GENERATION OF RECOMBINANT HEAT-STABLE TOXIN FROM ENTEROTOXIGENIC *Escherichia coli* using SYNTHETIC GENES

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Enterotoxigenic *E. coli* (ETEC) strains produce separately or simultaneously two types of enterotoxins, the heat labile (LT) and the heat-stable (ST). LT is a large (84 kDa) oligomeric AB_5 toxin, considered a good mucosal adjuvant . As consequence, several recombinant LT forms have been described. On the other hand, ST toxin is a non-immunogenic cysteine-rich peptide synthesized as a pre-pro-peptide of 72 amino acids that are processed during export to produce a 2 kDa mature active toxin of 18 or 19 amino acids. The production of immunogenic recombinant ST forms has faced a number of challenges, especially due to small size and complex structure of this toxin. Consequently, in this study, we describe the construction of a recombinant ST employing two synthetic genes aiming the increase of the immunogenicity for antibody production. In order to achieve this, it was used the prototype ETEC strain (H10407) *stA*1 and *stA*2 genes sequence as template for the design of two synthetic genes, one with two *stA*1 gene copies and other with two *stA*1 and *stA*2 copies joined by a glycine/serine linker. The synthetic genes were subcloned into pET28a, pET20b and pAE expression vectors and confirmed by sequencing. The recombinant proteins were expressed either into *E. coli* BL21(DE3), or BL21(DE3)pLysE or BL21 (DE3)CD43 cells. After induction in presence of 1 mM of IPTG, the recombinant protein codified by double *stA*1 gene generated a protein with apparent molecular weight of 18 kDa and the second hybrid containing *stA*1 and *stA*2 produced a protein with apparent molecular weight of 34 kDa. Both proteins were recognized by anti-His monoclonal antibodies through immunoblotting. Herein, for the first time we generate two ST recombinant toxins hybrid types with promising features for increase both purified recombinant ST obtainment and the toxin immunogenicity.

Keywords: Heat-stable toxin, ETEC, synthetic genes, Recombinant proteins

Financial support: CAPES, FAPESP

Animal ETEC strains are known to produce enterotoxins similar to those of human strains. The LT from animal strains, designated LTI, is similar to the LT produced by human ETEC; however, another variety designated LTII is only found in animals and is not associated with clinical human disease. Also, animal strains produce two major types of ST, designated STa (STI) and STb (STII). As in humans, both STh (STIb) and STp (STIa) may be produced by animal strains [6]. Herein, both fragments of antibodies were capable to detect LTI and STIb and STIa from ETEC strains.