

Probiotic Characterization and Bacteriological Analysis

The probiotic properties of the four bacterial species were characterized, as presented in Table S1. All four bacterial species were positive for catalase activity, casein hydrolysis, and starch hydrolysis, but negative for DNase activity. In the amino acid (AA) utilization test, serine was the only amino acid utilized by all bacterial species, while histidine was used only by *Exiguobacterium profundum*.

Table S1: Morphological and biochemical characteristics of four different bacterial species isolated from the biofloc system.

Note: Growth (+) or no growth (–) after incubation for 24 h at 30 °C.

Parameters	<i>Bacillus megaterium</i>	<i>Pseudomonas balearica</i>	<i>Exiguobacterium profundum</i>	<i>Pseudomonas stutzeri</i>
Gram staining	Positive	Positive	Positive	Positive
Cell morphology	Rod	Rod	Rod	Rod
Spore formation	+	+	+	+
Motility	+	+	+	+
Catalase	+	+	+	+
DNase activity	–	–	–	–
Proline	–	–	–	–
Lysine	–	–	–	–
Ornithine	–	–	–	–
Serine	+	+	+	+
Histidine	–	–	+	–
Arginine	–	–	–	–
Casein hydrolysis	+	+	+	+
Starch hydrolysis	+	+	+	+
Gelatin hydrolysis	+	+	+	–
Citrate utilization	–	–	–	–
Growth at 5% NaCl	+	+	+	+

In the present study, *B. megaterium* and *Exiguobacterium profundum* exhibited maximum inhibition zones of 14 mm against *V. parahaemolyticus*, followed by *Pseudomonas balearica*, with an inhibition zone of 13 mm (Table S2).

Table S2: Determination of antagonism of selected bacterial species against shrimp pathogens (*Vibrio parahaemolyticus*).

Bacterial sp.	VP (mm)
<i>Bacillus megaterium</i>	14
<i>Pseudomonas balearica</i>	13
<i>Exiguobacterium profundum</i>	14
<i>Pseudomonas stutzeri</i>	12