ASS1 SERVES AS A KEY TARGET FOR GENE-EDITING TO ENHANCE AMMONIA TOLERANCE IN TELEOST

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Abstract

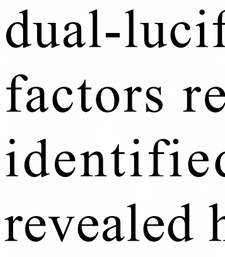
Ammonia toxicity is a key factor limiting the sustainable development of aquaculture under intensive farming conditions, and it is of great importance to develop ammonia-tolerant fish strains. However, the specific mechanisms regulating ammonia tolerance in fish remain unclear. In this study, ammonia stress induced serious injuries in fish brain with cerebral oedema, intraventricular hemorrhage, and astrocyte swelling, which was accompanied by the enhanced argininosuccinate synthase 1 (*ass1*) expression. Further gene-editing experiments confirmed the *ass1* as a key gene in fish ammonia detoxification. Subsequently, specific transcription factors were characterized using DNA pull-down and dual luciferase assays. Further knock-down and over-expression experiments in ZF4 cells elucidated the regulatory mechanisms of these transcription factors in enhancing fish ammonia resistance. This provides an important theoretical basis and potential gene targets for developing new strains of ammonia-tolerant aquaculture species.

Introduction

Ammonia toxicity is a major challenge facing sustainable aquaculture. However, the mechanisms by which fish tolerate ammonia remain unclear, and investigating these mechanisms is crucial for the development of ammonia-tolerant fish strains. This study investigated the damage caused to fish by ammonia stress and identified arginine succinate synthase 1 (*ass1*) as a key gene involved in ammonia detoxification. Through experiments involving gene editing, DNA pull-down assays and dual-luciferase assays, the specific transcription factors regulating ass1 expression were further identified. Functional validation in ZF4 cells revealed how these factors enhance fish tolerance to ammonia. The findings of this study provide a theoretical basis and potential genetic targets for the development of aquaculture strains tolerant to ammonia.

Methods

Development of animal models



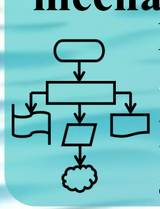
Establishment of an induced injury model in largemouth bass subjected to controlled ammonia stress.

Gene expression analysis and gene editing validation



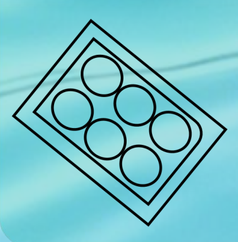
qPCR analysis of *ass1* expression in brain tissue → Knockout of *ass1* using CRISPR-Cas9 technology → Verification of its core role in ammonia detoxification.

Identification of transcriptional regulatory mechanisms



DNA pull-down / MS for binding proteins → Yeast one-hybrid assays, EMSA, and dual luciferase assays to validate the regulatory activity of transcription factors on *ass1*.

Functional validation at the ZF4 cell level



Knockdown or overexpression of candidate transcription factors to assess their regulatory role in ammonia tolerance.

Results

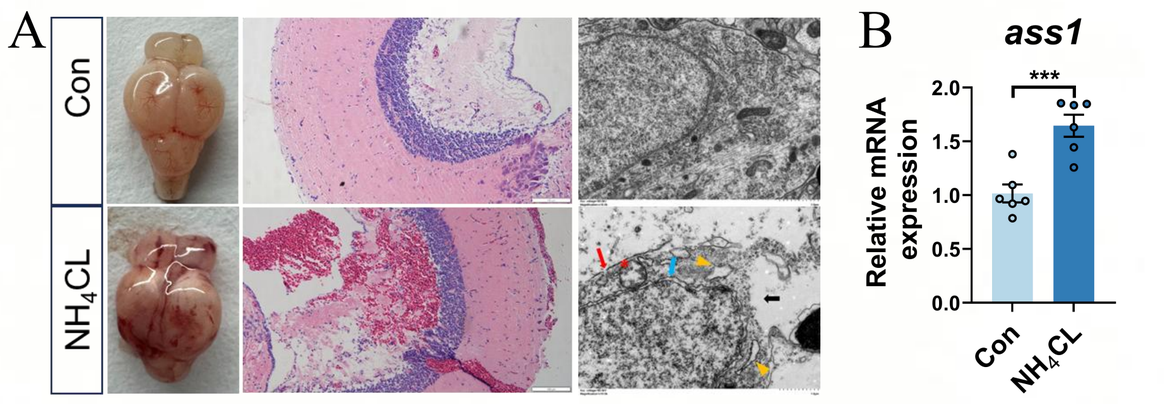


Fig. 1 Ammonia stress induced the brain injury, with cerebral oedema, intraventricular hemorrhage, and astrocyte swelling with significant upregulation of *ass1*.

