Bacteriocinlike Activity within the Genus Thermus

RICHARD J. BECKER, DEBORAH A. BECKER, AND MARVIN J. STARZYK*

Molecular and Microbiological Section, Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115

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Members of the genus Thermus were examined for the presence of bacteriocinlike inhibitory activity. Testing was done by the deferred antagonism technique. Antagonistic activity, as evidenced by zones of inhibition, was expressed by Thermus rubens against all other Thermus strains tested. T. rubens itself was immune to this activity. Plasmid analysis of T. rubens revealed the presence of one plasmid of approximately 64 megadaltons.

Examples of antagonism between particular bacterial strains are abundant in the literature. The mediating agent is often a bacteriocin. Fundamentally, bacteriocins are protein or glycoprotein substances produced by microorganisms which exert bactericidal effects on closely related strains or species (8). Since the first report of colicins by Gratia (10), many instances of bacteriocinogeny in both gram-positive and gram-negative organisms have been reported. For the most part, these studies have dealt primarily with the bacteriocins of mesophilic bacteria and have been the subject of several excellent reviews (11, 14, 21).

Perhaps the first report of a bacteriocinlike substance in a thermophile was that of Shafia (18), who reported on the thermocins of Bacillus steaothermophilus. Since that time there have been relatively few reports of bacteriocin activity or other antagonistic interactions in thermophiles, with the exception of several studies on the purification and characterization of thermocins (8, 19).

Organisms of the genus Thermus are gram-negative, nonsporulating, nonmotile, extremely thermophilic rods present in a wide variety of both natural and man-made thermal environments (3, 5, 16, 17, 20). Some members of the genus characteristically form unusual morphological aggregates called rotund bodies (1, 4, 9, 15). Previous efforts directed at the detection of bacteriocinlike activity in this genus have been limited primarily to Thermus flavus and T. thermophilus (12, 22, 23) and have been unsuccessful.

It has been suggested that examination of a sufficiently large number of strains or species of a given group of organisms will generally be rewarded with some evidence of bacteriocinlike antagonism (14). To this end we have examined the majority of Thermus species reported to date and a number of laboratory-derived strains. We report here the presence of antagonistic activity by T. rubens against other Thermus species, as well as the isolation of extrachromosomal DNA from T. rubens.

Potential producer strains of Thermus spp. were grown in TAM broth (Castenholz medium D [6] supplemented with 0.1% tryptone [Difco Laboratories, Detroit, Mich.] and 0.1% yeast extract [Difco]) at 63°C. Indicator organisms, consisting of Thermus strains and B. steaothermophilus, were grown as described above, and Escherichia coli and Staphylococcus aureus were grown in nutrient broth (Difco) at 37°C.

Base medium for plating consisted of TAM broth solidified with 1% Gelrite (Kelco, San Diego, Calif.) and 0.1% MgSO₄ · 7H₂O (Fisher Scientific Co., Fair Lawn, N.J.). Top medium for overlays consisted of TAM broth (for Thermus and Bacillus strains) or nutrient broth (for Staphylococcus and Escherichia strains) solidified with 0.75% Gelrite and 0.1% MgSO₄ · 7H₂O.

Potential producer strains were grown to a concentration of approximately 3.0 × 10⁸ CFU/ml and plated on base medium at an appropriate dilution to yield 10 to 100 colonies per plate. Plates were wrapped in plastic bags (to prevent desiccation) and incubated for 48 h at 63°C. Following incubation, base plates containing potential producer colonies were overlaid with 10 ml of tempered (60°C) top medium seeded with 0.2 ml of indicator strain cultures (at approximately 3.0 × 10⁶ CFU/ml). Overlaid base plates were wrapped in plastic bags and incubated at 63°C for 48 h (Thermus and Bacillus species) or at 37°C for 48 h (E. coli and S. aureus).

Plasmid DNA was isolated by the procedure of Birnboim and Doly (2), except that ethanol precipitation was done at room temperature. Plasmid DNA preparations were separated electrophoretically on 0.8% agarose gels in Tris-borate buffer (7) with gels (13.5 by 14 by 0.4 cm) in a submerged gel apparatus.

