobium roseum (91.2%). DNA–DNA hybridization between Thermorudis peleae DSM 27169T and WKT50.2T was less than the species-level threshold of 70% DNA–DNA relatedness (Wayne et al., 1987), confirming that WKT50.2T is a member of a novel species. In addition, WKT50.2T was able to utilize several substrates that Thermorudis peleae DSM 27169T could not, including sucrose, glycerol, ethanol, methanol and organic acids such as acetate, pyruvate and succinate. WKT50.2T was unable to utilize any monosaccharides tested (Table 1). Growth of WKT50.2T was promoted by cultivation in the presence of lasalocid A, metronidazole and trimethoprim, whereas Thermorudis peleae DSM 27169T and Thermomicrobium carboxidum DSM 27067T exhibited growth using all monosaccharides tested (Table 1). Growth of WKT50.2T was promoted by cultivation in the presence of lasalocid A, metronidazole and trimethoprim, whereas Thermorudis peleae DSM 27169T and Thermomicrobium carboxidum DSM 27067T exhibited only resistance to metronidazole and trimethoprim and not promoted growth. In contrast to Thermomicrobium roseum DSM 27067T, S. thermophilus DSM 27169T and Thermomicrobium Table 1. Differential characteristics of WKT50.2T and selected strains of the class Thermomicrobia

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<td>Xanthan</td>
<td>–</td>
<td>–</td>
<td>NR</td>
<td>+</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Antibiotic resistance‡</td>
<td>las§, met§, tri§</td>
<td>met*, tri*</td>
<td>met*, tri*</td>
<td>chl, met§*</td>
<td>str, tri§</td>
<td>met§*</td>
</tr>
<tr>
<td>Primary fatty acid(s)†</td>
<td>12-Me C18 : 0</td>
<td>C18 : 0</td>
<td>12-Me C18 : 0</td>
<td>12-Me C18 : 0</td>
<td>12-Me C18 : 0</td>
<td>12-Me C18 : 0</td>
</tr>
<tr>
<td>Primary quinone(s)§</td>
<td>MK-8, MK-9</td>
<td>NR</td>
<td>NR</td>
<td>MK-8, 2,3-epoxide*</td>
<td>MK-8</td>
<td>MK-8</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>58.4</td>
<td>60.7</td>
<td>66.0</td>
<td>64.3</td>
<td>66.0</td>
<td>62.6</td>
</tr>
</tbody>
</table>

*Data generated in this study.
†Result confirmed in this study.
‡chl, Chloramphenicol; las, lasalocid A; met, metronidazole; str, streptomycin; tri, trimethoprim.
§Stimulates growth.
| Me, Methyl. |
carboxidum DSM 27067^T, WKT50.2^T was unable to use carbon monoxide as an energy source or oxidize it constitutively. It is interesting to note that this inability is also shared with Thermorudis peleae (King & King, 2014b), and it is therefore tempting to infer that this observation could be used as a diagnostic tool to differentiate species of the genus Thermorudis from those of the genus Thermomicrobium. However, we believe that this observation is probably coincidental, particularly as the ability of other members of the Chloroflexi such as species of the genus Thermogemmatispora (King & King, 2014a) to oxidize carbon monoxide appears to be independent of 16S rRNA gene phylogeny. These findings indicate that strain WKT50.2^T is phylogenetically and physiologically distinct from other species within the class Thermomicrobia and we therefore propose that it represents a novel species within the genus Thermorudis, Thermorudis pharmacophila sp. nov. The descriptions of the species Thermomicrobium roseum and Sphaerobacter thermophilus are emended on the basis of new fatty acid, polar lipid, antibiotic resistance, quinone and substrate utilization data. Emended descriptions of Thermomicrobium carboxidum and Thermorudis peleae are also provided.

**Description of Thermorudis pharmacophila sp. nov.**

*Thermorudis pharmacophila* [phar.ma.co’phi.la. Gr. neut. n. pharmakon poison; N.L. adj. philus -a -um (from Gr. adj. philos -é -on) loving; N.L. fem. adj. pharmacophila poison-loving].

