Acta vet. scand. 1977, *18*, 266—273.

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THE BIOCHEMICAL ACTIVITY OF ENTEROTOXIN AND NON-ENTEROTOXIN PRODUCING STAPHYLOCOCCI

By

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DANIELSSON, MARIE-LOUISE and BO HELLBERG: *The biochemical activity of enterotoxin and non-enterotoxin producing staphylococci.* Acta vet. scand. 1977, *18*, 266—273. — One hundred and sixty-nine staphylococcal strains of human origin have been tested for production of enterotoxin A, B or C_1, coagulase activity, DNase activity, typical growth on ETGP-agar, hemolysin production and the breakdown of mannitol under aerobic conditions. Very good correlation was observed between enterotoxin production and coagulase activity, in that 82 % of the enterotoxin producing strains also synthesized coagulase. The correlation between DNase activity and positive reaction in mannitol to enterotoxin production was also good (80 % of the enterotoxic strains produced both DNase and aerobic acid from mannitol). Of the enterotoxin producing strains 66 % hemolysed bovine erythrocytes and 61 % were ETGP-positive. However, the frequency of hemolysing respectively ETGP-positive but non-enterotoxin producing strains was very high, viz. 46 % respectively 32 %. It is concluded that enterotoxin production can not to a satisfactory degree of security be predicted by means of the other biochemical characters.

staphylococci; enterotoxin.

Enterotoxin producing staphylococci are regarded as one of the most common causes of food poisoning. Since the identification of the staphylococcal enterotoxin is laborious and time-consuming, many attempts have been made to relate enterotoxin production to some other property of the staphylococcal cell (*Minor & Marth* 1971). There is, however, a lack of information concerning the relation between enterotoxin production and the biochemical activities most often utilized in diagnostic food microbiology.

In the present investigation the biochemical properties of enterotoxin and non-enterotoxin producing staphylococcal strains are compared. The aim has been to map the biochemical characters of enterotoxin producing strains with special reference to the biochemical tests usually used in food bacteriology.

MATERIALS AND METHODS

A total of 169 staphylococcal strains of human origin were examined. The strains were isolated from noses (110), throats (43) and wounds (16) of meat factory workers as described by *Danielsson & Hellberg* (in preparation). The identification of the bacteria was done according to *Bergey's Manual of Determinative Bacteriology* (1974).

The strains were tested for coagulase activity, DNase activity, typical growth on egg yolk tellurite glycine pyruvate agar (ETGP-agar), hemolysin production and aerobic acid from mannitol, which are the biochemical tests most often used in routine laboratory work.

Enterotoxin determination. The strains were tested for production of enterotoxin A, B and C_1 using antisera and reference enterotoxins from Serva Feinbiochemica, Heidelberg. To obtain a concentrated enterotoxin solution the "cellophane sac culture technique" described by *Donnelly et al.* (1967) was used. Enterotoxins were determined serologically using a microslide gel double diffusion technique (*Crowle* 1958, *Zehren & Zehren* 1968). All biochemical tests as well as the determination of enterotoxin production were done in duplicates.

Coagulase test. Coagulase production was determined in tubes with fresh rabbit plasma diluted 1:4 in 0.85 % NaCl. The plasma was inoculated with a single surface colony from a 24-hrs. blood agar culture and then incubated at 37°C. The plasma was examined for clotting after 2, 4, 6, and 24 hrs. Any degree of clotting was considered to be a positive reaction.

DNase activity. Extracellular deoxyribonuclease production was determined using DNase agar (Oxoid). The cultures were streaked on the surface of the agar and incubated for 24 hrs. at 37°C. After incubation the plates were flooded with 1 M-HCl. Transparent zones greater than 0.5 mm around the colonies were regarded as positive for DNase production.

Aerobic acid from mannitol. The ability to produce acid

aerobically from mannitol was determined in mannitol broth using bromcresol purple as indicator. The tubes were incubated for 5 days at 37°C. Only tubes in which the colour had changed to yellow throughout the whole medium were regarded as positive.

Growth on ETGP-agar. The ETGP-agar was prepared according to *Baird-Parker* (1962). The inoculated plates were incubated at 37°C for 24 and 48 hrs. Black, shiny, convex colonies with narrow, white entire margins and surrounded by clear zones extending 2—5 mm into the medium were regarded as positive.

