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Isolation and characterization of *Thermus thermophilus* Gy1211 from a deep-sea hydrothermal vent

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Abstract We examined a single, non-spore-forming, aerobic, thermophilic strain that was isolated from a deep-sea hydrothermal vent in the Guaymas Basin at a depth of 2000 m and initially placed in a phenetic group with *Thermus scotoductus* (X-1). We identified this deep-sea isolate as a new strain belonging to *Thermus thermophilus* using several parameters. DNA–DNA hybridization under stringent conditions showed 74% similarity between the deep-sea isolate and *T. thermophilus* HB-8^T (T = type strain). Phenotypic characteristics, such as the utilization of carbon sources, hydrolysis of different compounds, and antibiotic sensitivity were identical in the two strains. The polar lipids composition showed that strain Gy1211 belonged to the genus *Thermus*. The fatty acids composition indicated that this strain was related to the marine *T. thermophilus* strain isolated from the Azores. The new isolate *T. thermophilus* strain Gy1211 grew optimally at 75°C, pH 8.0, and 2% NaCl. A hydrostatic pressure of 20 MPa, similar to the in situ hydrostatic pressure of the deep-sea vent from which the strain was isolated, had no effect on growth. Strain HB-8^T, however, showed slower growth under these conditions.

Key words *Thermus thermophilus* · Thermophiles · Hydrothermal vents · Taxonomy · Marine

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Introduction

Most strains of *Thermus* have been isolated from inland, continental hot springs that are neutral or alkaline and have very low salinity, but strains of this genus have also been isolated from shallow water marine hot springs in Iceland (Hjörleifsdóttir et al. 1989; Kristjánsson et al. 1986), the Azores (Manaia and da Costa 1991), and Fiji (Hudson et al. 1986). These springs normally occur on the seashore or at shallow depths where the vent water is mixed with seawater. *Thermus* strains isolated from these areas have been compared to strains isolated from nonsaline hot springs and shown to be much more halotolerent than most of the strains originating from terrestrial hot springs. The strains have been extensively characterized and identified as *Thermus thermophilus.* This species was originally isolated in Japan (Oshima and Imahori 1971) and validly described (Oshima and Imahori 1974), although the name *T. thermophilus* was not included in the Approved List of Bacterial Names (Skerman et al. 1980). This species was only recently validated (Manaia et al. 1994; Williams et al. 1995) and includes strains "*Thermus flavus*" AT-62 (Saiki et al. 1972) and "*Thermus caldophilus*" GK-24 (Taguchi et al. 1982). Interestingly, the terrestrial hot springs where *T. thermophilus* species have been isolated are all located close to the sea and are described as saline, except for one spring in Iceland where the salt concentration is unknown (Manaia et al. 1994).

Recently, we isolated aerobic nonsporulating heterotrophic bacteria from deep-sea hydrothermal vents in Guaymas Basin (Gulf of California at 2000 m depth), and made a preliminary characterization (Marteinsson et al. 1995). In this article, we characterize *T. thermophilus* strain Gy1211, isolated from a deep-sea hydrothermal vent at a depth 2000 m. This is the first description of *Thermus thermophilus* isolated from this unique environment.

Materials and methods

Isolates and reference strains

Samples were collected by the manned submersible *Nautile* during the cruise "Guaynaut" in 1991. Enrichments of samples for aerobic microorganisms were performed as described by Marteinsson et al. (1995). The reference strain *Thermus thermophilus* HB-8^T (DSM 579)^T was obtained from the Deutsche Sammlung von Mikroorganismen, Braunschweig-Stöckheim, Germany. The following reference strains were used for fatty acid analysis: *T. thermophilus* HB-8^T, AT-62 (= ATCC 33923), GK-24 (kindly donated by R.A.D. Williams, London, UK), B (= NICIMB 11247), Fiji3 A1 (kindly donated by H. Morgan, Hamilton, New Zealand), and the strains designated PRQ-21, PRQ-23, and PRQ-25 (Manaia et al. 1994).