Has the following characteristics in addition to those given in the genus description by King & King (2014b). Cells are rods, approx. 0.9–1.1 μm in diameter and 2.1–2.8 μm long. Exhibits an oxidative, heterotrophic metabolism. No growth is observed on monosaccharides. Sucrose, trehalose and raffinose act as sole carbon sources; lactose, maltose and cellobiose do not. Avicel, CM-cellulose, starch and xylan are hydrolysed, but chitin, dextrin, gellan gum, galactomannan, glucomannan, pectin and xanthan are not. Cells also grow on Whatman filter paper, glycerol, sorbitol and 0.05% (v/v) methanol or ethanol. Sodium acetate, formate, fumarate, lactate, pyruvate and succinate act as sole carbon sources; sodium citrate, mannitol, galacturonic acid and 0.05% 1-propanol and 2-propanol do not. Cells grow readily on yeast extract, peptone, tryptone and Casamino acids. Growth is inhibited by ampicillin, chloramphenicol, kanamycin, neomycin, polymyxin B, streptomycin and vancomycin. Lasalocid A, metronidazole and trimethoprim stimulate growth. Ammonium ions, nitrate ions and yeast extract can act as sole nitrogen sources. Optimal growth at 68–73 °C (range 53–76 °C) and pH 6.8–7.0 (range pH 5.9–8.2). NaCl tolerance up to 1% (w/v). Growth on solid gellan-based medium produces pink, opaque colonies with circular and entire edges. The principal quinone detected is MK-8, with minor concentrations of MK-9. The major fatty acids are 12-methyl C_{18:0} and C_{18:0} with minor amounts of C_{16:0}, C_{16:1}, C_{18:1}, C_{18:0} and C_{14:0}. Polar lipids are diol-linked and include diolPI, diolP-acylMan, diolPI-acylMan and diolPI-Man.

The type strain, WKT50.2^T (=DSM 26011^T=ICMP 20042^T), was isolated from geothermally heated soils at Waikite, New Zealand. The DNA G+C content of the type strain is 58.4 mol%.

**Emended description of Thermomicrobium roseum Jackson et al. 1973**

The description is as given by Jackson et al. (1973) with the following amendments.

Growth occurs on simple sugars: D-xylose, D-galactose, D-mannose, D-fructose, cellobiose, maltose, sucrose, trehalose and raffinose. Dextrin, Phyt agile, starch, xylan and xanthan act as sole carbon sources, but Avicel and CM-cellulose do not. Grows on short-chain alcohols including methanol, ethanol, 1-propanol, 2-propanol and mannitol, but not sorbitol. Cells also grow on glycerol and sodium acetate, formate, fumarate, lactate, pyruvate and succinate but not citrate. Peptone, tryptone and Casamino acids act as sole carbon sources. Ammonium ions, nitrate ions and yeast extract can act as sole nitrogen sources. Growth is stimulated by metronidazole and trimethoprim. The predominant quinones are MK-8, MK-8 2,3-epoxide and MK-9, and the major fatty acid is 12-methyl C_{18:0}. Fatty acids are linked to diols. Optimal growth at 65–70 °C (range 52–77 °C) and pH 8.0–8.4 (range pH 6.0–9.4). NaCl tolerance up to 1.5% (w/v). Cells possess cell envelopes composed largely of protein, with only small amounts of peptidoglycan, and ornithine is present.

**Emended description of Sphaerobacter thermophilus Demharter et al. 1989**

The description is as given by Demharter et al. (1989) with the following amendments.

Growth occurs on D-fructose and cellobiose but not D-xylose, D-galactose, D-mannose, D-fructose, cellobiose, maltose, sucrose, trehalose and raffinose. Avicel, CM-cellulose, Phyt agile, starch and xylan act as sole carbon sources but dextrin and xanthan do not grow. Grows on some short-chain alcohols including ethanol, 1-propanol, 2-propanol and mannitol but not sorbitol or methanol. Cells also grow on glycerol and sodium acetate, citrate, formate, fumarate, lactate, pyruvate and succinate. Peptone, tryptone, Casamino acids and nutrient broth act as sole carbon sources but yeast extract does not. Ammonium ions, nitrate ions and yeast extract can act as sole nitrogen sources. Growth is inhibited by chloramphenicol, neomycin, kanamycin and trimethoprim, but growth is stimulated by metronidazole. Optimal growth at 55–60 °C (range 49–67 °C) and pH 8.0–8.4 (range pH 6.1–9.0). NaCl tolerance up to 1% (w/v). The major fatty acids are 12-methyl C_{18:0} and C_{18:0} with minor amounts of C_{16:0}, C_{16:1}, C_{18:0} and C_{18:1} n9. Polar lipids comprise diolPI, diolP-acylMan, diolPI-acylMan and diolPI-Man.
Emended description of *Thermomicrobium carboxidum* King and King 2014

The description is as given by King & King (2014b) with the following amendments.

Growth is inhibited by chloramphenicol, neomycin, streptomycin and kanamycin; resistant to metronidazole and trimethoprim.

Emended description of *Thermorudis peleae* King and King 2014

The description is as given by King & King (2014b) with the following amendments.

Growth is inhibited by chloramphenicol, neomycin, streptomycin and kanamycin; resistant to metronidazole and trimethoprim. Xanthan, dextrin and CM-cellulose do not support growth.

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