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Brief Communication

IDENTIFICATION OF AEROCOCCUS VIRIDANS BY MEANS OF CO-AGGLUTINATION

Gaffkemia, which has recently been reviewed by *Stewart & Rabin* (1970) and by *Stewart* (1975), is a fatal bacterial disease of the American and European lobster (Homarus americanus and Homarus vulgaris) (*Snieszko & Taylor* 1947). The causal agent, first classified as Gaffkya homari (*Hitchner & Snieszko* 1947), is now classified as Aerococcus viridans (*Williams et al.* 1953, *Buchanan & Gibbons* 1974).

According to *Williams et al.* A. viridans morphologically, culturally and biochemically resembles to some extent the streptococci and particularly the Lancefield group D streptococci or enterococci. As to serology these authors, using acid and formamide extracts of A. viridans, antisera to the streptococci, and the precipitation test, found no relationship between A. viridans and the group D streptococci. An antiserum prepared against A. viridans showed no reaction with extracts of the homologous strain, other aerococci or enterococci. Nor was any agglutination observed between A. viridans and antisera against various Lancefield groups of streptococci. Faced with the problem of the similarity between A. viridans and the enterococci and having previously achieved successful results with grouping of streptococci by means of co-agglutination (*Saxegaard* 1977), an attempt was made to see if the co-agglutination test could be applied to the identification of A. viridans.

Two strains of A. viridans (NVI* 1030 and 1032) were cultured on 5 % horse serum agar and the growth harvested in saline with 0.3 % formalin. The bacteria suspension was adjusted to McFarland tube No. 3 or 9×10^8 cells per ml. Rabbits were immunized by six intravenous injections, starting with 1 ml and increasing to 2 ml. Two injections were given per week and the rabbits were bled one week after the last injection. A 10 % suspension, in phosphate-buffered saline, of formaldehyde and heat-treated protein A-containing Staphylococcus aureus strain Cowan I (NCTC** 8530) was prepared according to the method of *Kron-*

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vall (1973) at the National Institute of Public Health, Oslo. The coating procedure was performed as described by *Christensen et al.* (1973). Prior to co-agglutination, serum agar cultures of A. viridans, which scarcely grew in Todd Hewitt broth, were harvested in saline, three petri dishes per culture and 2 ml saline per dish, centrifuged and resuspended in 0.5 ml trisbuffer*, 0.2 M, pH = 8.0. Three drops of a 5 % solution of trypsin were added and the suspension left in a water bath at 37°C for 1 hr. To test for cross-reactivity, streptococci were cultured for 24 hrs. in Todd-Hewitt broth before trypsinization. The co-agglutination test was performed by mixing one drop of trypsinized culture of A. viridans with one drop of coated staphylococcal reagent on a glass slide. The slide was tilted and observed for co-agglutination for 1 min. Later trypsinization of A. viridans was omitted and co-agglutination performed directly with cultures from blood or serum agar plates. Both strains of A. viridans showed distinct reaction in the co-agglutination test. No cross-reactivity was observed between coated staphylococcal reagent and trypsinized streptococci of Lancefield's groups A, B, C, D, E, G and L.

The comprehensive conventional biochemical tests hitherto employed to differentiate between A. viridans and streptococci are laborious and time consuming. The co-agglutination test when applied to A. viridans has so far proved to be specific. Furthermore, the test is simple, rapid and easy to perform, especially when cultures of suspected strains can be taken directly from agar plates and mixed with coated staphylococcal reagent. For these reasons the co-agglutination test offers great advantages to the biochemical tests.

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