The role of aroA and ppk1 in Aeromonas veronii pathogenicity and the efficacy evaluation of mutant strain AV-ΔaroA/ppk1 as a live attenuated vaccine

aroA 和 *ppk1* 在 维氏气单胞菌致病性中的作用以及突变株 AV-Δ*aroA*/*ppk1* 作为减毒活疫苗的效力评估

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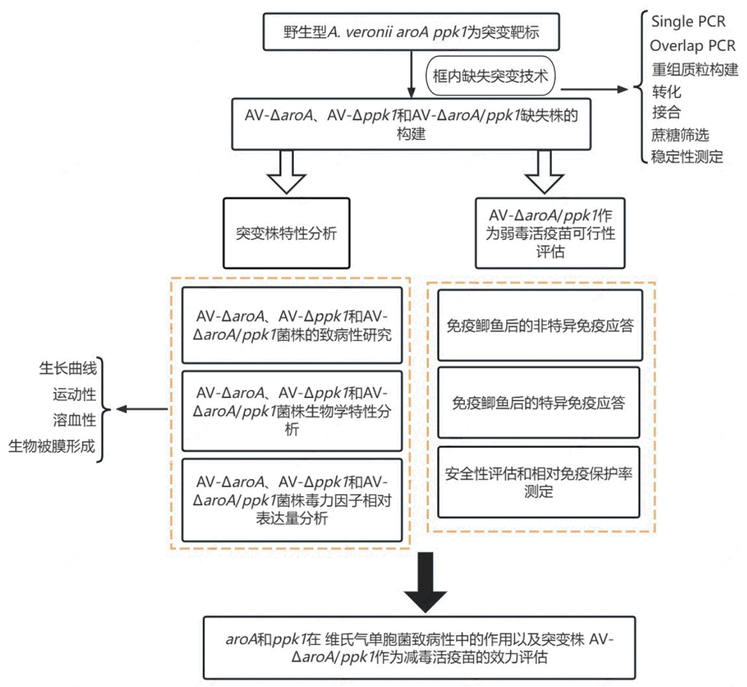
Introduction

- The ***aroA*** gene encodes 5-enolpyruvyl oxalylmanganate-3-phosphate synthase, which is vital for the synthesis and metabolism of bacterial aromatic compounds.
- Polyphosphate kinase, encoded by the ***ppk1*** gene, is a key enzyme that catalyzes bacterial ATP or GTP to form high-energy phosphate compound polyphosphates and plays an important role in bacterial growth, biofilm formation, adhesion and invasion, expression of virulence-associated factors and bacterial retention
- In this study, mutant strains with genetic stability, including single gene deletion strains (AV-Δ*aroA*, AV-Δ*ppk1*) and double gene deletion strain (AV-Δ*aroA*/*ppk1*) were constructed. Firstly, the effects of *aroA* and *ppk1* on *Aeromonas veronii* pathogenicity were investigated. Secondly, the changes in pathogenicity of the mutants were explained in terms of growth, motility, hemolysis, biofilm formation ability and the expression level of virulence-related genes. Finally, AV-Δ*aroA*/*ppk1*, which was most attenuated, was selected as live attenuated vaccine (LAV) candidate. After immunization in crucian carp, its efficacy was comprehensively evaluated from safety, nonspecific immune response levels, specific immune response levels and relative percentage survival. These results will shed light on the functional roles of *aroA* and *ppk1* in *A. veronii* pathogenicity, provide a good candidate of LAV for controlling *A. veronii* infection in aquaculture.

Technical Scheme



Results

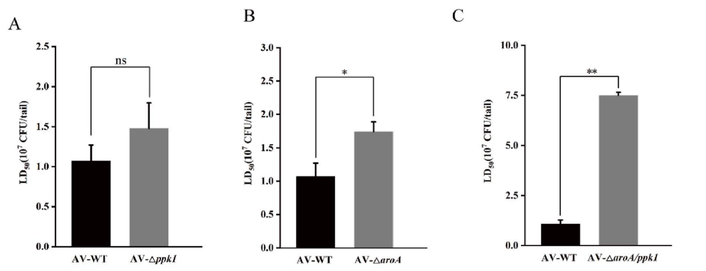


Figure 1. Pathogenicity of wild and mutant strains in crucian carp

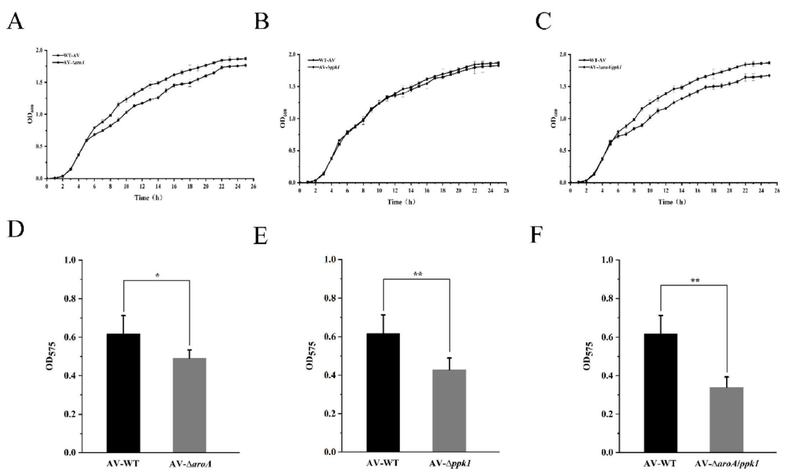


Figure 2. Growth and biofilm formation of wild and mutant strains.

