Abstract

Cholera is a severe diarrheal disease caused by the Gram-negative bacterium *Vibrio cholerae* O1 and O139 serogroups. Although vaccinesare available for O1 and O139 serogroups, but their protection is limited due to the short life of protective immunity and more doses are required to prolong the protection against cholera. The cost of the vaccines is high and not many countries afford to buy the vaccines for large scale vaccination. Therefore, there is a need to develop a vaccine at lowest cost possible and also able to provide long term protection. The objective of this study is to create an attenuated *Vibrio cholerae* O1 El Tor vaccine strain by mutating the *ctx*A gene, deleting the *ace* and *zot*genes, rendering it non-toxigenic but retaining its immunogenicity. Selection of vaccine candidate *Vibrio cholerae* O1 was done by genotypic and phenotypic characterization. Vibrio cholera O1 El Tor KMS368 was chosen as the vaccine candidate for this study based on its sensitivity to antimicrobial agents and the presence of a repertoire of virulent genes. The plasmid pWMcODmAKan, derived from pWM91, was used in this study, which contains the*ctx* operon with mutated *ctx*Agene and with deletion of *ace* and *zot*genes. Chloramphenicol resistant gene flanked withflippase recognition target(FRT) sites was inserted into the *ctx*operon of pWMcODmAKan, replacing kanamycin resistant geneas the selectable marker. *Vibrio cholerae* O1 El Tor mutant, VCAET-02, was obtained through conjugation with BW20767-λpir*E. coli* harboring pWMcODmA FRT *cat* carrying respective inserts, and by sucrose selection. The chloramphenicol resistant gene was removed using FRT/FLP recombination system. VCAET-02, the *ctx*A mutant obtained, was further characterized by genotypic and phenotypic methods.It still

possesses similar traits as its parental strain, such as biofilm-forming, low protease activity, hemolytic and high in motility.VCAET-02 still has the ability of producing cholera toxin (CT) which was demonstrated by CT ELISA. Thus in this study *ctx*A mutant of *V. cholerae* O1 El tor was successfully constructed and characterized.