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THE RELATIONSHIP BETWEEN VARIOUS BIOCHEMICAL REACTIONS AND ENTEROTOXIGENICITY OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM MILK

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SUMMARY: 53 out of 81 isolates of staphylococci recovered from 353 milk samples positive to Schalm test were coagulase positive. All coagulase positive isolates were positive in DNase and phosphatase tests, fermented glucose aerobically and anaerobically as well as mannitol aerobically and liquefied gelatine. 94.3 % of the isolates fermented mannitol anaerobically, 71.7 % were positive in hyaluronidase test and 60.4 % showed lecithin activity. 66 % produced β -haemolysin, 20.8 % α -haemolysin and 13.2 % α - β haemolysin. 14 out of the 53 coagulase positive isolates (26.4 %) were enterotoxigenic, of which 5 produced enterotoxin A, 2 enterotoxin C and 7 enterotoxin AD. 85.7 % of the toxigenic isolates were positive in all tests. However, only 48 % of isolates positive in all biochemical tests were found to produce enterotoxins.

INTRODUCTION

Staphylococcus aureus has been reported frequently as one of the cases of subclinical mastitis (Davidson, 1961; Gedek, 1972; Singh and Baxi, 1982 and Habem *et al.*, 1984). The consumption of such milk or its products would represent a health hazard if this organism is capable of producing enterotoxins (Bergdoll, 1970). Although coagulase-positive Staphylococci are considered pathogenic only 0-41% of the isolates from bovine mastitis were found to be enterotoxigenic (Casman *et al.*, 1976; Olson *et al.*, 1970; Hajek and Marsalek, 1973 and Amtsberg, 1980).

Because the detection and identification of *Staphylococcus aureus* enterotoxins are laborious many laboratories depend on the isolation of the organism on selective media as Baird-Parker and Chapman media and for confirmation coagulase or DNase tests are performed (Hobbs and Gilbert 1982).

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The present work was initiated to study various biochemical reactions of coagulase positive *Staphylococcus aureus* in relation to their ability to produce enterotoxins.

MATERIAL AND METHODS

Eighty one isolates of staphylococci could be isolated by the use of Staphylococcus Medium No. 110 (Oxoid) from 353 milk samples, positive to Schalm test (Schalm et al., 1971) and were examined by coagulase test (Gillespie, 1943). The coagulase positive isolates were subjected to the following tests:

1. Deoxyribonuclease (DNase) production (Blair et al., 1967).
2. Hyaluronidase activity (Murray and Pearce, 1949).
3. Phosphatase activity (Barber and Kuper, 1951).
4. Lecithinase test (Gillespie and Adler, 1952).
5. Acid production from glucose and mannitol, both aerobically and anaerobically (Cruickshank et al., 1975).
6. Gelatine liquefaction (Chapman, 1946).
7. Haemolysin production (Massen and Wulkow, 1968).
8. Enterotoxin production (Donnelly et al., 1967).
9. Typing of enterotoxins was carried out by optimal sensitivity plate (OSP) method (Robbins et al., 1974). The reference enterotoxins (A-E) and their corresponding antisera were kindly supplied by Prof. Dr. M.S. Bergdoll, Food Research Institute, Wisconsin University, Madison, U.S.A.

RESULTS

53 out of the 81 examined isolates were found to be coagulase positive *Staphylococcus aureus*. As shown in **Table 1** all isolates possessed deoxyribonuclease (DNase) and phosphatase enzymes, fermented glucose aerobically and anaerobically as well as mannitol aerobically and liquefied gelatine. 94.3 % of the isolates fermented mannitol anaerobically, 71.7 % were positive in hyaluronidase test and

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only 60.4% showed lecithinase activity. 8 isolates were negative in both hyaluronidase and lecithinase tests. All coagulase positive isolates produced haemolysin, of which 35 (66%) produced B-haemolysin, 11 (20.8%) α -haemolysin and 7 (13.2%) α - β -haemolysin.

14 out of the 53 (26.4%) coagulase positive *Staphylococcus aureus* produced enterotoxins, of which 5 produced enterotoxin type A, 2 type C and 7 type AD. It is interesting to note that 85.7 % of the toxigenic isolates were positive in all biochemical tests done. However, only 48 % of the isolates positive in all biochemical tests were enterotoxigenic. On the other hand, only 2 out of the 27 (7.4%) isolates showing negative reaction in one or more of the tests were toxigenic (Fig. 1)

