Metachromatic Agar-Diffusion Microslide Technique for Detecting Staphylococcal Nuclease in Foods

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The metachromatic agar-diffusion (MAD) microslide technique was shown to detect nanogram quantities of staphylococcal thermonuclease in various foods without prior extraction, purification, or concentration.

Chesbro and Auborn (2) use a standard spectrophotometric method to detect thermostable nuclease in foods which are heavily contaminated with *Staphylococcus aureus*. The method is laborious for it involves prior purification of the enzyme to reduce to low levels the background of naturally occurring oligonucleotides and nucleotides.

Attempts have been made to eliminate the need for prior purification by using deoxyribonucleic acid labeled with 14C as a substrate for the enzyme (9; W. R. Chesbro, and K. R. Dunlap, Bacteriol. Proc., p. 11, 1971). Cost and the extra precautions necessary in handling radioactive materials offset the advantage of this method.

The present study describes a simple technique for detecting the thermonuclease, i.e., heat-stable nuclease, of *S. aureus* in foods without prior extraction, purification, or concentration. Three categories of foods were examined: (i) foods to which measured amounts of thermonuclease were added, (ii) foods inoculated with enterotoxigenic staphylococci, and (iii) samples of food responsible for food poisoning.

The method used for detection of thermonuclease activity in foods is essentially the microslide technique described by Lachica et al. (6) for the detection of thermonuclease activity in staphylococcal cultures (7). Since the method is based on the metachromatic property of Toluidine Blue and agar diffusion, it is designated as the metachromatic agar-diffusion (MAD) microslide technique.

Measured amounts of purified staphylococcal nuclease (Worthington Biochemical Corp.) were thoroughly mixed with various foods to attain a uniform distribution. The foods investigated were homogenized whole milk, colby cheese, butter, custard, banana cream pie filling, chocolate cream filling, cake icing, corned beef spread, deviled ham, chicken spread, turkey spread, corn, green beans, and peas. To determine thermonuclease activity, wells cut in the MAD microslide were filled by means of a platinum loop with ca. 3 \( \mu \)litters of liquid food samples which were previously heated to 97 C for 15 min. Small particles of solid food samples (ca. 5 mg), previously steamed for 15 min, were placed on the surface of the MAD microslide for testing. After the cover was replaced, the microslide was incubated for 3 hr at 37 C. A positive test was indicated by a bright pink halo around the wells or food particles. Quantities as low as 0.005 \( \mu \)g/g were detected in all of the food samples, except the chocolate pie filling, in which the lower limit of detection was 0.2 \( \mu \)g/g. None of the foods yielded a bright pink halo without the addition of staphylococcal thermonuclease, i.e., there were no false-positive reactions. Greenish halos were observed around the high-protein food particles without added enzyme.

Two food products (chicken and turkey) naturally contaminated with enterotoxin were generously provided by R. W. Bennett of the Food and Drug Administration. Small portions of the samples (ca. 5 mg) were placed on the MAD microslide after being steamed for 15 min. Bright pink halos were observed after incubation for 3 hr at 37 C, thereby indicating thermonuclease activity.

To determine the association of nuclease and endotoxin production, beef and pork...
processed at different pH values and salt concentrations were inoculated with various (10 to 10⁹) numbers of cells of S. aureus S-6 (a strain that elaborates enterotoxin B). The procedure is described in detail elsewhere (3, 4). After an incubation period of 7 days at 30°C, the meats were homogenized in sterile water by using a total volume of 10 ml for the beef samples and 20 ml for the pork samples. Samples of 0.5 ml of each homogenate, serially diluted 10-fold, were used to inoculate blood-agar plates which were incubated overnight at 37°C. Enterotoxin B was determined by the microslide and single gel diffusion technique (3) after the remaining homogenates were centrifuged, dialyzed, lyophilized, and finally hydrated with 0.6 ml of phosphate-buffered saline (4). Thermolysin activity was then assayed as described above.

Table 1 shows that all of the meat samples supported good growth and thermolysin production. With inocula of 10³ and 10⁴, staphylococcal growth correlated perfectly with thermolysin and enterotoxin production. With smaller inocula, four samples were negative for enterotoxin B.

The present study demonstrates the convenience of using the MAD microslide test for thermolysin activity as an indication of staphylococcal contamination in foods. Additional studies are needed to determine the relationship of nuclease production with enterotoxin formation and growth of various strains of S. aureus under varying conditions. Moreover, the specificity of the thermolysin method is not yet fully proven. Recent studies have demonstrated that nearly all strains of S. aureus produce thermolysin (7, 8), whereas strains of S. epidermidis and Micrococcus sp. produce nuclease which are sensitive to heat (7). Although various kinds of bacteria have been shown not to produce thermolysin (2, 5), our survey of microorganisms continues.

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**Table 1. Growth and production of thermolysin and enterotoxin B by Staphylococcus aureus S-6 in processed beef and pork meat**

| Meat   | Inoculum (cells/sample) | pH | Percent NaCla | Growth | Thermolysin | Enterotoxin |
|--------|-------------------------|----|---------------|--------|-------------|-------------|
| Beef (1 g) | 10⁴                        | 5.8 | 3.75          | 10⁴    | +           | +           |
|        | 10³                        |    |               | 10³    | +           | +           |
|        | 10²                        |    |               | 10²    | +           | +           |
|        | 10¹                        |    |               | 10¹    | +           | +           |
| Pork (10 g) | 10⁴                        | 6.35 | 6.92          | 10⁴    | +           | +           |
|        | 10³                        |    |               | 10³    | +           | +           |
|        | 10²                        |    |               | 10²    | +           | +           |
|        | 10¹                        |    |               | 10¹    | +           | +           |
|        | 10⁰                        | 6.75 | 6.18          | 10⁰    | +           | +           |

a Per cent in brine.