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RESEARCH COMMUNICATION

AEROBIC METABOLISM OF TREHALOSE IN THE TAXONOMY OF COAGULASE NEGATIVE STAPHYLOCOCCI

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ABSTRACT

ERASMUS, J. A., 1988. Aerobic metabolism of trehalose in the taxonomy of coagulase negative staphylococci. *Onderstepoort Journal of Veterinary Research*, 55, 73–74 (1988).

Comparative assays demonstrated that the probability of error was 12,1 % when testing the aerobic fermentation of trehalose by coagulase negative staphylococci when this carbohydrate was added to the medium prior to sterilization whereas the error was only 2,7 % in media if filtered trehalose was added subsequent to steam sterilization of the medium.

INTRODUCTION

Baird-Parker (1975) classified the coagulase negative staphylococci as *Staphylococcus epidermidis* and *S. saprophyticus*. The former, which was subdivided into 4 biotypes, are known to be sensative to novobiocin. In contrast, isolates of *S. saprophyticus* are known to be resistant to this antibiotic.

According to the subcommittee on the taxonomy of staphylococci and micrococci (Anon, 1976), the aerobic metabolism of trehalose could also be used as a criterion in the differentiation between *S. epidermidis* (trehalose negative) and *S. saprophyticus* (trehalose positive). In her study on the taxonomy of coagulase negative staphylococci from bovine milk, Botha (1986) found that 33,9 % of the isolates resisted novobiocin and metabolized trehalose aerobically. Simultaneously, 4,6 % of the novobiocin sensitive isolates were trehalose negative. As these findings were at variance with the recommendations of the subcommittee (Anon., 1974), it was evident that the trehalose test for the taxonomy of the coagulase negative staphylococci should be investigated.

MATERIALS AND METHODS

Milk samples, which were received for examination for mastitis, were routinely cultured on mannitol salt agar plates, as described by Erasmus (1983, 1985). Suspect colonies were subcultured twice on tryptose blood agar (TBA) plates. After 24 h incubation of the last subculture, Gram's smears were prepared from single colonies from these plates. A culture was taken as a coagulase negative *Staphylococcus* if the smears revealed gram positive cocci and if colonies taken from the same plates reacted catalase positive, oxidase negative and coagulase negative in the presence of human and rabbit plasma, grew anaerobically in thioglycollate medium, and fermented glucose.

Trehalose metabolism was tedted in Hugh & Leifson's OF (HLOF) medium[1] containing 1 % (v/v) horse serum and 1 % (m/v) trehalose. The medium was prepared in 2 different ways; firstly (Medium A), HLOF and trehalose were added to the prescribed volume of distilled water. After sterilization at 105 °C for 15 min, sterile horse serum was added and the medium dispensed into sterile tubes. Secondly, Medium B was prepared by the addition of filter sterilized trehalose and sterile horse serum to heat sterilized HLOF, and dispensed into sterile tubes.

Twenty-five randomly selected isolates of coagulase negative staphylococci, isolated from bovine milk, were inoculated in triplicate into each of the mediums A and B. After an incubation period of 72 h at 37 °C, an organism was taken as trehalose positive when the medium changed from a light green to a definite yellow.

RESULTS AND DISCUSSION

The relevant data are summarized in Table 1.

TABLE 1 Aerobic metabolism of trehalose by 25 isolates of coagulase negative staphylococci. (All tests performed in triplicate)

Isolate No.	Trehalose metabolism in Medium A			Medium B			Isolate No.	Trehalose metablism in Medium A			Medium B		
1	+	+	+	+	+	+	14	+	+	+	+	+	+
2	+	—	—	+	+	+	15	+	+	+	—	—	—
3	+	—	—	+	+	+	16	+	+	+	+	+	+
4	+	+	—	+	+	+	17	+	+	+	+	+	—
5	+	—	—	—	—	—	18	+	+	+	+	+	+
6	—	—	—	—	—	—	19	—	—	+	—	—	—
7	+	+	+	+	+	+	20	—	—	+	—	—	—
8	—	—	—	—	—	—	21	—	—	—	—	—	—
9	—	—	—	—	—	—	22	+	—	—	+	—	—
10	—	—	—	—	—	—	23	—	—	—	—	—	—
11	+	+	+	—	—	—	24	+	+	+	—	—	—
12	—	—	—	—	—	—	25	+	—	—	—	—	—
13	+	+	+	+	+	+							
[a]S_A^2 = 0,107							[a]P_A = 12,1 %						
S_B^2 = 0,027							P_B = 2,7 %						

[a] See text for formulae

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[1] Biolab Chemicals (Pty) Ltd, P.O. Box 15849, Lynn East, Pretoria 0039

Variances in individual tests between triplicate organisms can be calculated from the equation:

$$S_i^2 = n/3t$$

where S_i^2 corresponds to the variance, n to the number of discrepancies when a number (t) of isolates are tested (Sneath & Johnson, 1972). The probability of error for an individual test is given by $P = \frac{1}{2}[1 - \sqrt{1 - 4S_i^2}] \times 100\ \%$ (Sneath & Johnson, 1972). When P is greater than 10 %, there may be serious problems with the reliability of such a test when the data are to be used in numerical taxonomy (Austin & Priest, 1986).

The probability of error, when aerobic trehalose metabolism was tested in Medium A, was about 12,1 %. This figure decreased to 2,7 % when the same isolates were tested in Medium B (Table 1). Furthermore, when tested in Medium A, isolates No. 11, 15 & 24 (Table 1) could either completely (3/3 tests positive) or partially (2/3 tests positive) metabolize trehalose aerobically. In Medium B the same isolates gave completely negative results.

When identifying *S. epidermidis* and *S. saprophyticus*, growth of the organisms in the presence of novobiocin and the aerobic metabolism of trehalose must both be tested. According to the above findings, the latter test should be performed in a medium where filter sterilized trehalose is added after heat sterilization of the basal medium.

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