Media and culture conditions

The bacteria were routinely cultured on medium 162 (Degryse et al. 1978) containing 2% (w/v) agar and 1% (w/v) NaCl (designated 162-1) (Alfredsson et al. 1988), but other concentrations of NaCl were used when appropriate. Strains were grown at several temperatures, pH values, and NaCl concentrations to determine their basic physiological characteristics. Growth on single carbon sources was tested on minimal-agar plates inoculated with automatic multipoint inoculator (Mast sampler; Mast, Liverpool, UK) as previously described (Marteinsson et al. 1995). All growth tests were performed at 65°C. Growth on agar plates was scored positive if clearly visible and confluent growth had occurred after 7 days incubation. In liquid medium, growth was followed by measuring the increase of OD_{600} in a spectrophotometer (Spectronic 20D; Milton Roy, Rochester, NY, USA), Growth rates were calculated from points along the logarithmic portion of the resulting growth curves, using linear regression analysis. Hydrolysis of gelatine, starch, casein, and hippurate were performed as described by Marteinsson et al. (1995). Arbutin and aesculin degradation were determined on 162-1 agar medium. These media were supplemented with 0.5% (w/v) filter-sterilized arbutin (Sigma) and $5\,mg^{-1}$ ammonium ferric citrate to detect arbutin degradation. Aesculin, 0.01% (w/v) (Merck) and $5\,mg\,l^{-1}$ ferric citrate were added into the medium to examine aesculin degradation.

Antibiotic sensitivities

Antibiotic sensitivities were tested with disks (Bio Mérieux, Charbonnières-les-Bains, France). Exponentially growing cells were spread uniformly on the surface of agar plates and the disks were then applied automatically with a dispenser. Diameters of growth inhibition were measured after 24 h incubation at 65°C.

Electron microscopy

The cell were grown overnight on agar, scraped off, and fixed for 1 h in minimal medium containing 3% (w/v) glutaraldehyde, and postfixed for 2 h with (w/v) OsO_4 in the same solution. After dehydration with increasing ethanol concentration, cells were embedded in Spurr's resin, thin sectioned, contrasted with 1% (w/v) uranyl acetate and 1% (w/v) lead citrate (EM grade; Bio-Rad S.A., Ivry, France), and examined with a Phillips model EM201 transmission electron microscope (Eindhoven, The Netherlands).

High-pressure cultures

Cells cultured under hydrostatic pressure were grown in 162-1 medium buffered with 20 mM PIPES buffer (Sigma) instead of the usual phosphate buffer. Cultivation was performed in sterile plastic syringes that were sealed by plunging needles into rubber stoppers before the inoculated medium was dispensed in the syringes. To supply oxygen to the cultures, fluorinert (F.C.72; 3M, Paris, France) was saturated with the gas and added to the cultures (25% of total volume) (Kato et al. 1995). Finally, the pistons were put in place and the gas phase was expelled, before tightening the seal on each syringe. The syringes were then transferred into the high-pressure and temperature incubation system (custom-built by Top Industrie S.A.; Zac "Le Plateau de Bière," rue Ampère, BP 95, France). The system consists of four stainless steel pressure vessel incubators that are heated in four vertically positioned ovens (Marteinsson et al. 1997). Bacterial densities were determined by measuring the turbidity at 600 nm using a spectrophotometer (Spectronic 20 D; Milton Roy). All cultures were performed in duplicate.

Polar lipid and fatty acid composition

Cultures for polar lipid analysis were grown in 1-l erlenmeyer flasks containing 200 ml of *Thermus* medium (Williams and da Costa 1992) at 70°C in a reciprocal water bath shaker until the late exponential phase of growth. Harvesting of the cultures, extraction of the lipids, and two-dimensional thin-layer chromatography (TLC) were performed as previously described (Donato et al. 1990).

The strains for fatty acids analysis were grown in *Thermus* medium solidified with 2.0% agar (Difco), spread on the plates as recommended by the Microbial ID (MIDI, Newark, DE, USA), and incubated in sealed plastic bags in a water bath at 70°C for 24 h. Fatty acid methyl esters (FAMEs) were obtained from fresh wet biomass by saponification, methylation, and extraction as described previously (Kuykendall et al. 1988). The FAMEs were separated using a Hewlett-Packard model 5890 gas chromatograph (Palo Alto, USA) with a flame ionization detector fitted with a 5% phenyl-methyl silicone capillary column (0.2 mm × 25 m; Hewlett-Packard). The carrier gas was high-purity H_2; the column head pressure was 60 kP; septum purge was $5\,ml^{-1}$; column split ratio was 55:1; and the injection port temperature was 300°C. The temperature of the oven was programmed from 170° to 270°C at 5°C min^{-1}. The identification and quantification of the FAMEs, as well as the numerical analysis of the fatty acid profiles, were

performed using the standard MIS Library Generation Software (Microbial ID).

DNA base composition

The G + C content of the DNA was determined from the melting point in 0.1 × SSC according to Marmur and Doty (1962) using *Escherichia coli* DNA (57% G + C; Sigma) as the standard.