Each Thermus species was tested for antagonistic activity against the following organisms: T. aquaticus ATCC 25104, T. aquaticus 3904 (lab-derived strain cured of plasmids pTA1, pTA2, and pTA4), T. caldophilus (from T. Oshima, Japan), T. flavus ATCC 33923, T. rubens ATCC 31556, T. ruber (from L. G. Loginova, USSR), T. thermophilus ATCC 27634, B. steaothermophilus (Northern Illinois University stock strain), E. coli Carolina 15-5065, and S. aureus ATCC 12600.

FIG. 1. (A) Inhibition zones in a T. aquaticus lawn produced by colonies of T. rubens. (B) Immunity exhibited by a T. rubens lawn plated over T. rubens colonies.
bacteriocin production and of host cell bacteriocin immunity; (iii) presence of an essential, biologically active protein moiety; (iv) a bactericidal mode of action; (v) attachment to specific cell receptors; and (vi) production by lethal biosynthesis (i.e., commitment to bacteriocin production will ultimately lead to cell death). The following evidence lends support to the possibility that the agent in question is a bacteriocin.

First, the agent does not appear to be bacteriophage-like in nature. Inhibitory zones picked to fresh T. aquaticus lawns in an attempt to propagate possible bacteriophage activity gave negative results. Further, dilution series of T. rubens broth culture supernatants plated on T. aquaticus lawns never produced discrete plaques. Testing by the reverse overlay technique (13) still resulted in the production of inhibitory zones in T. aquaticus lawns. In this technique there is no direct contact between the producer and indicator strains, and thus phage particles cannot reach the indicator bacteria. Production of inhibitory zones must therefore be attributed to a freely diffusible product.

Preliminary data support a narrow spectrum of inhibitory activity. T. rubens inhibitory activity appears to be confined to the genus Thermus. No activity was noted when it was tested against B. stearothermophilus, E. coli and S. aureus.

We have established the presence of plasmid DNA in the producer organism, lending support to the possibility of plasmid-encoded production of the inhibitory substance. Curing of the plasmid with concomitant loss of inhibitory activity would support the idea of plasmid-encoded production. We have had previous success in curing plasmids from members of the genus Thermus through the use of novobiocin at suboptimal growth temperatures (D. A. Kyl- lonen and M. J. Starzyk, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, H152, p. 33); however, attempts to cure the 64-MDa plasmid in T. rubens by using novobiocin, acridine orange, and ethidium bromide at a variety of temperatures have been unsuccessful.

Biochemical studies are under way in our laboratory to isolate and further characterize the inhibitory substance with respect to composition, molecular weight, and mode of action. Until completion of these studies, the antagonistic activity exhibited by T. rubens must be described as bacteriocin-like rather than as bacteriocin mediated.

The current taxonomy of thermophilic, gram-negative Thermus and Thermus-like isolates is in a state of some confusion. Requirements for complex growth medium and thermophilic incubation temperatures often make classical biochemical testing techniques difficult if not impossible. We hope that further analysis of inter- and intra species antagonism in this area will provide a useful tool for determination of the taxonomic relationships among these organisms.

LITERATURE CITED

FIG. 2. Plasmid profiles of Thermus species. Lanes: 1, T. aquaticus YT-1 with plasmids pTA1 (5.7 MDa), pTA2 (8.9 MDa), pTA3 (10.3 MDa), and pTA4 (11.4 MDa); 2, T. rubens, showing one plasmid (64 MDa); 3, E. coli VS17 standards.

Of the Thermus species tested, T. rubens was the only species that exhibited antagonistic activity. Inhibitory zones typically measured 4 to 5 mm in diameter, as shown in Fig. 1A, which illustrates inhibition of T. aquaticus by T. rubens. T. rubens exhibited this activity against all other Thermus species tested, but was itself immune to antagonism (Fig. 1B). No Thermus species, including T. rubens, exhibited inhibitory activity against B. stearothermophilus, E. coli, or S. aureus.

Plasmid analysis of T. rubens by agarose gel electrophoresis revealed a single plasmid, approximately 64 megadaltons (MDa) in size (Fig. 2). This plasmid is the largest plasmid yet reported for the genus Thermus, and the first reported in this species.

The following criteria have been proposed for defining a particular substance as a bacteriocin (21): (i) a narrow inhibitory spectrum of activity centered about the homologous species; (ii) plasmid-borne genetic determinants of
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