Incidence of Plasmids in *Thermus* spp. Isolated in Yellowstone National Park

MICHAEL J. MUNSTER,* ANN P. MUNSTER, AND RICHARD J. SHARP

Microbial Technology Laboratory, Public Health Laboratory Service Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 OJG, United Kingdom

Received 29 May 1985/Accepted 19 August 1985

Forty-eight strains of *Thermus* spp. were isolated from thermal sites in Yellowstone National Park, Wyo., and 62.5% showed evidence of plasmid DNA. Attempts to assign function to the plasmid DNA were unsuccessful, and the presence of plasmid DNA could not be correlated with antibiotic or heavy metal resistance. A number of these cryptic plasmids are now being investigated for their potential as vectors for molecular cloning in *Thermus* spp.

Members of the genus *Thermus* are described as thermophilic, gram-negative, aerobic, yellow-pigmented heterotrophs with an optimum growth temperature of 70°C and a pH optimum between 7 and 8 (1). *Thermus aquaticus* YT-1, designated as the type strain of the genus, was first isolated from a hot spring in Yellowstone National Park, Wyo. (3). Since this initial isolation, *Thermus* species have been isolated from other habitats, e.g., hot springs in Japan (9), USSR (8), Iceland (10), New Zealand (4), streams receiving thermal pollution (11), and manmade hot water systems (2, 10). Previous work has mainly concentrated on biochemical and physiological aspects of *Thermus* species, and little has been done to elucidate the genetics of this genus. However, some progress has been reported recently towards the development of an in vitro recombinant DNA system in *Thermus* species (13). In this communication, we report the incidence of plasmids in 56 *Thermus* strains which may serve as vectors for the construction of recombinant DNA molecules in these extreme thermophiles.

Four thermal areas in Yellowstone were sampled; Mud Volcano and Norris Geyser Basin were almost exclusively acidic areas, and White Creek (Lower Geyser Basin) and Potts Hot Spring Basin (West Thumb) included mostly neutral to alkaline springs. Approximately 65 hot springs and effluent channels were sampled, and the pH and temperature profiles are given in Fig. 1. The isolation of *Thermus* spp. from these mud and water samples was as previously described (M. J. Munster, Ph.D. thesis, Council for National Academic Awards, London, United Kingdom, 1984). As expected, *Thermus* spp. were only isolated from the neutral to alkaline areas with one exception. Firecracker Spring in the Norris Geyser Basin yielded four *Thermus* strains but was the only alkaline pool recorded in this area (pH 9.0; temperature, 76.5°C). The ease of isolating *Thermus* spp. would suggest that they are probably present in considerable numbers in all hot springs with temperatures of 55°C to 80°C and pHs of 6.0 and above. Several strains were isolated which were able to grow at pH 10.5 (Fig. 1); this is unusual and has been observed previously only with certain Icelandic strains (7). A total of 48 strains of *Thermus* spp. were finally isolated from the samples.

The 48 isolates were screened for the presence of plasmid DNA by a rapid single-colony method (6). Eight reference strains were also included in the screen: *Thermus* sp. strain T2 (ATCC 27737), *Thermus* sp. strain X-1 (11), *T. aquaticus* YT-1 (3), *T. aquaticus* B (14), *T. aquaticus* Y-VII-51B (3), *Thermus flavus* AT62 (12), *Thermus thermophilus* HB8 (9), and *Thermus ruber* (donated by R. A. D. Williams).

Of the 48 strains isolated from Yellowstone, 30 (62.5%) showed the presence of distinct plasmid bands on agarose gels. Figure 2a gives a diagrammatic representation of the banding patterns. The screening technique, however, appears not to be completely suited for use with *Thermus* spp., and the resolution of many of the gels is poor, examples of photographed gels being given in Fig. 2b. Many of the plasmid-carrying strains showed multiple bands, for example, YS034, which shows five bands. However, since one plasmid can generate at least three molecular species (closed circular, open circular, and linear DNA), we cannot state at present how many separate plasmids the bands represent. The approximate size of the plasmids can be estimated by comparison with the known weights of pCKL (5.6 megadaltons [MDa]) from *Thermus* sp. strain B (C. L. Kho, Ph.D. thesis, University of London, United Kingdom, 1981), pYS45-1 (3.8 MDa) from YS045 (N. D. H. Raven and R. A. D. Williams, 60th Biochemical Society Meeting, 18 to 20 July 1984, Leeds, United Kingdom), and pTT1 (6.0 MDa) from *T. thermophilus* HB8 (5). *T. thermophilus* HB8 has been reported to have only one plasmid, but our work showed two bands; therefore, we can only assume that we observed two molecular species of one plasmid. Also, *T.

![Temperature and pH profile of the sample sites used in Yellowstone National Park. Samples from which *Thermus* sp. were isolated (○) and could not be isolated (□) are shown.](http://aem.asm.org/)

* Corresponding author.
flavus AT62 is reported to contain plasmid pTF62 (6.8 MDa) (13), but this could not be demonstrated during this work. However, recent work with YS045 has shown the existence of two plasmids, pTYS45-1 (3.8 MDa) and pTYS45-2 (7.8 MDa), the smallest of which can be cured by freeze-drying (N. D. H. Raven, personal communication). This may possibly explain the absence of pTF62 in the T. flavus AT62 samples which were obtained from a freeze-dried culture.

All the strains were tested for resistance to a large number of antibiotics and three heavy-metal salts (As, Sb, and Bi) with a view to assigning functions to the plasmids detected. The results showed considerable homogeneity between the strains, with little evidence to correlate resistance to the presence of plasmid DNA. T. ruber, however, did show resistance to kanamycin (5-µg disks, Oxoid Ltd., London, United Kingdom) and has been identified as a plasmid-bearing strain. Whether this resistance can be assigned to the plasmid is not yet known.

This work represents the largest reported study of the incidence of plasmid DNA in Thermus spp. It would appear that plasmids are ubiquitous among Thermus spp., as over 60% of those tested here contained plasmid DNA, but at present all remain cryptic. Attempts to correlate the presence of plasmid DNA with pool location, temperature, and pH were unsuccessful. However, strains YS039, YS002, YS040, and YS041 all isolated at pH 10.5 did not show any evidence of plasmid DNA. As yet it is unclear whether this is significant. A restriction map of pTYS45-1 has been constructed, and it would appear to be potentially useful as a vector for molecular cloning in Thermus spp., having three single restriction sites (Raven and Williams, 609th Biochemical Society Meeting). Other suitable vectors may exist within this group of strains, and further work will be carried out to examine them in greater detail.

This work was supported by a Health and Safety Executive Grant.

We also thank Fred Hirschman and Milada Vachuda for their help and extensive tour of the Norris Geyser Basin during our visit there in August 1982 and Tony Atkinson and Neil Raven for helpful suggestions and comments on this work.

LITERATURE CITED