Ignisphaera aggregans gen. nov., sp. nov., a novel hyperthermophilic crenarchaeote isolated from hot springs in Rotorua and Tokaanu, New Zealand

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Consortia containing a novel coccus-shaped, anaerobic heterotroph together with *Pyrobaculum* rods were cultivated from geothermal environments in New Zealand. Pure cultures of the cocci were only obtained from one such consortium, despite extensive attempts. Cells of this strain (AQ1.S1^T) were regular to irregular cocci in morphology and occasionally formed large aggregates, especially when utilizing polysaccharides such as konjac glucomannan as a carbon source. Strain AQ1.S1^T is a hyperthermophile, with an optimal temperature for growth between 92 and 95 °C (range 85–98 °C), and a moderate acidophile, with optimal growth occurring at pH 6.4 (range 5.4–7.0). Growth was inhibited by the addition of sulphur and NaCl (optimal growth occurred without addition of NaCl) and an electron acceptor was not required. Strain AQ1.S1^T utilized starch, trypticase peptone, lactose, glucose, konjac glucomannan, mannose, galactose, maltose, glycogen and β -cyclodextrin as carbon sources. The G + C content was 52.9 mol%. Based on 16S rRNA gene sequence analysis and physiological features it is proposed that isolate AQ1.S1^T (=DSM 17230^T=JCM 13409^T) represents the type strain of a novel species of a new genus within the *Crenarchaeota*, *Ignisphaera aggregans* gen. nov., sp. nov.

According to 16S rRNA gene analysis and phenotypic characteristics, the cultured representatives of the *Crenarchaeota* within the archaeal domain group into three orders, the *Thermoproteales*, *Sulfolobales* and *Desulfurococcales* (Burggraf *et al.*, 1997). The *Thermoproteales* are rod-shaped organisms that group into the families *Thermoproteaceae* and *Thermofilaceae*, whereas members of the *Sulfolobales* are coccoid-shaped, lobed acidophiles. The *Desulfurococcales* are all coccoid to disc-shaped, neutrophilic or weakly acidophilic and strict anaerobes with fermentative metabolism or anaerobic respiration, with the exception of *Aeropyrum pernix* and *Aeropyrum camini* (Sako *et al.*, 1996; Nakagawa *et al.*, 2004). Phenotypic and 16S rRNA gene phylogenetic analyses divide the members of the *Desulfurococcales* into two families, the *Pyrodictiaceae* and the *Desulfurococcaceae*. The *Pyrodictiaceae* forms a distinct group [genera *Pyrodictium*

(Stetter *et al.*, 1983), *Hyperthermus* (Zillig *et al.*, 1991) and *Pyrolobus* (Blöchl *et al.*, 1997)] within 16S rRNA phylogenetic trees, whereas the *Desulfurococcaceae* consists of a diverse range of cultured representatives (Burggraf *et al.*, 1997). These two families can also be distinguished phenotypically by growth temperatures, where the *Pyrodictiaceae* consists of organisms with optimal temperatures above 100 °C and members of the *Desulfurococcaceae* are characterized by maximal growth temperatures of up to 100 °C. The *Desulfurococcaceae* consists of cocci to disc-shaped micro-organisms of the genera *Desulfurococcus* (Zillig *et al.*, 1982), *Aeropyrum* (Sako *et al.*, 1996; Nakagawa *et al.*, 2004), *Ignicoccus* (Huber *et al.*, 2000), *Staphylothermus* (Fiala *et al.*, 1986; Arab *et al.*, 2000), *Stetteria* (Jochimsen *et al.*, 1997), *Sulphobococcus* (Hensel *et al.*, 1997), *Thermoplasma* (Stetter, 2003), *Thermosphaera* (Huber *et al.*, 1998) and the distantly related 'Acidilobus group' consisting of *Acidilobus aceticus* (Prokofeva *et al.*, 2000), *Caldisphaera lagunensis* (Itoh *et al.*, 2003) and strain NC12 ('*Caldococcus noboribetus*'; Aoshima *et al.*, 1996).

