

TITLE: RAPID METHOD FOR SCREENING OF ANAEROBIC BACTERIAL ISOLATES FROM RUMINAL SAMPLES USING MALDI-TOF MASS SPECTROMETRY

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ABSTRACT:

Many microorganisms found in ruminal samples are naturally specialized in plant cell wall solubilization, because of this valuable trait these microorganisms are sought after by researchers for industrial applications (e.g., biofuels production). A rapid and feasible screening method for bacterial isolates identification needs to be developed to facilitate the selection of strains to further molecular and biochemical studies. Currently, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a powerful tool for identification and grouping of microorganisms. This method is based on the detection of mainly ribosomal protein fractions of microorganisms, which are the most abundant and conserved. This work aimed to analyze, group and select by MALDI-TOF MS, anaerobic bacterial isolates from cattle's rumen based on their ribosomal protein profile. A total of 46 ruminal bacteria were isolated in agar medium in anaerobic conditions containing cellulose and starch as carbon source. The isolates were cultured in liquid medium and proteins were extracted from cell pellet (absolute alcohol, formic acid 70% v/v, and acetonitrile) to be analyzed by mass spectrometer. The supernatants of the extracted proteins were overlaid with 1 µl of matrix solution on plate and were air-dried. The mass spectrometer used was MALDI-TOF (Bruker Daltonics), and spectra generated from samples were compared against ribosomal protein spectra of the reference library using a matching algorithm, these were performed automatically using MALDI Biotyper 3 software containing references of 5302 bacterial species. Analysis of spectra generated by MALDI-TOF MS allowed to identify the bacterial isolates and grouping them based on their ribosomal protein profile (bacterial isolates with similar protein patterns formed a group). The dendrogram analysis grouped 46 isolates into seven groups. A total seven isolates (one from each group) were selected for further molecular and biochemical characterization. MALDI-TOF MS has been shown to be an excellent tool for the rapid screening of bacterial isolates for prospection of ruminal microorganisms.

Keywords: MALDI-TOF mass spectrometry, screening, ruminal bacteria, ribosomal proteins

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