***Thermococcus guaymasensis* sp. nov. and *Thermococcus aggregans* sp. nov., two novel thermophilic archaea isolated from the Guaymas Basin hydrothermal vent site**

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***Thermococcus* strains TYS^T and TY^T isolated from the Guaymas Basin hydrothermal vent site and previously described were compared by DNA–DNA hybridization analysis with the closest *Thermococcus* species in terms of physiology and nutritional aspects. On the basis of the new data and taking into consideration the molecular, physiological and morphological traits published previously, it is proposed that strains TY^T and TYS^T should be classified as new species named *Thermococcus aggregans* sp. nov. and *Thermococcus guaymasensis* sp. nov., respectively. The type strain of *T. aggregans* is strain TY^T (= DSM 10597^T) and the type strain of *T. guaymasensis* is strain TYS^T (= DSM 11113^T).**

Keywords: *Thermococcus*, archaea, hydrothermal vents

INTRODUCTION

During the past 3 years, six additional species belonging to the genus *Thermococcus* have been described. Presently, 10 species represent this taxonomic group: *Thermococcus celer* (Zillig *et al.*, 1983), '*Thermococcus litoralis*' (Neuner *et al.*, 1990), *Thermococcus stetteri* (Miroshnichenko *et al.*, 1989), *Thermococcus profundus* (Kobayashi *et al.*, 1994), *Thermococcus peptonophilus* (Gonzalez *et al.*, 1995), *Thermococcus chitonophagus* (Huber *et al.*, 1995), *Thermococcus alcaliphilus* (Keller *et al.*, 1995), *Thermococcus fumicolans* (Godfroy *et al.*, 1996), *Thermococcus hydrothermalis* (Godfroy *et al.*, 1997) and '*Thermococcus zilligii*' (Ronimus *et al.*, 1997). All species are organotrophic, thermophilic anaerobes growing preferentially on proteinaceous compounds and utilizing carbohydrates as carbon sources rarely. Elemental sulphur considerably stimulates growth with the concomitant formation of H₂S. Typical optima temperatures range

from 85 to 88 °C (75 °C for *T. stetteri*) and the G + C content ranges approximately between 42 and 57 mol %, except for *T. litoralis* (38 mol %). According to the most recent investigations on the microbiology of hydrothermal vent sites, *Thermococcus* species appear to be present in significant numbers in these ecosystems (Antoine *et al.*, 1995; Marteinsson *et al.*, 1995).

Two novel deep-sea *Thermococcus* strains (TYS^T and TY^T) previously isolated from the Guaymas Basin (Gulf of California) and extensively described (Canganella & Jones, 1994a; Canganella *et al.*, 1997) showed a relatively high similarity of 16S rDNA sequences with other *Thermococcus* species but exhibited some different physiological properties (Canganella *et al.*, 1997). Compared with the most recently described *T. hydrothermalis* and *T. fumicolans*, our strains are able to grow on starch, there is no absolute requirement for elemental sulphur, and they are more sensitive to NaCl concentrations higher than 5.0 % (w/v). DNA–DNA hybridization experiments were necessary to determine the taxonomic standing of these two strains. Based on the results of these analyses, both strains were found to represent new species of

Abbreviation: TOR, temperature of renaturation.

The EMBL accession numbers for the partial 16S rDNA sequences of strains TY^T and TYS^T are Y08384 and Y08385, respectively.

Thermococcus, and their complete taxonomic description is reported in this paper.

METHODS

Source of micro-organisms. Strains TYS^T and TY^T were isolated from sediments collected at the Guaymas Basin hydrothermal vent site (2000 m depth) in the Gulf of California. On the basis of nutritional, physiological and molecular features, they were previously described as *Thermococcus* species by Canganella & Jones (1994a, b) and Canganella *et al.* (1997).

T. celer, '*T. litoralis*', *T. stetteri* and *T. profundus* strains were obtained from DSMZ, Braunschweig, Germany (see Table 1). *T. peptonophilus* strain OG-1^T (= JCM 9653^T) was kindly provided by Chiaki Kato (JAMSTEC, Yokosuka, Japan) and *T. hydrothermalis* and *T. fumicolans* were a gift from Anne Godfroy (IFREMER, Plouzane, France).