As part of a project investigating the microbial ecology of New Zealand's high-temperature geothermal habitats, a novel coccoid-shaped archaeum (isolate AQ1.S1^T) was isolated into pure culture from co-culture with a strain of *Pyrobaculum*. The isolated coccus was similar in morphology and 16S rRNA gene sequence to a previously identified

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Abbreviation: DDGE, denaturing gradient gel electrophoresis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Ignisphaera aggregans* AQ1.S1^T and strains Tok10A.S1, Tok37.S1 and Tok1 are DQ060321, DQ060322, DQ060323 and DQ060320, respectively.

archaeon, which was also in co-culture with a strain of *Pyrobaculum* and was obtained from a New Zealand hot spring (Götz, 1998). Other thermal habitats of New Zealand and Yellowstone National Park (WY, USA) were screened for the presence of these novel cocci by using culturing methods. In this study, we describe the novel isolate AQ1.S1^T and, based on 16S rRNA nucleotide sequence and phenotypic analysis, propose that it represents a novel genus and species, *Ignisphaera aggregans* gen. nov., sp. nov., within the *Crenarchaeota*.

Samples from hot springs and mud pools situated in the Rotorua and Tokaanu thermal areas of New Zealand were collected in sterile containers and transported back to the laboratory under ambient conditions. The medium used for enrichment and isolation contained (g l⁻¹): (NH₄)₂SO₄, 1.3; CaCl₂, 0.074; MgSO₄·7H₂O, 0.28; KH₂PO₄, 0.28; yeast extract, 0.1; trypticase peptone, 2.0; soluble starch, 2.0; and cystine, 0.6. Resazurin (0.1 %, w/v), FeCl₃ (0.28 g l⁻¹) and trace elements (mg l⁻¹): MnSO₄, 2.2; ZnSO₄·7H₂O, 0.5; H₃BO₃, 0.5; CuSO₄, 0.016; Na₂MoO₄·2H₂O, 0.025; and CoCl₂·6H₂O, 0.046) were added at 1 ml l⁻¹. The pH of the medium was adjusted to 7.0 at room temperature, and the medium was then boiled and dispensed into Hungate tubes under an N₂ atmosphere, autoclaved and reduced with 10 % Na₂S·9H₂O as required. Growth was monitored using phase-contrast light microscopy. Approximately 1 ml pool water from hot springs in Yellowstone National Park was used to inoculate media which were incubated *in situ* in the pool from which the inoculum originated. The media used included the enrichment medium outlined above and a modified enrichment medium, where cystine was removed and starch was replaced with konjac glucomannan (Shintoa Koeki Kaisha). The tubes were incubated in the pools for 4–7 days, after which they were transported to the laboratory under ambient conditions and microbial growth was checked using phase-contrast light microscopy.

Cells were examined under phase-contrast light microscopy. For scanning electron microscopy, cells were grown to late exponential phase, filtered through a 0.22 µm filter and fixed using 2.5 % glutaraldehyde. The filter was then exposed to four changes of 0.1 M sodium cacodylate buffer, rinsed in water and dehydrated in increasing concentrations of ethanol (50, 75 and 90 %, respectively), followed by four changes of absolute ethanol. The filter was then critical point dried, sputtered with platinum and viewed using a Hitachi S-4100 field emission scanning electron microscope.

For metabolic studies, all experiments were undertaken at 90 °C unless otherwise stated and growth was documented using phase-contrast microscopy with a Thoma counting chamber (depth 0.02 mm). Unless otherwise stated, carbon utilization studies involved the addition of substrates at 0.2 % (w/v) final concentration to modified enrichment medium. The modified enrichment medium had the starch supplement omitted, reduced concentrations of yeast extract and trypticase peptone (0.05 and 1.0 g l⁻¹, respectively), MOPS added to a final concentration of 25 mM and the pH

adjusted to approximately 7.0 at room temperature. A positive growth response to a substrate was only recorded when a similar cell density was achieved on three successive transfers in the same medium. For the growth response to pH, buffers were added to the medium to a final concentration of 25 mM. The buffers used and the corresponding pH ranges were: MES, pH 4.5–6.5; MOPS, pH 6.5–7.5; Tris, pH 7.5–9.0; CHES, pH 9.0–10.0; and CAPS, pH 10.0–10.5. The pH was adjusted at a temperature of 75 °C and the corresponding pH value (at 90 °C) was extrapolated using the appropriate d(pK_a)/dt coefficient. Various electron acceptors were tested by replacing the cystine of the standard enrichment medium. Alternative electron acceptors were used at final concentrations of both 2.5 and 10 mM in medium containing 25 mM MOPS. Elemental sulphur (approx. 20 mg) was added to individual Hungate tubes containing 9 ml medium prior to autoclaving. Utilization of electron acceptors was recorded as positive when the same cell density was achieved after at least two transfers in the medium. Salt tolerance was determined by adding NaCl directly to the standard enrichment medium. Autotrophic growth was tested in modified enrichment medium (starch omitted and both trypticase peptone and yeast extract at 0.05 g l⁻¹) under an H₂/CO₂ atmosphere (80:20, v/v). Autotrophic medium without an electron acceptor was tested and cystine, sulphite, thiosulphate and elemental sulphur were trialled as electron acceptors.

