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Evaluation of effects and genomic analysis of aquaculture probiotic strains with antioxidant and enzymatic activity

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During a screening for *Bacillus* strains with probiotic properties for aquaculture, we isolated five strains with proteolytic and amylolytic activity and five strains with high antioxidant activity. Antioxidant activity was assessed *in vitro* using bacterial LUX-biosensors [1].

Based on tests of cross-antagonistic activity and the ability of strains to form joint biofilms, two pairs of strains that did not inhibit each other's growth during co-cultivation were selected. Probiotic preparations were developed using solid-state fermentation of soybeans. Strains *Bacillus velezensis* MT14 and MT42 (Preparation 1) exhibited proteolytic and amylolytic activity, while strains *Bacillus subtilis* MT48 and MT74 (Preparation 2) demonstrated antioxidant and amylolytic activity.

The preparations were incorporated into starter feeds for sterlet larvae at a concentration of 0.1% of feed weight (final concentration: 10^6 CFU/g). Fish larvae were fed for two months. Both experimental groups showed weight gain compared to the control group receiving a commercial probiotic. However, the enzymatic probiotic performed better than the antioxidant one. This can be attributed to its proteolytic properties, which aid in protein digestion and absorption which is critical during early development and rapid growth stages.

Interestingly, gene expression analysis in sterlet tissues revealed a more pronounced effect in Group 2 (antioxidant probiotic). The antioxidant probiotic based on strains MT48 and MT74 demonstrated statistically significant protective effects on sterlet larvae. A reduction in il-1b gene expression was observed in both experimental groups, indicating reduced inflammation in fish receiving probiotics. Additionally, Group 2 showed decreased expression of *igf-1*, *hsp-70*, and *gst* genes expression. The downregulation of *gst* (associated with oxidative stress response) and *hsp-70* (linked to protein damage response) may suggest a positive impact of the antioxidant probiotic in mitigating stress and damage. Notably, the most significant effects were observed in larvae at the beginning of the experiment, implying that these probiotics are more effective during early developmental stages than in adult fish.

Genomic sequencing of the isolated strains revealed that those with antioxidant activity possessed genes responsible for pulcherriminic acid synthesis, absent in other strains. This suggests that pulcherriminic acid may act as an intermediate contributing to the antioxidant properties of these strains. Pulcherriminic acid is known for its antioxidant potential and is commonly found among *Bacillus* species [2]. Furthermore, all analyzed strains carried genes for bacilysin and bacillaene synthesis, and for a synthesis of variety of non-ribosomal products.

This study highlights the potential of *Bacillus*-based probiotics for aquaculture applications, demonstrating their ability to enhance growth performance and provide protective effects against oxidative stress in sterlet larvae. Our findings also emphasize the importance of strain-specific properties when developing probiotics tailored to aquaculture needs.

References

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