TYPIFICATION OF RHODOCOCCUS (CORYNEBACTERIUM) EQUI FROM BOVINE, OVINE AND CAPRINE SOURCES

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Summary

There exists considerable discrepancy in the literature as to the most important biochemical and colonial morphological criteria to be used for the identification of Rhodococcus equi. In the present study using R. equi from non-equine sources, we found the most important criteria for identifying this organism to be synergistic hemolytic reactions with beta toxin of S. aureus and with the organisms L. monocytogenes, C. pyogenes and C. pseudotuberculosis.

Resumo

Tipificação de Rhodococcus (Corynebacterium) equi de origem bovina, ovina e caprina

Existe considerável discrepância na literatura com relação aos principais critérios bioquímicos e de morfologia das colônias empregados para a identificação do Rhodococcus equi. Utilizando cepas de R. equi de origem bovina, ovina e caprina, concluímos que os critérios mais importantes para a identificação destas cepas foram as reações de hemólise sinérgica observadas com a toxina beta de S. aureus e com os organismos L. monocytogenes, C. pyogenes e C. pseudotuberculosis.


Criteria for identifying this organism has been somewhat less than satisfactory as reflected in the recent change of names from Corynebacterium equi to Rhodococcus equi (Goodfellow, M. & Alderson, G. – J. Gen. Microbiol., 100:99-122, 1977). There exists discrepancies in the literature as to the most important criteria adopted for laboratory identification of this organism. Although nitrate reduction has been suggested as a useful criteria (Multimer, M.D. & Woolcock, J.B. – Vet. Microbiol., 6:331-338, 1981) for example, there seems to be both nitrate negative (Whitford, H.W. & Jones, L.P. – S. West Vet., 27:261-262, 1974) as well as nitrate positive (Norse, E.V. – Cornell Vet., 40:49-55, 1950) strains. Similarly, various authors have reported the occurrence of both urease positive (Marsh, J.C. & von Graevenitz, A. – Cancer, 32:147-149, 1973) as well as urease negative strains (Whitford, H.W. & Jones, L.P. – S. West Vet., 27:261-262, 1974). Also, depending on the methodology used both hydrogen sulfide producing and non-producing R. equi have been described (Barton, M.D. & Hughes, K.L. – Vet. Bull., 50:65-80, 1980). Whether these variations represent different biotypes or are reflective of the species from which the organism originated is not yet known.

The objective of this paper was to study biochemical and cultural characteristics as well as synergistic hemolytic reactions of R. equi with beta toxin of S. aureus and with the organisms L. monocytogenes, C. pyogenes and C. pseudotuberculosis.

Bacterial strains of R. equi were isolated from three cases of bovine mastitis, one case of bovine mastitis, one case of ovine lymphadenitis and one case of caprine pneumonia. All bacterial strains were cultured in brain heart broth (Difco) and incubated aerobically at 37°C for 24 hours. Identification of R. equi was based on morphological, cultural, biochemical and hemolytic synergistic reactions using standardized bacteriological procedures. The following biochemical tests were performed as outlined by Carter (Carter, G.R. – Diagnostic procedures in veterinary bacteriology and mycology, 4.ed., USA, Charles C. Thomas, chap. 27, 1984): catalase, oxidase, nitrate reduction, glucose oxidation, hydrogen sulfide and indol production using sulfide indol motility medium, urease (Urea broth base-Oxoid or urea agar base-Difco, supplemented with filter sterilized urea solution to a final concentration of 2%). Proteolytic activity was studied using bovifiers medium, casein agar and nutrient gelatin. Synergistic hemolytic reactions were performed by streaking the suspect R. equi at right angles and through the following: beta toxin of S. aureus, or colonial streak growth of L. monocytogenes, C. pyogenes and C. pseudotuberculosis on blood agar base containing 5% sheep erythrocytes. Positive synergistic reactions were identified as areas of increased hemolytic activity (Fraser, G. – J. Path. Bact., 88:43-53, 1964).

Morphologically, all strains were Gram positive, nonmotile and coccoid to coccoabacillus type organisms. Colonies of all strains were mucoid, coalescent, nonhemolytic and developed a pink pigment. This pink pigment was especially observable when stärch agar was used. Biochemically all strains were catalase positive, oxidase negative, and did not produce H2S and indol, lacked proteolytic activity and failed to oxidize glucose. All four bovine strains were nitrate negative and urease positive. In contrast, the ovine and caprine strains were nitrate positive and urease negative. All strains potentiated hemolysis produced by the beta toxin of S. aureus as well as participated in synergistic hemolysis with L. monocytogenes, C. pyogenes and C. pseudotuberculosis.

Various authors have described considerable variations in the colonial morphology of R. equi, which are thought to be due to either colonial dissociation with development of rough variants (Jensen, H.L. – Proc. Linn. Soc. N.S.W., 59:19-61, 1964) or due to differences in culturing methods (Barton, M.D. & Hughes, K.L. – Vet. Bull., 50:65-80, 1980). In the present study, we found colonial morphology to be in accordance with the findings of Multimer & Woolcock (Multimer, M.D. & Woolcock, J.B. – Vet. Microbiol., 6:331-338, 1981) who observed only slight colonial variations.

Biochemically, these strains reacted to those of the literature (Multimer, M.D. & Woolcock, J.B. – Vet. Microbiol., 6:331-338, 1981), with several differences. The ovine and caprine strains were nitrate positive and all four bovine strains were negative even after 15 days of incubation. These negative tests were confirmed by attempts to reduce residual nitrate with zinc. Thus, similar to C.
pseudotuberculosis there seems to be nitrate positive as well as nitrate negative strains. Some authors (Multimer, M.D. & Woolcock, J.B. - Vet. Microbiol., 6:331-338, 1981) suggested that nitrate reduction should be used as a major criteria for the identification of R. equi. However, careful analysis of their work reveals strains of equine and non-equine origin which were nitrate negative. Furthermore, nitrate reduction has been recorded by others to be variable (Jensen, H.L. - Proc. Linn. Soc. N.S.W., 59:19-61, 1934) to negative (Woolcock, J.B. & Rudduck, H.B. - Aust. Vet. J., 49:319, 1973).

There also was a difference in the ability of these organisms to hydrolyse urea. Whereas the four bovine strains were urease positive the ovine and caprine strains were negative. Again various authors have noted variability in the ability of R. equi to hydrolyse urea (Natarajan, C. & Nilakantan, P.R. - Indian J. Anim. Sci., 44:329-333, 1974). Some authors have noted isolates which totally lacked the ability to hydrolyse urea (lacerda, J.P.G. & Veiga, S.M., UEP, 6321-327, 1959), whereas others reported isolates consistently positive for urease activity (Marsh, J.C. & von Graevenitz, A. - Cancer, 32:147-149, 1973).

A consistent finding with our R. equi isolates were their positive synergistic hemolytic reactions with beta toxin of S. aureus and with the organisms C. pyogenes, Listeria monocytogenes and C. pseudotuberculosis. It should be noted that we also found positive synergistic hemolytic reactions with strains of L. monocytogenes that were not hemolytic alone.

Although the incidence of R. equi infection in cattle, ovine and caprine is probably low the possibility of R. equi as a causative agent should not be overlooked. Proper bacteriological criteria need to be developed to identify its presence.