ABSTRACT

Cholera is a lethal diarrheal disease caused by toxigenic Gram-negative *Vibrio cholerae* O1 and *Vibrio cholerae* O139 serogroups. A dynamic 4.5 kb core region termed the virulence cassette has been found only in toxigenic *V. cholerae* O1 and O139 strains. The virulence cassette contains a set of toxin producing genes including *ctx*AB, *cep*, *zot*, *ace* and *orfU*. The cholera toxin genetic element CTX is made up of the core region together with flanking RS sequences. Genome of the filamentous bacteriophage CTXΦ make up the entire CTX element. CTXΦ integrates site-specifically into the larger of the two *V. cholerae* chromosomes is the principal virulence factor of the diarrheal-causing bacterium *V. cholerae*. During infection by CTXΦ, *xer*C gene is required by the bacteriophage, which uses host XerC and XerD proteins to integrate into the bacterial genome. Mutation of the *xer*C gene prevents the infection of CTXΦ phage thus preventing the conversion of *V. cholerae* back to its virulence form. Hence, the present study was focused towards developing a genetically safe vaccine candidate by incorporating mutation in the *xer*C gene in VCUSM14P. VCUSM14P is a potential O139 vaccine candidate developed from VCUSM14 which has mutation in the *ctx* gene and was found to be immunogenic and non-reactogenic in animal model. The mutation was carried out through two different methods of site-directed mutagenesis such as λ Red recombinase system and also by using suicide plasmid allele replacement method rendering it non-toxigenic while retaining its immunogenicity. The λ Red method involves the deletion of target gene via homologous recombination between the chromosomal region of interest and a polymerase chain reaction (PCR) product that contains an antibiotic resistance cassette flanked by sequences homologous to the target DNA, *xer*C gene. This method utilizes the λ Red *gam, bet*, and *exo* gene products, which encode an efficient homologous recombination system. PCR product was obtained through one-step PCR using primers that contain 90 nucleotide (nt) homologous extensions of the *xer*C gene. Suicide plasmid vector (pWM91) was used for allele replacement where the mutated *xer*C gene carried by the suicide plasmid was conjugated into VCUSM14P strain. Merodiploid colonies which have both the wild type *xerC* gene from VCUSM14P and also mutated *xer*C gene from pWM91 Δ*xerC*::*aph*A was successfully obtained by this method. The merodiploids were verified phenotypically by

microbiological methods and genotypically by PCR. This merodiploid will form a base for the development of *xerC* mutant of *V. cholerae* vaccine candidate, VCUSM14P.