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Caldivirga maquilingensis gen. nov., sp. nov., a new genus of rod-shaped crenarchaeote isolated from a hot spring in the Philippines

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Two novel hyperthermophilic, rod-shaped crenarchaeotes were isolated from an acidic hot spring in the Philippines. Cells were mostly straight or slightly curved rods 0·4–0·7 μm in width. Bent cells, branched cells, and cells bearing globular bodies were commonly observed. The isolates were heterotrophs and grew anaerobically and microaerobically. The addition of archaeal cell extract or a vitamin mixture to the medium significantly stimulated growth. The isolates grew over a temperature range of 60–92 °C, and optimally around 85 °C and grew over a pH range of 2·3–6·4, and optimally at pH 3·7–4·2. The isolates utilized glycogen, gelatin, beef extract, peptone, tryptone and yeast extract as carbon sources. They required sulfur, thiosulfate or sulfate as electron acceptors. The lipids mainly consisted of various cyclized glycerol-bisdiphytanyl-glycerol tetraethers. The G+C content of the genomic DNAs was 43 mol%. The 16S rDNA contained two small introns. The comparison of the 16S rDNA exon sequences revealed that they represented an independent lineage in the family *Thermoproteaceae*. The two strains were included in a single species because of high levels of DNA–DNA relatedness. From these results, *Caldivirga maquilingensis* gen. nov., sp. nov. is proposed in the family *Thermoproteaceae* to accommodate these isolates. The type strain of *C. maquilingensis* is strain IC-167^T (= JCM 10307^T = MCC-UPLB 1200^T = ANMR 0178^T).

Keywords: *Caldivirga maquilingensis*, *Thermoproteaceae*, hyperthermophile, crenarchaeotes

INTRODUCTION

The domain *Archaea* (archaeobacteria) is composed of two major lineages, the kingdoms *Euryarchaeota* and *Crenarchaeota* (Woese *et al.*, 1990). The kingdom *Euryarchaeota* comprises phenotypically diverse groups of micro-organisms such as methanogens, extreme halophiles and thermophiles; whereas all the species of the kingdom *Crenarchaeota* isolated to date are extreme thermophiles or hyperthermophiles. Since the first crenarchaeote *Sulfolobus acidocaldarius* was reported in 1972 (Brock *et al.*, 1972), many new crenarchaeotes have been isolated from terrestrial and submarine geothermal habitats (reviewed by Stetter, 1996). Within the kingdom *Crenarchaeota*, the order *Thermoproteales* is represented by two families of rod-shaped strains, *Thermoproteaceae* and *Thermofilaceae* (Burggraf *et al.*, 1997). At present, three genera, *Thermoproteus* (Zillig *et al.*, 1981), *Pyrobaculum* (Huber *et al.*, 1987) and *Thermocladium* (Itoh *et al.*, 1998a) are known in the family *Thermoproteaceae*. The three genera can be differentiated, for example, by their optimal growth temperature or DNA base composition. Nevertheless, all these genera form rod-shaped cells of more than 0·4 μm thickness. In contrast, members of the genus *Thermofilum* (Zillig *et al.*, 1983), the sole genus of the family *Thermofilaceae*, form thinner cells (0·15–0·35 μm thickness). These rod-shaped crenarchaeotes are thought to be widely distributed in terrestrial hot springs and submarine hydrothermal systems. In this paper, we report the isolation and the characterization of novel rod-shaped crenarchaeotes from a hot spring in the Philippines.

The DDBJ accession number for the 16S rDNA sequence of *Caldivirga maquilingensis* IC-167^T is AB013926.

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METHODS

Isolation procedure. Hot spring water, solfataric soils and muds were collected from a hot spring called 'Mud Spring' located on the mountainside of Mt Maquiling, Laguna, the Philippines. The samples were transported in sterile plastic tubes without temperature control. The composition of enrichment medium was the same as described previously (Itoh *et al.*, 1998a). The medium was reduced with $Na_2S.9H_2O$ (final concn 0·5 g l^{-1}) and the pH was adjusted to 5·0, at room temperature, under a N_2 atmosphere. Inoculated cultures were incubated at 85 °C for 1 week.

