Types and Distribution of Obligate Thermophilic Bacteria in Man-Made and Natural Thermal Gradients

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The types and distribution of obligate thermophilic bacteria were found to be similar in a thermal gradient resulting from man-made thermal pollution and the thermal gradients of two natural hot springs located in Colorado.

It has been proposed (2) that the presence of a gram-negative, extremely thermophilic bacterium such as *Thermus aquaticus* (5) may be used as an indication of thermal pollution from either natural (13) or man-made causes (6). We have recently had an opportunity to examine the types and distribution of the obligate thermophilic bacteria found in man-made thermal gradients and to compare them with the obligate thermophilic bacteria found in natural hot spring thermal gradients (2).

The Indiana University physical plant releases a constant discharge (2 to 3 liter/min) of slightly alkaline (pH 7.8) hot water (90°C) onto the side of the stream bed of a small stream (Jordan River) that flows through the Indiana University campus at Bloomington, Indiana. This discharge flowed down a partially shaded stream bed for 8 m before it joined the Jordan River during which time the water temperature decreased to 30°C. Thus, a thermal gradient is formed (Fig. 1) similar to that found in the effluent channels of naturally occurring hot springs (1, 7).

The types and distribution pattern of thermophilic bacteria in the thermal gradient were repeatedly investigated during the summer of 1972 by removal of core samples of the microbial mat including sediment and gravel from the indicated locations in the stream bed; procedures to avoid contamination were used. The samples were suspended with four volumes of water collected at the same point and agitated vigorously for 1 min. The debris was allowed to settle for 5 min and 0.1 ml of the liquid was spread onto dilute yeast extract-tryptone agar plates (11). The agar plates were wrapped with commercial food film or placed into plastic food storage bags to avoid drying of the agar. The plates were incubated at 60°C for 48 h. The results presented in Table 1 are expressed as pink colonies (K-2 type isolate) (R. F. Ramaley et al., Int. J. Syst. Bacteriol., accepted for publication), yellow colonies (*T. aquaticus*) (5), cream colonies (*Thermus X-1*) (11) and spore formers (chiefly *Bacillus stearothermophilus*). The identity of representative colonies was established by microscopic observation and physiological properties of the colony in pure culture.

The distribution pattern of the various thermophilic bacterial types was quite stable. Samples removed during the summer of 1972 and one set of samples taken in December 1972 showed the same distribution pattern as shown in Table 1 even though the slope of the winter gradient was steeper due to lowered ambient temperature. At 65 to 70°C and 58°C, the major thermophilic bacteria are *Thermus X-1* and *T. aquaticus*. However, at lower temperatures the K-2 isolate and *B. stearothermophilus* make up a larger proportion of the thermophilic bacterial flora. The shift in population is statistically significant at the 0.05 level (>95% probability) and would be consistent with the lowered optimum growth temperatures of the K-2 isolate (60°C) and *B. stearothermophilus* (55 to 65°C) (for different strains) in comparison to the optimum growth temperature of 70°C for *Thermus X-1* and *T. aquaticus*.

Similar analysis of samples from the thermal gradient of slightly alkaline hot springs at Yellowstone National Park showed only *T. aquaticus* (9) and a low level of *B. stearothermophilus* (10) and other spore-forming bacteria. However, the thermal gradients at Yellowstone National Park receive very high levels of direct sunlight (7, 9) and it was possible that the *Thermus X-1*, which lacks the yellow carotenoid photoprotective pigments of *T. aquaticus*, might be selected against these yellow water mediastinal growth.
FIG. 1. Map of the man-made thermal gradient on the Indiana University campus in Bloomington, Ind. The cut stone bridge shown crosses the Jordan River to the east of Jordan Avenue.

TABLE 1. Number of thermophilic bacteria in sample taken from the man-made thermal gradient shown in Fig. 1*

| Sample station | Temp (C) | No. of thermophilic bacteria per 0.1 ml |
|----------------|---------|---------------------------------------|
|                |         | Yellow                               | Cream | Pink |
|                | Mean    | %                                    |       |
| I              | 65-70       | 12                                   | 19 ± 12| 50  |
| II             | 58       | 74                                   | 27 ± 15| 158 |
| III            | 43       | 38                                   | 14 ± 8 | 86  |
| IV             | 38-40    | 12                                   | 10 ± 6 | 39  |
| V              | 27-31    | 19                                   | 13 ± 10| 31  |

|                | Mean    | %                                    |       |
|                |         | Spore-formers                         |       |
|                | Mean    | %                                    |       |
| I              | 65 ± 14| 5                                    | 6 ± 5 | 3   |
| II             | 63 ± 12| 14                                   | 5 ± 4 | 11  |
| III            | 46 ± 13| 52                                   | 26 ± 9| 20  |
| IV             | 46 ± 10| 30                                   | 28 ± 8| 15  |
| V              | 47 ± 13| 21                                   | 25 ± 6| 12  |

*The standard error of the mean percentage was used to calculate significance levels with the Student t test. The difference in the population distribution between the two high temperature stations I and II and the three lower temperature stations (III to V) is significant at the 0.05 level.

*Mean of five separate samples taken at each sample station.

*The mean percentage and the standard deviation from each of the five separate samples.

already been demonstrated in mixed laboratory cultures of Thermus X-1 and T. aquaticus grown in high light [J. Hixson and R. Ramaley, unpublished observations].

Thus, during July and August 1973, we examined the thermal gradients of natural hot springs in central and northern Colorado that did not receive as much direct sunlight as the thermal gradients in Yellowstone Park for the presence of Thermus X-1. There were two naturally occurring thermal gradients that showed the same type of thermophilic bacteria found in the man-made thermal gradient on the Indiana University campus (Table 2). One of these natural gradients was at Routt Hot Springs, 7 miles north of Steamboat Springs, Colorado. The main springs at this location are on a north-facing slope and receive a reduced level of direct sunlight. The other gradient was at Poncha Springs, outside of Poncha Springs, Colo. This thermal gradient is shaded by the sides of a concrete pool normally used for
bathing. The water from both of these springs is slightly alkaline with no detectable sulfide (8).

The thermal gradient in low sulfide springs such as Waunita Hot Spring (east of Gunnison, Colo.) which contained 0.4 μg of hydrogen sulfide per ml (8) had a considerably altered thermophilic bacterial flora, whereas a high sulfide spring such as Hot Sulfur Springs (Hot Sulfur Springs, Colo.; 40 μg of hydrogen sulfide per ml; [8]) did not yield any isolates of Thermus or B. steaothermophilus.

Thus it appears that the presence of other gram-negative thermophilic bacteria besides T. aquaticus in thermal environments may be more common than originally supposed. Also, given similar types of aquatic thermal environments (pH, light levels, and water chemistry; [4]), the same types of thermophilic bacteria may be cultured from both man-made and natural thermal environments.

The determination of the number of viable thermophilic bacteria by colony formation is presently restricted to those thermophilic bacteria that can be easily cultured at 60 to 70 C in the laboratory. Brock and his associates (1, 2, 4) have demonstrated that there are additional thermophilic bacteria in thermal gradients with higher optimum growth temperatures than the 60 C incubation temperatures used in these studies. However, these bacteria have not yet been maintained in laboratory culture and do not give rise to colonies under our present conditions or at higher incubation temperatures. The rapid colonization of man-made thermal environments (3, 11) far removed from natural thermal areas (8) also indicates the relative environmental abundance of Thermus and similar types of gram-negative thermophilic bacteria.

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