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Probiotic Potential of Intestinal *Bacillus* for Sustainable Aquaculture of Marine Species

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Abstract

Bacillus is found not only in terrestrial and marine environments but also in the intestines of humans, animals, and marine species. Due to its ability to produce these valuable bioactive compounds, *Bacillus* is widely utilized as a key material for developing new probiotics. In an experiment using strains isolated from the intestines of sea cucumbers, the strains were tested for their ability to break down agar, alginic acid, carrageenan, cellulose, and starch-major polysaccharides in seaweed consumed by marine organisms-along with chitin. The results showed that all strains capable of degrading at least two of these macromolecules belonged to the genus *Bacillus*. Notably, *B. proteolyticus* strain SCT64 and *B. halotolerans* strain SCT118 demonstrated the ability to degrade all the tested macromolecules. Therefore, intestinal *Bacillus* strains derived from marine organisms, with their ability to produce various bioactive compounds, hold great potential as probiotics for sustainable aquaculture, particularly for farmed sea cucumbers, fish, and other marine species.

Keywords: Aquaculture; Intestinal microorganism; Maine Bacillus; Polysaccharides; Probiotics

Introduction

Bacillus is widely distributed in various aerobic and anaerobic environments, including terrestrial and marine habitats, the intestines of humans, animals, and seafood. It can also be found in extreme environments, such as those with high pH, high temperatures, and high salt concentrations. In 2020, the *Bacillus* genus was reported to include 293 species, including subspecies [1]. However, based on analyses of their physiological, biochemical, and genetic characteristics, some species have been reclassified into new genera or species.

Bacillus strains are believed to have diversified into bacteria with highly varied capabilities depending on their habitats. Certain *Bacillus* are known to produce enzymes which are of highly valuable in an industrial application: proteases are used in detergents, leather processing, and food industries; amylases in textile desizing, baking, and bioethanol production; lipases in biodiesel production and food processing; chitinases for waste management and biocontrol in agriculture [2-6]. A variety of biopolymers [7,8], bioactive substances like antifungal and antibacterial compounds against plant pathogens [9], and biopesticides and insecticides [9,10] are produced in *Bacillus* species. Accordingly, they are widely used in livestock [11], food [12], pharmaceutical [13,14], environmental [15-18], energy [19] and the cosmetics industry [20].

Bacillus species are fermentation bacteria commonly found in anaerobic environments, such as the intestines of animals. With the growing recognition of the close relationship between the gut microbiota and human health, research on probiotics derived from gut microbiota has been actively pursued. Probiotic microorganisms are live, non-pathogenic microorganisms known to play a crucial role in maintaining the balance of intestinal microbiota [21-24].

Lactic acid bacteria (LAB) and *Bifidobacteria* have long been the most commonly used bacteria for probiotic development. In contrast, *Bacillus* species, despite being commonly found in the gut, have received relatively less attention in probiotic research compared to these two bacterial groups. Recently, *Bacillus* has been used in the development of various fermented foods and as a key material in probiotics aimed at promoting health. Several *Bacillus* species, including *B. laterosporus, B. subtilis, B. clausii, B. licheniformis, B. cereus, B. coagulans,* and *B. pumilus*, have been reported as microorganisms with potential for use as human probiotics [25].

Recently, there has been growing interest in marine microorganisms that can be utilized to enhance the aquaculture of marine organisms used as food, such as fish, sea cucumbers, and abalone, as well as the industrial applications of marine algae. Marine algae, in particular, produce various polysaccharides such as agar, alginic acid, carrageenan, cellulose, and starch. Since these polysaccharides are macromolecules, converting them into low-molecular-weight substances is essential for increasing their industrial applicability and effectiveness. Microorganisms play a crucial role in breaking down these macromolecular polysaccharides into various forms of oligosaccharides [26]. Therefore, the discovery and utilization of marine microorganisms capable of decomposing high-molecular-weight substances of seaweeds hold significant value in terms of enhancing the industrial usability of raw materials.

Marine *Bacillus* species can convert marine algae into versatile and useful materials through bioconversion, enzymatic hydrolysis, and fermentation. They can be used to develop probiotics for fish aquaculture by improving fish health, enhancing digestion, and protecting against pathogens [21-23]. Marine *Bacillus* probiotics are beneficial bacteria added to fish feed or water to promote a healthy gut microbiome and overall aquaculture sustainability [21]. Marine *Bacillus* probiotics reduce feed conversion ratio (FCR), making aquaculture more cost-effective. As an example, marine *Bacillus cereus* enhances protein digestion and absorption in shrimp and fish. *Bacillus* species also produce growth-promoting factors such as vitamins, short-chain fatty acids, and antioxidants that enhance fish growth, as well as help fish tolerate environmental stress such as salinity changes, temperature fluctuations, resulting in the reducing the need for antibiotics and lowering the risk of antibiotic resistance in fish farms. All these mentioned above ensures organic aquaculture certification for environmentally friendly fish farming.

Recently, our research team conducted an experiment using sea cucumber intestines as samples to isolate superior probiotic bacteria that can be used in the aquaculture of marine organisms such as sea cucumbers and fish. A total of 169 bacterial strains grown on the MA medium were tested for their ability to degrade six polysaccharides(agar, alginic acid, carrageenan, cellulose, and starch), which are the main components of seaweed consumed by fish and sea cucumbers, as well as chitin. As a result, 50 strains that exhibited the ability to degrade at least two of the seven tested substances were identified, and all of them were found to belong to the genus *Bacillus*.