Growth conditions. The two novel *Thermococcus* strains as well as the reference micro-organisms were grown on a mineral medium containing the following constituents per litre of distilled water: 0.33 g KCl; 2.7 g MgCl₂·2H₂O; 3.4 g MgSO₄·7H₂O; 0.25 g NH₄Cl; 0.14 g CaCl₂·2H₂O; 0.14 g K₂HPO₄; 0.01 mg Na₂SeO₃; 0.01 mg NiCl₂·6H₂O; 1.0 g NaHCO₃; 18.0 g NaCl; 0.001 g resazurin; 0.5 g cysteine·HCl·H₂O; 0.5 g Na₂S·9H₂O. The medium also contained Bacto-yeast extract (0.2 g l⁻¹) and 10 ml l⁻¹ each of trace metal and vitamin solutions (Balch *et al.*, 1979).

Anaerobic techniques used to prepare and dispense the medium, the enrichment experiments, isolation procedures, as well as the physiological characterization of the new isolates have been described elsewhere (Canganella & Jones, 1994a, b).

Phylogenetic analysis. DNA of harvested cells was extracted according to the procedure of Marmur (1961), digested to nucleosides, and the G+C content was determined after nucleoside analysis by HPLC as described by Mesbah *et al.* (1989).

Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of PCR products was carried out as described previously (Barns *et al.*, 1994; Rainey *et al.*, 1992; Rainey & Stackebrandt, 1993).

The 16S rDNA sequence was manually aligned against representative sequences of members of the domain *Archaea*. The following sequences from the EMBL database were analysed: M21529 (*T. celer*), Z75240 (*T. stetteri*), D37982 (*T. peptonophilus*), Z70252 ('*T. litoralis*'), Z70244 (*T. hydrothermalis*), Z70250 (*T. fumicolans*), Z75233 (*T. profundus*), X99570 (*T. chitonophagus*) and U20163 (*P. furiosus*). Pair-wise evolutionary distances were computed using the correction factor of Jukes & Cantor (1969). The least-squares distance method of De Soete (1983) was used in the construction of the phylogenetic dendrogram from distance matrices.

DNA isolation and hybridization. DNA was isolated by column chromatography using hydroxyapatite according to the procedure of Cashion *et al.* (1977).

DNA-DNA hybridization was performed according to the methods of De Ley *et al.* (1970) using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermo-programmer and plotter.

The DNA concentration was 45 µg ml⁻¹. Owing to the high G+C content of *Thermococcus* DNA (mean value of 50 mol %), 20% (v/v) DMSO was added to 2.5 × SSC to depress the DNA melting point (*T*_m) (Escara & Hutton, 1980). The resulting buffer was 2 × SSC + 20% DMSO. The mean *T*_m was determined from the DNA melting curves and the optimal temperature of renaturation (TOR) was calculated as *T*_m + 25.

Renaturation rates were calculated by regression analysis between 10 and 30 min after the reaction start using the computer program TRANSFER.BAS (Jahnke, 1992).

RESULTS AND DISCUSSION

Morphology of *Thermococcus* strains TYS^T and TY^T

The morphological features of isolates TYS^T and TY^T have been reported previously (Canganella & Jones, 1994a; Canganella *et al.*, 1997). Under light microscopy, cells of TYS^T and TY^T usually appear as irregular cocci with a variable width of 0.5–3.0 µm. Strain TYS^T is similar to other *Thermococcales* species in that a dark granule often forms on the outer cell surface. Strain TYS^T usually exists as single cells, whereas strain TY^T typically forms cell aggregates up to 50–100 cells under optimal growth conditions. Both strains are non-motile.