To minimize temperature degradation in experiments to test for sensitivity to antibiotics (Peteranderl *et al.*, 1990), antibiotics were added to cells in exponential growth (90 °C) to a final concentration of 100 µg ml⁻¹.

H₂S was determined qualitatively (Huber *et al.*, 1986). The breakdown products of growth on konjac glucomannan were tested by size exclusion chromatography through a Biogel P2 (2.6 × 95 cm) size exclusion column. Size standards of monosaccharide (glucose) and disaccharide (maltose) were run through the column at 1 % (w/v) in pure water (Milli-Q; Millipore). Growth medium containing konjac glucomannan with and without an inoculum of AQ1.S1^T cells was incubated at 90 °C. The medium was removed after approximately 3 days incubation, growth in the inoculated tubes was recorded by using light microscopy and the control tubes were confirmed as sterile. The medium was then passed through a 0.22 µm filter and 10 ml of the filtrate was snap-frozen in liquid N₂ and freeze-dried. It was then resuspended in 4–5 ml 100 % methanol, incubated for 30 min in an ultrasonic cleaner and then centrifuged at 2000 g for 7 min. The supernatant was transferred to a clean tube and the methanol was removed by exposure to N₂ gas flow, with the tube in a 40 °C heat block. The residue was resuspended in 2 ml Milli-Q water, sonicated for 5 min and passed through a 0.22 µm filter. Finally, 2 ml was loaded onto the column for analysis. In addition, konjac glucomannan (1 %, w/v) suspended in Milli-Q water was processed as described for the growth medium (without the 90 °C incubation) and passed through the column.

Determination of G + C content was undertaken in duplicate as described by Gonzalez & Saiz-Jimenez (2002) using a Smart Cycler II (Cepheid). DNA from *Escherichia coli* (strain DH5 α) was used as a control.

The 16S rRNA gene was amplified by PCR utilizing the primers A5F (5'-CCGTTGATCCTGCCGG-3') and U1522R (5'-AAGGAGGTGATCCARCCGCA-3'). Each PCR consisted of 1 μ M of each primer, 200 μ M dNTPs, 1 $\times$ PCR buffer, 1.5 mM MgCl₂, 1.25 units *Taq* polymerase and approximately 20 ng template DNA, in a final reaction volume of 25 μ l. The thermocycling conditions consisted of initial denaturation at 94 °C for 3 min and 30 s, then 32 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 2 min, with a final extension at 72 °C for 6 min. The 16S rRNA gene was sequenced by using the MegaBACE DNA analysis system (Amersham Bioscience) using the primers A5F, A347F (5'-CCAGGCCCTACGGGGCGCA-3'), A915F (5'-AGGAATTGGCGGGGAGCAC-3'), A907R (5'-CCGTCAATTCCTTTGAGTTT-3'), 519R (5'-GWATTACCGCGGCKGCTG3-'), A1335R (5'-GTGTGCAAGGAGCAGGGAC-3') and U1522R. The 16S rRNA gene sequences for the coccal members of the Tok10A and Tok1 consortia were determined by using DNA extracted from a coccal-dominated culture for Tok10A.S1 and gene-cloning for Tok1 (Götz, 1998). Both strands of the 16S rRNA gene were sequenced for the Tok37.S1 and AQ1.S1^T isolates, with single-strand sequencing being undertaken for Tok10A.S1 and Tok1. Sequences were checked for chimeric artefacts using the CHIMERA_CHECK online tool of the Ribosomal Database Project (Maidak *et al.*, 2001). Phylogenetic analysis and alignment of 16S rRNA gene sequences were performed using the ARB software package (Ludwig *et al.*, 2004). The phylogenetic positions of the sequences were determined using the PHYLIP package with analysis of sequences undertaken using the programs DNADIST, DNAML, DNAPARS, FITCH, NEIGHBOR and SEQBOOT (Felsenstein, 1993).

