Distribution of *Thermus* spp. in Icelandic Hot Springs and a Thermal Gradient

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The growth range in nature of bacteria belonging to the genus *Thermus* was investigated by sampling 55 different hot springs in Iceland. The springs ranged in temperature from 32 to 99°C, and in pH from 2.1 to 10.1. Viable counts of *Thermus* spp. ranging from 10 to 10^6 CFU/100 ml of spring water were found in 27 of the springs sampled. The temperature range for these bacteria was found to be 55 to 85°C, and the pH range was from about 6.5 to above 10. *Thermus* spp. were found in springs containing up to 1 mM dissolved sulfide and having conductivity up to 2,000 μS/cm. The distribution of *Thermus* spp. in a hot spring thermal gradient was also investigated and found to agree well with the overall distribution in individual springs.

Bacteria belonging to the genus *Thermus* are thermophilic, aerobic heterotrophs which have been extensively studied in recent years (5, 6, 15, 16, 18, 20, 23, 29). *Thermus* strains have been isolated from various thermal sources, including hot springs (9, 16, 29), laundry heaters (6), hot tap water (22), and thermally polluted rivers (5, 15). The main emphasis of previous work has been on the physiology and taxonomy of these bacteria (15, 18, 21), and relatively little work has been done on the ecology of the genus *Thermus* (25, 28). Considerable attention has been given to the ecology of chemoo- and photoautotrophic microorganisms found in hot springs (5, 13), but less to the heterotrophs. Several other heterotrophic caldoactive (i.e., extremely thermophilic) bacteria, both aerobic and anaerobic, have been isolated from hot springs around the world (8, 10, 17, 19, 30), but few have been studied as much as the *Thermus*-type bacteria (5, 15, 16, 21, 29).

In this paper, we report the first systematic study of the temperature and pH range for *Thermus* spp. in natural hot springs. The distribution of *Thermus* spp. in a hot spring thermal gradient was also studied.

MATERIALS AND METHODS

Study sites. The areas chosen for this study were Krisuvik, the Hveragerdi-Hengill area, and the Bis-kupstungur-Geysir area, all in southern Iceland. These areas are all located within a 2-h driving distance of the laboratory (in Reykjavik) and have also been extensively studied by others (2, 5, 11, 12, 14, 19, 23, 27-30). In the Krisuvik area, the springs are mostly acidic and with high sulfide content, whereas in the other two areas, the springs are neutral to alkaline and with low sulfide content. Samples were only collected from well-separated springs and taken close to the source to exclude the possibility that the presence of *Thermus* spp. could be due to contamination from other springs.

Hot spring thermal gradient. One spring was chosen in which to study the distribution of *Thermus* spp. along a thermal gradient. This spring, designated HV-11, is located in the Graendalur valley above the village of Hveragerdi, about 1.5 km from the mouth of the valley and on the west side. It had a pH of 9.6 and a steady source temperature of 98°C. The effluent formed a narrow stream with a flow speed of about 0.25 m/s. The total flow from this spring was about 0.4 liters/s. The stream runs down a steep hill about 40 m into a small river. Along this distance a steady thermal gradient from 98 to 30°C was established. The distribution profiles of sulfide and pigment content per unit area in the thermal gradient were also determined and are shown in Fig. 2.

Sampling and water analysis. Water samples for viable counts, pH, and conductivity measurements were taken in sterile 250-ml screwcap bottles. The bottles were filled completely and then closed tightly to prevent the loss of dissolved gases. The water samples were brought back to the laboratory and analyzed within 24 h. Water samples (1, 2, and 10 ml) were filtered on sterile 0.45-μm Millipore membrane filters. The filter was then transferred to an agar plate and incubated at 72°C for 2 days before the colonies were counted. The filter method was compared with the plate method, using pure cultures of several strains, and was found to give very similar results. The culture medium used was medium 162 of Degryse et al. (15), containing 0.3% yeast extract (Oxoid), 0.3% tryptone (Oxoid), and 3% agar. The pH was measured with a Radiometer pH meter (model PHM 71) and the conductivity with a Schott Geräte digital conductivity meter (model CG 857). Temperature was measured in situ with a mercury bulb thermometer or with a Sika digital thermometer (model TT7070). For sulfide analysis, 15-ml water samples were taken in the field and mixed with 2 ml of 20% zinc acetate, pH 5.5. Within 24
h, the ZnS precipitate was redissolved and titrated with \( I_2 \). (1).

