Application of Compound Microbial Agents in Aquaculture

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Abstract

Global consumption of edible fish is growing rapidly every year, and the contribution of aquaculture to global fish production is growing rapidly. The growth of aquaculture production demand is accompanied by many challenges, among which disease outbreak is the main factor hindering the development of aquaculture. In the past decades and even today, antibiotics are often used to control and prevent diseases in aquaculture, resulting in two major hazards: antibiotic residue and antibiotic resistance. Feed microbial preparations as a green and safe substitute for antibiotics have attracted great attention in recent years. This kind of preparation is prepared by fermentation, purification and other processes of natural beneficial microbial strains. It has the advantages of improving feed utilization, improving nutrition, promoting animal growth, maintaining intestinal microecological balance and enhancing the immunity of cultured products. In this experiment, two strains of probiotics with safety, protease ability, inhibition effect on common pathogenic bacteria in aquaculture and good adaptability to fish intestinal environment were selected to prepare two kinds of composite microbial agents, and *Sebastes schlegelii* was selected for feeding test. The results showed that the two probiotics had no hemolysis, had certain protease production ability, could inhibit common pathogenic bacteria in aquaculture, had good in vitro tolerance, and promoted the growth of fish. Therefore, the compound microbial agents mixed with aquaculture feed can enrich the nutrition, facilitate the digestion and absorption of aquatic animals, significantly improve the growth performance of aquatic animals, and have broad application prospects.

Purpose of Experiment

- **Two bacterial strains were selected with potential application value. Initially, strain identification was performed via phylogenetic tree analysis. Hemolytic activity was assessed using blood agar plates. Several virulence genes in *Bacillus* strain with PCR technology to detect.**
- **The amylase and protease activities were screened by using the selective medium, followed by quantitative enzymatic activity assays. The survival rates of the two strains were tested by under different pH conditions in vitro, as well as in the simulated gastric fluid, the simulated intestinal fluid, and the bile salt environments. Antimicrobial activity against five pathogenic bacteria was also evaluated in this research.**
- **The compound microbial agent was administered to *Sebastes schlegelii* in a feeding trial to examine its effects on fish.**

Result

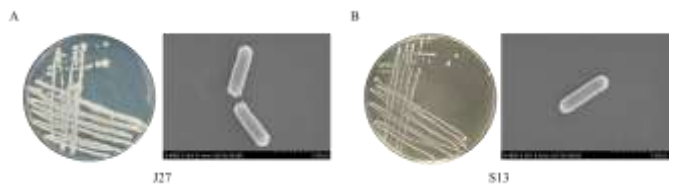

Fig. 1 Probiotic strains J27 and S13: colony morphology and scanning electron micrographs.
(A) Probiotic colony morphology;
(B) Scanning electron micrographs of probiotics.

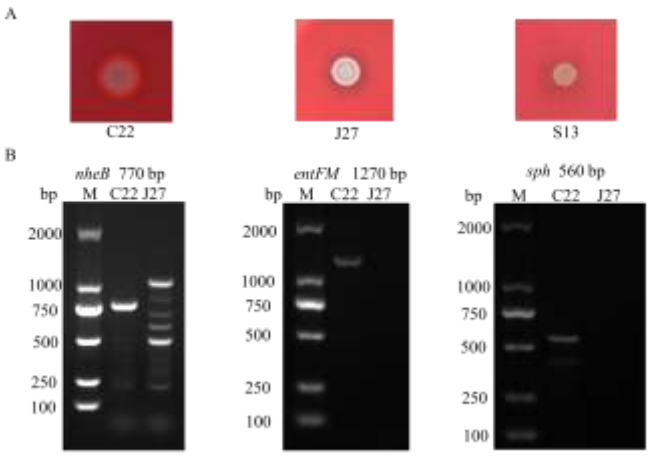

Fig. 2 Safety assessment of probiotic strains J27 and S13. Using *Bacillus cereus* C77 as a positive control.
(A) Hemolytic activity of probiotics.
(B) Detection of *Bacillus* virulence factors.
No hemolytic activity was detected in the strains, and no virulence factors were identified in the *Bacillus* strains.

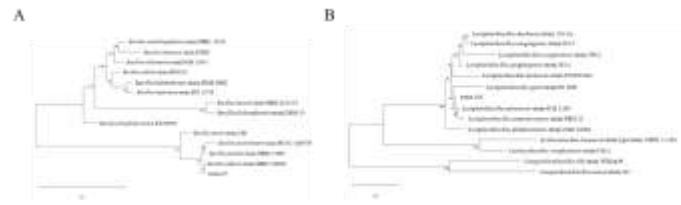

Fig. 3 Phylogenetic tree of probiotic strains.
(A) Strain J27; (B) Strain S13.
Strains J27 were identified as *Bacillus safensis*, while strain S13 was identified as *Lactiplantibacillus plantarum*.

Table. 1 Antibiotic resistance of probiotic strains J27 and S13.

