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Brief Communication

HEAT STABLE NUCLEASE IN MASTITIC MILK

The production of a heat stable nuclease (deoxyribonuclease) by Staphylococcus aureus was first reported by *Cunningham et al.* (1956). The detection of the enzyme was simplified by *Lachica et al.* (1971), who developed an agar diffusion test based on the metachromatic properties of toluidine blue.

In the present work, the agar medium described by *Lachica et al.*, TDA (Toluidine Blue DNA agar), with a pH of 9.0, was used for investigation of staphylococcal nuclease activity in milk. In order to dissolve the DNA completely, the DNA solution was heated to 100°C with constant stirring. The agar was poured into Petri dishes or into glass trays, and wells with a diameter of 10 mm were punched in the agar, which was 2 mm thick. The diameters of the pink zones produced by the enzyme dilutions after incubation at 37°C for 18 hrs. were measured, and based on these figures, the enzyme concentrations were expressed in diffusion units as described by *Sandvik* (1962).

In teat samples of foremilk from cows with an acute or peracute mastitis, nuclease could sometimes be detected at concentrations from 50,000 to 150,000 diffusion units per 0.1 ml. According to information from the veterinary surgeons, the cows from which these samples originated were generally heavily affected, anorexia was predominant, the temperature was 40.0—41.5°C and the local symptoms were severe. Pure cultures of Staphylococcus aureus were isolated in all cases of high concentrations of nuclease. The concentrations of staphylococci were generally high, as estimated by the primary isolations.

In milk samples from cows with subacute and chronic mastitis, detection of the enzyme was rare. When it was found, the concentration was generally less than 1000 units per 0.1 ml. By heating the samples in a boiling water bath for 10 min., nuclease activity was, however, demonstrated in nearly all samples from cows with clinical mastitis due to S. aureus. In visually normal milk from which S. aureus was isolated, the nuclease could also be detected after boiling. The detection of the heat stable nuclease in these samples was most frequent in milk with an increased cell content indicating a subclinical mastitis.

The heat stable nuclease was even found in some samples from cows with subclinical mastitis according to the California Mastitis Test (CMT) (*Schalm & Noorlander* 1957) from which no mastitis bacteria could be isolated. These observations may indicate that the abnormal milk was a result of an inflammation caused by S. aureus.

The preliminary results of this investigation indicate that the heat stable nuclease produced by S. aureus may have significance for the pathogenesis of staphylococcal mastitis and that the detection of the enzyme may be an aid in diagnosing mastitis. A more detailed report on the present study will be published.

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