Culture conditions. The isolates were routinely cultivated at 85 °C in modified TCD medium (Itoh *et al.*, 1998a), which consisted of 1·0 l salt base of the *Sulfolobus* medium (Brock *et al.*, 1972), 0·5 g yeast extract (Difco), 10 ml vitamin mixture (Balch *et al.*, 1979), 1·0 mg resazurin and 10·0 g sulfur powder. The basal salt medium and yeast extract [a 10·0% (w/v) solution] were separately autoclaved at 121 °C for 15 min, and sulfur was autoclaved at 105 °C for 1 h. The vitamin mixture was filter-sterilized (pore size 0·2 μm, Minisart; Sartorius). After all the components, except for $Na_2S.9H_2O$, were mixed in culture vessels, the gas phase was replaced with H_2/CO_2 (4:1, v/v), and sealed with butyl rubber stoppers. The medium was reduced by the addition of $Na_2S.9H_2O$ (final concn 0·5 g l^{-1}), and the pH was adjusted to 4·0, at room temperature, with 0·5 M H_2SO_4 which was autoclaved under an N_2 atmosphere. After inoculation, the culture was pressurized to 100 kPa H_2/CO_2 (4:1, v/v).

Phenotypic characterization and nucleic acid studies. Morphology, growth characteristics, carbon sources utilized, possible electron acceptors, antibiotic sensitivity, lipid composition, DNA base composition and DNA–DNA relatedness were determined as described previously (Itoh *et al.*, 1998a). The archaeal cell extract, which was added to the basal medium in the previous study, was replaced by the vitamin mixture. Unless otherwise indicated, the isolates were grown in 5 ml test medium in a 10 ml vial (or 10 ml medium in a 20 ml vial) under a H_2/CO_2 (4:1, v/v, 100 kPa) atmosphere without shaking. To determine the effects of gas phases on growth of the isolates, serum bottles (120 ml) containing 10 ml of the culture medium were incubated without shaking. The effect of pH on growth was examined with either 10 mM trisodium citrate (pH 1·7–6·0, at room temperature) or 10 mM MES (pH 5·3–7·0, at room temperature) as a buffer. The pH values of the culture media were adjusted, after reduction, with 0·5 M H_2SO_4 at room temperature as described above. Growth rates at different temperatures or at different pH values were determined for cultures (500 ml medium in a 1·2 l bottle) grown in the modified TCD medium supplemented with 10 mM trisodium citrate, with reciprocal shaking (80 r.p.m.). Sensitivities to the following antibiotics were determined at a concentration of 100 μg ml^{-1}: ampicillin, chloramphenicol, erythromycin, kanamycin, novobiocin, oleandomycin, rifampicin, streptomycin and vancomycin. To test for degradation of the antibiotics during cultivation, paper discs (8 mm; Advantech) impregnated with the cultures were placed on plates inoculated with 1·0% (v/v) *Bacillus sphericus* JCM 2502 or *Bacillus subtilis* JCM 2499 followed by incubation at 37 °C for 12 h.

16S rDNA analysis. Almost the whole 16S rDNAs were amplified by PCR with primers A-20F (5′-TCCGGTTGATCCTGCCG, positions 8–24 in *Escherichia coli* numbering system) and A-1530R2 (5′-GGAGGTGATCCGRCCG, positions 1540–1525). Sequencing of the DNA fragments and phylogenetic analysis were conducted as described previously (Itoh *et al.*, 1998b). The 16S rDNA sequence of strain IC-167^T has been deposited in the DNA Database of Japan (DDBJ) under the accession number AB013926.

