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Bacterial white patch disease caused by *Bacillus cereus*, a new emerging disease in semi-intensive culture of *Litopenaeus vannamei*

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ABSTRACT

The *Litopenaeus vannamei* aquaculture industry in India has faced a severe problem with a new type of bacterial disease namely white patch disease (WPD). Day by day the disease causes gradual mortalities and once the severe disease outbreak comes, the farm faces high mortality of more than 70% within 3 to 5 days. The major symptoms are white opaque patches in the carapace, necrosis, whitish blue coloration, loss of appetite and pale white muscles. Infected shrimp subjected Gram staining found that the rod shaped bacteria was the pathogen. Genomic identification also confirmed that the causative pathogen is *Bacillus cereus* WPD (GenBank No: KF673474.1). Also *B. cereus* WPD had the ability of having higher virulence factors including hemolytic activity, lipase activity and high mortality to the *L. vannamei* and Artemia when challenging at the rate of 10^4 to 10^8 cfu ml^{-1}.

1. Introduction

Shrimp production in India, with main contribution from *Penaeus monodon*, drastically declined to 75,000 mt in 2008 from a moderately high value of 125,000 mt in 2004. The sharp decline was mainly due to exclusive *P. monodon* culture and disease problems associated with it. The recession caused in the Indian shrimp industry urged the government of India to allow the import of specific pathogen-free (SPF) Pacific white shrimp, *Litopenaeus vannamei* to India, as this species is reported to be more disease-resistant, has high growth rate and is tolerant to high stoking densities, low salinities and temperature (Remany et al., 2010). The farmers attained a tremendous success and high profit by farming, *L. vannamei* culture which has turned the other farmers' attention to culture this alien species (Palanikumar et al., 2011).

Unfortunately, the *L. vannamei* aquaculture industry in India faced a serious problem related with a new type of bacterial disease in Nellore, Prakasam, Gundur, Krishna, West Kodavari, East Kodavari districts of Andhra Pradesh and Nagapattinam, Sirkali, Cudalore, Velankanni, Pudhukottai and Poneri region of Tamilnadu. The disease cause running or slow mortality at day by day in the cultured shrimps. Once this disease comes, the shrimps are getting low survival rate and high FCR. Sometimes the outbreaks cause heavy mortality within two to three days forced premature harvest makes economic losses to *L. vannamei* culture farmers. The important symptoms include focal to extensive necrotic area in striated tail muscle tissues and abdominal muscles tissues; necrotic areas appearing as white opaque patches and in later stage the white patches changed into black spot or splinter; whitish blue discoloration of infected shrimp body; loss of appetite; sometimes roughness on whole surface of infected shrimp with or without red discoloration; pale white muscles; many of these dead shrimp displayed intact empty exoskeletons with large portions of internal tissue and tail muscle absent as if eaten or degraded from the inside outwards and in acute stage, mortality was up to 70%.

Histological and genomic identification results revealed that, the causative agent for the white patch disease was confirmed as the Gram positive rod shaped bacterial pathogen, *Bacillus cereus.* Several *B. cereus* strains have been identified as the causative agent for different types of food poisoning (Ehling-Schulz et al., 2004). The present work intends to identify and characterize the novel bacterial pathogen causing white patch disease in *L. vannamei* culture.

2. Materials and methods

2.1. Litopenaeus vannamei samples

The moribund Pacific white shrimp *L. vannamei* samples were collected from the semi-intensive shrimp farms of Tanuvtur, Prakasam district in Andhra Pradesh, wherein moss mortalities were due to bacterial infection. The moribund shrimps were aseptically transported to the nearest microbiology lab at Ongole, Andhra Pradesh within 1 h using sterile polythene bag containing oxygenated farm water at the water temperature 26 ± 28 °C. The major symptoms of the moribund shrimps

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were white opaque patches in the carapace, necrosis, whitish blue coloration, loss of appetite and pale white muscles.

2.2. Water quality and other physicochemical parameters during disease outbreaks

The water quality parameters including salinity, dissolved oxygen (DO), pH, alkalinity, hardness and ammonia were analyzed during sampling in the infected pond. The water quality parameters were analyzed by the standard protocols described by Bhaskaran (1964) and tabulated in Table 1.

