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Equi Factors in the Identification of *Corynebacterium equi* Magnusson

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The production of *equi* factor(s) by 173 serologically verified *Corynebacterium equi* isolates was tested by streaking strains at right angles to a culture of *Corynebacterium pseudotuberculosis* or *Staphylococcus aureus* on a cattle blood agar plate. All strains produced *equi* factor(s). This characteristic was more specific than other tests used on the strains.

Corynebacterium equi (11) produces soluble factors which interact with the phospholipase D (PLD) of *Corynebacterium pseudotuberculosis*, the β-toxin of *Staphylococcus aureus*, and an uncharacterized partial hemolysin of *Listeria monocytogenes* to give an area of complete hemolysis with sheep erythrocytes (2, 4). In the absence of the soluble factors produced by these other organisms, *C. equi* is not hemolytic. Linder and Bernheimer and their co-workers have demonstrated at least two activities associated with *C. equi* which enhance the tendency of sheep erythrocytes to undergo lysis in the presence of phospholipase D produced by *C. pseudotuberculosis*: (i) a phospholipase active in hydrolizing ceramide phosphate generated by the action of phospholipase D on the erythrocyte membrane and (ii) a cholesterol oxidase which converts membrane cholesterol to cholest-4-en-3-one (5).

C. equi is at times difficult to identify in routine diagnostic laboratories since its pattern of activity in common bacterial tests is very limited. There is disagreement as to its behavior in certain biochemical tests (1). The organism is usually identified on the basis of the source of isolation, the characteristic mucoid-teardrop colony, the delicate salmon-pink color which often develops with time, catalase activity, urea hydrolysis, and failure to produce acid from carbohydrates (1, 8), but some isolates sent to our laboratory as *C. equi* by these criteria have not been *C. equi*. A recent report on variation in *C. equi* colony morphology showed that a small proportion of isolates did not develop the classical mucoid appearance and consequently might not be recognized as *C. equi* (8).

It occurred to us that *equi* factor(s) might serve as a valuable adjunct property in the identification of *C. equi*. Here we report the production of *equi* factor(s) by a large number of serologically verified (7, 9) strains of *C. equi*. We also list the variable behavior of *C. equi* in a battery of biochemical tests.

A total of 173 isolates of *C. equi* from different sources was tested; 94 of these isolates were from a collection in the Ontario Veterinary College (OVC) and were the subject of a recent study of capsular antigens of *C. equi* (9). They belonged to the following capsular serotypes (the number of isolates of each serotype is shown in parentheses): 1 (56), 2 (24), 3 (1), 4 (4), 5 (3), 6 (5), and 7 (1). Forty-nine of the OVC strains were isolates from disease processes in horses, 34 were from pigs, and the remaining 11 were from disease processes in humans, cattle, dogs, and a cat. The other 79 isolates came from the collection of J. B. Woolcock and M. D. Mutimer, University of Queensland, Australia (8). They belonged to the following serotypes: 1 (41), 2 (16), 3 (1), 5 (5), 6 (15), and 7 (1). Thirteen of these isolates were from disease processes in horses and pigs, and the remainder were isolated from the intestinal contents or feces of horses, pigs, kangaroos, koala bears, cattle, and sheep or from soil. ATCC strains 6939 and 7699 and NTCC strain 1621 were included in the Australian strains: these belong to capsular serotype 1.

The 173 strains tested were subcultured from storage agar slopes onto cattle blood agar plates and purified if contaminated. Pure cultures were streaked with a loop onto a tryptic soy agar (Difco Laboratories, Detroit, Mich.) plate, containing 5% washed cattle erythrocytes, at right angles to a culture streak of *C. pseudotuberculosis* on one side of the plate and an *S. aureus* streak on the other side. These two organisms were laboratory isolates. Between one and six *C. equi* isolates were streaked onto each blood agar plate. Plates were incubated for 48 h at 37°C

