

TITLE: EXPRESSION, PURIFICATION AND DERIVATIZATION OF TWO RECOMBINANT SURFACE PROTEINS OF *LEPTOSPIRA INTERROGANS*

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ABSTRACT

Leptospirosis is a worldwide important and emergent disease caused by pathogenic bacteria *Leptospira* spp., which affects humans and animals. Genus *Leptospira* has pathogenic and saprophytic species, and the lipopolysaccharide (LPS) is claimed to be responsible for its antigenic diversity. *Leptospira* surface proteins capable of binding to extracellular matrix (ECM), a fundamental step in the infection, are described in the literature. Amongst them are Lsa45 and Lsa63, which interact with ECM components and are capable of inducing a humoral immunological response in mice. Therefore, this work aimed to clone, express and purify these two surface proteins and evaluate their derivatization efficiency for further chemical conjugation with *L. biflexa* LPS. Lsa45 was cloned into vector pET28a with N-terminal 6x His-Tag and transformed in *E. coli* BL21(DE3) with chaperone plasmid pGro7 that contains groES-groEL. Cells were grown at 37°C in Tunair™ shake flask in auto-induction medium with antibiotic and L-arabinose until glucose depletion, when temperature was changed for 22°C. Lsa63 was cloned into pET101, transformed in *E. coli* BL21 (SI) cells and stir-cultivated in LBON medium with antibiotic at 30 °C in Erlenmeyer flasks until OD₆₀₀ reached 0.6-0.8. Protein expression was induced with 300 mM NaCl. Cells were harvested by centrifugation, resuspended and disrupted. Supernatants were loaded into nickel affinity columns, followed by size exclusion (SEC) or cation exchange (CEX) chromatography, monitoring their purity by SDS-PAGE and densitometry. Purified recombinant proteins were reacted with adipic acid dihydrazide (ADH) and purified by SEC; amine and protein contents were evaluated. The final yields for Lsa45 and Lsa63 were 15.44 mg/L (29% recovery, 92,5% relative purity) and 6.29 mg/mL (14% recovery, 94% relative purity), respectively. The molar ratio after derivatization between -NH₂ and Lsa45 was 13, with final amine content thrice the initial, indicating successful introduction of molecular spacer. Data regarding Lsa63 derivatization are still in analysis. Recombinant proteins' expression and purification were successfully accomplished under bench-scale, and culture as well as expression parameters are being optimized. The derivatization reaction to introduce the molecular spacer appears to be well-succeeded for Lsa45, providing a final amine content three times higher than the unmodified protein, which will help the conjugation between Lsa45 and *L. biflexa* LPS.

Keywords: *Leptospira*, Lsa45, Lsa63, recombinant protein, derivatization.

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