DNA–DNA hybridization

DNA concentration and purity were estimated using a spectrophotometer with a Genequant II (Pharmacia Upsala, Sweden). The DNA probes were labeled using a Nick Translation kit (Amersham, Buckinghamshire, England). The nuclease S1 method for quantitative DNA–DNA hybridization was carried out as described by Popoff and Coynault (1980) using DE8 filters (Whatman, Madistone, UK) and a scintillation counter (Kontron, Mogtigny le Bretonneux, France). The hybridizations were performed under stringent conditions during 24 h in 0.42 M NaCl.

Results and discussion

Isolation and morphology

A *Thermus*-like strain, Gy1211, was isolated in 1995, as described by Marteinsson et al. (1995). Eight new deep-sea isolates were isolated from the same sample source in this study. The strain Gy1211 was used here as representative strain for the deep-sea isolates. All the deep-sea isolates formed pleomorphic rods, about 0.5–1.0 μm in diameter and about 5–10 μm long. Spores were never observed and long filaments were observed in liquid cultures. Both vesicular and aggregate type of rotund bodies, as described for *Thermus* strains by Brock and Freeze (1969) and Becker and Starzyk (1984), were often observed. Thin section showed that cells of strain Gy1211 had an envelope consisting of three layers.

Polar lipid and fatty acid composition

The polar lipid composition of the strains of the species of *Thermus*, with the exception of *T. scotoductus* X-1 (colony type t1), is composed of one major phospholipid, designated phospholipid-2, and one major glycolipid, designated glycolipid-1 (Donato et al. 1990; Tenreiro et al. 1995). The polar lipid composition of strain Gy1211 and the eight new deep-sea isolates clearly identifies these organisms as strains of the genus *Thermus* (results not shown). The polar lipids of strain Gy1211 and *T. thermophilus* HB-8^T were composed of one major glycolipid and one major phospholipid; a minor glycolipid and a minor phospholipid were also detected by two-dimensional TLC.

The fatty acid composition of the strains of the genus *Thermus* is very variable and cannot be used to identify any particular species, except for *T. aquaticus* and *T. oshimai* (Nobre et al. 1996). The fatty acid composition of the strains of *T. thermophilus* is particularly variable; however, the fatty acid composition of strain Gy1211 is identical to strain PRQ-21, PRQ-23, and PRQ-25, isolated from marine hot springs on the island of Sao Miguel, Azores, which have been classified as *T. thermophilus* (Manaia et al. 1994; Nobre et al. 1996). The fatty acid composition indicates that the deep-sea strain Gy1211 is probably closely related to the Azorean strains.

Physiological properties

The strain Gy1211 grew on minimal medium plates with several single compounds added as sole carbon and energy source. The growth pattern on these single carbon sources was identical for the strain and *T. thermophilus* HB-8^T. Both strains grew on galactose, glucose, maltose, sucrose, phenylalanine, arginine, casamino acids, proline, serine, acetate, butyrate, glutamate, and pyruvate. Neither strain grew on lactose, starch, glycerol, malate, alanine, aspargine, glutamine, leucine, ornithine, threonine, valine, aspartate, citrate, fumarate, gluconate, succinate, gelatine, or α-ketoglutarate.

Antibiotic sensitivity was the same for the strains Gy1211 and HB-8^T. Both organisms were sensitive to ampicillin (10 μg), erythromycin (15 μg), gentamycin (10 μg), kanamycin (30 μg), penicillin G (10 U), tetracyclin (30 μg), vancomycin (30 μg), virginamycin (15 μg) streptomycin (10 μg), nalidixic acid (30 μg), lincomycin (15 μg), and chloramphenicol (30 μg). Resistance to polymyxin-B (300 U), rifampicin (30 μg), sulfamides-G (250 μg), colistin (10 μg), and fusidic acid (10 μg) was exhibited by the strains. Both strains were unable to hydrolyze casein, arbutin, and hippurate. Hydrolysis of aesculin was observed in both strains.

Temperature range for growth and effects of pH, salt concentration, and pressure

The deep-sea isolate Gy1211 and *T. thermophilus* HB-8^T grew at temperatures between 50° and 80°C, and their optimum temperatures were about 75° and 70°C, respectively (Fig. 1A). *T. thermophilus* Gy1211 and *T. thermophilus* HB-8^T grew at pH values between 6 and 9, with pH optimum of 8.0. Both strains were able to grow without NaCl. Strain Gy1211 grew slowly if 4% (w/v) NaCl was added to the medium, but strain HB-8^T did not grow at this salt concentration. Compared to *T. thermophilus* HB-8^T, higher salt concentrations (2% NaCl) were required for the optimal growth of strain Gy1211 (Fig. 1B).