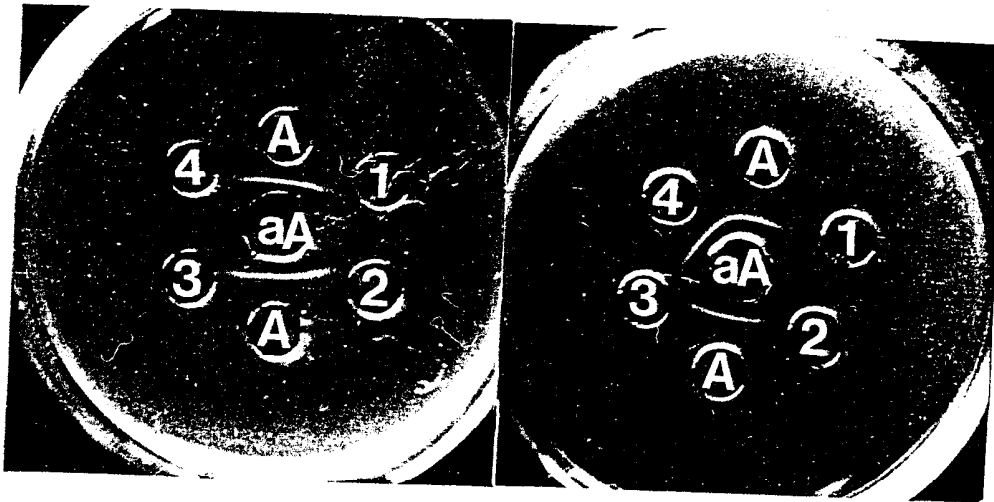


Fig. 1: *Staphylococcus aureus* enterotoxin assay by OSP technique
 A = reference enterotoxin A, aA= anti-enterotoxin A, 1,2,3 and 4 preparations under test left figure: negative results, right figure: positive result in preparation no. 4.

Table 1: Relation between the enterotoxigenicity and the biochemical characteristics of coagulase positive Staphylococci isolated from milk.

Groups	No. of coagulase positive isolates	Biochemical reactions										Enterotoxin types									
		DNase	Hyal.	Phosph.	Lecith.	Glucose		Mannitol		S.T.	Haemolysin			No. of Toxicogenic isolates	A	B	C	D	E	AD	
						aerob.	aer-ob.	aerob.	anarob.		α	β	$\alpha\beta$								
I	25	+	+	+	+	+	+	+	+	+	-	+	-	3	-	-	2	-	-	-	7
II	10	+	+	+	-	+	+	+	+	+	-	+	-	2	-	-	-	-	-	-	-
III	3	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	-
IV	7	+	-	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-
V	8	+	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Total	53	53	38	53	32	53	53	50	53	53	11	35	7	14	-	-	2	-	-	-	7
(%)		100	71.7	100	60.4	100	100	94.3	100	20.8	66.0	13.2	26.4								

-DNase = Deoxyribonuclease
 Phosph. = Phosphatase
 Aerob. = Aerobic fermentation
 Anaerobic. = Anaerobic fermentation

Hyal. = Hyaluronidase
 Lecith. = Lecithinase
 Gel. Liq. = Gelatin liquefaction

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DISCUSSION

It has been reported by several authors (Hallander and Körlof, 1967, Casman et al., 1967 and Gilbert, 1974) that most enterotoxin-producing Staphylococci are coagulase positive. However, not all coagulase positive Staphylococci are necessarily enterotoxigenic (Angelotti, 1969 Frazier and Westhoff, 1978). Casman et al. (1967) found 2% of coagulase positive *Staphylococcus aureus* isolated from mastitic milk were enterotoxigenic. 4 % enterotoxin producers were reported by Untermann and Sinell (1970) and Untermann et al. (1973), 9 % were recorded by Ghosh et al. (1970). The highest incidence of enterotoxigenic *Staphylococcus aureus* of mastitic origin were 34.4 % and 41.4 % reported by Kato and Kume (1980) and Niskanen and Koiranen (1977), respectively. In the present work a relatively high incidence (26.4 %) was reported. The commonly isolated types are A, C and D (Niskanen and Koiranen, 1977, Kato and Kume, 1980). Other reports indicated that staphylococci isolated from mastitic milk have usually produced enterotoxins C or D (Casman et al., 1967, Untermann et al., 1973, Kajek and Marsalek, 1973 and Wieneke, 1974). In the present study strains producing enterotoxin A in combination with D were the most common followed by those producing enterotoxin A and then C.

The comparison of the biochemical reactions with the ability to produce detectable enterotoxins showed that 85.7 % of the enterotoxigenic isolates were able to produce coagulase DNase, phosphatase and lecithinase enzymes. This is almost in agreement with the observation of Victor et al. 1969 and Untermann (1980). On the other hand, the results obtained in the present work demonstrate clearly that none of the biochemical tests used, singly or combined, can be trusted as indicative of enterotoxigenicity. The detection of enterotoxins is therefore the only reliable test to determine the role of *Staphylococcus aureus* in food poisoning of the consumer.

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