

TITLE: BIOPROSPECTION OF *Corynebacterium pseudotuberculosis* BIOVAR OVIS IN SMALL RUMINANTS DIAGNOSED WITH CASEOUS LYMPHADENITIS FROM FARMS LOCATED IN THE STATE OF PARÁ

AUTHORS: MOURA V.A.G.; DA COSTA W.L.O.; LIMA A.C.; PAIXÃO C.T.M.; ARAÚJO C.L.A.; CASTRO Í.R.R.; MATTOS-GUARALDI A.L.; SILVA A.; FOLADOR A.R.C.; MARQUES J.M.

INSTITUTION: UNIVERSIDADE FEDERAL DO PARÁ

ABSTRACT:

Corynebacterium pseudotuberculosis is a Gram-positive, non-spore forming, pleomorphic, intracellular microorganism of great veterinary interest, mainly known as agent causative of caseous lymphadenitis (CL) in small ruminants. This disease is characterized by inflammation and enlargement of superficial or internal lymph nodes that untreated can lead the animal to death, causing great economic loss for livestock. The state of Pará was recently highlighted by the large number of small ruminants herds in relation to the rest of the country, consequently the economic losses related to this microorganism became a notorious problem. Thus, the objective of this study was to perform the bioprospection of *C. pseudotuberculosis* biovar *ovis* in small ruminants diagnosed with CL from farms located in the state of Pará. For this purpose, eight samples of caseous material from different animals previously diagnosed with this illness were collected and analyzed by microbiological techniques of culture and isolation. Among these samples, three were characterized as belonging to the species *C. pseudotuberculosis* considering the morphological profile they presented. After being extracted and submitted to an electrophoretic race proving its integrity, the DNA was subjected to a polymerase chain reaction using three specific genes for this bacterium: gene encoding specific 16S ribosomal RNA, *rpoB*, gene encoding the beta subunit of RNA polymerase, and the gene encoding the exotoxin phospholipase D, the *pld*, which revealed that the band pattern verified was consistent with *C. pseudotuberculosis*. The three strains already confirmed as belonging to the species (PA05, PA06 and PA07) were sequenced to phylogeny study through the construction of a concatenated phylogenetic tree using the *rrs* and *rpoB* genes. In addition, these genes of other species belonging to the genus *Corynebacterium* were selected from public database as outgroups. The isolated strains clustered apart from bacterial strains of the same genus, but from different species, and aligned together with other species of *C. pseudotuberculosis*. Thus, it was possible to observe of two different clusters according to biovars (*ovis* and *equi*). PA05, PA06 and PA07 were also submitted to Denaturing gradient gel electrophoresis (DGGE) and Box-PCR techniques along with six other strains collected in other studies; where it was possible to observe band profiles suggesting only one profile between the strains.

Key words: *Corynebacterium pseudotuberculosis*, Caseous lymphadenitis; small ruminants; phylogenetic studies; fingerprint technics.

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