RESULTS

Isolation

Seven samples (hot spring water, solfataric soil and mud) were taken from a hot spring called 'Mud Spring'. The *in situ* temperature ranged from 70 to 90 °C, and pH values of samples determined at ambient temperature were 2·2–5·1. After enrichment at 85 °C under an N_2 atmosphere for 1 week, all samples yielded rod-shaped cells accompanied by a lesser number of coccoid cells. On the other hand, coccoid micro-organisms predominated in the enrichment cultures at 85 °C, pH 5·0, under a H_2/CO_2 (4:1, v/v, 100 kPa) atmosphere. Applying the serial dilution method as described previously (Itoh *et al.*, 1998a), three rod-shaped strains (strains IC-162, IC-164 and IC-166) were isolated with the medium used for the enrichment, and one rod-shaped strain (strain IC-167^T) was obtained with the enrichment medium supplemented with 1% (v/v) archaeal cell extract solution (Itoh *et al.*, 1998a). Strains IC-162 and IC-164, which were isolated from mud samples, were later identified as *Thermoproteus* spp. Strains IC-166 and IC-167^T were isolated from samples of solfataric soil (70 °C, pH 5·0) and hot spring water (70 °C, pH 2·1), respectively. The purity of both strains was confirmed by microscopic observations of the cultures grown under various growth conditions (e.g. temperature range of 50–100 °C, pH range of 1·7–7·0)

Morphology

Cells of strain IC-166 and IC-167^T were mostly regular, straight or slightly curved rods of 0·4–0·7 μm in width (Fig. 1a). The length of cells was highly variable, mostly ranging from 3 to 20 μm, and occasionally up to 50 μm. Bent cells, branched cells and cells bearing globular bodies were commonly observed (Fig. 1a–c, 2a). The globular bodies, which measured 2–5 μm in diameter, were usually formed at one end of cells (Fig. 2a), and sometimes at both ends or laterally. Some globular bodies were accompanied by only short rods, as shown in Fig. 2c. Bulged rods and inflated rods with the globular bodies were rarely observed (Fig. 1d, 2b). Cells appeared to divide by constriction (Fig. 2d). Cells had pili attached terminally, laterally or peripherally (Fig. 2a, c, d). No motility was observed.

Growth characteristics

Strains IC-166 and IC-167^T grew under strictly anaerobic conditions with N_2, H_2/CO_2 (4:1, v/v, 100 kPa) or N_2/CO_2 (4:1, v/v, 100 kPa) in the gas phase. To estimate the effect of the gas phase on the growth of the isolates, growth yields were determined by measuring of the cellular protein contents after 1 week incubation, as described previously (Itoh *et al.*,

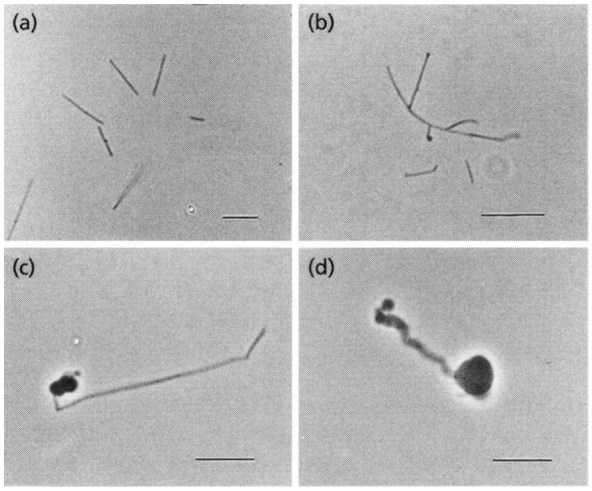

Fig. 1. Phase-contrast micrographs of strain IC-167^T. Cells were mostly rod-shaped (a), and occasionally branched (b) or possessed globular bodies (b, c). Inflated rods with globular bodies were rarely observed (d). Bars, 10 μm.

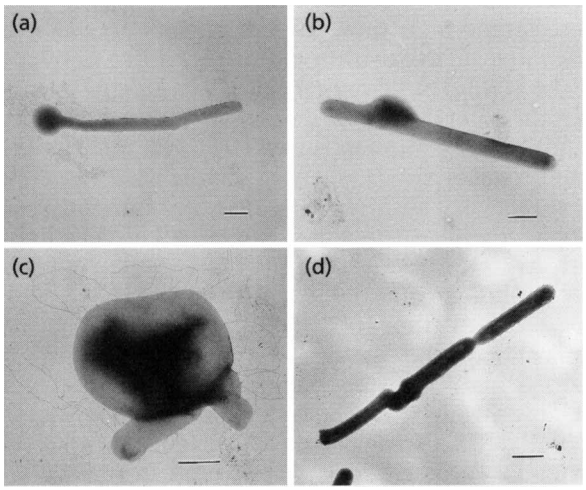

Fig. 2. Transmission electron micrographs of strains IC-166 (d) and IC-167^T (a–c). Cells formed globular bodies (a, c), and bulges (b). Cells seemed to divide by constriction (d). Pili were attached to cells. Bars, 1 μm.

