

1978 FIELD STUDIES OF THE PINK BACTERIAL MASSES
WHICH GROW AT 90°C IN THE OUTFLOW CHANNEL
OF SOME YELLOWSTONE HOT SPRINGS

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Objectives

The presence of masses of pink bacteria growing at 90° in the outflow channel in some hot springs in Yellowstone National Park was observed at least as early as 1899 (unpublished work of W. A. Setchell, cited in Brock, 1978, pp. 47-49). When Brock began his extensive microbiological studies of Yellowstone thermal areas in 1965, he also found "pink, gelatinous, stringy bacterial masses" growing at 90°C in the upper end of the runoff channel of Octopus Spring and published phase micrographs of these bacterial masses (Brock, 1978, pp. 45-46). However, Brock and his associates were never able to grow these bacteria in laboratory culture and beyond the determination of the fatty acid composition of the bacterial masses by Bauman and Simmond (1969) no other biochemical studies have been conducted with these extremely thermophilic bacteria.

Our 1978 field studies centered on two objectives: (1) to see if the amount of the bacterial mass could be increased without causing any adverse effect on the natural microbial population or the runoff channel habitat and (2) to find other locations of these bacteria since the runoff channel of Octopus Spring was the major known habitat of these extremely thermophilic bacteria (Brock, 1978).

Procedures

Increasing the amounts of pink bacterial mass was investigated by placement of artificial material in the runoff channel in August of 1977 to act as a support for the growth of the bacterial mass. Sterilized cotton string loops or a single loop with trailing string, 1.5 feet long, was placed across the runoff channel at various points (attached with concrete nails) and left until the following August. We also placed masonite strips in the runoff channel rough side up (similar to those used by Zuber in Icelandic hot spring runoff channels for the growth of the thermophilic algae). String and masonite strips were placed in the runoff channel of Octopus Spring and Twin Butte Vista Spring (located on top of the hill behind Octopus Spring).

Samples for enrichment culture or biochemical analysis were placed in sterile plastic bags iced and transported back to Omaha. Samples taken in 1978 were transported on ice to the Field Station. Samples for enrichment culture were refrigerated and those to be used for future biochemical analysis were frozen. Enrichment cultures were made in liquid tube culture and some samples were also directly streaked out on solid media. Four different

media were used.

1. Standard nutrient broth (e.g., 0.8% nutrient broth, pH 6.5) (for B. stearo., etc.)
2. Castenholz salts medium (0.1% yeast extract, 0.1% tryptone) (Ramaley and Hixson).
3. Octopus Spring medium (0.1% yeast extract, 0.1% tryptone with spring water.
4. High pH Castenholz salts medium (same as #2 but pH of 9.5).

A search for the presence of additional pink bacterial mass growing in the effluent channel of other Yellowstone hot springs was also conducted in August of 1978.

Results

The cotton strings placed in the runoff channel of Octopus Spring were extremely effective as an artificial support for the growth of the pink bacterial masses. The strings were not obtrusive and the concrete nail showed no discoloration of the silica. In fact, if you didn't know that the string had been introduced, you might not notice it at all or think that it was a natural growth. The masonite strips were very visible and since they did not support bacterial growth as well, no further use was made of them.

The bacterial mass was about 95% harvested (to serve as a typical sample of the steady state bacterial mass) and the strings were allowed to remain in the runoff channel for another week (the second harvest represents a selection of the more actively growing bacteria). Analysis of these two harvests is still in progress.

Enrichment cultures of these pink bacterial masses showed a few Thermus type isolates and an apparently new bacteria showing the best growth in high pH Castenholz salts medium (characterization of this bacterium is still incomplete).

The pink bacterial masses growing at 90°C are apparently limited to only a few Yellowstone Hot Springs (Octopus, Butte Vista and Firehole Pool) (the latter two have bacterial masses more color characteristic of the 75-80°C section of the Octopus Spring runoff channel. Thus actually there was really only a single source of the pink bacterial mass.

This was a potential problem because Octopus Spring, while it is not directly visible from the Firehole Loop Road, is accessible to tourists from the Great Fountain Geyser parking lot. Octopus Spring has a nearby hillside covered with rocks and it is obvious that tourists have been dropping those rocks and even boulders into the spring. Thus, it was not impossible that Octopus Spring might eventually be blocked or might even change its temperature naturally as did Mushroom Spring (Brock, 1978, p. 18).

Thus, in August of 1978, I began to search for other sources of the pink bacterial masses and, after considerable effort, was fortunate enough to

find two extensive growths of the pink masses in a most unlikely spot. The masses were found in one of the former tourist areas (Artist's Paint Pot) which is across from Sylvan Spring and south of Norris Geyser Basin (both acidic thermal areas) (Fig. 1).

The two springs in the Artist's Paint Pot area that have the pink bacterial mass are (like Octopus Spring) slightly alkaline (pH 8.5 at 25°C ~ pH 7.0-7.5 at 90°C) and have even more extensive pink bacterial masses than does Octopus Spring.

This area is a very good research area because it is no longer heavily visited by tourists. (The bacterial/algae mat colors are no longer as spectacular as they were a decade ago, due to shifting of thermal activity following the last Yellowstone earthquake). The Park Service has actually taken down the sign on the road that identifies it as the Artist's Paint Pot area.

Samples were taken of each of the pink bacterial masses in the area and these are currently being used for enrichment cultures and biochemical studies. Cultures from these and the Octopus Spring effluent channel pink bacterial masses and additional samples from other locations have been sent to J. J. Perry (University of North Carolina) and delivered to R. S. Wolfe of the University of Illinois.

Thus having an alternative source of the pink bacterial masses for future studies and with the observed rapidity of colonization of the cotton string, the strings were removed in August of 1978.

Conclusions

From thin section electron micrographs (Fig. 2) of these pink bacterial masses growing at 90°C, it is clear that these masses are made up of at least two different gram negative bacteria and since they probably represent new types of extremely thermophilic bacteria, their natural habitat should be carefully protected. This is especially essential since they have not yet been grown in laboratory culture. The 1977-78 field studies showed that it was possible to increase the mass of pink bacteria growing in the upper end of the runoff channel of Octopus Spring by providing a support for the attachment and growth of the bacteria. Use of the support will provide sufficient material for biochemical studies without disturbing the natural bacterial population (attached to silica and rocks in the runoff channel) and the presence of cotton string in the runoff channel is practically undetectable from even a few feet away.

One of the most important results of the 1978 study was the finding of pink bacterial masses in the runoff channel of two of the hot springs in the old Artist Paint Pot area will also facilitate the comparative study of these extremely thermophilic bacteria.

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Figure 2. Thin section electron micrograph of pink bacterial masses growing at 90°C.