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Study on Correlation Between Coagulase and Lipase Production in Staphylococci.

It follows from the literature that pathogenic staphylococci can produce an opacity zone around the colonies, at their growth on agar media containing egg yolk, while the enzymatic factor causing the above reaction is defined as an "egg yolk factor" (1,3,5,6,8,10,12,13,14,15).

Alder et al. (1) suggest that "staphylococcus egg yolk factor", similarly as coagulase, is connected with pathogenicity of this microorganism, while Richou et al. (13) at determining pathogenic properties of staphylococci, regard the above factor as equivalent to the coagulase production. On the other hand, there are some reports on a lack of correlation between coagulase and lipase production by staphylococci (4,8,9,11).

Alford et al. (2) observed that the activity of egg yolk factor, which, according to the opinion of many authors, is lipase, depended on temperature. Vadehra and Harmon (16) investigated the temperature influence upon the activity of this lipase and stated that an optimal temperature for the above enzyme was 45°C with an intensified activity between 30-60°C. Raj and Liston (12) stated also that the staphylo-

cocci incubation at 45°C instead of 37°C on medium with egg yolk accelerated the "opacity" response. This has been confirmed also by the authors' own observations, carried out at diagnosing staphylococci strains isolated from men and food. At the incubation of lipase-positive staphylococci at 37°C, sometimes no opacity zones appeared on the media with egg yolk, or they were distinctly smaller than in the case of incubation at 44°C. The above observations led to a supposition that at 44°C a closer correlation can occur between lipase and coagulase production in staphylococci. In this connection the present study has been carried out, aiming at determination of this correlation degree at the incubation temperature of 44°C, under particular consideration of the strains isolated from cured meat.

Material and methods.

In total 1232 staphylococcus strains were examined, including 616 lipase-positive strains, i.e. producing "egg yolk factor", and 616 lipase-negative strains. These strains were isolated from cured meat designed for production of sausages and canned meats (1174 strains), from raw meat (18 strains) and from ice cream (28 strains) as well as from nasal-laryngal cavity and from skin of men (12 strains). From the above kinds of samples inoculations were made on the Staphylococcus No. 110 medium with an egg yolk addition (3), and incubated at 44°C for 48 hours. From each plate showing the growth of staphylococci, by one lipase positive and lipase-negative colony were selected at random and reinoculated on the same kind of medium, so as to obtain a pure strain. Thus obtained strains were investigated on their coagulase production ability. The coagulase test was carried out on the following way: from the pure culture on the Staphylococcus No. 110 medium with egg yolk addition several colonies were taken, inoculated into nutrient broth and incubated at 37°C for 3 hours. Then 0,2 ml. of the broth culture were taken into test tubes containing by 0,5 ml of freshly prepared rabbit

plasma and afterwards brought into water bath and incubated at 37°C for 3 hours. The readings were taken every hour. After 3-hour incubation the tubes were transferred into room temperature for 18 hours and then the last reading was taken. All lipase-positive and lipase-negative strains were also investigated on their catalase production ability as well as on glucose and manitol fermentation.

Results.

A complete correlation between lipase production at 44°C and coagulase production has been stated. All the 616 strains forming a precipitation zone on Staphylococcus No. 110 agar with egg yolk addition, i.e. lipase-positive ones, produced coagulase, while the remaining strains, non-forming any zones on the above medium, were coagulase-negative ones. It must be stressed that, similarly as in the investigations of some other authors, not all the coagulase-positive staphylococcus strains fermented mannitol, while, on the other hand, some coagulase-negative strains showed a positive response with mannitol.

Discussion.

The authors' own investigations, carried out on 1232 strains, showed a complete correlation between lipase production by staphylococci at 44°C and coagulase production. Consequently, it can be supposed that all or most of the lipase-positive strains in meat or other food, at the incubation at 44°C are able to produce coagulase, while lipase-negative strains do not show such ability. The applied method of recognizing lipase-positive staphylococci on the medium with egg yolk addition presents no difficulties both in research and routine investigations. Moreover, it renders possible easy recognition of count of lipase-positive staphylococcus colonies, even in the cases of examination of material heavily contaminated with other microorganisms. On the contrary, the coagulase test is more labour-consuming one, does not enable

any immediate and quick recognition of coagulase-positive strains on agar media, while obtaining the rabbit plasma is sometimes connected with difficulties in conditions of laboratories carrying out routine tests. While taking into consideration the obtained investigation results, it seems that at the estimation of pathogenic abilities of staphylococci in food products, the coagulase test can be successfully replaced by the lipase test.

Conclusions.

1. In the investigations in question a complete correlation has been proved between lipase ("egg yolk factor") production by staphylococci at 44°C and coagulase production.
2. At estimating pathogenic abilities of staphylococci and at counting these bacteria in food products, the coagulase test can be replaced by the lipase test.

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