1998a). The growth yields under a H_2/CO_2 (4:1, v/v, 100 kPa) or a N_2/CO_2 (4:1, v/v, 100 kPa) gas phase were 234–272 μg protein ml^{-1}, while the growth yields under a N_2 gas phase were 204–206 μg protein ml^{-1}. Thus, the presence of CO_2 in the gas phase slightly promoted growth. Sulfide was detected following growth. Under a gas phase of 5% air, growth was observed after 1 week incubation even though the medium remained oxidized (as judged by colour of resazurin). The growth yields of the microaerobic cultures were less than those of the cultures with a N_2 gas phase. No growth was observed under an air atmosphere. The isolates did not grow autotrophically, in the absence of a carbon source, under a H_2/CO_2

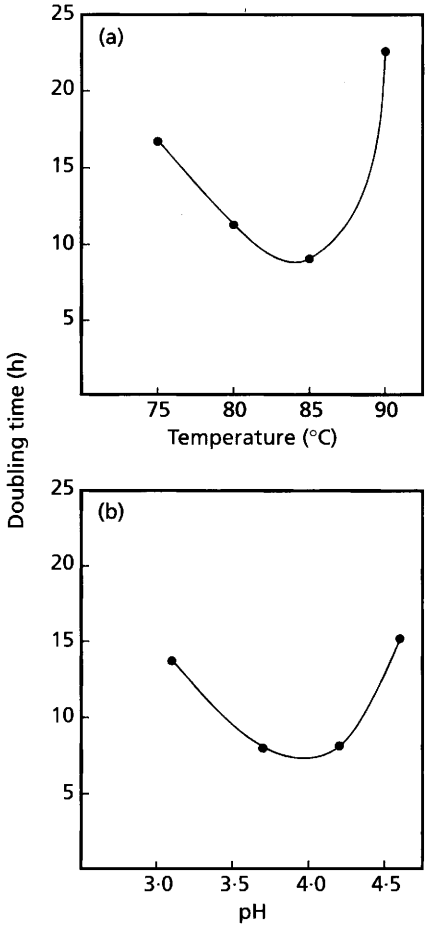

Fig. 3. Effect of temperature (a; pH 3·5) and pH (b; 85 °C) on the growth of strain IC-167^T.

(4:1, v/v, 100 kPa) or a N_2/CO_2 (4:1, v/v, 100 kPa) atmosphere.

Effects of cell extract solutions (prepared from *Sulfolobus acidocaldarius* JCM 9062, *Methanosarcina barkeri* JCM 10043^T, *Halobacterium salinarum* JCM 8978^T, *Thermoplasma acidophilum* JCM 9062^T and *Escherichia coli* JCM 1649^T) and a vitamin mixture of Balch *et al.* (1979) on the growth of isolate IC-167^T were determined as described previously (Itoh *et al.*, 1998a). Each of the cell extract solutions or the vitamin solution alone did not support growth when yeast extract was omitted. The growth yield of the culture with the vitamin mixture was 261 μg protein ml^{-1}, while those values of the cultures with the archaeal cell extracts ranged from 164 to 255 μg protein ml^{-1}. In contrast, the culture with *E. coli* cell extract and the culture without any additive yielded 89 and 65 μg protein ml^{-1}, respectively. Similar results were obtained for strain IC-166 (data not shown).

