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# NOTES

## Simple Method for Detecting *Bacteroides* spp. Bacteriocin Production

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*Bacteroides* isolates were grown anaerobically on a 0.22-μm membrane filter on an agar plate for 48 h. Cultures of an indicator strain were grown as a lawn on the agar after removal of the filter, and bacteriocin sensitivity was detected by zones of inhibition.

Bacteriocinogeny and sensitivity to bacteriocin have been successfully applied as an epidemiological tool in several species of bacteria, e.g., *Shigella sonnei* (1), *Pseudomonas aeruginosa* (6), and *Escherichia coli* (8). There have been few reports on the production of bacteriocins by *Bacteroides* spp. (2-4). None have described a typing system based on bacteriocin production, although Booth et al. (4) suggested it was a possibility. While in the process of developing a bacteriocin typing system for *Bacteroides* spp. (manuscript submitted for publication), it became apparent that none of the established methods were readily adaptable to this group of organisms. This paper describes a simple and effective method for detecting bacteriocin production by *Bacteroides* isolates that is adaptable to screening a large number of isolates in an epidemiological study.

In preliminary experiments, three typing schemes were compared. The overlay technique described by Booth et al. (4) was unsatisfactory with *Bacteroides* test strains since many grew very poorly, thereby making the inhibition zones difficult to visualize. The standard cross-streaking method based on the technique of Abbott and Shannon (1) was found to be inadequate, particularly with mucoid strains. The growth-in-broth method described by Farmer (5) for bacteriocin typing of *Serratia marcescens* was also tried. However, this was very time consuming, particularly when dealing with a large number of unknown strains. During the course of these experiments, another possibility arose. It was found that bacteriocins produced by *Bacteroides* spp. could pass freely through a 0.22-μm membrane filter without detectable loss of activity.

Hence, the following method for detection of bacteriocin production in *Bacteroides* spp. was developed and used in a screen for bacteriocin sensitivity in 50 strains. A 90-mm diameter membrane filter (0.22-μm average pore size) (Millipore) was placed onto the surface of a brain heart infusion agar plate supplemented with 0.0005% hemin, 0.002% menadione, and 0.5% yeast extract. Overnight cultures of *Bacteroides* spp. in supplemented brain heart infusion broth were spotted onto the surface of the membrane filter with the aid of a 25-prong multipoint inoculator. After incubation at 37°C for 48 h in an anaerobic jar charged with a GasPak (BBL Microbiology Systems, Cockeysville, Md.), the membrane filter was removed, and the surface of the plate was flooded with 1 ml of an overnight culture of the test organism diluted to an opacity equivalent to a McFarland no. 1 tube. The excess was removed, and the plates were allowed to dry. Zones of inhibition were visible after overnight incubation in an anaerobic jar. By using the above method, 50 clinical *Bacteroides* isolates, identified according to the criteria of Holdeman et al. (7), were screened fr bacteriocin sensitivity against the same 50 strains. Results were recorded as positive when there was a clear inhibition zone and negative when there was no discernible inhibition. All reactions fell into these two categories, and there were no intermediate results. Of the 50 strains, 27 (54%) were bacteriocinogenic.

Sensitivity to at least one bacteriocin was found in 45 (90%) of the 50 strains, whereas 42 isolates (84%) were sensitive to two bacteriocins, and 40 (80%) were sensitive to three or more bacteriocins. Thus, by sensitivity testing of *Bacteroides* isolates, it should be possible to type all or most isolates.

To test the reproducibility of the method, 10

colonies of a *Bacteroides* isolate were picked from a pure culture, and each was used as an indicator strain against 9 producer strains chosen from the original 27 producer strains. All 10 colonies gave the same typing reactions.

Although this method has been developed primarily for detecting bacteriocin production in *Bacteroides* spp., it has applications in bacteriocin detection and typing systems for other bacteria, providing the bacteriocin produced can be shown to pass through a 0.22-μm membrane filter. It may be particularly useful, for example, in the bacteriocin typing of *Klebsiella* spp., where the mucoid nature of the organism may preclude the use of more conventional techniques.

## LITERATURE CITED

1. **Abbott, J. D., and D. G. Shannon.** 1958. A method for typing *Shigella sonnei*, using colicine production as a marker. J. Clin. Pathol. **11**:71-77.
2. **Beerens, H., and G. Baron.** 1965. Mise en évidence de bacteriocines élaborées par les bactéries anaérobies a gram negatief appartenant au genre *Eggerthella*. Ann. Inst. Pasteur Paris **108**:255-256.
3. **Beerens, H., G. Baron, and M. M. Tahon.** 1966. Mise en évidence de substances inhibitries élaborées par les bactéries anaérobies non sporulees. Ann. Inst. Pasteur Lille **17**:1-20.
4. **Booth, S. J., J. L. Johnson, and T. D. Wilkins.** 1977. Bacteriocin production by strains of *Bacteroides* isolated from human feces and the role of these strains in the bacterial ecology of the colon. Antimicrob. Agents Chemother. **11**:718-724.
5. **Farmer, J. J., III.** 1972. Epidemiological differentiation of *Serratia marcescens*: typing by bacteriocin production. Appl. Microbiol. **23**:218-225.
6. **Gillies, R. R., and J. R. W. Govan.** 1966. Typing of *Pseudomonas pyocyanea* by pyocine production. J. Pathol. Bacteriol. **91**:339-345.
7. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed).** 1977. Anaerobe laboratory manual, 3rd ed. Virginia Polytechnic Institute and State University, Blacksburg.
8. **Linton, K. B.** 1960. The colicine typing of coliform bacilli in the study of cross-infection in urology. J. Clin. Pathol. **13**:168-172.