Recombinant system for expression of cholera toxin B subunit in *Escherichia coli*

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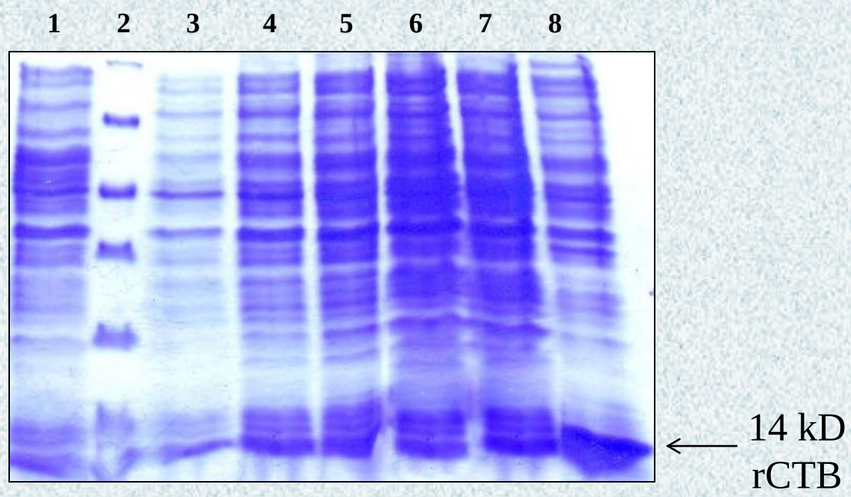
Background

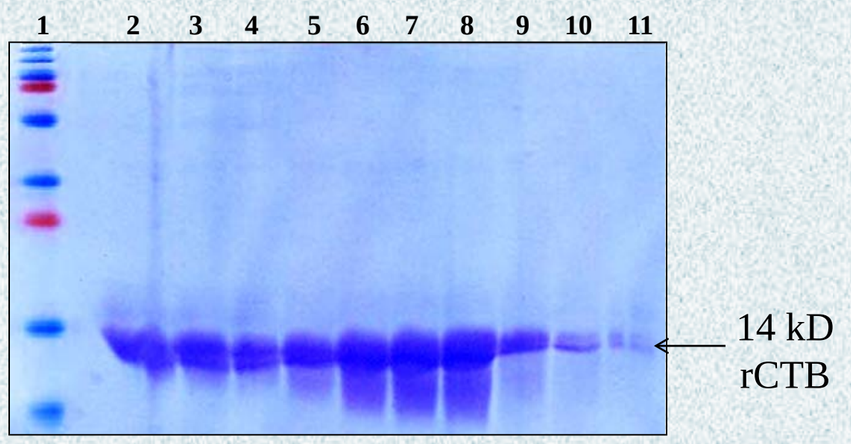
Cholera toxin (CT) is the key virulence factor of *Vibrio cholerae*, which is encoded by the *ctx*AB operon, which resides in the genome of a filamentous bacteriophage (CTX) that specifically infects *V. cholerae*. The symptoms of cholera are mainly caused by cholera toxin (CT), B subunit of which binds to the GM1 ganglioside and promote the endocytosis of CT. The aim of this study was to clone and express ctxB and to purify therecombinant protein using pAE as an efficient expression vector.



SDS-PAGE analysis of expression of recombinant CTB in *E.coli* BL21 (DE3) using pAE_*ctx*B as expression vector. Lane 1, BL21 (DE3) containing pAE plasmid as the negative control; lane 2, protein size marker; lane 3, BL21(DE3) containing pAE-*ctx*B before induction with IPTG; lane 4-8, indicate 1-5 hours after induction respectively.

Methods

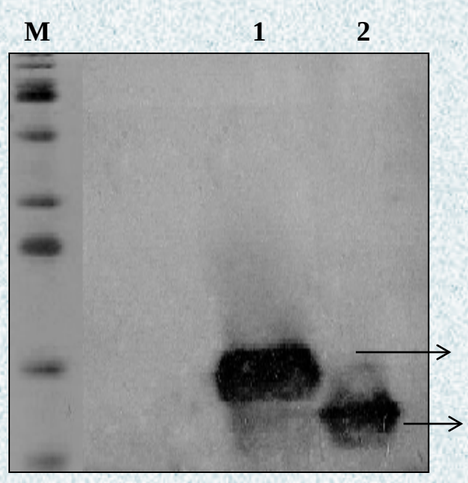
The recombinant pAE-CTB was transformed to the competent *E.coli* BL21 to express CTB protein. The system was induced by IPTG after which cells were harvested from LB medium by centrifugation and analyzed by 15% SDS-PAGE. Western blotting performed using cholera toxin-specific antibody. Recombinant CTB was expressed in this system with 6XHis tag at N-terminus and was purified through Ni2+-charged column chromatography. Concentration of protein measured with Bradford assay. The functionality of the CTB pentamers was assessed by GM1-ELISA assay.