The phenotypic diversity of the yellow and nonpigmented "high-temperature" *Thermus* strains has not been useful in defining taxonomic groups (Hudson et al. 1989; Williams 1989). However, it has been shown by DNA–DNA hybridizations and by 16 S rRNA gene sequencing (Saul et al. 1993) that strains able to grow in 3% NaCl

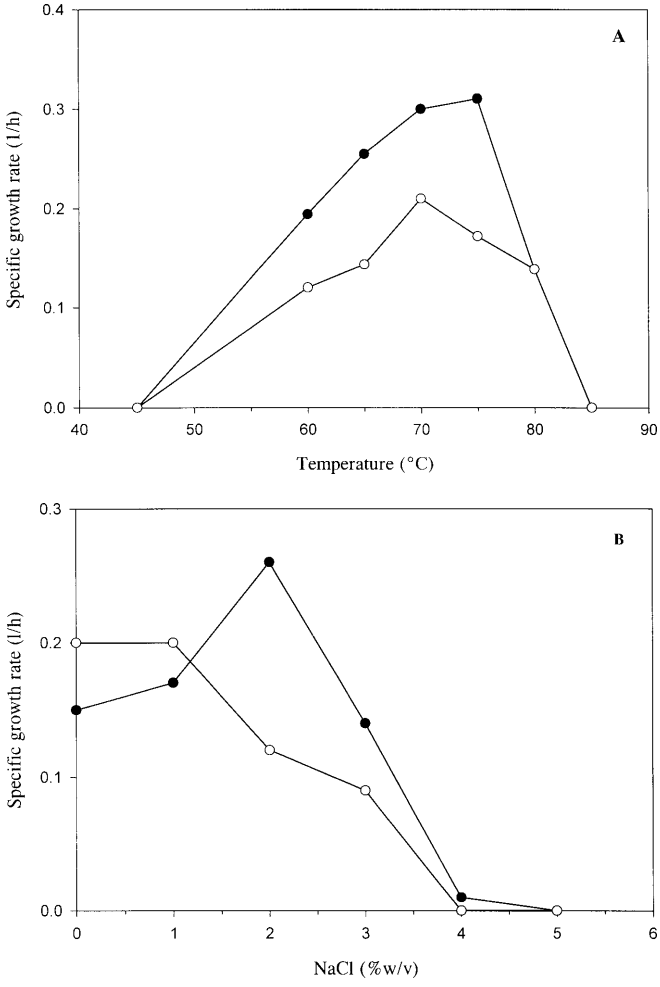

Fig. 1A,B. Effects of temperature and NaCl on the growth of the new deep-sea isolate Gy1211 and *Thermus thermophilus* HB-8^T. **A** Temperature range for growth of Gy1211 (*solid circles*) and *T. thermophilus* HB-8^T (*open circles*) in 162-1 medium at pH 7.0. **B** Effect of NaCl on growth of Gy1211 (*solid circles*) and *T. thermophilus* HB-8^T (*open circles*) at 75°C

and at 80°–85°C belong to *T. thermophilus*. The result of DNA–DNA hybridization studies showed that the halotolerant strains, isolated from shallow marine hot springs in the Azores, and the terrestrial Azorean strain RQ-1 belonged to the species *T. thermophilus*, and that this species constitutes a single DNA homology group with *"T. flavus"* AT-62, *"T. caldophilus"* GK-24, and *Thermus* strain B, among other strains (Manaia et al. 1994). Both these characteristics are very rare in terrestrial *Thermus* isolates, but always appear to occur together and are found in all marine isolates examined (Manaia and da Costa 1991).

Strain Gy1211 and *T. thermophilus* HB-8^T were able to grow at 3 and 200 atm, respectively. The optical density at 600 nm after 19 h of growth at 75°C was 0.3. Unlike strain Gy1211, *T. thermophilus* HB-8^T had a slower growth rate (1.6 times) under a hydrostatic pressure of 200 atm.

DNA base composition and DNA–DNA hybridization

The G + C content of DNA was determined to be 63.7 mol% and 62.9 mol% for Gy1211 and *T. thermophilus* HB-8^T, respectively. The strains showed 74% similarity under stringent DNA–DNA hybridization conditions. Strain Gy1211, as labeled DNA probe, showed 75% homology to *T. thermophilus* HB-8^T; 73% homology was found with *T. thermophilus* HB-8^T as the labeled DNA probe.

Conclusion

Thermus thermophilus strains are known to occur in widely separated areas, including Icelandic terrestrial and marine hot springs (Kristjánsson et al. 1986), Sao Miguel in the Azores (Manaia and da Costa 1991), and Fiji (Hudson et al. 1986). Our results show that *T. thermophilus* is also present in deep-sea hydrothermal vent environments, and we confirm previous results indicating that *T. thermophilus* is widely distributed in thermal areas around the world.

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