

## STUDY OF *MYCOPLASMA MYCOIDES* SUBSP. *MYCOIDES* XOPOLYSACCHARIDES

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*Mycoplasma mycoides* subsp. *mycoides* (Mmm) is responsible for contagious bovine pleuropneumonia, a severe lung disease of cattle and buffaloes. Mmm belongs to the class Mollicutes, grouping wall-less bacteria that have evolved by major gene losses and are considered the smallest self-replicating organisms.

Mmm cells are known to be surrounded by a galactan pseudo-capsule, which has been associated to Mmm pathogenicity. Mmm is also able to secrete free exopolysaccharides (EPS) both *in vitro* and *in vivo*. However, these EPS have not been characterized yet because of technical difficulties due to the complexity of the mycoplasma growth medium.

The aim of this study was to develop a suitable methodology to produce and purify Mmm EPS, to measure their carbohydrate content and to compare them biochemically to the capsular polysaccharide.

Transfer of Mmm cells from complex to defined culture medium in controlled conditions allowed extraction of pure EPS fractions. IEHPLC and NMR analyses showed that the secreted EPS had the same characteristics as capsular galactan, i.e. a  $\beta(1 \rightarrow 6)$  galactofuranose polymer. The EPS extraction method was applied to intraclonal Mmm variants that produce opaque/translucent colonies on agar related to ON/OFF switch regulation of the glucose phosphotransferase system permease gene. Opacity was correlated to the presence of capsular EPS, as demonstrated by electron-microscopic examinations. All this, added to *in silico* analyses of candidate EPS biosynthetic pathway, suggested that capsular and secreted galactan may be produced independently. These results pave the way for future studies on Mmm pathogenicity linked to EPS production.

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