

Farm-scale evaluation of a fermented probiotic–rice bran formulation on pond conditions, gut bacteria, immunity, and production performance of *Penaeus monodon* in semi-intensive shrimp ponds

Saroj Kumar Mistry^{1,2} • Syed Hafizur Rahman¹ • Md. Golam Sarower³

¹ Department of Environmental Sciences, Jahangirnagar University, Dhaka 1342, Bangladesh

² Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka 1205, Bangladesh

³ Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna 9260, Bangladesh

Article link: <https://doi.org/10.17017/j.fish.1510>

Citation:

Mistry SK, Rahman SH, Sarower MG (2027) Farm-scale evaluation of a fermented probiotic–rice bran formulation on pond conditions, gut bacteria, immunity, and production performance of *Penaeus monodon* in semi-intensive shrimp ponds. Journal of Fisheries 15(1): 151207. DOI: 10.17017/j.fish.1510

TABLE S1 Pond-soil physicochemical properties by treatment and sampling stage.

Treatment	Sampling stage	Soil pH	Electrical conductivity (dS m ⁻¹)	Organic matter (%)	Total nitrogen (%)	Phosphorus (µg g ⁻¹)	C:N ratio
SIS	Pre-culture	6.63 ± 0.06 ^{aB}	23.73 ± 1.40 ^{aA}	1.437 ± 0.246 ^{aA}	0.087 ± 0.015 ^{aB}	16.23 ± 1.97 ^{aB}	9.45 ± 0.14 ^{aA}
	During culture	7.23 ± 0.23 ^{bA}	7.43 ± 0.29 ^{aB}	1.187 ± 0.172 ^{bB}	0.097 ± 0.006 ^{aAB}	24.93 ± 1.31 ^{aA}	7.09 ± 0.70 ^{bB}
	Post-harvest	7.10 ± 0.17 ^{bAB}	3.93 ± 0.25 ^{aC}	1.087 ± 0.120 ^{cB}	0.117 ± 0.015 ^{aA}	17.45 ± 3.70 ^{aB}	5.40 ± 0.56 ^{cB}
SIP	Pre-culture	6.80 ± 0.17 ^{aB}	22.47 ± 1.10 ^{aA}	1.157 ± 0.040 ^{aB}	0.067 ± 0.006 ^{aB}	20.25 ± 3.26 ^{aA}	9.85 ± 0.88 ^{aA}
	During culture	8.13 ± 0.15 ^{aA}	7.60 ± 0.60 ^{aB}	1.597 ± 0.196 ^{aA}	0.093 ± 0.012 ^{aBA}	24.92 ± 2.03 ^{aA}	9.89 ± 0.01 ^{bA}
	Post-harvest	8.07 ± 0.15 ^{aA}	3.70 ± 0.44 ^{aC}	1.460 ± 0.095 ^{bA}	0.083 ± 0.006 ^{bAB}	21.80 ± 2.29 ^{aA}	10.15 ± 0.99 ^{bA}
SIC	Pre-culture	7.00 ± 0.26 ^{aB}	23.67 ± 2.11 ^{aA}	1.227 ± 0.081 ^{aB}	0.073 ± 0.006 ^{aA}	20.50 ± 1.12 ^{aA}	9.68 ± 0.05 ^{aC}
	During culture	8.17 ± 0.06 ^{aA}	7.13 ± 1.05 ^{aB}	1.690 ± 0.101 ^{aA}	0.070 ± 0.010 ^{bA}	21.87 ± 3.50 ^{aA}	14.14 ± 1.56 ^{aB}
	Post-harvest	8.07 ± 0.21 ^{aA}	3.77 ± 0.31 ^{aC}	1.800 ± 0.050 ^{aA}	0.063 ± 0.015 ^{bA}	20.57 ± 2.24 ^{aA}	16.90 ± 3.33 ^{aA}

Note: Values are means ± SD of three independent ponds per treatment and sampling stage ($n = 3$). Different lowercase superscripts indicate differences among treatments within the same sampling stage, whereas different uppercase superscripts indicate differences among sampling stages within the same treatment (Bonferroni-adjusted $p < 0.05$). SIS, semi-intensive synbiotic; SIP, semi-intensive probiotic; SIC, semi-intensive control.

TABLE S2 Additional pond-soil physicochemical properties by treatment and sampling stage.