From an in-depth study of the microbial ecology of a hot pool (designated AQ1) situated in Kuirau Park, Rotorua, New Zealand, a consortium of rod- and coccoid-shaped organisms was obtained as an enrichment from pool water incubated at 90 °C. Typically, rods dominated the cultures within the first days of incubation, with an overgrowth of cocci occurring after 4–7 days. Therefore, serial dilution (1:10) of a nascent consortium culture was undertaken to obtain a pure culture of the rod-shaped organism. A pure culture of the coccoid-shaped organism was eventually achieved after many successive dilutions (1:10) from an older consortium culture in which cocci dominated. Both cultures were maintained by weekly transfer of serial dilutions (1:10) for nearly a year prior to characterization. Sequencing of the 16S rRNA gene identified the rod as a strain of *Pyrobaculum* (results not shown) and the coccus (isolate AQ1.S1^T) matched very closely (16S rRNA gene sequence and morphology) another previously identified coccus (Tok1) that had been isolated from a hot spring in Tokaanu, New Zealand (Götz, 1998). Tok1 was also enriched

as a co-culture with a strain of *Pyrobaculum*; however, extensive attempts to obtain a pure culture of Tok1 failed (Götz, 1998). Other geothermal habitats within New Zealand and Yellowstone National Park were then screened by enrichment for the presence of these novel cocci (Table 1). Samples from New Zealand hot springs and mud pools were inoculated into anaerobic enrichment medium and incubated at 93 °C. A duplicate enrichment was incubated at 80 °C if the *in situ* pool temperature was below 90 °C (results not shown). No cocci were enriched from springs in Yellowstone National Park. However, for the New Zealand springs, as was the case for the AQ1 consortium, rod-shaped organisms were commonly observed in the medium after 2–4 days incubation, followed by an overgrowth of cocci (Table 1). Conversely, cells with a coccoid morphology dominated rod-shaped organisms in the Tok10A enrichment (coccus member designated Tok10A.S1) and an enrichment containing only cocci was obtained from pool Tok37 (coccus designated Tok37.S1). Comparison of denaturing gradient gel electrophoresis (DGGE) profiles of the enrichments and AQ1.S1^T suggested that all coccal-containing consortia contained AQ1.S1^T-related members (results not shown). All attempts to isolate pure cultures of cocci from enrichments of pools AQ2, AQ5, Tok10A and Tok13 failed, including attempts at isolation using solid Gelrite medium (Hungate, 1969). The 16S rRNA genes of the coccal isolates from pools Tok10A and Tok37 were sequenced and were found to match closely those of AQ1.S1^T and Tok1 (results presented below). However, the Tok37 coccal enrichment also contained an atypical 16S rRNA gene PCR product that contained introns, indicating that it may be a mixed culture of at least two different coccal species. Isolate AQ1.S1^T was therefore used for further characterization.

Cells of strain AQ1.S1^T were regular to irregular cocci, with a diameter of 1–1.5 μ m when grown on starch. The cells occurred singly, in pairs or as aggregates of many cells. Aggregation of cells was common when AQ1.S1^T was grown on mono-, di- or polysaccharides, as shown in Fig. 1. A web-like array of extracellular material appeared to hold cells together when cell aggregates were examined using scanning electron microscopy (Fig. 2). Attempts to stain this material using Alcian blue- and copper sulphate-based capsule/polysaccharide staining methods were unsuccessful. Cells of Tok1 also formed aggregates, but this was not observed for either the Tok37.S1 or Tok10A.S1 cultures.

Strain AQ1.S1^T grew at 85–98 °C and pH 5.4–7.0. No growth was observed at 80 or 100 °C, or at pH 4.9 or 7.4. The optimum temperature and pH for growth on starch were between 92 and 95 °C and 6.4, with a doubling time of approximately 7.5 h. Cultures of AQ1.S1^T grew to densities of approximately 2.5×10^7 cells ml⁻¹. Strain AQ1.S1^T utilized starch, trypticase peptone, lactose, glucose, konjac glucomannan, mannose, galactose, maltose, glycogen and β -cyclodextrin. Weak growth was evident on beef extract and sucrose. Growth was not observed on yeast extract, cellobiose, methanol, ethanol, trehalose, pyruvate, acetate,

Table 1. Summary of results from hot springs of New Zealand (NZ) and Yellowstone National Park (YNP)

