Proteomic profiles of nucleoid-assotiated proteins isolated from Mycoplasma gallisepticum in different growth phases

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Mycoplasma gallisepticum (MG) belongs to the class Mollicutes. It is characterized by a lack of cell wall and reduced genome size. As a result of genome reduction, MG has a limited variety of DNA-binding proteins (DBPs) and transcription factors. The study of the dynamic changes of the proteomic profile of MG nucleoid may assist in revealing its mechanisms of functioning, regulation of chromosome organization, and stress adaptation.

For the first time, we isolated the nucleoid of MG with synchronized cell cycles in the logarithmic phase (LP) and in the stationary phase (SP). The method includes soft lysis and fractionation by centrifugation in sucrose gradient.

2D-DIGE reveals enrichment of DBPs against overall MG proteome. Proteomic profiling was performed by LC-MS/MS (Thermo Scientific Q Exactive Plus) in DDA mode followed by LFQ data analysis.

First, the resulting proteomic profiles of LP and SP and corresponding whole cell lysates were analyzed. Proteins considered to be nucleoid-associated (NAPs) were enriched in the nucleoid samples in comparison to the samples of cell lysate ($Log2FC > 1$, $p\text{-value} < 0.05$). 61 NAPs were identified in both growth phases, along with 18 unique to LP and 12 unique to SP. Among all identified NAPs are DBPs HU1, HU2, and Dps, transcription factors MraZ and Fur, DNA-directed RNA polymerase subunits RpoA, RpoB, RpoE, and sigma factor RpoD. 22 and 18 enriched proteins with unknown function (UFP) were identified in LP and SP, respectively.

A quantitative comparison of LP and SP proteomic profiles was also performed. In SP in comparison to LP were enriched 40 proteins, including some of the DBPs (HU2, Dps, Tuf) but only 2 UFPs, whereas in LP in comparison to SP 29 proteins were enriched including 7 UFPs.

As a result of this study, we obtained detailed proteomic profiles and determined two sets of unique proteins of *M. gallisepticum* nucleoid in different growth phases.

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