2.3. Bacterial isolation

The microbiological examination was carried out by randomly picking up ten numbers from each pond (PI, PII and PIII) including moribund and normal shrimps. Known weight of outer and inner tissues including muscle, hepatopancreas, gills, gut, eye stalk and tail of the infected shrimp was dissected out aseptically, homogenized in phosphate buffered saline (PBS) and serially diluted in the same buffer up to 10^{-7} dilutions. Triplicate samples were plated on nutrient Agar, zobell marine agar and thiosulfate citrate bile salt sucrose (TCBS) agar (HIMEDIA, India) for specific and differential bacterial counts. The inoculated plates were incubated at 28 °C until appearance of colonies (approximately 24 h).

2.4. Screening for WSSV

Due to the presence of white patches in the shrimps, they were screened for WSSV for confirming the presence of WSSV. In this regards, genomic DNA was extracted from hemolymph following the method described by Chang et al. (1999). Two step PCR amplification was performed from the genomic DNA template using the primer designed by Namita et al. (2004) using WSSV VP28 primers. The negative samples detected in the first step PCR were further subjected to second step PCR analysis. Triplicate samples from each sampling area were analyzed for the diagnostic PCR.

2.5. Virulence factors

2.5.1. Cumulative mortality

In order to study the virulence of the causative bacterial pathogen, challenges were made against healthy *L. vannamei* PL, adult and *Artemia franciscana* adults using the bacterial micro biota isolated from the infected shrimps. The dominant isolates were incubated in Zobell Marine Broth 2216 (Himedia, India) at 28 ± 2 °C for 24 h, and the harvested colonies were propagated overnight in Tryptic Soy Broth (TSB: Himedia, India) supplemented with 1% NaCl in a shaking culture (100 rpm). The cultures were centrifuged at 5000 ×*g* for15 min at 15 °C and resuspended in sterile saline (1.5% NaCl) water. The bacterial cell density of 10^8 cfu ml^{-1} which diluted in saline water and densities were calculated by counting the cells in hemocytometer and adjusted. Ten microliters of the bacterial cells was intraperitoneally injected in the second abdominal segment to *L. vannamei* adult weighed of 8 ± 2 g in triplicate ($n = 20 \times 3 = 60$) in 1000 l glass tanks (Saeed and Plump, 1986). The control group was injected with 10 μl of saline instead of bacterial cells. *L. vannamei* post larvae (PL-15) were also challenged orally with a density of 10^8 cfu ml^{-1} in triplicate ($n = 50 \times 3 = 150$) in 100 l glass tanks. *A. franciscana* measuring 8 ± 0.5 mm were also orally challenged ($n = 50 \times 3 = 150$) with the cell density mentioned above. The survival and other pathological signs were observed until 10 days after challenge.

2.5.2. Extra cellular virulence factors

Proteolytic activity was determined by production of a clear zone of proteolysis around the colonies on skim milk agar plates incubated at 37 °C for 24 h. Hemolytic activity was determined by producing a zone of hemolysis around the colonies on blood agar plates containing 2% (v/v) human blood (Creti et al., 2004). Gelatinase assay was detected qualitatively by inoculating the *bacterial* cells onto gelatin agar and incubating at 35 °C for 24 h. The growth of the isolates on the plate was then flooded with mercuric chloride solution. A clear zone around the colonies indicated the digestion of gelatin and production of gelatinase by the organism. For lipase assay, bacterial isolates were cultured on nutrient agar plates containing olive oil (2.5%), Victoria blue (0.4 mg/l) and appropriate salt concentration with an initial pH of 7.2–7.4. The plates were incubated at 37 °C for 48 h and the colonies with blue color zones were identified.

2.6. Bacterial identification protocol

2.6.1. Phenotypic identification

The bacterial micro biota such as PI-3, PII-2 and PIII-2 were selected for phenotypic identification based on higher virulence. The identification protocols including morphology, biochemical and physiological confirmative tests were based on the methodologies described by Bergey's Manual of Systematic Bacteriology (Vos et al., 2009) and the Bacteriological Analytical Manual (Tallent et al., 2012). The identifications include Gram staining, motility, endospore forming, growth on different media, indole production, methyl red, Voges Proskauer, citrate utilization, catalase test, oxidase, urease, nitrate reduction, carbohydrate fermentation, lysozyme production, hemolysis, toxin production, etc.

2.6.2. Gram staining of shrimp tissue samples

The different tissue samples were fixed in 10% formalin fixative for 24 h. The fixed tissue was washed with water and then the sample was subjected to the dehydration process, which was accomplished by passing the tissue through a gradient of isopropyl alcohol from 70% to 100%, followed by clearing the isopropyl alcohol using xylene and

Table 1
Water quality parameters of the sampling sites of white patch disease (WPD) affected shrimp farm in the Prakasam district of Andra Pradesh.

