

Supplementary Materials

Antibacterial Activity of *Bacillus* Strains against Acute Hepatopancreatic Necrosis Disease-Causing *Vibrio campbellii* in Pacific White Leg Shrimp

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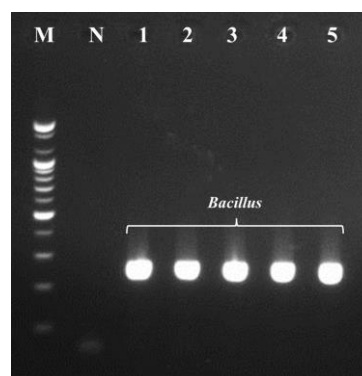
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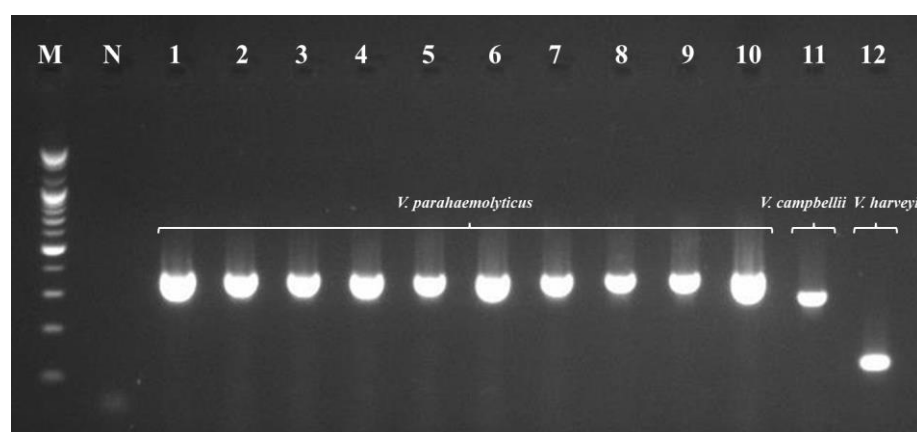
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a)



b)

Figure S1. a) PCR analysis for *Bacillus* identification (263 bp). Lane M: 100-bp ladder, Lane N: Negative control (DDH₂O), Lane 1: B1, Lane 2: B3, Lane 3: B5, Lane 4: B7, and Lane 5: B8. b) PCR analysis for *Vibrio* spp. identification (*V. parahaemolyticus*: 349 bp, *V. campbellii*: 294 bp, *V. harveyi*: 121 bp). Lane M: 100-bp ladder, Lane N: Negative control (DDH₂O), Lane 1: 13-028/A3, Lane 2: 15-250/20, Lane 3: CH49, Lane 4: CH50, Lane 5: CH51, Lane 6: CH52, Lane 7: CH53, Lane 8: 19-021D1, Lane 9: 19-022A1, Lane 10: NSU116, Lane 11: 16-904/1, and Lane 12: LB4.

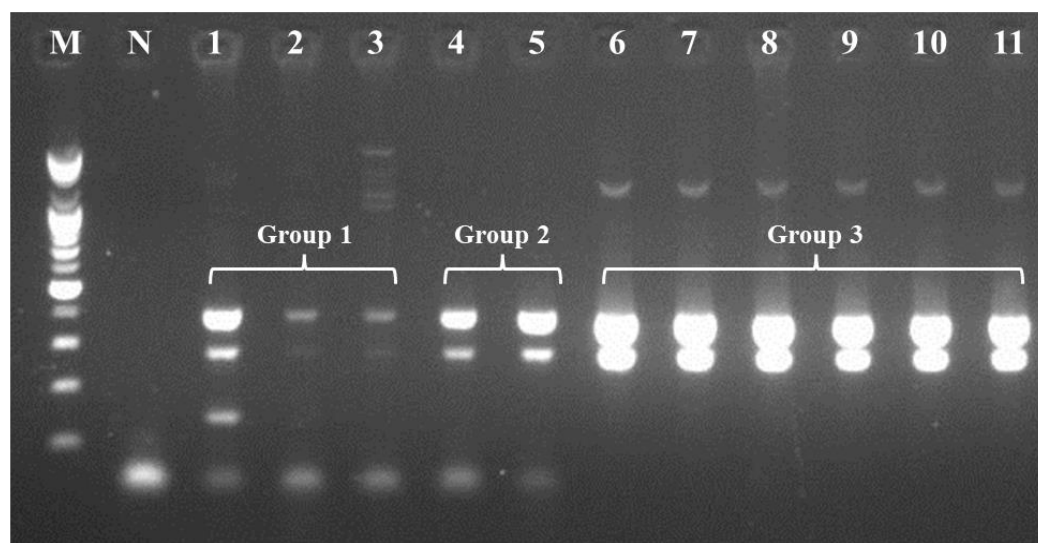



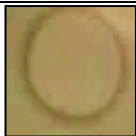







Figure S2. PCR analysis was performed to identify the AHPND toxin genes (*pirA*: 284 bp, *pirB*: 392 bp) in dead shrimp. Lane M: 100-bp ladder, Lane N: Negative control (DDH₂O), Lane 1–3: dead shrimp in group 1 (*V*_{CAHPND} immersion after B1 treatment), Lane 4–5: dead shrimp in group 2 (*V*_{CAHPND} immersion after B3 treatment), and Lane 6–11: dead shrimp in group 3 (*V*_{CAHPND} immersion without B1 and B3 treatment).