The isolates grew between 60 and 92 °C, but failed to grow at 55 or 94 °C. When strain IC-167^T was cultivated between 75 and 90 °C, it grew optimally around 85 °C (Fig. 3a). Strains IC-166 and IC-167^T

grew in pH ranges of 2·3–5·8 and 2·7–5·8, respectively, when the medium was supplemented with 10 mM trisodium citrate. Alternatively, they grew up to pH 6·4, but not at pH 6·6, with 10 mM MES as the buffer. When strain IC-167^T was cultivated at various pH values (3·1, 3·7, 4·2 and 4·6) with 10 mM trisodium citrate, it grew optimally at pH 3·7–4·2 (Fig. 3b). At the optimal condition (85 °C, pH 3·7), strain IC-167^T grew with a doubling time of 8 h, and the stationary cultures contained 230–250 μg protein ml^{-1}, and approximately $2·5 \times 10^7$ cells ml^{-1}. In the media supplemented with NaCl (0·25, 0·5, 0·75, 1·0 or 1·25%, w/v), strains IC-166 and IC-167^T grew up to 0·75 and 0·5% (w/v) NaCl, respectively.

Utilization of carbon sources

The isolates grew well in the presence of yeast extract (Difco) as a carbon source. Glycogen, gelatin (Difco), beef extract (Difco), peptone (Difco), and tryptone (Difco) supported moderate growth. No or negligible growth was obtained on D-arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-ribose, sucrose, D-xylose, soluble starch (Difco), acetate, butyrate, citrate, formate, fumarate, lactate, L-malate, propionate, pyruvate, succinate, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, methanol, ethanol, formamide, monomethylamine, trimethylamine, Casamino acids (Difco) or malt extract (Difco). By adding yeast extract as a carbon source at various concentrations (0·02, 0·05, 0·1, 0·2, 0·5 and 1·0%, w/v), both strains grew up to 0·5%, but not 1·0% yeast extract.

Possible electron acceptors

The two strains showed no or negligible growth on the basal medium without an electron acceptor. Addition of sulfur, thiosulfate and sulfate supported growth, and nitrate and $FeCl_3$ supported faint growth. No growth was obtained in the medium supplemented with sulfite, cystine, oxidized glutathione, malate or fumarate.

Antibiotic sensitivity

The two strains were insensitive to chloramphenicol, kanamycin, oleandomycin, and streptomycin. Antimicrobial activities of noboviocin and rifampicin were drastically reduced after 2 d in the medium; nevertheless, growth did not occur even after 2 weeks. No antimicrobial activity of ampicillin, erythromycin and vancomycin remained after 2 d incubation. In the presence of erythromycin, growth was initially inhibited, however, it recovered after the inactivation of erythromycin. The presence of ampicillin or vancomycin had no effect on the growth of the two isolates.

Lipid analysis

The two strains contained large amounts of tetraether core lipids and trace amounts of diether core lipids. The core lipid fractions contained various, at least 5, cyclized glycerol-bisphytanyl-glycerol tetraethers.

G+C contents of DNAs

The G+C contents of DNAs from strain IC-166 and IC-167^T were 43·3 and 43·0 mol%, respectively.

DNA–DNA hybridization

Strains IC-166 and IC-167^T showed high similarity values of DNA–DNA relatedness to each other (94–105%), while both strains showed low similarity values (less than 2%) to *Thermoproteus tenax* JCM 9277^T.