Nutritional requirements

Strains TYS^T and TY^T grew chemo-organotrophically on complex substrates, e.g. yeast extract, trypticase, casein, starch, dextrose and maltose. In the presence of elemental sulphur, higher growth yields were observed with proteinaceous compounds, accompanied by H₂S production (Canganella & Jones, 1994a). No growth was detected in medium supplemented with chitin, organic acids (formate, acetate, propionate, butyrate; 0.5%, w/v), alcohols (methanol, ethanol, propanol; 0.5%, v/v), or Difco Casamino acids (0.4%, w/v) as primary carbon sources (Canganella & Jones, 1994a).

Phylogenetic analysis and DNA hybridization

The G+C content of DNA extracted from *Thermococcus* isolates TY^T and TYS^T was 42.0 and 46.0 mol %, respectively.

The nearly complete sequences consisting of 1301 bases of the amplified 16S rDNA of isolates TY^T and TYS^T were previously determined. The sequences of both isolates were therefore aligned and compared with all the available 16S rDNA sequences of *Thermococcus* species, including *Pyrococcus furiosus*. On the basis of these data, the 16S rDNA sequence of strain TY^T showed a similarity of 98.4% with strain TYS^T, 96.7% with *T. chitonophagus*, 97.7% with *T. hydrothermalis*, 97.0% with *T. fumicolans* and 93.9% with *P. furiosus*. The 16S rDNA sequence of strain TYS^T showed a similarity of 96.6% with *T. chitonophagus*, 98.4% with *T. hydrothermalis* and 96.0% with *T.*

Fig. 1. Unrooted phylogenetic tree indicating the phylogenetic position of *T. guaymasensis* and *T. aggregans* compared with other *Thermococcus* species and *P. furiosus*.

fumicolans. The phylogenetic tree constructed from all similarity data is shown in Fig. 1.

Thermococcus strains TYS^T and TY^T are distinct species as indicated by a DNA hybridization value of 41.2% (Table 1). They also exhibit DNA hybridization values less than 70% with the other *Thermococcus* species, indicating that they are new and distinct species in accordance with the recommendations of the Committee on reconciliation of approaches to bac-

terial systematics (Wayne *et al.*, 1987). Strains TYS^T and TY^T are named *Thermococcus guaymasensis* sp. nov. and *Thermococcus aggregans* sp. nov., respectively. *T. alcaliphilus* was not included in this analysis because its primary physiological feature (optimal growth at elevated pH) represents a unique taxonomical trait; moreover, its 16S rDNA sequence is not available on GenBank. '*T. zilligii*' was also not included in the present work because its isolation source (terrestrial freshwater hot pool), its inability to grow at NaCl concentrations higher than 1.2% (w/v) and its unusual membrane lipid composition clearly differentiate it from the other *Thermococcus* species.

Description of *Thermococcus aggregans* sp. nov.

Thermococcus aggregans (ag'gre.gans. L. v. *aggregare* to stick together, to aggregate; L. pres. part. *aggregans* assembling, aggregating).

Coccoid archaeon with cell diameters of 1.0–1.5 µm. Cells are Gram-positive, non-motile and occur as single cells, diploid forms or (particularly when cultivated on yeast extract/tryptone plus sulphur) aggregates of many cells (up to 50). Colonies are circular, convex and pale-grey in colour, and colony diameters range from 0.5 to 1.0 mm after 48–72 h incubation at 70 °C under strictly anoxic conditions. Chemo-organotrophic and strictly anaerobic. Utilizes preferably proteinaceous substrates such as yeast extract and trypticase in the presence of 0.5–1.0% (w/v) elemental sulphur. Casein, dextrose, maltose and starch are also utilized. The end products of metabolism on medium containing yeast extract plus tryptone and sulphur are acetate, propionate, isobutyrate, isovalerate, CO₂ and H₂S, whereas, after growth on dextrose or maltose plus sulphur, only acetate, CO₂ and H₂S are formed. The pH range for growth is 5.6–7.9; the optimum pH is 7.0. Grows between 60 and 94 °C, with no growth occurring at 95 °C. The optimum temperature is 88 °C. The NaCl range for growth is 1.0–3.0% (w/v) with the optimum at 2.5%. The G + C content of the DNA is 42.0 mol%. Lipids are mainly represented by one phospholipid

