

Presence of Thermophilic Bacteria in Laundry and Domestic Hot-Water Heaters

THOMAS D. BROCK AND KATHRYN L. BOYLEN

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 15 September 1972

Thermophilic bacteria resembling *Thermus aquaticus* were isolated from hot water taken from domestic and commercial hot-water tanks. Cold water from the same locations never yielded thermophilic bacteria, suggesting that the bacteria were growing in the tanks. In contrast to the *T. aquaticus* isolates from hot springs, the present isolates were rarely pigmented. In general, the hotter sources more frequently yielded bacteria.

Recent work on the bacterial flora of natural geothermal habitats, such as hot springs, has revealed that a wide variety of bacteria thrive at temperatures of from 70 to 90 C (2, 6, 7). In the present work we report that thermophilic bacteria apparently related to naturally occurring bacteria can be isolated from hot water taken from hot-water heaters and that these organisms are apparently living in these man-made thermal habitats.

Brock and Freeze (9) isolated from hot springs and characterized a new organism, *Thermus aquaticus*, which has an optimal temperature for growth of from 70 to 75 C and a range from 45 to 80 C. The isolates of this organism were gram-negative nonsporulating rods which formed a yellow pigment. The fine structure of *T. aquaticus* has been reported by Brock and Edwards (8), who noted that this organism produced a characteristic spherically shaped structure called a "rotund body" formed as a result of the association of a number of separate cells. In their initial studies Brock and Freeze (9) noted that *T. aquaticus* could also be isolated from hot water taken from the hot-water tap. Later Ramaley and Hixson (11) isolated from a man-made hot-water source an organism called by them X-1 which appeared closely related to the isolates of *T. aquaticus* obtained by Brock and Freeze (9) but which lacked the yellow pigment. Strain X-1 grew slightly faster than the yellow-pigmented isolates, and Ramaley (*personal communication*) postulated that the yellow pigment of hot spring isolates of *T. aquaticus* was an adaptation to survival in sunlight, whereas in hot-water heaters this adaptation would not be necessary and nonpigmented

strains, if they grew faster, would have a selective advantage.

Two studies have been carried out to see whether *T. aquaticus* could be used as an indicator of the input of thermal water into natural streams. Zeikus and Brock (12) studied the distribution of this organism in the cold and heated portions of the Firehole River in Yellowstone National Park and showed that the organism was absent from the unheated portion of the river but was present in river water that had received thermal additions. Brock and Yoder (Proc. Indiana Acad. Sci. 1970, 80:183-188, 1971) studied the distribution of *T. aquaticus* in streams arising from cold springs in southern Indiana and from the Jordan River, a spring-fed stream which received considerable thermal input from hot water arising from Indiana University operations. *T. aquaticus* was always absent from the unheated spring-fed streams but was readily isolated from Jordan River water. These two studies thus suggest that *T. aquaticus* can be an indicator of thermal input to natural waters; the organism probably grows in the man-made thermal sources and is carried into the receiving waters.

It thus seemed of interest to examine further the distribution of *T. aquaticus* in man-made hot-water sources. In the present paper we present results of a study on the presence of *T. aquaticus* and other thermophilic bacteria in water derived from the hot-water heaters of laundries and residences in Madison, Wisconsin. Thermophilic isolates were obtained from about one-half of the sources studied. The isolates have been characterized, and the organisms were found to be predominantly non-

pigmented varieties of *T. aquaticus*, although a few other types were also found. The results thus suggest that hot-water heaters are a suitable habitat for the growth of thermophilic bacteria and that a consideration of possible harmful effects of these organisms is in order.

MATERIALS AND METHODS

Culture media. A basal salts medium initially designed for the growth of thermophilic algae was used (10). The salts medium had the following composition (in milligrams per liter of deionized water): nitrioltriacetic acid, 100; $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 60; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 100; NaCl , 8; KNO_3 , 103; NaNO_3 , 689; Na_2HPO_4 , 111; FeCl_3 , 0.28; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 2.2; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; H_2BO_3 , 0.5; CuSO_4 , 0.016; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.025; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.046; pH was adjusted to 8.2 with NaOH . This salts medium was supplemented with 0.1% tryptone and 0.1% yeast extract, both pH 8.2, after autoclaving. A 3-g amount of agar (Difco) was added per each 100 ml of medium. Starch agar was prepared by the addition of 0.1% yeast extract, 1% soluble starch, and 3% agar to the basal salt solution.

Incubation conditions. Covered water baths were used for all incubations. The temperatures of the water baths were checked frequently with mercury thermometers.