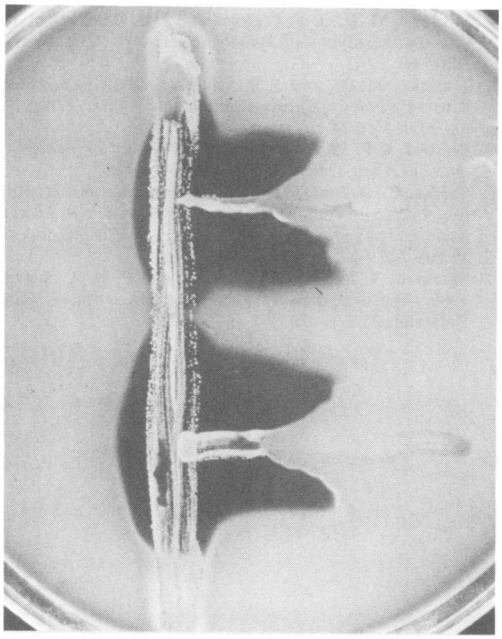

FIG. 1. *Equi* factor(s) demonstrated after 48 h of incubation at 37°C in air of two *C. equi* isolates streaked at right angles to the right of a *C. pseudotuberculosis* streak culture on a cattle blood agar plate.

in air and were examined 24 and 48 h after incubation.

Biochemical and biological tests were done on the 94 strains in the OVC collection. These tests were for catalase, cytochrome *c*, urea hydrolysis, fermentation of dextrose and xylose, and sodium hippurate and esculin hydrolysis. The tests were done by standard methods, and appropriate positive and negative controls were included (3, 6).

All isolates tested produced *equi* factor. This was seen after 24 h with the *S. aureus* streak culture as a clear enhancement of the beta-hemolysin, and after 24 h of incubation with the *C. pseudotuberculosis* streak as a broad zone of hemolysis which developed with time (Fig. 1), producing a winglike effect of the hemolytic zone farthest from the *C. pseudotuberculosis* streak after 48 h of incubation.

The 94 OVC isolates all produced catalase and with one exception strongly hydrolyzed urea within 48 h. The urease-negative isolate (number 102), which was capsular type 6, also strongly hydrolyzed esculin. Two other isolates hydrolyzed esculin weakly, but the remainder failed to do so. Five isolates produced cytochrome *c*. Only one isolate hydrolyzed sodium hippurate (weakly); this was number 7, capsular serotype 3, which also produced cytochrome oxidase. Other isolates failed to hydrolyze sodium hippurate. All isolates failed to produce acid from dextrose or xylose. When test results differed from those of the majority of isolates, the isolates were again checked for purity and tests were repeated.

The finding that all isolates produced catalase and failed to ferment dextrose and xylose was consistent with the results of most other workers (1, 8). Other investigators have also found that a few strains hydrolyze esculin and that the majority of strains hydrolyze urea (8). There is disagreement as to the hydrolysis of hippurate. Most workers have found that their isolates fail to produce cytochrome *c* (1).

All strains of *C. equi* tested produced *equi* factor(s), and all belonged to established capsular serotypes. A recent review of *C. equi* indicated that there was a clear need to develop better discriminatory tests to establish whether isolates and strains called *C. equi* are a single species or a more heterogeneous group (1). This opinion was supported by a study of *C. equi* isolates recovered from soil or animal sources which concluded that identification was difficult because of variability in colony morphology and in biochemical reactions (8). The original description of the *equi* factor(s) by Fraser (4) implied that it would be useful in the identification of *C. equi*; none of the descriptions of the identifying characteristics of *C. equi* published by later workers has included this simple test. Our results indicate that production of *equi* factor(s) is a reliable characteristic of *C. equi* which should be useful in the practical matter of the identification of the organism.

Reliance on this characteristic alone for the identification of *C. equi* would be wrong, since other organisms may produce similar effects; we have not tested this. There may also be a small number of *C. equi* strains which do not produce *equi* factor(s). Nevertheless, *equi* factor(s) has been shown to be produced by all the serologically verified *C. equi* strains tested, and the production of *equi* factor(s) should be recorded in future descriptions of alleged *C. equi* isolates.

The *equi* factor(s) can be demonstrated by using sheep, cattle, goat, rabbit, or chicken erythrocytes, but only occasionally with horse erythrocytes (4). *L. monocytogenes* has been substituted for *C. pseudotuberculosis* or *S. aureus* (10).

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