The identification test based on 16S rRNA gene sequencing revealed ten *Bacillus* species capable of degrading at least two of seven tested macromolecules: *B. haynesii*, *B. licheniformis*, *B. proteolyticus*, *B. safensis*, *B. pumilus*, *B. altitudinis*, *B. stratosphericus*, *B. halotolerans*, *B. stercoris*, and *B. velezensis* [27]. In particular, both *B. proteolyticus* strain SCT64 and *B. halotolerans* strain SCT118 were able to degrade all six tested macromolecules, suggesting that these two *Bacillus* species have high potential as probiotics for aquaculture, including the farming of sea cucumbers and other marine organisms, indicating the need for further study [28] (Figure 1).

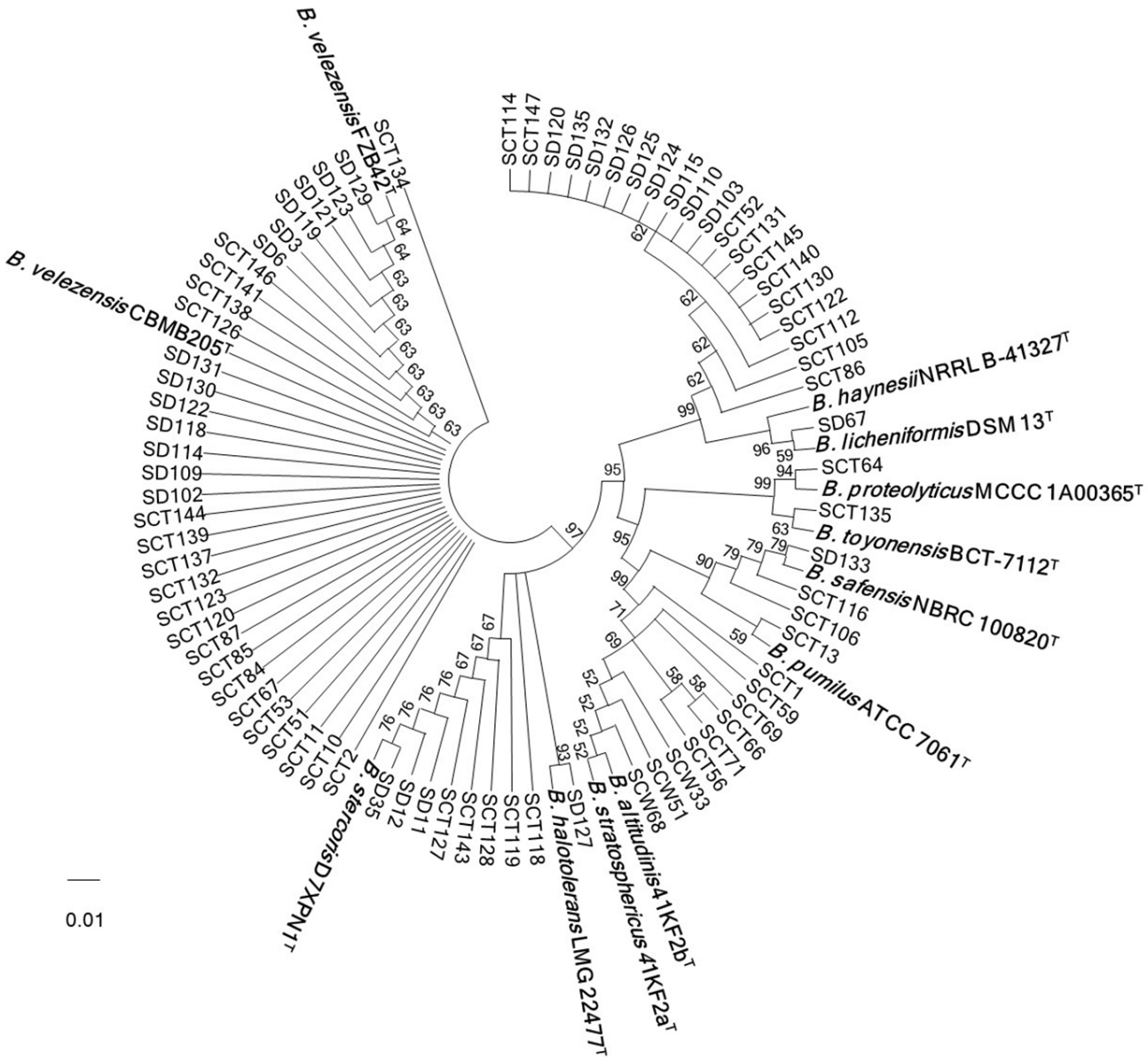

Figure 1: Neighbour-joining phylogenetic tree constructed from based on 16S rRNA gene sequences showing the relationships of *Bacillus* from sea cucumbers and sea cucumber aquaculture farm of South Korea and the type strains of species of the genus *Bacillus*. Bootstrap percentages (based on 1000 replicates) greater than 50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position

Conclusion

In conclusion, intestinal *Bacillus* from marine species could be a promising innovation in sustainable aquaculture, improving fish health, promoting growth, reducing disease, and enhancing water quality. These species also provide a natural, cost-effective alternative to antibiotics, making fish farming more efficient and eco-friendly.

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