Treatment	Sampling stage	Soil physicochemical properties			
		Organic carbon (%)	Potassium (meq 100 g ⁻¹)	Calcium (meq 100 g ⁻¹)	Magnesium (meq 100 g ⁻¹)
SIS	Pre-culture	0.820 ± 0.151 ^{aA}	0.500 ± 0.173 ^{aA}	27.37 ± 1.09 ^{aB}	6.08 ± 0.06 ^{aA}
	During culture	0.687 ± 0.096 ^{bAB}	0.653 ± 0.035 ^{aA}	31.70 ± 2.72 ^{aA}	5.55 ± 0.02 ^{aA}
	Post-harvest	0.627 ± 0.070 ^{cB}	0.533 ± 0.117 ^{aA}	31.81 ± 0.45 ^{aA}	5.40 ± 0.41 ^{aA}
SIP	Pre-culture	0.653 ± 0.015 ^{aB}	0.587 ± 0.121 ^{aA}	26.56 ± 1.45 ^{aB}	6.20 ± 1.02 ^{aA}
	During culture	0.923 ± 0.115 ^{aA}	0.603 ± 0.118 ^{aA}	31.34 ± 1.04 ^{aA}	5.50 ± 0.06 ^{aA}
	Post-harvest	0.843 ± 0.055 ^{bA}	0.497 ± 0.083 ^{aA}	29.41 ± 1.56 ^{aAB}	4.67 ± 0.49 ^{aB}
SIC	Pre-culture	0.710 ± 0.052 ^{aB}	0.393 ± 0.179 ^{aA}	25.89 ± 1.20 ^{aB}	5.95 ± 0.39 ^{aA}
	During culture	0.980 ± 0.056 ^{aA}	0.620 ± 0.030 ^{aA}	31.71 ± 2.95 ^{aA}	5.51 ± 0.05 ^{aA}
	Post-harvest	1.037 ± 0.035 ^{aA}	0.463 ± 0.091 ^{aA}	28.81 ± 2.78 ^{aAB}	5.35 ± 0.11 ^{aA}

Note: Values are means ± SD of three independent ponds per treatment and sampling stage ($n = 3$). Different lowercase superscripts indicate differences among treatments within the same sampling stage, whereas different uppercase superscripts indicate differences among sampling stages within the same treatment (Bonferroni-adjusted $p < 0.05$). Means sharing at least one letter do not differ significantly. SIS, semi-intensive synbiotic; SIP, semi-intensive probiotic; SIC, semi-intensive control.

TABLE S3 Type III tests of fixed effects for additional pond-soil physicochemical properties.

Soil parameter	Treatment effect	Sampling-stage effect	Treatment × sampling-stage interaction
Organic carbon	F(2, 6) = 6.611; p = 0.030	F(2, 12) = 13.411; p = 0.001	F(4, 12) = 19.232; p < 0.001
Potassium	F(2, 6) = 0.753; p = 0.511	F(2, 12) = 4.820; p = 0.029	F(4, 12) = 0.905; p = 0.491
Calcium	F(2, 6) = 1.044; p = 0.408	F(2, 12) = 21.686; p < 0.001	F(4, 12) = 0.696; p = 0.609
Magnesium	F(2, 6) = 0.417; p = 0.677	F(2, 12) = 15.182; p = 0.001	F(4, 12) = 1.652; p = 0.225

Notes: Treatment, sampling stage, and their interaction were fitted as fixed effects. Linear mixed-effects models were fitted by restricted maximum likelihood with pond as a random intercept and Kenward–Roger denominator degrees of freedom. Bold values indicate p < 0.05.

TABLE S4 Welch’s one-way ANOVA results for pond-level mean water-quality parameters among microbial treatments.

Variable	Welch F	df ₁	df ₂	p	η ²
Depth	7.714	2	4.000	0.042	0.750
Temperature	1.153	2	3.210	0.419	0.185
Salinity	8.666	2	3.870	0.037	0.747
pH, morning	8.221	2	3.780	0.042	0.732
pH, evening	50.025	2	3.850	0.002	0.959
ΔpH	55.389	2	3.181	0.003	0.923
DO	67.659	2	3.877	0.001	0.970
Alkalinity	347.357	2	4.000	<0.001	0.993
Nitrate	166.147	2	2.867	0.001	0.978
Ammonia	—	—	—	—	—

The η² values were calculated from the conventional one-way ANOVA sums of squares. In a single-factor ANOVA, η² and partial η² are numerically equivalent.

TABLE S5 Effect of treatments on presumptive *Vibrio* colony abundance.