Location	Hot spring	Temp. (°C)	pH	Enrichment	Novel cocci
NZ – Kuirau Park, Rotorua	AQ1	91	7.5	Rods and cocci	AQ1.S1 ^T
	AQ2	92	8.0	Rods and cocci	
	AQ3	87	7.5	Rods	
	AQ5	86	8.0	Rods and cocci	
NZ – Ohinemutu, Rotorua	OH2	94	9.0	No growth	
NZ – Whakarewarewa, Rotorua	WH1	96	8.5–9.0	No growth	
NZ – Tokaanu Thermal Park	Tok3	85	6.5	No growth	
	Tok10A	84	7.0	Rods and cocci	Tok10A.S1 (consortium)
	Tok13	97	6.0	Rods and cocci	
	Tok37 (mud pot)	99	7.5	Cocci	Tok37.S1 (probable consortia)
YNP – GOPA*	Tok13	90	Neutral	Rods and cocci	Tok1 (consortium)
	Obsidian pool	73.5–76	6.0	Rods and fervidobacterial morphology	
	South Obsidian pool	75.5–80	6.0	Fervidobacterial morphology	
YNP – Sentinel Meadows	OH	75–85	5–6	Rods	
	EM	81–85	7–8	No growth	
	BP	87–92.5	7–8	No growth	
	Boulder spring	86	8	No growth	
YNP – Sylvan	Sylvan spring	75–78	5–6	Few rods	
YNP – South Sylvan	JTP	89	6–7	No growth	
	FL	81	6	Rods	

*Greater Obsidian pool area.

malate, Casamino acids (0.1, % w/v), carboxymethylcellulose, amylopectin (corn), xanthan gum, locust gum (bean), guar gum, dextran, xylan (oat spelts, larch or birch), xylitol, xylose or amylose (corn and potato). The strain could also grow in the standard growth medium without the addition of starch. Addition of a vitamin solution (Wolin *et al.*, 1963)

weakly promoted growth. Cultures grew in the presence of up to 0.5 % NaCl (optimally with no added NaCl), but not at 0.75 % NaCl. An electron acceptor was not required and the removal of cystine from the growth medium did not affect cell numbers significantly. Other electron acceptors trialled included thioglycolate, nitrate, nitrite, sulphite, thiosulphate and elemental sulphur. The electron acceptors

Fig. 1. Phase-contrast micrograph of an aggregation of cells of strain AQ1.S1^T grown in enrichment media. Bar, 5 µm.

Fig. 2. Scanning electron micrograph of a dehydrated AQ1.S1^T cell aggregate grown in enrichment medium with konjac glucomannan as a carbon source. Bar, 1 µm.

were inhibitory at a concentration of 10 mM (including cystine) and at 2.5 mM both sulphite and thioglycolate also inhibited growth of AQ1.S1^T. Growth of AQ1.S1^T, Tok37.S1, Tok10.S1 and Tok1 was inhibited by sulphur. No growth was detected in serum bottles under autotrophic conditions. All isolates were strict anaerobes; even trace quantities of oxygen (just sufficient to turn resazurin pink) inhibited growth. Mono- and disaccharides accumulated in AQ1.S1^T cultures grown in medium containing konjac glucomannan, but not in sterile medium that had been exposed to the same temperature as the inoculated medium or the stock of konjac glucomannan. This most probably indicates that the konjac glucomannan is being hydrolysed enzymically by AQ1.S1^T into sugars for metabolism. Hydrogen sulphide was also detected in AQ1.S1^T cultures grown in enrichment medium. Cells of AQ1.S1^T were resistant to novobiocin and streptomycin but sensitive to erythromycin, chloramphenicol and rifampicin.

A near-full-length 16S rRNA gene sequence for strain AQ1.S1^T (1491 bp) was obtained and had a G+C ratio of 66%. The 16S rRNA gene sequences of the other novel coccal isolates matched very closely that of AQ1.S1^T, being 99% similar to Tok37.S1 (1423 bp), 98% to Tok10A.S1 (1433 bp) and 98% to Tok1 (1191 bp), and all sequences were free of chimeric artefacts. Sequence similarities between strain AQ1.S1^T and members of the *Pyrodictiaceae* ranged from 91.5 to 94% and from 87 to 94.5% for the *Desulfurococcaceae*, with the closest sequence match being that with *Staphylothermus marinus* F1^T (GenBank accession no. X99560). The G+C content of strain AQ1.S1^T was 52.9 mol%. Phylogenetic analysis revealed that AQ1.S1^T and the other novel coccal isolates formed an independent group within the *Crenarchaeota* (Fig. 3). The topology of the phylogenetic tree did not change when DNADIST (FITCH and NEIGHBOR), DNAML or DNAPARS analysis was used, or when the shorter 16S rRNA gene sequence of Tok1 (1195 bp) was removed. Likewise, the structure of the phylogenetic tree did not change with the inclusion of *Methanopyrus kandleri* DSM 6324^T (GenBank accession no. M59932), *Archaeoglobus fulgidus* DSM 4304^T (Y00275) and *Methanocaldococcus jannaschii* DSM 2661^T (M59126) as additional outgroups.