16S rDNA analysis

Almost the entire 16S rDNAs of the two strains were amplified by PCR with primers A-20F and A-1530R2. The resulting DNA fragments were approximately 1·7 kb, judging from electrophoretic mobility, which were larger than those of the normal 16S DNAs of *Thermoproteaceae* strains (approx. 1·5 kb). Sequencing of the 16S rDNA fragment of strain IC-167^T revealed that the 16S rDNA contained two additional inserts of 37 and 140 bp after positions 901 and 908 (in *E. coli* numbering system), respectively. These two inserts were regarded as introns because they had the putative intron core structures that exist in all archaeal rRNA introns so far discovered (Lykke-Andersen & Garrett, 1994: Itoh *et al.*, 1998b). For strain IC-167^T, 1465 positions of the 16S rDNA exon sequence were determined, and its G+C content was 66·4%. The exon sequences were compared with some crenarchaeote strains and *Thermococcus celer*. All the small subunit rRNA sequence signatures defining the crenarchaeotes (Woese, 1993) were found in the sequences from strain IC-167^T. To calculate the similarities between strain IC-167^T and other crenarchaeotes, 1238 positions of the each sequence were compared. The phylogenetic tree derived from the 16S rRNA/DNA sequences is shown in Fig. 4. Strain IC-167^T and members of the family *Thermoproteaceae* formed a cluster with a high bootstrap value (100%). Within this cluster, strain IC-167^T formed a separate lineage. Although the lineages of strain IC-167^T and *Thermocladium modestius* were joined together, it gave a lower confidence (bootstrap value, 71·4%). Similarity values between strain IC-167^T and the other *Thermoproteaceae* species ranged from 92·7 to 93·7%. In addition, strain IC-167^T exhibited 91·1, 89·9–90·8 and 83·5–85·9% sequence similarities with *Thermofilum pendens*, the representatives of the families *Desulfurococcaceae*/*Pyrodictiaceae* ('*Igneococcales*'), and the representatives of the order *Sulfolobales*, respectively. The partial sequence of strain IC-166 (positions 442–658, in *E. coli* numbering system) was identical with that of strain IC-167^T.

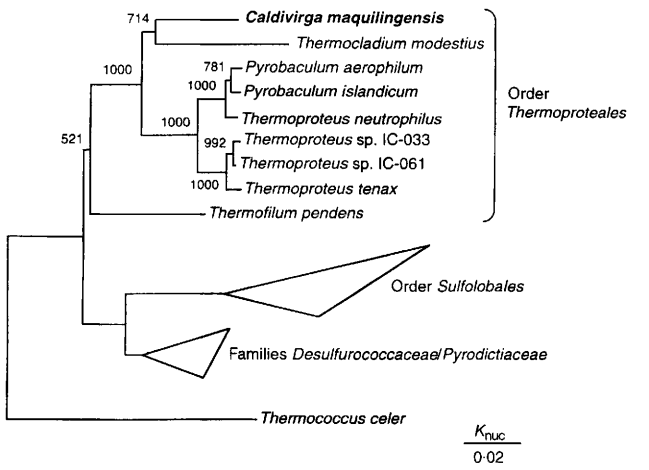

Fig. 4. Phylogenetic tree of the *Caldivirga maquilingensis* strain IC-167^T 16S rRNA gene. The tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) based on the 16S rRNA/rDNA sequences. The numbers indicate the bootstrap scores of 1000 trials. Accession numbers of nucleotide sequences cited are *Pyrobaculum aerophilum* im2^T (L07510), *Pyrobaculum islandicum* geo3^T (L07511), *Thermocladium modestius* IC-125^T (AB005296), *Thermococcus celer* DSM 2476^T (M21529), *Thermofilum pendens* DSM 2475^T (X14835), *Thermoproteus neutrophilus* JCM 9278^T (AB009618), *Thermoproteus* spp. IC-033 (AB009616) and IC-061 (AB009617), and *Thermoproteus tenax* (M35966). The clade of the order *Sulfolobales* comprises *Acidianus brierleyi* DSM 1651^T (X90477), *Acidianus infernus* DSM 3191^T (X89852), *Metallosphaera sedula* DSM 5348^T (X90481), *Sulfolobus acidocaldarius* ATCC 33909^T (D14876) and *Sulfolobus metallicus* DSM 6482^T (X90479). The clade of the families *Desulfurococcaceae* and *Pyrodictiaceae* comprise *Aeropyrum pernix* K1^T (D83259), *Desulfurococcus mobilis* (M36474), *Pyrodictium occultum* PL19^T (M21087) and *Staphylothermus marinus* F1^T (X99560).

DISCUSSION

Analyses of the 16S rDNA sequences, by means of the signature and the similarity analyses, support that the two new isolates IC-166 and IC-167^T are classified in the kingdom *Crenarchaeota*. In addition to the phylogenetic analysis, the characteristic rod-shape, presence of cyclic glycerol diphytanyl tetraether lipids, and the cell thickness (0·4–0·7 μm) support that the two isolates are related to the family *Thermoproteaceae*. To date, three genera *Pyrobaculum* (Huber *et al.*, 1987; Völkl *et al.*, 1993), *Thermocladium* (Itoh *et al.*, 1998a) and *Thermoproteus* (Zillig *et al.*, 1981; Zillig, 1989; Bonch-Osmolovskaya *et al.*, 1990) are known in this family. Therefore, our isolates are compared with these three genera.

