

**TITLE:** GENOTYPIC AND PHENOTYPIC IDENTIFICATION OF STRAINS OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* ISOLATED FROM SMALL RUMINANTS IN BRAZILIAN AMAZON

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**ABSTRACT:** Mainly known as the causative agent of caseous lymphadenitis (CL) in small ruminants, *Corynebacterium pseudotuberculosis* is a gram positive, non-spore forming, pleomorphic bacteria of a substantial veterinary interest. CL is a disease that is characterized by inflammation and enlargement of superficial or internal lymph nodes that untreated can lead the animal to death causing great economic losses. Thus, the main objective of this study was to collect samples of caseous material from animals that presented clinical symptoms of caseous lymphadenitis on farms located in different regions of the Amazon for later isolation, phenotypic and genotypic identification of the circulating strains in the region. For this purpose, 34 samples of caseous material from different animals previously diagnosed with this disease were collected and for the initial characterization of the isolated bacteria, phenotypic parameters were used through morphological tests such as the absence of turbidity, presence of bacterial flocculation and a bacterial surface when inoculated in enriched liquid medium, as well as growth rate, colony staining, Gram staining test and hemolytic standard to be presented when sown in culture media supplemented with sheep blood. For genotypic analyzes, the strains that presented satisfactory results were submitted to DNA extraction and then a polymerase chain reaction was performed using four specific genes (Quadruplex-PCR) for the target microorganism: *rrs*, *rpoB*, *pld* and *narG* for final identification. At the end of the phenotypic and genotypic analyzes, among the 34 samples collected, 21 strains showed morphotintorial characteristics, response to biochemical tests, and pattern of growth consistent with the organism of interest. The 21 strains that presented satisfactory results for the phenotypic analyzes were submitted to Quadruplex PCR and 16 strains presented the result consistent with the amplification of three specific genes: *rrs*, *rpoB*, *pld*. The amplification of these three genes characterizes the isolated strains as belonging to species *C. pseudotuberculosis*. The absence of the band corresponding to the *narG* gene, allows to affirm that the isolated strains in this work are from biovar *ovis* which reinforces studies that indicate the predominance of this biovar in diseases caused by *C. pseudotuberculosis* in small ruminants.

**Key words:** *Corynebacterium pseudotuberculosis*, Caseous lymphadenitis; Small ruminants ; Amazon

**Development agency:** National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES), Foundation for Supporting Research in the State of Pará (FAPESPA), and by the Genome and Proteome Network of the State of Pará (RPGP)