Hemolysis. Hemolysis was determined in tryptone glucose extract agar (TGA-agar) with 5 % defibrinated bovine blood added. The hemolytic activity was observed after 24 and 48 hrs. incubation at 37°C. Colonies surrounded by a clear or partly clear zone of at least 1 mm were regarded as hemolysing.

RESULTS

The results are given in Tables 1 and 2, and illustrated in Figs. 1 and 2. Out of the 169 strains examined 34 strains produced enterotoxin A, 4 strains enterotoxin B, 4 strains enterotoxin C_1, 1 strain both A and C_1 and 1 strain B and C_1; giving a total of 44 enterotoxin producing strains. The biochemical activity of the 169 strains was as follows: 58 strains were positive for coagulase, 60 strains produced DNase, 65 strains gave positive reaction in mannitol, 67 strains were positive on ETGP-agar and 86 strains hemolysed bovine erythrocytes.

Table 1. Summary of the reactions of 44 enterotoxin producing staphylococci in the tests for coagulase, ETGPA-reactions, DNase, mannitol oxidation and hemolysis.

Test	Combinations of reactions							
Coagulase	+	+	+	—	—	—	+	+
ETGPA	+	—	+	—	+	—	—	—
DNase	+	+	+	—	—	—	+	—
Mannitol	+	+	+	—	—	—	+	—
Hemolysis	+	+	—	—	+	+	—	+
Total strains	16	9	9	5	2	1	1	1
% strains	36	20	20	11	5	2	2	2

Table 2. Summary of the reactions of 125 non-enterotoxin producing staphylococci in the tests for coagulase, ETGPA-reactions, DNase, mannitol oxidation and hemolysis.

Test	Combinations of reactions												
Coagulase	—	—	—	+	+	—	+	—	—	—	—	—	—
ETGPA	—	—	+	+	+	+	—	—	+	—	+	+	—
DNase	—	—	—	+	+	—	+	—	—	+	+	—	+
Mannitol	—	—	—	+	+	—	+	+	+	—	+	+	+
Hemolysis	—	+	+	+	—	—	+	—	+	—	—	—	—
Total strains	50	27	13	12	7	4	3	3	2	1	1	1	1
% strains	40	22	10	10	6	3	2	2	2	1	1	1	1

Correlation between enterotoxin production and biochemical activity

As demonstrated in Fig. 1 the enterotoxin producing strains generally were biochemically more active than the non-enterotoxin producing strains. Of the biochemical characteristics investigated, not one was found to be absolutely linked to enterotoxin production. Coagulase activity was found to be the biochemical reaction best correlated to enterotoxin production. The

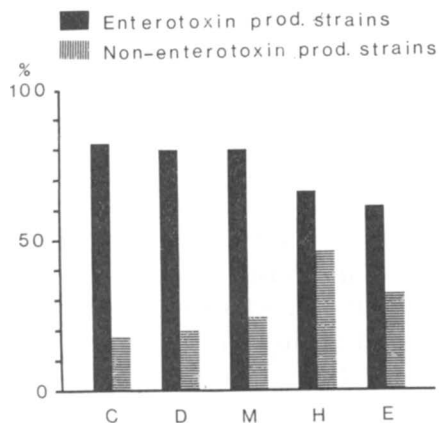

Figure 1. The biochemical activity of enterotoxin and non-enterotoxin producing staphylococci.
C = coagulase positive. D = DNase positive. M = mannitol oxidating. H = hemolysing. E = ETGPA positive.

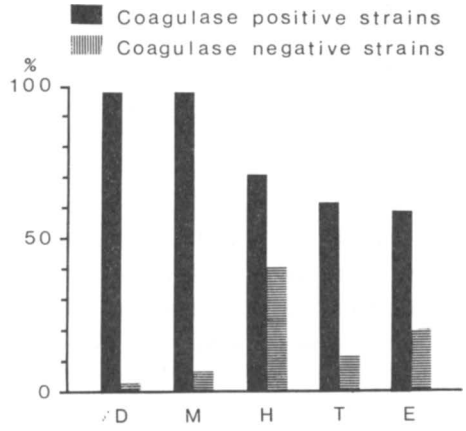

Figure 2. The biochemical activity of coagulase positive and negative staphylococci.
D = DNase positive. M = mannitol oxidating. H = hemolysing. T = enterotoxin producing. E = ETGPA positive.

correlation between DNase activity and mannitol oxidation ability to enterotoxin production was nearly as good. All enterotoxin producing DNase positive strains also were mannitol positive and all DNase negative enterotoxin producing strains were mannitol negative (Table 1). Among the enterotoxin negative strains a similar relation between aerobic acid from mannitol and DNase activity was not observed (Table 2).