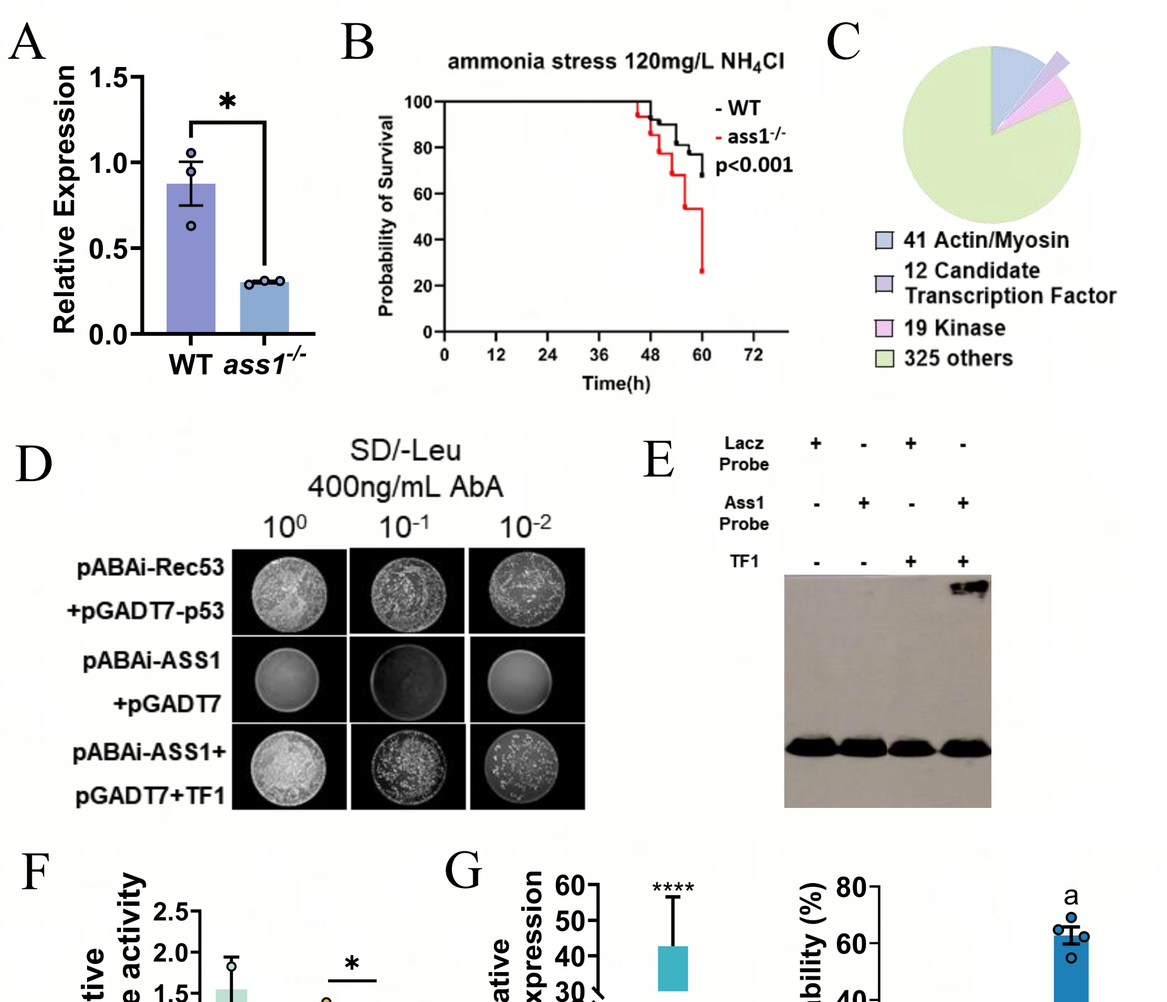


Fig. 2 TF1 plays a crucial regulatory role in fish resistance to ammonia stress.

Conclusion

This study identified the *ass1* gene and its upstream transcription factor, TF1, as key regulators in the ammonia detoxification process in fish, thereby providing potential genetic targets for the development of ammonia-tolerant aquaculture strains.

Unpublished work

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