Colony type	SIC (CFU mL ⁻¹)	SIP (CFU mL ⁻¹)	SIS (CFU mL ⁻¹)	Statistic	p-value
Yellow colony	9900.00 ± 5875.43	10521.21 ± 3322.90	1095.45 ± 59.09	F _{2,6} = 5.49	0.044
Green colony	875.76 ± 275.94	669.70 ± 585.95	45.45 ± 56.77	F _{2,2.80} = 11.74	0.044
Black colony	12.12 ± 20.99	33.33 ± 18.92	6.06 ± 5.25	F _{2,6} = 2.23	0.188

Values are presented as mean ± SD (n = 3 independent ponds per treatment). Standard one-way ANOVA was used for yellow and black colony morphotypes, whereas Welch’s ANOVA was used for green colonies because the homogeneity of variance assumption was not met. Although the omnibus tests were significant for yellow and green colonies (p = 0.044 for both), no individual post-hoc pairwise comparison was significant at p < 0.05. Therefore, numerical differences among individual treatments should not be interpreted as statistically significant pairwise differences.

TABLE S6 Effect of treatments on gut bacterial populations.

Bacterial group	SIC	SIP	SIS	F-value	p-value
Bacillus (log ₁₀ CFU g ⁻¹ gut)	7.22 ± 0.30 ^a	7.06 ± 0.47 ^a	8.78 ± 0.19 ^b	22.987	0.002
Lactobacillus (log ₁₀ CFU g ⁻¹ gut)	4.74 ± 0.14	4.52 ± 0.66	4.63 ± 0.28	0.200	0.824
Vibrio (log ₁₀ CFU g ⁻¹ gut)	9.06 ± 0.47 ^a	8.99 ± 0.48 ^a	6.79 ± 0.07 ^b	32.830	0.001
Streptococcus (log ₁₀ CFU g ⁻¹ gut)	6.91 ± 0.04	6.92 ± 0.05	6.84 ± 0.22	0.386	0.695
Total bacteria (log ₁₀ CFU g ⁻¹ gut)	12.88 ± 1.89	11.85 ± 2.12	10.34 ± 0.10	1.813	0.242

Values are presented as mean ± standard deviation, n = 3 per treatment. Different superscript letters within the same row indicate significant differences between treatments at p < 0.05 according to Tukey HSD post-hoc comparison.

TABLE S7 Innate immune responses of *Penaeus monodon* under different microbial treatments.

Parameter	SIC	SIP	SIS	F _{2,6}	p-value	η ²
THC (×10 ⁵ cells mL ⁻¹)	42.37 ± 1.11 ^b	44.20 ± 1.45 ^b	61.47 ± 1.11 ^a	217.929	<0.001	0.986
Lysozyme (U mL ⁻¹)	19.80 ± 0.60 ^c	21.80 ± 1.08 ^b	28.05 ± 0.30 ^a	103.059	<0.001	0.972
Pro-PO (U mL ⁻¹)	0.051 ± 0.002 ^b	0.052 ± 0.002 ^b	0.091 ± 0.001 ^a	812.529	<0.001	0.996
SOD activity (U g ⁻¹ tissue)	19.15 ± 0.70 ^c	21.74 ± 0.69 ^b	28.05 ± 0.72 ^a	126.804	<0.001	0.977

Values are mean ± SD (n = 3 replicate ponds per treatment). SIC, control; SIP, probiotic; SIS, synbiotic. Different superscript letters within a row indicate significant differences according to Tukey's HSD test (p < 0.05). η², eta-squared effect size.

Table S8 Summary of Pearson correlations among environmental, microbial, immune, and production variables.

Predictor variable	Associated variables	Correlation range (r)	Direction of association
Dissolved oxygen (DO)	THC, Lysozyme, ProPO, Production	0.972 to 0.979	Positive
Dissolved oxygen (DO)	FCR	-0.894	Negative
Δ Soil organic matter (Δ Soil OM)	THC, Lysozyme, ProPO, Production	-0.915 to -0.950	Negative
Δ Soil organic matter (Δ Soil OM)	FCR	0.942	Positive
Gut <i>Bacillus</i> -like abundance	THC, Lysozyme, ProPO, Production	0.854 to 0.928	Positive
Gut <i>Bacillus</i> -like abundance	FCR	-0.751	Negative
Gut <i>Vibrio</i> -like abundance	THC, Lysozyme, ProPO, Production	-0.910 to -0.960	Negative
Gut <i>Vibrio</i> -like abundance	FCR	0.823	Positive

Note: Values represent Pearson correlation coefficients (r) obtained from nine pond replicates. All reported correlations were significant (p ≤ 0.05).