**Identification of bacteria.** The medium currently in use for isolation (6, 9, 26) and enumeration (16, 28) of *Thermus* spp. is a dilute nutrient medium (containing 0.1 to 0.3% yeast extract and 0.1 to 0.3% tryptone) and therefore cannot be expected to be very selective. Also, no rapid identification scheme has yet been developed for the genus *Thermus*. In this study, *Thermus* spp. were identified by the criteria described by Brock and Freeze (9) and Degryse et al. (15). Frequent checks were done such that all colony types on one plate were picked, purified by streaking, and tested for the following characters (reaction for *Thermus* spp. in parentheses): Gram reaction (−); carotenoid pigments (>90% +); colony morphology (on a filter 2 to 4 mm in diameter, low convex with a smooth surface); flagellation (−); pellicle in liquid culture (+); presence of spores (−); anaerobic growth (−); oxidase (+); cell morphology (on plates at 72°C, thin rods a few micrometers long; in liquid at 75°C, long filaments). According to this scheme, over 95% of the colonies on the filters belonged to the genus *Thermus*. Aberrant colonies were mainly sporeformers or had different cell morphology (unpublished data).

**Determination of pigments in cores.** Cores of the undermat of spring HV-11 were taken with sharp-edged glass tubes having an internal diameter of 14 mm. The tubes had a small hole at the distal end to prevent pressure buildup. The core could be easily removed by closing the hole with a finger. In the laboratory, the organic fraction was removed from the silt, homogenized in a tissue grinder, and finally extracted with acetone as described previously by Brock and Brock (7). The chlorophyll and carotenoid content of the core was then estimated by measuring the absorbance at 662 and 478 nm, respectively. Absorbance was measured with a Hitachi spectrophotometer (model UV/VIS 100-20).

## RESULTS

**Temperature and pH range.** The 55 hot springs sampled represented a random selection from the areas studied. Results of pH measurements showed two clusters, i.e., acidic and alkaline; springs with a pH of 4 to 6 were rare (4). Temperature and pH values for each spring or effluent channel are shown in Fig. 1. In the case of effluent channels, the temperature was determined exactly at the point where the water samples were collected. The low-temperature springs from which *Thermus* spp. were not isolated all contained considerable algal growth in the source pools. The absence of *Thermus* spp. from those springs should therefore not have been due to lack of substrate. In this study, the temperature range found for the *Thermus* spp. was 55 to 85°C. The lower pH limit found was 6.5, but the higher pH limit could not be determined. The highest pH measured was 10.1 in a spring in the Geysir area, and the spring was rich in this bacterial type. The springs that harbored *Thermus* spp. contained 10 to 10⁶ cells per 100 ml. Culture-negative springs were sampled several times.

The water from *Thermus*-containing springs had a conductivity of 200 to 2,000 μS/cm, and five (19%) of those springs had sulfide concentrations between 0.1 and 1 mM.

**Distribution in a thermal gradient.** To study further the ecology of *Thermus* spp., we investigated its distribution in a typical hot spring thermal gradient. The main characteristics of this gradient are shown in Fig. 2. Over the first 16 m, the water flowed evenly in a single channel which resulted in exponential cooling. After about 18 m, the flow spread out and trickled over gravel, producing more accelerated cooling. The growth in the channel was typical of an Icelandic, sulfide-containing, alkaline spring (12, 14). In the first 5 m (section I) of the channel, no visible growth was observed. At 6 to 7 m (section II), a thin greyish bacterial layer was seen,
consisting mostly of rods and short filaments. At 8 to 10 m and down to 16 m (section III), Chloroflexus filaments formed thick orange streamers as demonstrated by the high carotenoid content of the cores (Fig. 2) (12). Underneath the orange layer was a thin green Mastigocladus layer. From 18 m and below (section IV), the mat was pale green to yellow and consisted of Mastigocladus laminosus and Phormidium spp. (24). As indicated by the pigment content, the amount of algal-bacterial biomass per unit area increased with decreasing temperature (3). The distribution of Thermus spp. over this gradient is shown in Fig. 3.

Thermus spp. could be easily isolated from the algal mat that covered rocks in the splash zone above the boiling water source. This explains the counts of Thermus spp. occasionally found in the 95°C hot effluent water at the top of the gradient. At about 85°C, the viable count increased abruptly and reached a maximum at 75°C. The viable count remained high down to about 50°C but declined after that.