Water quality parameters	(Pond-I)		(Pond-II)		(Pond-III)	
	Sampling time					
	7.00 AM	5.00 PM	7.00 AM	5.00 PM	7.00 AM	5.00 PM
Temperature (°C)	18.3 ± 0.46	28.4 ± 1.3	17.8 ± 0.55	27.0 ± 1.8	20.5 ± 0.84	28.9 ± 1.54
pH	8.1 ± 0.2	8.4 ± 0.9	7.8 ± 0.6	8.0 ± 0.1	8.2 ± 0.33	8.5 ± 0.2
DO (ppm)	3.2 ± 0.15	4.8 ± 0.43	4.2 ± 0.4	5.5 ± 0.7	3.8 ± 0.21	4.9 ± 0.2
Alkalinity (ppm)	180.6 ± 5.5	168.5 ± 2.8	194.8 ± 2.6	175.5 ± 2.8	140.4 ± 1.5	112.2 ± 1.3
Salinity (‰)	30.06 ± 1.8	30.2 ± 1.7	28.4 ± 2.8	27.3 ± 7.2	27.3 ± 0.82	25.0 ± 1.2
Hardness (ppm)	6540.5 ± 10.2	6320.8 ± 9. 7	5620.9 ± 22.9	5460.7 ± 14.5	5870.6 ± 25.3	4930.2 ± 10.11
Ammonia (ppm)	1.04 ± 0.03	0.52 ± 0.03	0.75 ± 0.02	0.35 ± 0.02	1.2 ± 0.02	1.05 ± 0.02

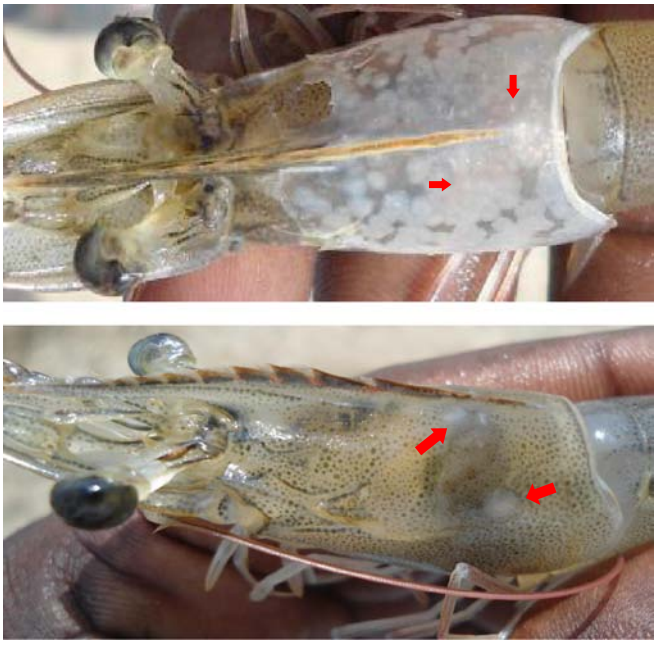

Fig. 1. White patch disease (WPD) caused by bacterial infection in Pacific white shrimp *L. vannamei* during massive disease outbreak. Arrows indicates white patches in carapace.

embedded with paraffin wax (Himedia, India). Then, a thin section of 7 μm thickness was obtained using a microtome (Besto). The section was processed and subjected to hematoxylin–eosin basic staining protocol. The slide was mounted with DPX (dis-trene plasticizer xylene) and observed under the microscope for documentation.

2.6.3. Genomic identification

The dominant micro biota (Bacillus sp) was genomically identified by 16SrRNA sequencing. To these 100 ng of genomic DNA which was extracted from the *Bacillus* sp. and the 16S rRNA gene was amplified using the universal primer with the standard PCR protocol. The PCR products were purified by a gel extraction kit (Medox Biotech India Pvt. Ltd) and sequenced (Amnion Biosciences, Bangaluru, India). The nucleotides of the 16SrRNA sequence were matched with the other microbes in the NCBI database using the BLAST program. The construction of phylogenetic tree was carried out by the Geneious Basic software and evolutionary history inferred using the neighbor joining method (Sneath and Sokal, 1973).

2.7. Data analysis

One-way ANOVA was carried out using the SPSS statistics data package and the means were compared at 0.001% level.