The genus *Thermoproteus* includes *T. tenax*, *T. neutrophilus* and '*T. uzoniensis*'. All of these species are non-motile, hyperthermophiles growing optimally at 85–90 °C, and use S° as an electron acceptor. Compared with our isolates, however, the *Thermoproteus* species have significantly higher DNA G+C composition (55–60 mol%) and optimal growth pH (5·0–6·5). The *Pyrobaculum* species are the only organisms that grow at over 100 °C within the family *Thermoproteaceae*, and their optimal growth pH values (5·0–7·0) and DNA G+C compositions (about 50 mol%; Itoh *et al.*, 1998a) are clearly different from those of our isolates. Furthermore, the *Pyrobaculum* species are motile and show relatively shorter cell-length (3–8 μm) than the other *Thermoproteaceae* species including our new isolates. The two strains IC-166 and 167^T are physiologically similar to *Thermocladium modestius*. They grow better at acidic pH (around pH 4·0) and tolerate a microaerobic atmosphere. Their growth is significantly enhanced by the presence of archaeal cell extracts or vitamin mixture. Nevertheless, our isolates can be differentiated from *Thermocladium modestius* by the growth temperature: strains IC-166 and IC-167^T are hyperthermophiles growing at 60–92 °C, while *Thermocladium modestius* is an extreme thermophile growing at 45–82 °C. Moreover, *Thermocladium modestius* has a distinctly higher DNA G+C composition (52 mol%).

Phylogenetic analysis of the 16S rDNA (exon) sequences places strain IC-167^T within the family *Thermoproteaceae* with a high bootstrap confidence (100%). Strain IC-167^T positioned distantly from the *Pyrobaculum/Thermoproteus* clade, or the lineage of *Thermocladium modestius* with similarity values of 92·7–93·7%. These values are significantly low when compared with the values between different genera, e.g. *Thermoproteus tenax* and *Pyrobaculum islandicum* (96·8%), or *Desulfurococcus mobilis* and *Pyrodictium occultum* (95·4%).

On the grounds of the phenotypic and phylogenetic differences described above, the two isolates should be recognized as a new genus in the family *Thermoproteaceae*. Strains IC-166 and IC-167^T are assigned in the same species because of the phenotypic similarities and high levels of DNA–DNA relatedness. Therefore, we propose the name *Caldivirga maquilingensis* gen. nov., sp. nov. for the two isolates; the type strain of the new species is IC-167^T.

Growth of *Caldivirga maquilingensis* is significantly stimulated by the presence of archaeal cell extract or the vitamin mixture of Balch *et al.* (1979), as is *Thermocladium modestius*. Strains of *Pyrobaculum* and *Thermoproteus* grew well in the growth medium without the archaeal cell extract or the vitamin mixture, although, addition of the vitamin mixture to the cultures of *Thermoproteus tenax* and *P. islandicum* increased their growth yields slightly (Itoh *et al.*, unpublished results). So far, among the vitamin components, vitamin B_{12} has been proved to stimulate the growth of *Caldivirga maquilingensis* significantly; however, each of other components alone did not stimulate growth significantly (Itoh *et al.*, unpublished results). A study of the effect of the vitamin components on *Caldivirga maquilingensis* and other *Thermoproteaceae* species is still in progress. Although *Thermofilum pendens* is known to require the cell extract of *Thermoproteus*, this preparation cannot be replaced by that of *Thermoplasma* or a vitamin mixture

(Zillig *et al.*, 1983). Therefore, the actual element contributing to the growth enhancement of *Thermofilum pendens* is possibly different from those of *Caldivirga maquilingensis*. Interestingly, another *Thermofilum* strain ('*Thermofilum librum*') does not require the cell extract of *Thermoproteus tenax* (Stetter, 1986). Therefore, such a requirement may depend on the species.