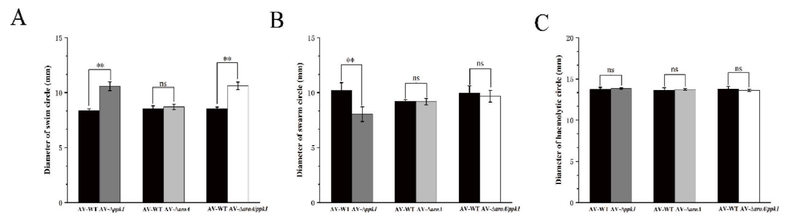


Figure 3. Determination of motility and hemolytic.

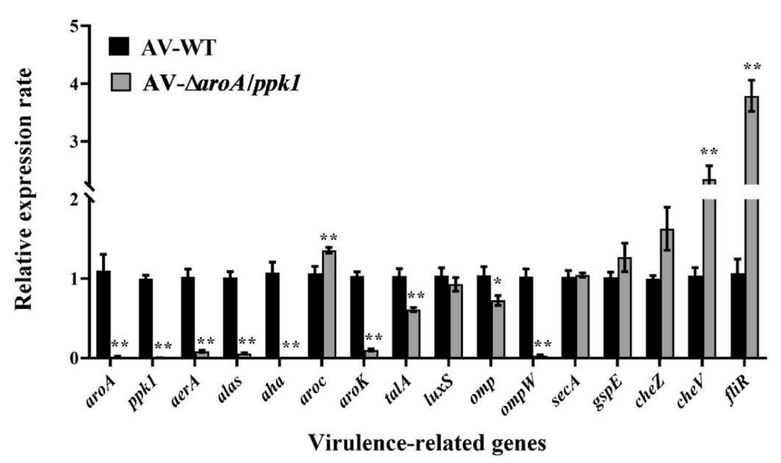


Figure 4. The relative expression of virulence-related genes by qRT-PCR

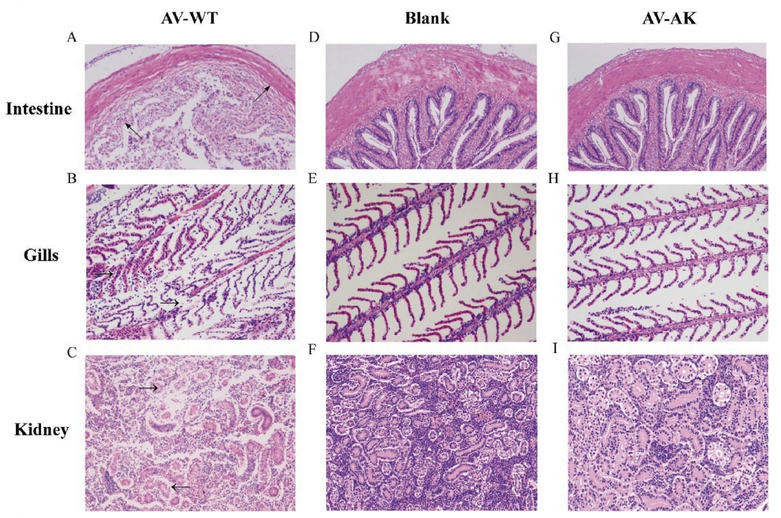


Figure 5. Histopathological observations.

Results

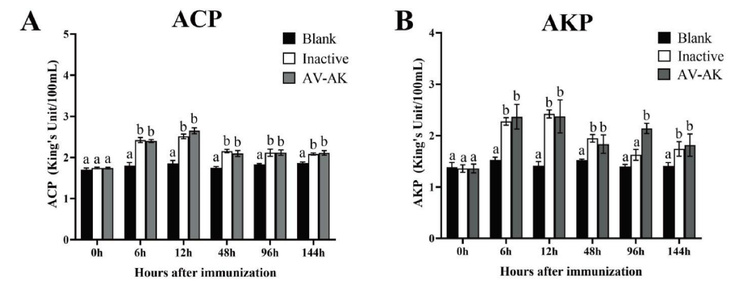


Figure 6. Nonspecific immune related enzyme activities.