3. Results

3.1. Pathology of infected L. vannamei

The *L. vannamei* had slow mortality day by day after the onset of white patch disease with high food consumption rate. The principle clinical signs of the affected shrimp were (a) focal to extensive necrotic area in striated tail muscle tissues and abdominal muscles tissues; (b) necrotic areas appearing as white opaque patches and in later stage white patches changed into black spot or splinter; (c) whitish blue discoloration of infected shrimp body; (d) loss of appetite; (e) sometimes roughness on whole surface of infected shrimp with or without red discoloration; (f) pale white muscles; (g) many of these dead shrimp displayed intact empty exoskeletons with large portions of internal tissue and tail muscle absent as if eaten or degraded from the inside outwards and in acute stage, mortality was up to 70% (Fig. 1).

3.2. Bacterial isolation

Four dominant bacterial genera were isolated from each of the ponds. They were namely PI-1, PI-2, PI-3 and PI-4 from pond-I; PII-1, PII-2, PII-3 and PII-4 from pond-II; PIII-1, PIII-2, PIII-3 and PIII respectively. The colonies PI-3, PII-2 and PIII-2 had identical morphology and this isolated all ten shrimps screened from the three ponds (Table 2).

3.3. Screening for WSSV

Double step PCR amplification performed from the genomic DNA of white patch infected *L. vannamei* revealed that, there are no positive PCR signals observed in the all tested shrimps from three different ponds (Table 3).

3.4. Virulence factor studies

Cumulative mortality (%) data of *L. vannamei* adult, PL and *A. franciscana* challenged with different bacterial isolates from WPD infected *L. vannamei* were given in Table 4. Among the different bacterial micro biota challenged against the shrimp and artemia, the bacterial micro biota of PI-3, PII-2 and PIII-3 effectively killed the tested shrimp and artemia. The cumulative mortality observed was 92.4%, 100% and 100% in *L. vannamei* PL, adult and *A. franciscana* against the PI-3 challenge. The *L. vannamei* PL, adult and A. *franciscana* challenged

Table 2
Dominant bacterial micro biota isolated from the white patch disease affected ponds (I to III) in the Prakasam district of Andra Pradesh.

Infected samples (numbers)	Dominant bacterial micro biota isolated											
	Pond-I				Pond-II				Pond-III			
	PI-1	PI-2	PI-3[a]	PI-4	PII-1	PII-2[a]	PII-3	PII-4	PIII-1	PIII-2[a]	PIII-3	PIII-4
1	+	−	+	+	−	+	+	+	+	+	+	+
2	−	+	+	−	−	+	−	+	+	+	+	+
3	+	+	+	+	+	+	−	+	−	+	−	−
4	−	+	+	+	+	+	−	−	+	+	+	−
5	−	+	+	−	+	+	+	+	+	+	−	+
6	+	+	+	+	+	+	+	+	+	+	+	+
7	+	−	+	+	−	+	+	+	+	+	+	+
8	−	+	+	−	−	+	−	+	+	+	−	+
9	+	−	+	+	+	+	+	−	+	+	+	+
10	−	−	+	+	−	+	−	+	−	+	+	−

[a] The dominant micro biota *Bacillus cereus* WPD isolated from all screened *L. vannamei* from different ponds.

Table 3
PCR screening for WSSV from white patch disease affected *L. vannamei* from different ponds in the Prakasam district of Andra Pradesh.

Infected samples (numbers)	Dominant bacterial micro biota isolated					
	Pond-I		Pond-II		Pond-III	
	Ist step	IInd step	Ist step	IInd step	Ist step	IInd step
1	–	–	–	–	–	–
2	–	–	–	–	–	–
3	–	–	–	–	–	–
4	–	–	–	–	–	–
5	–	–	–	–	–	–
6	–	–	–	–	–	–
7	–	–	–	–	–	–
8	–	–	–	–	–	–
9	–	–	–	–	–	–
10	–	–	–	–	–	–

the bacteria PII-2 attained the mortalities of 96.6, 100 and 98.50 % respectively. There was also higher mortality (95% to 100%) observed against the PIII-2 challenge. One-way ANOVA revealed that the data were statistically significant ($P \leq 0.001$) between different bacterial micro biota challenges against the shrimp and artemia.