Two introns were located in the 16S rDNAs of the two strains of *Caldivirga maquilingensis*. The rRNA introns are found, to date, only in the crenarchaeotic families *Thermoproteaceae* and *Desulfurococcaceae*, i.e. 23S rRNA introns for *Aeropyrum pernix* (Nomura *et al.*, 1998), *Desulfurococcus mobilis* (Kjems & Garrett, 1985), *P. organotrophum* (Dalgaard & Garrett, 1992), *Staphylothermus marinus* (Kjems & Garrett, 1991) and 16S rRNA introns for *Aeropyrum pernix* (Nomura *et al.*, 1998), *P. aerophilum* (Burggraf *et al.*, 1993), *Thermoproteus neutrophilus* (Itoh *et al.*, 1998b) and *Thermoproteus* spp. (Itoh *et al.*, 1998b). The rRNA introns distribute idiosyncratically among the same genera suggesting that the rRNA introns are mobile (Kjems & Garrett, 1988: Kjems *et al.*, 1992). Furthermore, the rRNA introns would evolve much more rapidly than the 16S rRNA exons (Itoh *et al.*, 1998a). Therefore, taxonomic significance of the rRNA intron is still unclear.

Description of *Caldivirga* gen. nov.

Caldivirga (Cal.di.vir'ga. L. adj. *caldus* hot; L. n. *virga* rod; M.L. masc. n. *Caldivirga* a hot rod).

Cells are straight or slightly curved rods, and occasionally bent and branched out singly or extensively. Most of cells are 0·4–0·7 × 3–20 μm. Often, spherical bodies protrude at the end of the cells, or laterally. Cells multiply by branching or budding, and divide by the constriction mode. Pili attach to cells terminally, laterally or peripherally. No motility is shown. Grows anaerobically or microaerobically. Prefer extremely high temperature and weakly acidic conditions for growth (e.g. 85 °C, pH 3·7–4·2). Insensitive to chloramphenicol, kanamycin, oleandomycin and streptomycin. Sensitive to erythromycin, novobiocin and rifampicin. Possess at least five, cyclized glycerol-bisphytanyl-glycerol tetraethers. The G+C content of genomic DNA is 43 mol%. The 16S rDNA is typical of a crenarchaeote in the sequence signature analysis. Phylogenetically represents an independent lineage in the family *Thermoproteaceae*. Inhabits an acidic hot spring. Monospecific at present. The type species is *Caldivirga maquilingensis*.

Description of *Caldivirga maquilingensis* sp. nov.

Caldivirga maquilingensis (ma.qui.lin.gen'sis. L. adj. suff. *-ensis* belonging to; M.L. adj. pertaining to Mt Maquiling, a volcano in the Philippines).

Grows anaerobically or microaerobically. Heterotrophic. Growth is stimulated significantly by archaeal cell extracts or a vitamin mixture. Growth occurs from 60 to 92 °C, and between pH 2·3 and 5·9 when buffered with 10 mM trisodium citrate. Alternatively, grows up to pH 6·4 by using 10 mM MES as buffer agent. Strain IC-167^T grows optimally around 85 °C, pH 3·7–4·2, with a doubling time of 8 h. Grows at low salinity (0–0·75% NaCl). Utilizes glycogen, gelatin, beef extract, peptone, tryptone and yeast extract as carbon sources. Requires sulfur, thiosulfate or sulfate as possible electron acceptors. Produces sulfide during growth. The G+C content of genomic DNA is 43 mol%. The 16S rDNA may contain introns. The type strain is strain IC-167, which was isolated from a hot spring at Mt Maquiling, Laguna, the Philippines. The two strains IC-166 and IC-167^T have been deposited in the Japan Collection of Microorganisms, The Institute of Physical and Chemical Resarch, Saitama, Japan, as JCM 10306 and JCM 10307^T, respectively. Also, the isolates have been deposited in the Microbial Culture Collection of the Museum of Natural History, University of the Philippines, Los Baños, the Philippines, as MCC-UPLB 1199 and MCC-UPLB 1200^T, respectively. In addition, both strains are registered as ANMR 0177 and ANMR 0178^T, respectively, as the strains belong to the 'Asian Network on Microbial Researches' project.

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