Table 1. DNA hybridization (%) between *T. guaymasensis* strain TYS^T, *T. aggregans* strain TY^T and other *Thermococcus* species

Strain	1	2	2	4	5	6	7	8	9
1 <i>T. guaymasensis</i> (DSM 11113 ^T)	100								
2 <i>T. aggregans</i> (DSM 10597 ^T)	42.1	100							
3 <i>T. celer</i> (DSM 2476 ^T)	44.4	33.9	100						
4 ' <i>T. litoralis</i> ' (DSM 5474)	37.1	52.4	38.2	100					
5 <i>T. stetteri</i> (DSM 5262 ^T)	48.7	41.5	44.9	39.9	100				
6 <i>T. peptonophilus</i> (JCM 9653 ^T)	48.3	31.1	49.5	33.9	47.3	100			
7 <i>T. profundus</i> (DT 5432 ^T)	45.9	36.8	48.4	32.7	46.8	50.7	100		
8 <i>T. fumicolans</i> (CIP 104680 ^T)	50.5	55.6	49.9	40.9	49.2	59.9	44.8	100	
9 <i>T. hydrothermalis</i> (CNCMI 1319 ^T)	51.6	43.4	53.9	39.8	39.3	43.6	40.3	57.2	100

and some minor components; core lipids are characteristically archaeol and caldarchaeol compounds in equal amounts. Isolated from marine sediments at the Guaymas Basin hydrothermal vent site (2000 m depth) in the Gulf of California. Type strain TY^T has been deposited in the DSMZ–German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, as strain DSM 10597^T and in the Japanese Collection of Microorganisms as JCM 10137^T.

Description of *Thermococcus guaymasensis* sp. nov.

Thermococcus guaymasensis (gua.i.masi'ensis L. gen. n. *guaymasensis* named after the Guaymas Basin, the name of the deep-sea site where the species was isolated).

Coccoid archaeon with cell diameters of 1.0–3.0 µm. Cells are Gram-positive, non-motile and occur mainly as single cells, but also as diploid forms. Colonies are circular, convex and pale grey in colour, and colony diameters range from 0.5 to 1.0 mm after 48–72 h incubation at 70 °C under strictly anoxic conditions. Chemo-organotrophic and strictly anaerobic. Utilizes preferably proteinaceous substrates such as yeast extract and trypticase in the presence of 0.5–1.0% (w/v) elemental sulphur. Casein, dextrose, maltose and starch are also utilized. The end products of metabolism on medium containing yeast extract plus trypticase and sulphur are acetate, propionate, isobutyrate, isovalerate, CO₂ and H₂S, whereas, after growth on dextrose or maltose plus sulphur, only acetate, CO₂ and H₂S are formed. The pH range for growth is 5.6–8.1, with the optimum at 7.2. Grows between 56 and 90 °C, with no growth occurring at 95 °C. The optimum temperature for growth is 88 °C. The NaCl range for growth is 1.0–5.0% (w/v), with the optimum at 3.0%. The G+C content of the DNA is 46.0 mol%. Lipids are mainly represented by one phospholipid and some minor components; core lipids are characteristically archaeol and caldarchaeol compounds in equal amounts with traces of tricyclized caldarchaeol. Isolated from marine sediments at the Guaymas Basin hydrothermal vent site (2000 m depth) in the Gulf of California. Type strain TYS^T has been deposited in the DSMZ–German Collection of Microorganisms and Cell cultures, Braunschweig, Germany, as strain DSM 11113^T and in the Japan Collection of Microorganisms as JCM 10136^T.

ACKNOWLEDGEMENTS

We thank Jutta Burghardt and Cathrin Spröer of DSMZ for DNA hybridization and 16S rDNA analyses, respectively. We also thank Eduardo Pagnotta of ICMIB (Arco Felice, Italy) for the lipid analyses. We are also grateful to J. Wiegel for revising the manuscript. This work was supported by EC grant on 'Extremophiles as Cell Factories', ERBBIO4CT960488 to G. Antranikian and '60%' and '40%' grants from the Italian Ministry of University and Scientific Research to F. Canganella.

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