Growth temperature optimum studies were performed by using 5 ml of medium in 16-mm culture tubes with stainless-steel caps. The tubes were incubated unshaken in covered water baths.

Agar plates were wrapped in Saran Wrap (Dow Chemical Co.) to prevent drying and incubated just above the surface of the water in a covered water bath.

Antibiotic sensitivity. Antibiotics were dissolved in water as stock solutions, and appropriate samples were added to the agar medium just before the plates were poured. The inocula were grown overnight in liquid medium, and plates were inoculated by depositing drops of the undiluted culture on the surface of the plates to give a spot about 1 cm in diameter. Five cultures were inoculated per plate. The plates were incubated for 2 days at 70 C; growth of the spots was estimated visually.

Actinomycin D sensitivities were determined by placing filter paper discs onto agar plates on which 0.1-ml amounts of an overnight liquid culture had been spread. Immediately after each disc was placed on the agar, a measured amount of the antibiotic was pipetted onto the disc.

Isolation and most probable number determination. Samples of water were added to the medium and incubated unshaken at 70 C. A 5-ml sample was added to 20 ml of medium, and 1, 0.1, and 0.01 ml was added to 9, 9.9, and 10 ml of medium, respectively. In many cases, the initial isolations were done in triplicate so that the most probable number could be calculated (1). Within 1 to 2 days visible turbidity appeared in the positive tubes, often as a surface pellicle or as large clumps. All positive cultures had grown within 3 days. All

samples negative at 3 days had not shown growth by 7 days and were then discarded.

Microbial examination of the bacterial growth revealed rods of various sizes as well as short and long filaments. Sphereoplasts of different sizes were seen frequently. No spores were seen in initial isolation cultures.

Pure cultures were isolated by streaking from tubes onto agar plates. Within 2 days of incubation at 70 C colonies were seen. One each of morphologically different colonies was inoculated into liquid medium, grown for 2 days, then restreaked, and checked for colonial uniformity. Stock cultures were carried by weekly transferring and were used as source of inoculum for the various tests.

RESULTS

Inoculum sources. Professional laundries and coin-operated laundromats were chosen for study, and a few private homes were also included. The data are summarized in Table 1. Water temperatures were measured with a thermistor probe and meter (Yellow Springs Instrument Co.). Samples were obtained by running tap water until the maximal temperature had been reached and then were collected into sterile bottles.

Isolates. Sixteen of the 28 sources surveyed contained thermophilic bacteria resembling *T. aquaticus*. The other 12 showed no growth after 7 days at 70 C, the isolation temperature. Although a total of 44 colonial types could be differentiated upon plating the initial 16 positive enrichments, morphological and cultural characteristics were very similar. In this report only one culture from each enrichment was examined, resulting in a total of 23 cultures from the 16 positive sources.

The water temperature of the positive enrichment cultures varied from 52.5 to 82.5 C, whereas temperatures of sources which did not yield isolates varied from 34 to 67 C. Samples of cold tap water from each source were also included in the enrichment procedures; all of these were negative. The most probable numbers of positive enrichments ranged from <40 to >2,400 bacteria per 100 ml of water.

The growth temperatures given in Table 1 show that all of the cultures appear to be obligate thermophiles. The upper temperature limit was 75 to 80 C, and the lower limits were usually 45 C, whereas the optimal growth temperatures were 65 C in a few cases and 70 C in the majority of the cultures. It should be noted, however, that since 70 C was used as the isolation temperature, isolates with optima at this temperature might be expected.

Morphologically, all of the cultures were gram-negative rods. Many frequently appeared

