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Optimizing identification of mycoplasma bovis by MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry).

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Abstract

Mycoplasma bovis is an important bovine pathogen causing primarily pneumonia, otitis and arthritis in calves, and pneumonia and mastitis in adult cattle. Mixed infections of *M. bovis* with other less or non-pathogenic Mycoplasma species in clinical samples are possible. While cultivation is inexpensive and allows strain typing after isolation, definite identification requires expensive and time-consuming techniques. Nevertheless, early detection of *M. bovis* is highly important in order to rationalize antimicrobial use. MALDI-TOF MS is a fast technique for identifying pathogens in routine diagnostics. However, quality and reproducibility of spectra can be influenced. Therefore, the objective of this study was to explore growth conditions and incubation time to optimize *M. bovis* identification. A single colony of three different M. bovis strains was inoculated several times in 25 mL of four different broths (B1-4). Basic broth (B1) consisted of pleuropneumonia-like organism broth, enriched with 25% horse serum and 0.07% yeast extract. B2, B3 and B4 were additionally supplemented with pyruvate (0.5%), polysorbate 80 (1.0%) and ampicillin (0.01%), respectively. Protein extraction was performed at 0, 24, 48, 72, 96 and 120 hours after incubation at 37°C in a 5% CO2-enriched atmosphere. Spotted supernatant was processed with an Autoflex III smartbeam MALDI-TOF MS (Bruker Daltonik). Identification, interpreted as reliable with a score ≥1.7, was best after 48 and 72h in B1 (93-100%) and B2 (100%). At 0h no identification was possible and B3 failed identification at all times. After 24h, 63-85% (B1, 2, 4) of the M. bovis isolates were identified, with significant higher identification rates in B2. After 72h identification rates reduced drastically in B1 and B3. Identification scores with B2 remained similar until the end of the experiment. In conclusion, starting from one colony, reliable and cheap *M. bovis* identification is possible after 48h with MALDI-TOF MS. Use of polysorbate 80 is discouraged, however adding ampicillin to the medium may be useful to avoid contamination. Adding pyruvate to the medium ensured fast reliable identification after 24 hours of incubation and persisted up to five days after inoculation. This information can be useful to improve MALDI-TOF MS assisted diagnosis of *M. bovis* in peripheral laboratories.

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