3.5. Identification of suspected bacterial pathogen

3.5.1. Phenotypic identification

The phenotypic identification protocol revealed that, the suspected bacterial micro biota (PI-3, PII-2 and PIII-2) were Gram positive, motile and have endospore forming ability. They were positive for methyl red, catalase test, starch hydrolysis, oxidase test, tyrosine agar and phenol red glucose broth etc. They were negative for Voges Proskauer test, indole production, citrate utilization, urease test and nitrate reduction etc. All the three strains had the ability of fermenting the carbohydrates, resistant against lysozymes, hemolysis and toxin producing abilities. Based on the morphological, biochemical and physiological confirmative tests, the suspected three strains were conformed as *Bacillus cereus* (Table 5).

3.5.2. Gram staining of shrimp tissue samples

Gram staining results indicated that, Fig. 2a and b clearly shows the presence of rod-shaped Gram positive bacteria inside the tissue sections of the abdominal muscles of the white patch infected *L. vannamei*.

Table 4
Cumulative mortality (%) of *L. vannamei* PL, adult and *A. franciscana* challenged with the bacterial pathogens at 10^8 cfu ml^{-1} isolated from WPD infected *L. vannamei*. Means with the same superscripts (a–i) do not differ from each other ($P < 0.001$) — one-way ANOVA.

Sl. no.	Bacterial micro biota	Percentage mortality after challenging		
		L. vannamei PL	*L. vannamei* adult	*A. franciscana*
1	PI-1	2.1 ± 0.05 ^{a}	3.2 ± 0.02 ^{a}	1.7 ± 0.01 ^{a}
2	PI-2	0.0 ± 0.0 ^{b}	3.4 ± 0.03 ^{a}	3.9 ± 0.07 ^{b}
3	PI-3*	92.4 ± 5.9 ^{c}	100 ± 0.0 ^{b}	100 ± 0.0 ^{c}
4	PI-4	3.7 ± 0.01^{d}	6.5 ± 0.14 ^{c}	5.3 ± 0.12 ^{d}
5	PII-1	3.5 ± 0.04 ^{d}	4.7 ± 0.06 ^{d}	3.8 ± 0.09 ^{b}
6	PII-2*	96.6 ± 3.3 ^{e}	100 ± 0.0 ^{b}	98.50 ± 2.6 ^{e}
7	PII-3	3.9 ± 0.02 ^{d}	5.8 ± 0.04 ^{e}	6.9 ± 0.02 ^{f}
8	PII-4	4.2 ± 0.07 ^{f}	6.5 ± 0.06 ^{c}	4.8 ± 0.06 ^{g}
9	PIII-1	2.3 ± 0.03 ^{a}	6.8 ± 0.04 ^{c}	3.1 ± 0.07 ^{h}
10	PIII-2*	95.2 ± 1.34 ^{g}	100 ± 0.0 ^{b}	100 ± 0.0 ^{c}
11	PIII-3	6.6 ± 0.05 ^{h}	8.8 ± 0.2 ^{f}	7.8 ± 0.03 ^{i}
12	PIII-4	2.7 ± 0.15 ^{a}	4.01 ± 0.09 ^{g}	3.06 ± 0.01 ^{h}

* The dominant micro biota *Bacillus cereus* WPD isolated from Pond-I, Pond-II and Pond-III.

Table 5
Phenotypic identification of selected virulent bacterial micro biota isolated from white patch disease (WPD) affected *L. vannamei* from different ponds.

Sl. no.	Biochemical tests	Bacterial micro biota isolated		
		PI-1	PII-2	PIII-3
1	Gram staining	Positive rod	Positive rod	Positive rod
2	Motility	M	M	M
3	Endospore forming	Ellipsoidal	Ellipsoidal	Ellipsoidal
4	Methyl red	+	+	+
5	Voges Proskauer	–	–	–
6	Indole production	–	–	–
7	Citrate utilization	–	–	–
8	Catalase test	+	+	+
9	Starch hydrolysis	+	+	+
10	Oxidase test	+	+	+
11	Urease test	–	–	–
12	Nitrate reduction	–	–	–
13	MYP agar	+	+	+
14	Tyrosine agar	+	+	+
15	Phenol red glucose broth	+	+	+
16	Lysozyme resistant	+	+	+
17	Hemolysis	+	+	+
18	Toxin production	+	+	+
19	Carbohydrate fermentation			
	Glucose	+	+	+
	Sucrose	+	+	+
	Fructose	+	+	+
	Lactose	+	+	+
	Maltose	+	+	+
	Galactose	+	+	+