Antibiotics	Chloramphenicol	Erythromycin	Streptomycin	Tetracycline	Vancomycin	Kanamycin	Gentamicin
J27	+	-	+	-	-	-	-
S13	-	-	+	-	+	+	+

Strain J27 exhibits low tolerance to chloramphenicol and lincomycin but is sensitive to other antibiotics; strain S13 is sensitive to chloramphenicol, erythromycin, and tetracycline.

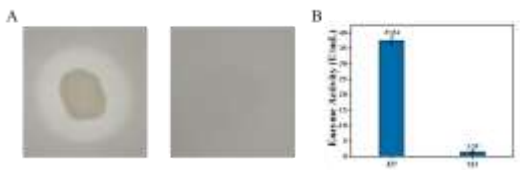

Fig. 4 Protease activity detection of strains J27 and S13.
(A) Preliminary screening of protease activity;
(B) Determination of protease activity.
The results show that strain J27 exhibits higher protease activity.

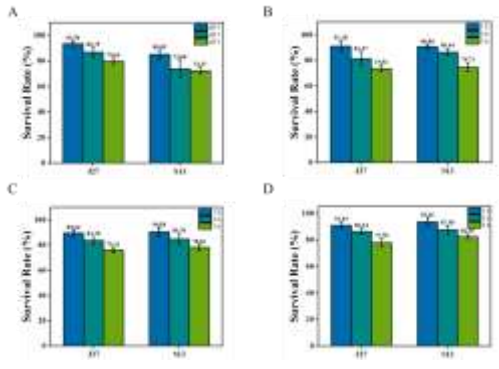

Fig. 5 In vitro tolerance of probiotic strains J27 and S13.
(A) Acid tolerance of probiotics;
(B) Gastric juice tolerance of probiotics;
(C) Intestinal fluid tolerance of probiotics;
(D) Bile salt tolerance of probiotics.
Strain J27 exhibits stronger acid tolerance capability, while strain S13 demonstrates enhanced tolerance to gastric juice, intestinal fluid, and bile salt.

Table. 2 Antibacterial activity of probiotic strains J27 and S13.

Pathogenic bacteria	*Escherichia coli*	*Staphylococcus aureus*	*Clostridium perfringens*	*Aeromonas hydrophila*	*Vibrio parahemolyticus*
J27	-	-	-	-	-
S13	+	+	+	++	++

Strain S13 exhibits inhibitory activity against all five tested pathogenic bacteria.

Table. 3 The effects of different feed groups on *Sebastes schlegelii*.

Parameters	Control	Group 1	Group 2	Group 3
IBL (cm)	9.38±0.38	9.33±0.39	9.60±0.35	9.55±0.40
IBW (g)	20.09±0.59	19.89±0.69	20.25±0.61	20.19±0.81
FBL (cm)	9.99±0.51	9.87±0.17	10.81±0.51	10.24±0.62
FBW (g)	25.50±3.31	23.68±2.44	29.66±4.17	28.99±4.74
WGR (%)	27±2.56	19±1.44	**46±3.49**	**44±3.26**

The control group was fed commercial feed, group 1 was fed commercial feed supplemented with strain J27 single-agent, group 2 was fed commercial feed supplemented with strain S13 single-agent, group 3 was fed commercial feed supplemented with a composite probiotic agent containing both strains.

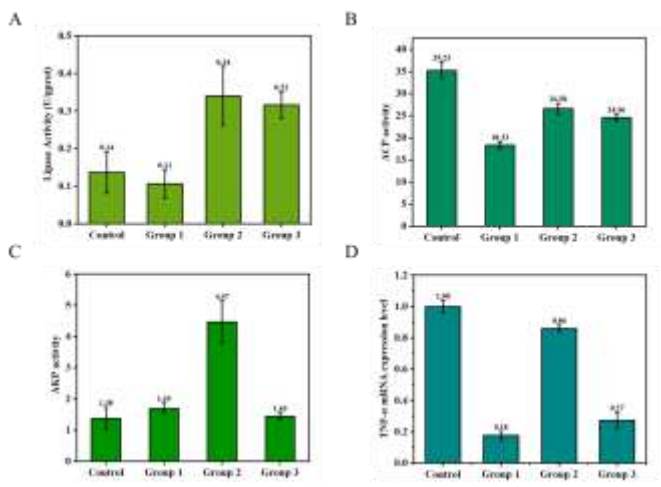

Figure 6 Fish index detection.
(A) Intestinal lipase activity; (B) Liver AKP activity;
(C) Liver AKP activity;
(D) Intestinal TNF-α mRNA expression level.

Conclusion

Two probiotic strains were screened in this research with certain safety. The protease activity was measured and conducted by vitro tolerance tests, followed by feeding trials on *Sebastes schlegelii*. The results demonstrated that the mixing compound microbial agents could significantly enhance their growth performance. The experiment indicated the broad application prospects with feeding aquaculture in the richer nutritional content, better facilitates the digestion and absorption in aquatic animals.

References

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[3] Xiao R, Wang L, Tang Z, et al. PloS one, 2024, 19.