These novel coccoid micro-organisms are widely distributed in high-temperature, near-neutral hot pools and mud pools in New Zealand. An unusual feature was that the pH range for growth for the type strain AQ1.S1^T determined in laboratory culture was between pH 5.4 and 7.0 (at 90 °C), yet isolation was regularly achieved from more alkaline springs. This was not the result of an aberrant pH reading, as the pH of the AQ1 pool was measured at regular intervals over at least a year and was consistently above pH 7.0 (range measured was 7.4–8.2). Moreover, we failed to isolate or detect molecular signatures of AQ1.S1^T-related organisms by DGGE analysis in pools where the pH was below pH 6.7 yet, ostensibly, the organism should be able to proliferate under such conditions. These novel coccus-shaped organisms

Fig. 3. Phylogenetic tree based on analysis of the 16S rRNA gene sequences of strain AQ1.S1^T, the other novel isolates Tok37.S1, Tok10A.S1 and Tok1 and representative species of the *Crenarchaeota*, showing the positions of the novel cocci. The dendrogram was produced with DNADIST (neighbour-joining) analysis using 1027 bp of aligned sequence. The tree was rooted with *Thermococcus celer* DSM 2476^T (GenBank accession no. M21529; not shown). Bootstrap values >65% are shown from 100 replicates. Bar, 10% sequence divergence.

were not enriched from pools with similar characteristics in Yellowstone National Park. Moreover, the use of 'Ignisphaera-specific' enrichment media, i.e. no electron acceptor and konjac glucomannan as a carbon source, did not yield consortia containing coccoid organisms.

Commonly, the novel coccus-shaped organisms grew commensally with species of *Pyrobaculum*, with the exception of Tok37.S1, and, although the *Pyrobaculum* sp. could readily be obtained as a pure culture, this proved impossible with the coccus-shaped organisms (with the exception of AQ1.S1^T) and may be indicative of a metabolic dependency of the cocci with *Pyrobaculum*. In fact, the outgrowth of cocci following the development of rods (*Pyrobaculum* sp.) within cultures may be due to the cocci utilizing a product excreted by the rod-shaped members of the consortium. Likewise, the presence of a second atypical 16S rRNA gene PCR product for Tok37.S1 may indicate another coccal member that Tok37.S1 might be dependent upon.

AQ1.S1^T is a coccoid-shaped, anaerobic, moderately acidophilic, heterotrophic hyperthermophile, with maximum growth occurring below 100 °C. These morphological and phenotypic attributes fit within the description of the family *Desulfurococcaceae* (Burggraf *et al.*, 1997). The ability of strain AQ1.S1^T to grow in media with no exogenous electron acceptor indicates its capacity to ferment polysaccharides and complex proteinaceous substrates. The fermentation of polysaccharides and/or peptides has also been noted for other members of the family *Desulfurococcaceae*, such as *Desulfurococcus* (Zillig *et al.*, 1982), *Thermosphaera* (Huber *et al.*, 1998), *Staphylothermus* (Fiala *et al.*, 1986), *Sulfophobococcus* (Hensel *et al.*, 1997) and both *Acidilobus* (Prokofeva *et al.*, 2000) and *Caldisphaera* (Itoh *et al.*, 2003) of the 'Acidilobus group'. The formation of cell aggregates that was typical of AQ1.S1^T and Tok1 has also been noted in cultures of members of the *Desulfurococcaceae*, for example, cells of *Caldisphaera lagunensis* clump together (Itoh *et al.*, 2003) and *Thermosphaera aggregans* (Huber *et al.*, 1998), *Staphylothermus marinus* and *Staphylothermus hellenicus* grow as grape-like aggregates (Fiala *et al.*, 1986; Arab *et al.*, 2000). However, the growth inhibition by sulphur of the novel cocci does not accord with the description of the *Desulfurococcaceae*, as its members typically use elemental sulphur for reduction or respiration (Burggraf *et al.*, 1997). However, *Aeropyrum camini* (Nakagawa *et al.*, 2004), *Thermosphaera aggregans* (Huber *et al.*, 1998) and *Sulfophobococcus zilligii* (Hensel *et al.*, 1997) are already exceptions within the *Desulfurococcaceae* in this regard.

