

Isolation of rapidly growing *Thermococcus* sp. TS2

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The environments of deep-sea hydrothermal vents are considered to be similar to those at the era when the origin of life emerged on the earth. At the early stage of evolution of life, microorganisms are thought to frequently fuse to exchange genetic substances. Recently, *Thermococcus coalescens* was isolated from a hot water from a hydrothermal vent of Suiyo Seamount located in the Izu-Bonin Arc. This species is devoid of cell wall at 8.5 h of growth and performs cell fusion at room temperature in the presence of DNA-intercalating dye. Cell fusion during growth at 87 C was strongly suggested from the appearance of peculiar large cells similar to those generated by the fusion at room temperature. From this characteristic, lateral gene transfer via cell fusion was postulated. The same microbial source contained *Thermococcus* sp. TS2 that could grow in a growth medium for *Thermotoga*, in which *T. coalescens* cannot grow. This species is interesting as a reference to *T. coalescens* in that to what extent possible lateral gene transfer might have occurred between these species. In the present study, we characterized isolate TS2 especially in comparison with *T. coalescens*.

Isolate TS2 was isolated by the dilution-to-extinction method and then by single colony isolation with 0.8% Gelrite plates containing the *Thermotoga* medium. The cells were 0.7 to 2 μm in diameter. Transmission electron microscopy (TEM) of 8 h-grown cells revealed that the cells possessed a thin (4 nm) but electron-dense cell envelope. This type of cell envelope is similar to that of 16 h-grown *T. coalescens* cells that cannot perform cell fusion. We have never observed fusion of isolate TS2, being consistent with the cell envelope type. TEM also showed that isolate TS2 has dense particles at the late growth stage similar to *T. coalescens* and *Thermococcus celer*. In the latter two species, the occurrence of dense particles is accompanied by the death of the cells. However, isolate TS2 remained alive with dense particles, showing rather normal growth curve. The cells grew at 60 to 90 C, pH 5.5 to 8.5, and NaCl concentrations of 2.0 to 4.0%, with the optima at 80 C, pH 7.0, and 3.0% NaCl, respectively. Under the optimal conditions, the cells grow at a growth rate of 3 per hour, the rapidest growth in the genus *Thermococcus*. The isolate was an anaerobic chemoorganotroph capable of growing on yeast extract or tryptone as the sole growth substrate. The G+C content of the genomic DNA was 54.6mol%. Phylogenetic analysis based on 16S rRNA gene sequence indicated that the isolate was most closely related to *Thermococcus siculi*, *T. coalescens*, and *T. celer*. However, no significant hybridization was observed between TS2 and the others in genomic DNA-DNA hybridization. Therefore, it is proposed that the isolate be described as a novel species.