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Construction of vaccine from Lactococcus lactis bacteria using Aeromonas hydrophila virulent Aerolysin gene.

ABSTRACT

In this study the forward and reverse primers were designated to amplify the segments (~250 bps and ~650 bps) of the gene coding domains 1 and 4 of aerolysin of Aeromonas hydrophila. These two domains are involved in pathogenesis of the aerolysin gene. Sequences for two restriction enzymes, Pst I and Hind III, were included in the forward and reverse primers respectively. These restriction enzyme sites were used because they are not present within the genes of interest but are available in the multiple cloning sites of plasmid pNZ8048. Amplified PCR products were analyzed with 1% agarose gel electrophoresis and results showed that amplifications were very specific. In comparison with the DNA marker, the sizes of the amplified PCR products were determined to be approximately ~250 bps and ~650 bps respectively. PCR products were then purified by the DNA purification kit, digested with REs and ligated with linearised pNZ8048 plasmid using T4 DNA ligase. Transformation of Lactococcus lactis NZ9000 cells was performed by the electroporation method. Verification for cloning of virulent genes was performed by REs digestion and also DNA sequencing. Since several antigens (bacterial and viral) and cytokines have been efficiently produced in L. lactis, constructing and expression and utilization of recombinant L. lactis harboring the aerolysin domains (virulent) genes from A. hydrophila may induce production of antibodies in fish against this pathogen.

Keyword: Vaccine; Lactococcus lactis; Aeromonas hydrophila.