DISCUSSION

The biochemical property most frequently associated with enterotoxicity is coagulase production (*Evans* 1950). Also the present investigation found coagulase activity to be the biochemical property best correlated to enterotoxin production in that 82 % of the enterotoxin producing strains also synthesized coagulase. Coagulase production, however, does not seem to be a reliable single criterion for enterotoxicity as 38 % of the coagulase positive strains were negative for enterotoxin production. It is also of interest to note that as much as 7 % of the coagulase negative strains produced enterotoxin. The literature gives only a few examples of food poisonings caused by coagulase negative staphylococcal strains (*Omori & Kato* 1959, *Bergdoll et al.* 1967, *Breckingridge & Bergdoll* 1971). A possible explanation for this may be that coagulase negative strains are neglected when outbreaks of food poisoning are investigated.

The DNase test, which was specially developed for the diagnosis of pathogenic staphylococci, is based on the ability of certain strains to split deoxyribonucleic acid in mono- and polynucleotides (*Weckman & Catlin* 1957). The correlation between enterotoxin and DNase production was also very good, in that 80 % of the enterotoxic strains produced DNase. A still better correlation was found by *Victor et al.* (1969), who studied both heat labile and stable DNase, in that 95 % of their enterotoxin producing strains produced heat stable DNase and 97 % were DNase positive when using unheated culture.

All the enterotoxin producing strains which were positive for DNase also gave positive reaction in mannitol. However, the DNase test seems to be more reliable in predicting enterotoxicity, as we found more mannitol positive strains among the non-enterotoxin producing ones. The advantage of the DNase test in routine laboratory work is obvious, as it is more convenient and requires less time than the mannitol breakdown test.

The ETGP-agar was developed by *Baird-Parker* (1962) as a diagnostic and selective medium for the isolation and enumeration of coagulase positive staphylococci in foods. In the present investigation, however, the staphylococcal strains of human origin were found not to give a satisfactory degree of correlation between coagulase synthesis and typical appearance on ETGP-agar. As demonstrated in Fig. 2 only 59 % of the coagulase producing staphylococci were ETGP-positive. The correlation to enterotoxin production (Fig. 1) was only slightly better, viz. 61 %. This finding is notable since staphylococcal strains involved in food poisonings generally are of human origin. *Casolari et al.* (1973), who tested enterotoxin B producing strains isolated from food, also found a low correlation between ETGP-positive and enterotoxin producing strains in that only 35 % of the enterotoxic strains were also ETGP-positive.

In ETGP-agar the ability of the bacteria to clear and precipitate egg yolk is used as one diagnostic criterion. *Reid & Wilson* (1959), who investigated the reaction in egg yolk medium of coagulase positive staphylococci isolated from bovine udders, found that 75 % of the staphylococci isolated from cows suffering from acute mastitis produced opacity compared with only 7 % of the strains isolated from normal udders or udders with chronic mastitis. The high frequency of coagulase positive but ETGP-negative strains in the present study may thus possibly depend on the fact that they were not involved in any acute infection when isolated.

CONCLUSIONS

It is obvious that none of the investigated biochemical properties can be used for a satisfactory differentiation of enterotoxic staphylococci from non-enterotoxin producing strains. This is especially true for the properties utilized in ETGP-agar.

ACKNOWLEDGEMENT

The authors wish to thank Mrs. Margareta Lagerberg and Mrs. Lilly Stridsberg for their skilful technical assistance.

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SAMMANFATTNING

Några biokemiska egenskaper hos enterotoxinbildande och icke enterotoxinbildande stafylokocker.

169 stafylokockstammar isolerade från näsa, svalg och sår på människa har undersökts avseende produktion av enterotoxin (A, B, C_1), koagulas, DNas och hemolysin, typisk växt på ETGP-agar samt mannitoloxidationsförmåga. 82 % av de enterotoxinproducerande

stammarna var koagulaspositiva, 80 % spjälkade DNA, 80 % oxiderade mannitol, 66 % hemolyserade bovina erytrocyter och 61 % var ETGP-positiva. Av de icke enterotoxinbildande stammarna var 46 % hemolyserande, 32 % ETGP-positiva, 24 % mannitolpositiva, 20 % DNas-positiva och 18 % koagulaspositiva.

(Received March 17, 1977).

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