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Mapping the presence of *Francisella tularensis* glycoproteins

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Aim: Our study seeks to discover the presence of glycoproteins in the bacterium *Francisella tularensis* (*F. tularensis*), in which the molecular mechanisms of virulence are still not understood.

Methods: Both detection and enrichment glycoproteomic strategies were undertaken to explore the occurrence of glycoproteins in *F. tularensis*.

The presence of glycoproteins was investigated using two detection systems, applied to 2D-PAGE-separated samples. First, carbohydrate-specific staining was used. *Cis*-diol carbohydrate groups of glycoproteins were oxidized, followed by reaction of the resulting aldehydes with the fluorescent dye. In the second method, various digoxigenin-labeled lectins were utilized. 2D-PAGE-separated proteins were blotted onto nitrocellulose membranes where putative glycoprotein-capturing lectins were detected with anti-digoxigenin-labeled alkaline phosphatase. The detected proteins were excised from gels, tryptically digested, and identified by various types of mass spectrometers.

Once the presence of glycosylation was disclosed, enrichment techniques, such as lectin-affinity chromatography and chromatography on phenylboronic acid-based resin, were employed. In lectin-affinity approach, the lectins Concanavalin A, *Sambucus nigra* agglutinin, Peanut agglutinin, and *Datura stramonium* agglutinin were utilized. Isolated glycoproteins were subjected to trypsin digestion and the digests were analyzed by cap RP-HPLC interfaced to a mass spectrometer. By using phenylboronic acid-based resin, glycoproteins containing *cis*-diol groups were bound to *m*-aminophenylboronic acid. Eluted glycoproteins were then tryptically digested and identified by mass spectrometry.

Results: Several putative glycoproteins were detected by the fluorescent reagent and identified as FTL1096, FTL0112, and FTL1328, etc. Moreover, putative glycoproteins FTL0112, FTH1463, FTL0949, and FTL1328 were found out by using labeled lectins. Lectin-affinity chromatography revealed the presence of several putative glycoproteins, e.g. FTL1096, FTH0414, and FTH0384. Finally, FTH0414 was observed as glycosylated using the phenylboronic acid-based resin.

Conclusions: In this study, the presence of glycosylation-modified proteins was determined. The differences in occurrence of glycosylation among the analyzed *F. tularensis* subspecies were observed.