

## DEVELOPMENT OF LIVE VACCINES FROM YERSINIA RUCKERI BY AROA AND AROC GENE MUTATIONS

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### RAZVIJANJE VAKCINE PROTIV YERSINIA RUCKERI POMOĆU AROA I AROC GENSKE MUTACIJE

#### *Apstrakt*

Jersinioza koju izaziva *Yersinia ruckeri* je osnovni uzrok visokog mortaliteta i velikih ekonomskih gubitaka u slatkovodnoj i marinskoj akvakulturi. Da bi se bolest sprečila razvijena je živa atenuisana vakcina. U tu svrhu su geni *Y. ruckeri aroA* i *aroC* umnoženi i DNK fragmenti mutirani korišćenjem PCR metode kojom su klonirani i ubačeni u plazmid. Plazmid je prenet u *Y. ruckeri* da bi zamenio divlji tip gena mutiranim *aroA* i *aroC* genima, putem homologe rekombinacije. Virulenca mutiranog *Y. ruckeri* je određivana inficiranjem mladi kalifornijske pastrmke mutiranim bakterijama i izračunavanjem mortaliteta i imunog odgovora riba. Efikasnost mutiranog *Y. ruckeri* je određivana vakcinacijom riba mutiranim bakterijama i izazivanjem obolenja kod iste ribe divljim tipom *Y. ruckeri* RB0708 6 meseci po vakcinaciji. Vakcinacija kalifornijske pastrmke sa *aroA* i *aroC* mutantima kao žive vakcine doprineo je značajnoj zaštiti protiv divljeg soja *Y. ruckeri*.

*Ključne reči: sinteza aromatičnih amino kiselina, aroA, aroC, PCR, živa atenuisana vakcina*

#### *Abstract*

Yersiniosis caused by *Yersinia ruckeri* is the main causes of high mortalities and severe economic losses in freshwater and marine aquaculture. To prevent this disease, a live attenuated vaccine was developed. For this purpose, *Y. ruckeri aroA* and *aroC* genes were amplified and DNA fragments mutated by overlap extension PCR was cloned into a suicide plasmid. This plasmid was transferred to *Y. ruckeri* for replacing wild type genes with mutated *aroA* and *aroC* genes via homologous recombination. Virulence of

mutant *Y. ruckeri* was determined by infecting juvenile rainbow trout with mutant bacteria and calculating fish mortalities and immune responses in fish. Similarly, efficacy of mutant *Y. ruckeri* was determined by vaccinating fish with mutant bacteria and then challenging the same fish with wild type *Y. ruckeri* RB0708 6 months after vaccination. Vaccination of rainbow trout with the *aroA* and *aroC* mutant as a live vaccine conferred significant protection against the wild-type *Y. ruckeri*.

*Keywords: Aromatic amino acid biosynthesis network (aro), aroA, aroC, overlap PCR, live attenuated vaccine*