Short Communication

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ISOLATION OF AN EXTREME THERMOPHILE AND THERMOSTABILITY OF ITS TRANSFER RIBONUCLEIC ACID AND RIBOSOMES

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An extreme thermophile has been isolated from a Japanese thermal spa. To elucidate the molecular basis of thermophily, chemical and physical natures of the cell constituents of the microorganism are under investigation. In this communication the bacterial properties of the thermophile and the heat stability of t-RNA and ribosomes will be described.

Aliquots (about 2 ml) of thermal water sampled from a hot spring at Mine Hot Spring, Shizuoka, Japan, were transferred into polypeptone-yeast extract-sodium chloride media (final 8, 4, and 3%) pH 7.0, and incubated at 75°. The thermophiles developed in the media were transplanted into fresh culture media. After several transplantations, isolation was carried out on agar plates. Among thermophilic isolates, one strain numbered HB8 has been investigated in detail. The cells are gram negative, yellow pigmented, and non-sporulating rods, $3.0 \times 0.5 \mu$ (Fig. 1). The yellow pigments produced were not soluble in the medium. The absorption spectrum of the pigments extracted with 90% acetone shows the maximum at 453 nm with shoulders at 473 nm and 430 nm, and suggests carotenoid compounds in nature as illustrated in Fig. 2. When glucose was added to the cell suspension at 75°, an acid but no gas was produced. The organism is aerobic and strictly thermophilic. Under vigorous aeration, the optimum temperature for the growth was observed to be 65 to 72° at the rate of 20 min per generation. The population of the cells at stationary state was estimated to be 8×10^8 per cm³ using a haematometer. The maximal growth temperature of the organism was 85°. No growth was observed at or below 45° after incubation for two days. The base composition of deoxyribonucleic acid of the microorganism was determined to be 69% in guanine plus cytosine content. From these properties, the thermophilic bacterium was tentatively identified as a new species of the

Fig. 1. Electron micrograph of *Flavobacterium thermophilum* HB8.

The picture was taken using a Hitachi scanning electron microscope, Model HSM-2, at Application Laboratory, Naka Works, Hitachi Ltd.

Fig. 2. Absorption spectrum of acetone extract of *Flavobacterium thermophilum* cells. The cells (0.15 g in wet weight) washed with 0.8% sodium chloride were suspended in 3 ml of 90% (v/v) acetone. The absorption spectrum of the yellow supernatant was recorded using a Beckmann DK-IIA Ratio Recording Spectrophotometer with 10-mm cuvettes.

genus *Flavobacterium* (1) and named *F. thermophilum* HB8 (sp. n.).

Ribosomes were extracted and purified from the cell extract by the method described by TAKANAMI (2). Ultracentrifugal analysis demonstrated that the ribosome preparation consisted of four components whose sedimentation coefficients were 30S, 50S, 70S and 100S, in the presence of 0.01 M magnesium ion. The ribosomes were dissociated into two subunits in the absence of magnesium ion and their sedimentation coefficients were 30S and 50S. These results indicate that the subunit structure of the thermophile ribosomes is similar to those of mesophilic procaryotes. This consideration was also supported by the fact that the thermophile was sensitive to chloramphenicol and tetracycline. The thermal denaturation profile of *F. thermophilum* ribosomes in the presence of 0.01 M Mg^{2+} was recorded by using a Gilford spectrophotometer (Model 240) equipped with a temperature readout assembly. The results are shown in Fig. 3 in comparison with those of *E. coli* ribosomes. From the figure, the melting temperature (T_m) of the thermophile ribosomes was determined to be 86° which is 15° higher than that of *E. coli* ribosomes.

Transfer RNA was purified according to literatures (3–5). The final preparation was ultracentrifugally homogeneous with a sedimentation coefficient of 4S. The temperature-260 nm absorbancy profile in the presence of 0.01 M Mg^{2+} is given in Fig. 4. The thermophile t-RNA had T_m of 87.5° which is about 9° higher than that of *E. coli* t-RNA.

Ribosomes and t-RNA of *F. thermophilum* HB8 are remarkably heat stable compared to *E. coli* counterparts. Since the melting temperatures of the ribonucleic acids and the nucleoproteins are close to the maximal growth temperature of the bacterium, it might be possible to speculate that they closely correlate to the limiting factors in thermal death. PACE and CAMPBELL (6) have reported a positive correlation between the maximal growth temperature and T_m of ribosomes of bacteria. The present results are quite consistent with their report. In contrast, it has been reported that t-RNA from moderate thermophiles belonging to *Bacillus stearothermophilus* shows similar heat stability to *E. coli* t-RNA (7–9). It was observed that *E. coli* t-RNA could be replaced by the thermostable t-RNA from *F. thermophilum* in the methionyl-t-RNA formation reaction catalyzed by *E. coli* methionyl-t-RNA synthetase (EC 6.1.1.10), suggesting the common structure around the interaction site to the enzyme (Unpublished data). Detailed comparison of structure of both the thermophilic and mesophilic t-RNAs would be a promising approach for studying the molecular requirements for synthetase-t-RNA interaction. Circular dichroic study of the thermophilic t-RNA suggests that the guanine-cytosine base pairs are richer in the *F. thermophilum* t-RNA than in the *E. coli* t-RNA (10). This could account for the higher thermostability of the thermophile t-RNA.

BROCK and FREEZE (11) have recently reported the isolation of extreme thermophiles from thermal waters in the United States. *Thermus aquaticus* YT-1 (gen. n and sp. n.), from Mushroom Spring in Yellowstone

Fig. 3. Heat stability of *F. thermophilum* ribosomes.

— *F. thermophilum*, ---- *E. coli*.

The buffer solution used was 0.05 M Tris-HCl, pH 7.5, containing 0.05 M ammonium chloride, 0.001 M dithiothreitol and 0.01 M magnesium acetate.

Fig. 4. Heat stability of *F. thermophilum* t-RNA.

— *F. thermophilum*, ---- *E. coli*.

The buffer solution used was 0.05 M Tris-HCl, pH 7.5, containing 0.2 M sodium chloride and 0.01 M magnesium acetate.

National Park, closely resembles to the present organism in both bacterial properties and thermal stabilities of ribosomes and t-RNA (12). It will be of particular interest to compare the molecular structures of the cell constituents of *F. thermophilum* with those of *T. aquaticus* in order to elucidate molecular alterations during evolution in aquatic thermal environments.

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