**DISCUSSION**

Since the isolation of Thermus aquaticus by Brock and Freeze (9), many different types of caldoactive microorganisms have been isolated (8, 10, 17, 19, 30). Few of these have been studied as much as bacteria belonging to the genus Thermus (5, 15, 21, 23, 29). Thermus spp. are heterotrophic aerobes, which are easy to grow in the laboratory (5, 15) and therefore ideally suited for biochemical research. Variations in optimum growth temperature, pigmentation, and other traits have been reported among Thermus isolates (5, 20, 26). Despite these differences, some workers regard the isolates as sufficiently related to be classified as one species (15). Thermus spp. are ubiquitous and can be isolated from both natural and man-made thermal sources (5, 6, 15, 16, 22, 25, 28, 29). It must be assumed, however, that the natural habitats of Thermus spp. are the geothermal areas of the world. These bacteria have been found both in neutral and alkaline hot springs around the world (9, 16, 20, 25, 26, 29). In some cases, they have been shown to be present in high numbers in the undermat of hot springs and effluent channels (9, 16). Thermus spp. can utilize a variety of organic substrates for growth (15) and may well be the most important heterotrophs in the hot spring ecosystem.

The aim of this investigation was to study the ecology of the genus Thermus in natural hot springs. Our results indicate that Thermus spp. are probably present in considerable numbers in all hot springs with temperatures of 55 to 85°C and pH values higher than 6.5. The temperature range for Thermus spp. found in this ecological study agrees well with published work on the temperature growth range of Thermus strains in laboratory cultures (9, 29). We found, however, that these bacteria are able to grow at considerably higher pHs in nature than in culture (9). All springs within the shaded area in Fig. 1 contained Thermus spp. Some of these Thermus-yielding springs had sulfide concentrations and conductivities as high as 1 mM and 2,000 μS/cm, respectively. Ramaley and Bitzinger (25) did not find Thermus spp. in high-sulfide springs (40 mg of H2S per liter) in Colorado, but our work indicates that Thermus spp. are not very much affected by high concentrations of sulfide or dissolved ions. In situations where the effluents of an alkaline and an acidic spring come together, Thermus spp. can be detected at a lower pH than shown in Fig. 1. Several Thermus strains were isolated in one study by liquid enrichment culture and subsequent plating from a spring with a pH lower than 6 (29). In our study, however, the acidic springs were always devoid of these bacteria, so presumably when found in mixed waters they all originated from the alkaline spring(s).

Our results for the distribution of Thermus spp. in a hot spring thermal gradient agree well with the results of our survey of different springs. It is also in fair agreement with the distribution of Thermus spp. in a man-made thermal gradient (25).

We have divided the thermal gradient into
four sections (Fig. 3) and can speculate about the presence of Thermus spp. in each section. The temperature in section I was probably too high to allow much multiplication of the bacteria, so when found they were probably washed from the algal growth in the splash zone above the source. The sudden rise in the curve in section I suggested that some growth was already occurring at 85°C. This is not so unreasonable since some Thermus strains have been reported to be able to grow at temperatures as high as 85°C (15). The maximum bacterial numbers were found in section II at 70 to 75°C, which is the reported optimum growth temperature for most Thermus strains so far isolated (15, 23, 29). In section II, the algal-bacterial biomass density was low, however, so the bacteria may have been substrate limited. The density of the algal-bacterial biomass (Fig. 2) (3) increased very much in section III, which probably provided for ample substrate, but here the lower temperature may have become growth limiting. This may explain the fact that the viable counts were high and similar in both sections II and III. In section IV, the algal-bacterial biomass density was even higher than in section III, but little growth of Thermus spp. should occur below 50°C (9, 23). That is in agreement with the low viable counts found in the bottom section of the gradient.

In rapidly flowing water as is found in this effluent channel, it is unlikely that the decrease in viable counts in section IV is due to bacterial death. Rather, this may be due to the trapping of free-floating bacteria, brought from the upper sections, in the extensive undermat of section IV in this particular spring. Because of the low temperature the trapped bacteria cannot grow and would slowly die off. The multiplication of the bacteria probably takes place in the algal-bacterial undermat but not in the flowing water. In the growth zone (sections II and III) the bacteria are then constantly being released into the passing water. Since this hot spring ecosystem is in a steady state, the concentration of free-floating bacteria should be in equilibrium with the amount of attached bacteria. The viable count of the water would therefore be a relative measure of the actual distribution of bacteria along this hot spring thermal gradient.

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LITERATURE CITED


DISTRIBUTION OF *THERMUS* SPP. IN HOT SPRINGS