By themselves, the phenotypic characteristics of the novel cocci suggest that they represent members of the *Desulfurococcaceae*; however, based on 16S rRNA gene analysis the novel coccal isolates form an independent lineage within the *Crenarchaeota* with a 100 % bootstrap value and do not group within any other lineage within the *Desulfurococcaceae*. Consequently, the 'Ignisphaera group' constitutes a novel deep-branching genus of the *Desulfurococcaceae*, branching independently from both the families *Desulfurococcaceae* and *Pyrodictiaceae*. Therefore, inclusion of the 'Ignisphaera group' and the proposal of strain AQ1.S1^T as representing a new genus and species within the *Desulfurococcales* may be controversial, as is the case for the 'Acidilobus group', because of its independent grouping based on 16S rRNA gene analysis and acidophilic growth optima (Itoh *et al.*, 2003). Despite the above considerations, with regard to the phylogenetic placement of strain AQ1.S1^T and related isolates, we propose a new genus within the *Desulfurococcales* as the most conservative origin for this lineage. However, as more representatives of the *Crenarchaeota* are cultured and characterized the position of the 'Ignisphaera-type' isolates may be resolved.

Description of *Ignisphaera* gen. nov.

Ignisphaera (Ig.ni.sphae'ra. L. n. ignis fire, L. fem. n. sphaera ball, N.L. fem n. Ignisphaera fire ball).

Cells are regular to irregular cocci that occur singly, in pairs or as aggregates. Hyperthermophilic anaerobe. Moderate acidophile. Heterotrophic. Electron acceptor is not absolutely necessary. Elemental sulphur and NaCl inhibit growth. The 16S rRNA gene groups within the *Crenarchaeota*. Habitat is terrestrial, near-neutral hot springs and mud pots. The type species is *Ignisphaera aggregans*.

Description of *Ignisphaera aggregans* sp. nov.

Ignisphaera aggregans (ag'gre.gans. L. part. adj. *aggregans* aggregate forming, aggregating clumping).

Cells are approximately 1–1.5 µm in size when grown on starch. Temperature and pH ranges for growth are 85–98 °C (optimum at 92–95 °C) and pH 5.4–7.0 (optimum pH 6.4). Obligate anaerobe. Ferments both poly- and disaccharides. Grows at low salinity (<0.5 % NaCl) and optimally without addition of NaCl. Resistant to novobiocin and streptomycin but sensitive to erythromycin, chloramphenicol and rifampicin. The G + C content of the DNA is 52.9 mol%.

The type strain is *Ignisphaera aggregans* AQ1.S1^T (=DSM 17230^T=JCM 13409^T), which was isolated from a near-neutral, boiling spring in Kuirau Park, Rotorua, New Zealand.

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References

- Aoshima, M., Nishibe, Y., Hasegawa, M., Yamagishi, A. & Oshima, T. (1996). Cloning and sequencing of a gene encoding 16S ribosomal RNA from a novel hyperthermophilic archaeobacterium NC12. *Gene* **180**, 183–187.
- Arab, H., Völker, H. & Thomm, M. (2000). *Thermococcus aegaeicus* sp. nov. and *Staphylothermus hellenicus* sp. nov., two novel hyperthermophilic archaea isolated from geothermally heated vents off Palaeochori Bay, Milos, Greece. *Int J Syst Evol Microbiol* **50**, 2101–2108.
- Blöchl, E., Rachel, R., Burggraf, S., Hafenbradl, D., Jannasch, H. W. & Stetter, K. O. (1997). *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113 °C. *Extremophiles* **1**, 14–21.
- Burggraf, S., Huber, H. & Stetter, K. O. (1997). Reclassification of the crenarchaeal orders and families in accordance with 16S rRNA sequence data. *Int J Syst Bacteriol* **47**, 657–660.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fiala, G., Stetter, K. O., Jannasch, H. W., Langworthy, T. A. & Madon, J. (1986). *Staphylothermus marinus* sp. nov. represents a novel genus of extremely thermophilic submarine heterotrophic Archaeobacteria growing up to 98 °C. *Syst Appl Microbiol* **8**, 106–113.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G + C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.