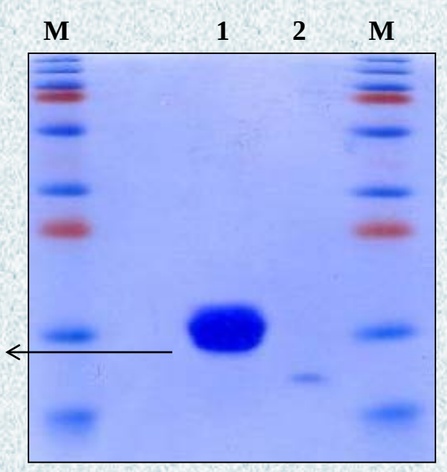
Inclusion bodied was laysed by urea and denatures protein was purified with Ni^{2+}-charged column chromatography. Lane 1, protein size marker, lane 2-11, show different fractions of purified proteins with chromatographic column

Results

SDS-PAGE analysis showed the expression of rCTB in the system and western blot analysis confirmed the presence of recombinant CTB in blotting membranes. Recombinant CTB was able to bind GM1 in a dose-dependent manner. Some part of rCTB may be expressed in the inclusion bodies so we also lubricated the inclusion bodies.



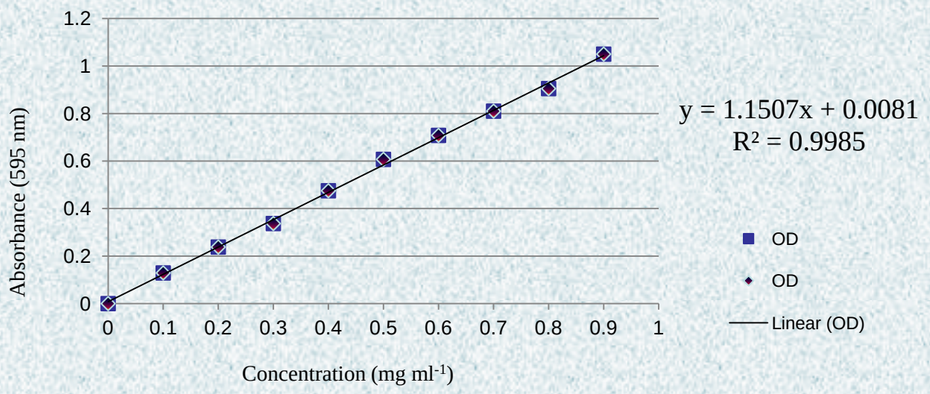
Western blotting analysis of rCTB. PVDF membrane were probed with rabbit anti-CTB antibodies and immunoconjugates and revealed with ECL reagent. Lane 1, shows rCTB and lane 2, shows commercial CTB used as positive control.



Mixture of different purified rCTB elutions analyzed with 15% SDS-PAGE gel. Lane 1, recombinant CTB after purification, lane 2, commercial CTB

Conclusion

Our results confirmed that although expressed in the inclusion bodies, 6XHis-tagged rCTB was properly refolded, easily purified, and as expected was free of possible CTA contaminants. This will enable us to study CTB immunological properties, oral tolerance, its use as mucosal adjuvant or in vaccine development.



Concentration of rCTB with Bradford assay. Ten different concentrations of bovine serum albumin (BSA) were assayed. The absorbances measured on 595 nm and were plotted and a best-fit line drawn through the points. Concentration of rCTB was about 700 µg ml^{-1}.

References

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2. Haryanti, T., Mariana, N.S., Latifah, S.Y., Yusoff, K., and Raha, A.R. 2008. Controlled Expression of Cholera Toxin B Subunit from Vibrio cholerae in Escherichia coli. Pak. J. Biol. Sci. 11(13): 1718-1722
3. Liang, W., Wang, S., Yu, F., Zhang, L., Qi, G., Liu, Y., et al. 2003. Construction and evaluation of a safe, live, oral *Vibrio cholerae* vaccine candidate, IEM108. Infect. immun. **71**(10): 5498–5504.