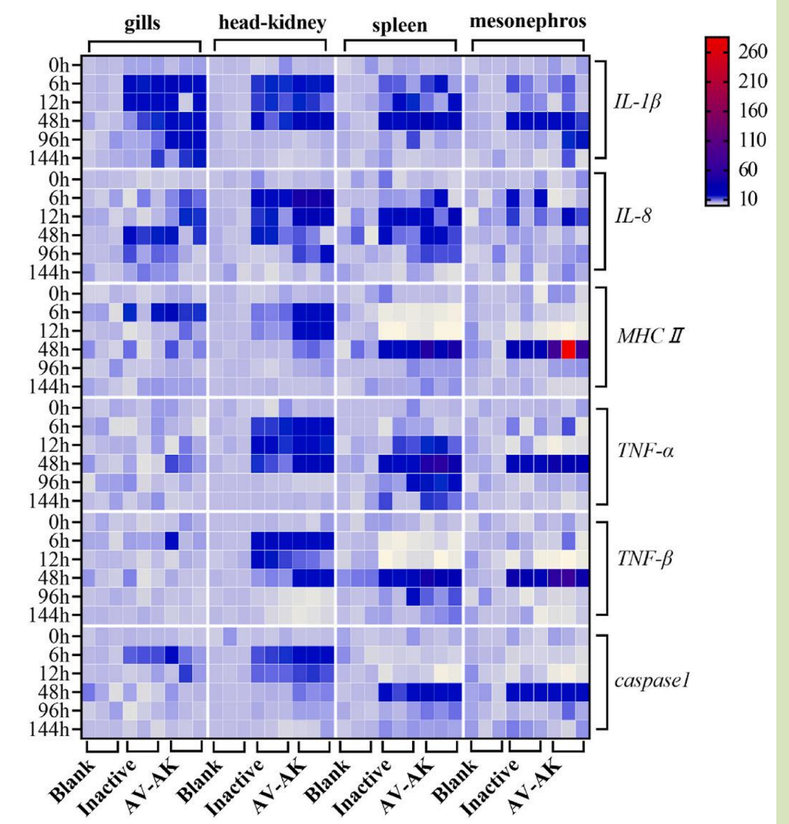


Figure 7. Heatmap of nonspecific immune-related gene expression.

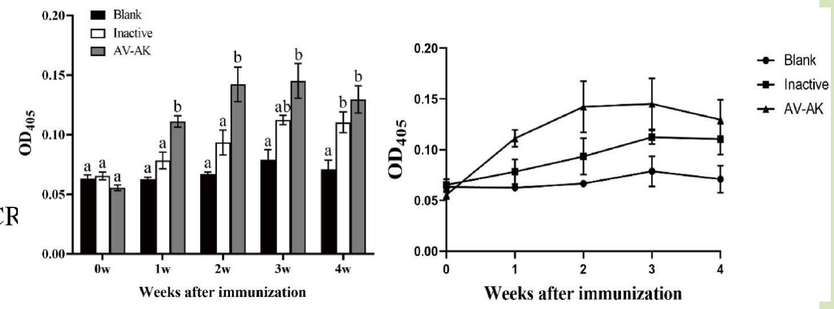


Figure 8. Changes in specific antibodies (IgM) after immunization.

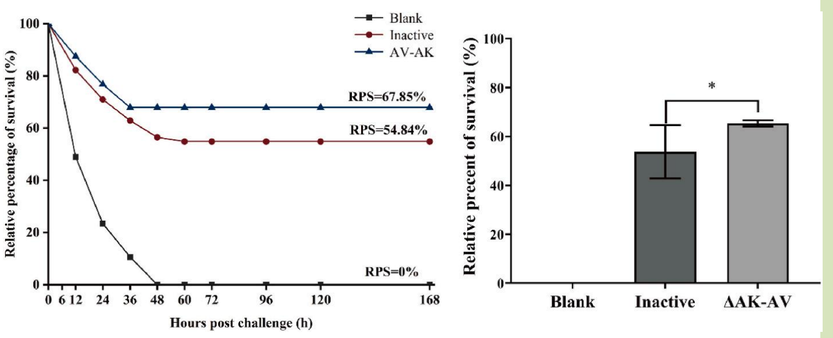
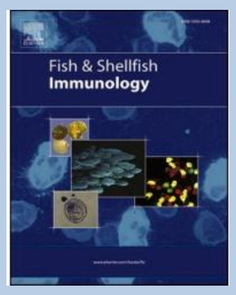


Figure 9. Survival rate of vaccinated crucian carp immunized with AV-Δ*aroA*/*ppk1*, inactivated or *A. veronii* PBS following the challenging tests of 168 h by live *A. veronii* at 28 days after immunization.

Conclusions

Compared to the AV-WT, mutants had reduced pathogenicity for reasons that may be related to decreased growth, swarming and biofilm formation abilities as well as low expression of virulence-related genes. The AV-ΔaroA/ppk1 as LAV was safe for C. carassius, can effectively increase immune-related enzyme activities, cytokine expression levels and produce more efficient antibody levels, which in turn has higher relative percentage survival (RPS). Therefore, this study provides a promising vaccine candidate for controlling A. veronii infection in aquaculture.



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