Table S1. Clear zone diameter (mm) illustrating the antibacterial activity of *Bacillus* strains used in this study against 12 *Vibrio* strains.

<i>Vibrio</i> strains	Type strain ^a (<i>B. velezensis</i>)	<i>Bacillus</i> strains				
		B1	B3	B5	B7	B8
16-904/1	<0.5	1.5	1.0	–	<0.5	0.5
13-028/A3	1.0	–	0.5	–	–	–
15-250/20	<0.5	–	0.5	<0.5	–	<0.5
CH49	<0.5	0.5	0.5	–	–	–
CH50	<0.5	–	0.5	–	–	<0.5
CH51	–	–	<0.5	1.0	–	–
CH52	<0.5	–	<0.5	–	–	–
CH53	–	2.0	2.0	1.5	0.5	0.5
19-021D1	<0.5	2.0	1.5-2.0	1.0	0.5	0.5
19-022A1	<0.5	2.0	2.0	1.0	0.5-1.0	0.5
NSU116	0.5–1.0	2.0	2.0	0.5	0.5	0.5
LB4	–	0.5	0.5	0.5	–	–

^a: CR-502^T (=NRRL B-41580^T). –: No clear zones were observed.

Table S2. Clear zone images of *Bacillus* strains (type strain, B1, and B3) against the representative *Vibrio* strains (16-904/1, 19-021D1, and 19-022A1).

<i>Vibrio</i> strains	<i>Bacillus</i> strains		
	Type strain ^a (<i>B. velezensis</i>)	B1	B3
16-904/1 (<i>V. campbellii</i>)			
19-021D1 (<i>V. parahaemolyticus</i>)			
19-022A1 (<i>V. parahaemolyticus</i>)			

^a: CR-502^T (=NRRL B-41580^T).

Table S3. The predicted secondary metabolite gene clusters in *Bacillus velezensis* NRRL B-41580^T using anti-SMASH.

Contig	Position		Type	Biosynthetic gene clusters	Substance	Similarity (%)
	From	To				
11	557,919	651,711	transAT-PKS	Polyketide + NRP	Difficidin	100
			RiPP-like	RiPP:head-to-tail cyclized peptide	Amylocyclacin	100
15	316,967	368,757	NRPS	NRP	Bacillibactin	100
					Paenibactin	100
				NRP:NRP siderophore	Bacillibactin	100
16	1	66,223	transAT-PKS	Polyketide + NRP	Bacillaene	85
	291,097	379,327	transAT-PKS	Polyketide	Macrolactin H	100
					Macrolactin H / B / 1c / E	100
	242,499	283,917	Other	Other	Bacilysin	100
17	440,952	464,140	Lanthipepti de-class-ii	RiPP:lanthipeptide	Mersacidin	100
18	2	100,307	transAT-PKS	Polyketide + NRP:lipo peptide	Bacillomycin D	100
				NRP + Polyketide	Mycosubtilin Iturin	100 88
			NRPS	NRP	Paenilarvins	100
					Fengycin	86

Table S4. The additional secondary metabolite gene clusters in *Bacillus* isolates B1 and B3 using anti-SMASH.

Region	Position		Type	Biosynthetic gene clusters	Substance	Similarity (%)
	From	To				
<i>Bacillus</i> isolate B1						
3	1,025,227	1,065,953	T3PKS	ND*	ND	-
4	1,131,293	1,151,419	Terpene	ND	ND	-
8	1,962,757	1,991,645	Lanthipeptide- class-ii	ND	ND	-
9	2,112,138	2,132,878	Terpene	ND	ND	-
10	2,214,922	2,256,166	PKS-like	Saccharide	Butiroid A / B	7
<i>Bacillus</i> isolate B3						
1	68,787	88,913	Terpene	ND*	ND	-
5	900,251	929,139	Lanthipeptide- class-ii	ND*	ND	-
6	1,049,632	1,070,372	Terpene	ND*	ND	-
7	1,152,416	1,193,660	PKS-like	Saccharide	Butiroid A / B	7
12	3,891,499	3,929,788	T3PKS	ND*	ND	-

*ND, No detection of the closest compound from the MiBIG database.