TITLE: PilS fimbriae from atypical enteropathogenic *Escherichia coli:* cloning, expression and biochemical characterization after refold at high pressure and alkaline pH

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ABSTRACT:

Pil fimbriae is a member of the type IV pilin, which is a remarkably versatile component with a widely variety of functions, including motility, attachment to chemically diverse surfaces, electrical conductance, acquisition of DNA, and secretion of a broad range of structurally distinct protein substrates. In enteroaggregative *Escherichia coli* C1096, the IncI1 plasmid encodes this pilin that contributes to plasmid conjugation, epithelial cell adherence, and adherence to abiotic surfaces; *pilS* gene also occurs in different pathovars of *E. coli* strains. The acquisition of a plasmid carrying its operon in typical enteropathogenic *E. coli* confer to other *E. coli* strains the ability to produce the aggregative-like pattern on HeLa cells. Therefore, more studies are required to understand the mechanisms involved in the regulation of Pil expression and production, guiding to the present study that comprises the generation of PilS recombinant protein. However, the production of recombinant protein using traditional refolding processes with high levels of denaturing reagents for PilS inclusion bodies (PilS-IBs) solubilization results in poor recovery and immunologically inactive probably due to improper refolding of the protein. Based on the assumption that PilS fimbriae may play some role in the adhesion process of atypical enteropathogenic *E. coli*, the aim of this work was to obtain a functional recombinant PilS, which was efficiently solubilized at high pressure, and in alkaline pH did not suffer reaggregation by decompression and pH reduction to 7.4 by dialysis. To confirm the production of recombinant PilS and its correct translation, a SDS/PAGE band was excised after purification and submitted to mass spectrometry analysis. We identified by trypsin digestion the protein: MTLLEVLGVMVVAAIVIGAVMGLMSDTLSSSDNQKELKNLQTIATKMKAQKFQGQYTG TDYVKILTESGLPADMIAGGNKAKNAWGGAVTIKVSSDKYSYVIESSNVPKKNCIDLVT SLRSSSMFTKINGNVTNKVDPSTVCNADKTTIKLETNS (with score of 494, coverage of protein sequence of about 48%) that corresponded to PilS protein, confirming its correct production and purification. To evaluate the effect of the refolding process on PilS structure, we conducted a circular dichroism analysis and a chemical denaturation, evaluated by measuring the intrinsic fluorescence. Both analyses allowed us to respectively confirm that PilS presented secondary and tertiary structures after the refolding process.

Keywords: PilS, E. coli, fimbriae, atypical EPEC, cloning

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