- Götz, D. K. (1998). *The characterisation of three hyperthermophilic archaea from New Zealand hot springs*. PhD thesis, University of Waikato, Hamilton, New Zealand.
- Hensel, R., Matussek, K., Michalke, K., Tacke, L., Tindall, B. J., Kohlhoff, M., Siebers, B. & Dielenschneider, J. (1997). *Sulfophobococcus zilligii* gen. nov. spec. nov. a novel hyperthermophilic archaeum isolated from hot alkaline springs of Iceland. *Syst Appl Microbiol* **20**, 102–110.
- Huber, R., Langworthy, T. A., König, H., Thomm, M., Woese, C. R., Sleytr, U. B. & Stetter, K. O. (1986). *Thermotoga maritima* sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90 °C. *Arch Microbiol* **144**, 324–333.
- Huber, R., Dyba, D., Huber, H., Burggraf, S. & Rachel, R. (1998). Sulfur-inhibited *Thermosphaera aggregans* sp. nov., a new genus of hyperthermophilic archaea isolated after its prediction from environmentally derived 16S rRNA sequences. *Int J Syst Bacteriol* **48**, 31–38.
- Huber, H., Burggraf, S., Mayer, T., Wyszchony, I., Rachel, R. & Stetter, K. O. (2000). *Ignicoccus* gen. nov., a novel genus of hyperthermophilic, chemolithoautotrophic Archaea, represented by two new species, *Ignicoccus islandicus* sp. nov. and *Ignicoccus pacificus* sp. nov. *Int J Syst Evol Microbiol* **50**, 2093–2100.
- Hungate, R. E. (1969). A roll-tube method for the cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.
- Itoh, T., Suzuki, K., Sanchez, P. C. & Nakase, T. (2003). *Caldisphaera lagunensis* gen. nov., sp. nov., a novel thermoacidophilic crenarchaeote isolated from a hot spring at Mt Maquiling, Philippines. *Int J Syst Evol Microbiol* **53**, 1149–1154.
- Jochimsen, B., Peinemann-Simon, S., Völker, H., Stüben, D., Botz, R., Stoffers, P., Dando, P. R. & Thomm, M. (1997). *Stetteria hydrogenophila*, gen. nov. and sp. nov., a novel mixotrophic sulfur-dependent crenarchaeote isolated from Milos, Greece. *Extremophiles* **1**, 67–73.
- Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Maidak, B. L., Cole, J. R., Lilburn, T. G. & 7 other authors (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**, 173–174.
- Nakagawa, S., Takai, K., Horikoshi, K. & Sako, Y. (2004). *Aeropyrum camini* sp. nov., a strictly aerobic, hyperthermophilic archaeon from a deep-sea hydrothermal vent chimney. *Int J Syst Evol Microbiol* **54**, 329–335.
- Peteranderl, R., Shotts, E. B., Jr & Wiegel, J. (1990). Stability of antibiotics under growth conditions for thermophilic anaerobes. *Appl Environ Microbiol* **56**, 1981–1983.
- Prokofeva, M. I., Miroshnichenko, M. L., Kostrikina, N. A., Chernyh, N. A., Kuznetsov, B. B., Tourova, T. P. & Bonch-Osmolovskaya, E. A. (2000). *Acidilobus aceticus* gen. nov., sp. nov., a novel anaerobic thermoacidophilic archaeon from continental hot vents in Kamchatka. *Int J Syst Evol Microbiol* **50**, 2001–2008.
- Sako, Y., Nomura, N., Uchida, A., Ishida, Y., Morii, H., Koga, Y., Hoaki, T. & Maruyama, T. (1996). *Aeropyrum pernix* gen. nov., sp. nov., a novel aerobic hyperthermophilic archaeon growing at temperatures up to 100 °C. *Int J Syst Bacteriol* **46**, 1070–1077.
- Stetter, K. O. (2003). *Thermodiscus maritimus* gen. nov., sp. nov. In *Validation of Publication of New Names and New Combinations Previously Effectively Published Outside the IJSEM*, List no. 89. *Int J Syst Evol Microbiol* **53**, 1–2.
- Stetter, K. O., König, H. & Stackebrandt, E. (1983). *Pyrodictium* gen. nov., a new genus of submarine disc-shaped sulphur reducing archaeobacteria growing optimally at 105 °C. *Syst Appl Microbiol* **4**, 535–551.
- Wolin, E. A., Wolin, M. J. & Wolfe, R. S. (1963). Formation of methane by bacterial extracts. *J Biol Chem* **238**, 2882–2886.
- Zillig, W., Stetter, K. O., Prangishvili, D., Schäfer, W., Wunderl, S., Janekovic, D., Holz, I. & Palm, P. (1982). *Desulfurococcaceae*, the second family of the extremely thermophilic, anaerobic, sulfur-respiring *Thermoproteales*. *Zentralbl Bakteriol Mikrobiol Hyg I Abt Orig C* **3**, 304–317.
- Zillig, W., Holz, I. & Wunderl, S. (1991). *Hyperthermus butylicus* gen. nov., sp. nov., a hyperthermophilic, anaerobic, peptide-fermenting, facultatively H₂S-generating archaeobacterium. *Int J Syst Bacteriol* **41**, 169–170.