POTENTIAL USE OF AUXOTROPHIC AROA AND AROC MUTANT YERSINIA RUCKERI AS A LIVE ATTENUATED VACCINE

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POTENCIJALNA UPOTREBA AUKSOTROFNIH *aroA I aroC MUTANATA YERSINIA RUCKERI* KAO ŽIVE ATENUIRANE VAKCINE

Abstract

Yersinia ruckeri causes yersiniosis in rainbow trout (*Oncorhynchus mykiss*). Yersiniosis is the main causes of high mortalities and severe economic losses in freshwater and marine aquaculture. To treat and prevent yersiniosis, antibiotics and inactive vaccines have been used. However, use of antibiotics can cause antibiotic resistance in bacteria, while inactive vaccines do not provide prolonged protection against bacterial fish diseases. Formation of antibiotic resistance in bacteria against antibiotics, and chemical contamination of the environment are some of the undesirable outcomes. For these reasons, prevention of fish diseases using vaccination strategies is important for ensuring the profitability and sustainability of aquaculture production.

The objective of this research is to develop live attenuated vaccines against yersiniosis. To reach this aim, *aroA* and *aroC* genes of *Y. ruckeri* have been mutated and virulence and efficacy of these mutants are characterized. Our main hypothesis is that *Y. ruckeri* with mutations in their aromatic amino acid biosynthesis network (*aro*) will lose their ability to cause infections in fish and these will be used as live vaccines. To accomplish the aim of this research, 5' and 3' regions of *Y. ruckeri aro*A (5-enolpyruvylshikimate-3-phosphate syntheses) and *aroC* (2, 3-dihydroxybenzoic acid) genes are amplified and DNA fragments mutated by overlap extension PCR will be cloned into a suicide plasmid (pDS132). This plasmid will be transferred to *Y. ruckeri* for replacing wild type genes with mutated *aroA* and *aroC* genes via homologous recombination. Successful completion of this phase is expected to yield live attenuated vaccine candidates. It is expected that these live attenuated vaccines will provide resistance against the wild type *Y. ruckeri* infections in trout, and thus, prevent onset and progress

of diseases. Successful completion of this study is expected to prevent fish losses due to *Y. ruckeri* infections and increase the profitability of aquaculture. Contend with fish diseases using traditional methods is generally ineffective and expensive.

Key words*: Aromatic amino acid biyosenthesis network* (*aro*), *aroA*, *aroC*, *overlap PCR, live attenuated vaccine*