TABLE 1. Sources and characteristics of thermophilic bacteria^a

Source (all addresses, Madison, Wis.)	Culture no.	Temp			MPN/100 ml	Growth temp			Yellow pigment	Rotund bodies	Shape
		11/22, 1971	12/8, 1971	1/25-1/27, 1972		Optimum	High	Low			
Yellowstone Park	YT-1	—	—	—	—	70	75	45	+	+	FR
Quality Service Laundry, 1202 Regent	20-4 36-21-1	82.5	79	81	ND	70	75	45	—	—	FR
					≥ 2,400	70	75	45	—	—	FR
Fleury's 3F Laundry, 1509 Emil	22-6-1 32-7	73	79	77	30	70	80	85	—	+	R
					< 30	70	75	45	+	+	FR
Spic & Span Laundry, 1002 E. Washington	22-9 36-17-1	82	80	ND	230	70	75	45	—	+	FR
					≥ 2,400	70	75	45	—	—	R
Suds Your Duds, 1817 S. Park	22-1 32-5-1	75	78	77	40	70	75	45	—	—	FR
					2,400	70	75	45	—	+	FR
Monroe Launderette, 1865 Monroe	22-2-1 26-5	70.5	55	73	< 30	65-70	75	45	—	+	FR
					230	65-70	75	45	—	+	R
EZ Wash, 908 E. Johnson	22-8-1	55	ND		90	70	80	45	+	+	FR
Quick Clean Center, 701 E. Johnson	22-10	52.5	60	65	230	65	75	45	—	—	FR
Quick as a Wink, 5920 Monona	32-3	ND	53	55	430	70	75	45	—	+	FR
Econ-O-Wash, 4500 Monona	32-9	ND	64	63	40	70	75	45	—	+	FR
Coin Wash-Ette, 1306 Mound	32-15	ND	56	53	2,400	65-70	75	37	—	+	R
Truax Plaza Laundromat, 3541 E. Washington	36-14-1	ND	56	55	≥ 2,400	70	75	55	—	—	FR
Coin Wash-Ette, 1205 E. Williamson	36-16-1	ND	79	80	≥ 2,400	65-70	75	45	—	+	FR
Queen's Way Launderette, 529 University	32-13-1 20-2	70	65	67.5	2,400	70	75	45	—	+	FR
					ND	70	75	45	—	+	FR
408 N. Pinckney	20-1-1 22-15-1	ND	65	ND	ND	70	75	45	—	—	FR
					230	65-70	75	45	+	+	FR
6306 Mound	22-3-1	61	ND	ND	430	70	75	45	—	+	FR
1227 Dartmouth	18-2	71.5	ND	ND	ND	70	75	45	—	—	R

^a The following samples did not yield thermophilic cultures: Wee Wash, 920 E. Johnson, 55 to 63 C; Middleton Quick Wash, 6321 University, 46 to 57.5 C; Hilldale Cleaners, 702 N. Midvale, 48.5 to 50 C; Queensway Self Service, 2801 Sherman, 52 to 59.5 C; Northgate Cleaning Center, 1131 N. Sherman, 55 to 57 C; Sampley's Coin Op Laundry, 2611 E. Johnson, 34 to 51 C; Oak Park Laundry, 3204 Packers, 54 to 57 C; Meadowood Laundromat, 5712 Raymond, 56 to 57 C; West Wind Wash, 4315 Avond, 65 to 67 C; Cottage Plaza Queensway, 917 Atlas, 57 to 59 C; Quick Clean Center, 151 S. Gorham, 55 to 58 C; University of Wisconsin, Bacteriology Building, 53 to 58 C. All of the isolates were gram negative and nonsporing, and none digested starch. They were all sensitive to 10 µg of novobiocin or penicillin per ml and to 0.8 µg of actinomycin D per ml. MPN, most probable number; ND, Not done; R, rods; F, filaments.

as pleomorphic rods, sometimes becoming filamentous. Most of the cultures formed the rotund bodies found in *T. aquaticus* as described earlier (8). A few cultures were pigmented in varying shades of gold, although most were nonpigmented. All cultures were

grown on starch agar plates and examined for amylase production by flooding the plates with Lugol's iodine; no starch hydrolysis was observed. Because spore formation is often favored by growth on starch agar, colonies were examined for the presence of spores. No spores

were ever found, thus confirming that the isolates are nonsporulating organisms.

Antibiotic sensitivities are reported in Table 1. *T. aquaticus* is especially sensitive to actinomycin D, a trait rare for gram-negative bacteria (9), so that the great sensitivity of all the isolates to actinomycin D is of considerable diagnostic importance. The isolates were also sensitive to low concentrations of penicillin and novobiocin, two other antibiotics active against *T. aquaticus* but not generally active against other gram-negative bacteria. However, because not all cultures formed rotund bodies, it is possible that not all of the isolates should be classified as *T. aquaticus*.

DISCUSSION

These data show that thermophilic bacteria related to *T. aquaticus* can be isolated from a wide variety of man-made hot-water sources. In general, the hotter sources more frequently yielded bacteria, although some of the cooler tanks also provided thermophilic isolates. However, in contrast to the *T. aquaticus* isolates from hot springs, the present isolates were rarely pigmented. In this respect the present isolates resemble the X-1 strain of Ramaley and Hixson (11), also isolated from a man-made hot-water source. If the carotenoid pigment serves a photoprotective purpose, it would be reasonable to expect that the hot-spring organisms, which are often exposed to bright sunlight, would be pigmented.

There were considerable differences in the temperatures of hot-water heaters in the different establishments. In general, the highest temperatures were found in commercial laundries. Some of the laundromats also had fairly high temperatures, whereas others were considerably lower. An individual hot-water tank should of course vary in temperature as a function of usage, and if hot water is used from it continuously, it should exist generally at a lower temperature. It did not seem appropriate in the present work to measure temperature variations in single hot-water heaters with time. Many of our temperature measurements were made early in the morning before usage became heavy, and these temperatures presumably reflect the long evening and night period at which constant high temperature should prevail.

It can be concluded that the thermophilic bacteria isolated in this study were actually growing in the hot-water heaters. Cold water never yielded thermophilic isolates, so that it is unlikely that the organisms merely were being carried to and through the tanks. Growth

probably does not take place in the free water, but on the walls of the hot-water heater. Most hot-water heaters are glass-lined, and the bacteria undoubtedly attach and grow on the glass lining in a manner similar to their growth on glass microscope slides immersed in aquatic habitats (3). The bacteria found free in the water thus probably represent organisms which have sloughed off the glass lining. The most probable number counts thus should give only a crude measure of the extent of bacterial growth in the heaters.

The source of nutrients for growth of these heterotrophic bacteria in the hot-water tanks is probably the water itself. The water supply for Madison, Wisconsin, is ground water derived from deep wells and contains no organic matter. However, the water is quite hard, and water softening is routine so that all of the laundries and residences undoubtedly had ion exchange soft-water systems. These ion exchange resins slowly degrade and leach organic matter into the water (G. Fred Lee, *personal communication*), and it is probably such organic matter which provides nutrients for the growth of the bacteria. It should be noted that high concentrations of organic matter are not necessary to maintain bacterial populations if the organisms are attached to surfaces and if frequent exchange of water occurs. Yellowstone hot-spring waters have only 1 to 2 parts per million of total organic content (4), yet maintain quite large bacterial populations in the effluent channels (5).

The hot-water heater is clearly an interesting habitat for thermophilic bacteria. The possible practical significance of these bacteria, either within the heater or after they leave it, is unknown. We have studied small hot-water heating systems in laundries because they were easy to sample. It is likely that large hot-water systems in industries would provide similar habitats for growth of thermophilic bacteria and could be a major source of these organisms. It seems reasonable to conclude that hot-water heaters may be a significant source of the thermophilic bacteria found in natural waters receiving treated or untreated domestic waste. Further work on growth of thermophilic bacteria in such habitats is warranted.

ACKNOWLEDGMENTS

This work was supported by Atomic Energy Commission grant COO-2161-2 and by the Wisconsin Alumni Research Foundation.

The assistance of Beverly Mejchar is gratefully acknowledged.

LITERATURE CITED

1. American Public Health Association. 1965. Standard methods for the examination of water and waste

- water. American Public Health Association, Inc., New York.
2. Bott, T. L., and T. D. Brock. 1969. Bacterial growth rates above 90 C in Yellowstone hot springs. *Science* **164**:1411-1412.
 3. Bott, T. L., and T. D. Brock. 1970. Growth and metabolism of periphytic bacteria: methodology. *Limnol. Oceanogr.* **15**:333-342.
 4. Brock, T. D. 1967. Relationship between standing crop and primary productivity along a hot spring thermal gradient. *Ecology* **48**:566-571.
 5. Brock, T. D. 1969. Microbial growth under extreme conditions, p. 15-41. *In* Microbial growth, 19th Symp. Soc. Gen. Microbiol., Cambridge University Press.
 6. Brock, T. D., M. L. Brock, T. L. Bott, and M. R. Edwards. 1971. Microbial life at 90 C: the sulfur bacteria of Boulder Spring. *J. Bacteriol.* **107**:303-314.
 7. Brock, T. D., and G. K. Darland. 1970. Limits of microbial existence: temperature and pH. *Science* **169**:1316-1318.
 8. Brock, T. D., and M. R. Edwards. 1970. Fine structure of *Thermus aquaticus*, an extreme thermophile. *J. Bacteriol.* **104**:509-517.
 9. Brock, T. D., and H. Freeze. 1969. *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. *J. Bacteriol.* **98**:289-297.
 10. Castenholz, R. W. 1967. Aggregation in a thermophilic Oscillatoria. *Nature (London)* **215**:1285-1286.
 11. Ramaley, R. F., and J. Hixson. 1970. Isolation of a nonpigmented, thermophilic bacterium similar to *Thermus aquaticus*. *J. Bacteriol.* **103**:527-528.
 12. Zeikus, J. G., and T. D. Brock. 1972. Effects of thermal additions from the Yellowstone geyser basins on the bacteriology of the Firehole River. *Ecology* **53**:283-290.