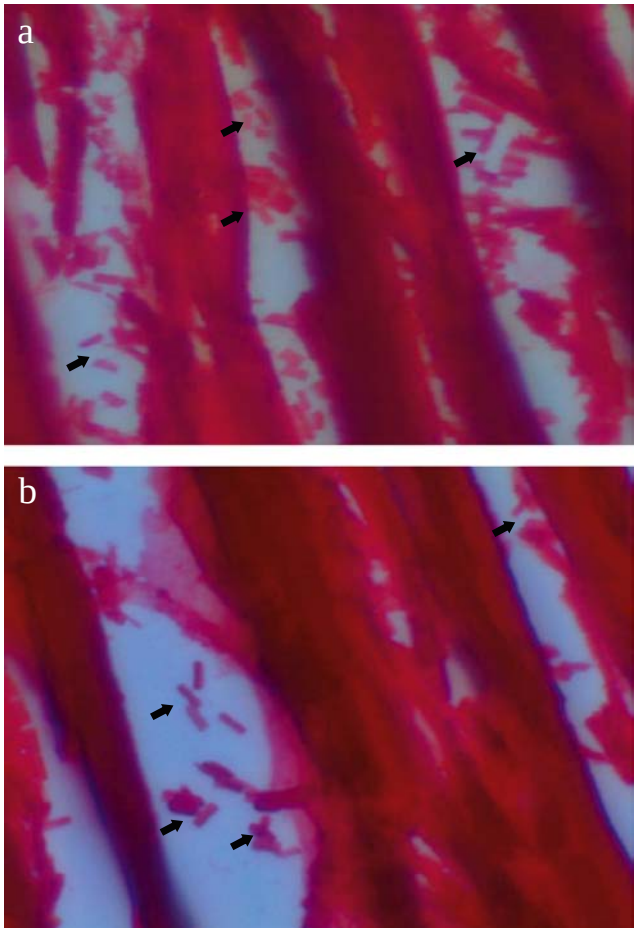

Fig. 2. a & b. Histopathological studies of the infected muscle tissue samples of WPD infected shrimp after Gram staining (40× magnification). Arrows indicate the presence of rod shaped bacteria.

3.5.3. Genomic level identification for the causative bacterial pathogen

NCBI Blast search analysis revealed that, the WPD causative pathogen conformed as *Bacillus cereus* WPD. Phylogenetic and evolutionary analysis of the 16S rRNA sequence revealed that, *B. cereus* WPD shared 98% similarity with other *B. cereus* such as *B. cereus* IAM. The higher similarity followed by the *Bacillus* strains of *B. cereus* Y-TSB18, *B. cereus* BAB2832 and *B. cereus* XX2010, etc. (Fig. 3). The sequence was deposited in the NCBI database and strain name and GenBank accession number was *Bacillus cereus* WPD; KF673474.1.

4. Discussion

The victim of the white patch disease started very slowly in the year 2012 at the southern part of Andra Pradesh, India and it gradually spread to a moderate level in the year 2013 to the other parts of Andra Pradesh including Nellore, Prakasam, Gundur, Krishna, West Kodavari, and East Kodavari districts of Andra Pradesh and Nagapattinam, Sirkali, Cudalore, Velankanni, Pudhukottai and Poneri region of Tamilnadu. Presently, the disease outbreak was found in the semi-intensive *L. vannamei* culture of Andra Pradesh and Tamilnadu. Earlier (i.e., 2012), the mortality due to white patch disease happened in summer season only. Later, in the year 2014, the disease outbreak happened in both the winter and summer season also. In the present study, the white patch affected *L. vannamei* first had the symptom of extensive necrotic area in striated tail muscle tissues and the necrotic areas appear as white opaque patches in later stage and at the end the white patches changed into black spot or splinter. The earlier work done by Wang et al. (2000), *Bacillus subtilis* is the causative bacterial pathogen for causing bacterial white spot syndrome (BWSS) disease in *P. monodon* in the commercial shrimp farms of Peninsular Malaysia. The infected shrimp showed white spots similar to those caused by the white spot syndrome virus (WSSV) and the presence of large numbers of bacilliform bacteria.

In the present study, twelve bacterial micro biota were isolated from three different ponds and virulence studies were performed to confirm the real culprit for the WPD in the *L. vannamei*. Double PCR screening for WSSV also confirmed that no PCR signals observed in the tested 10 shrimps from each pond. Among the tested shrimps (30 numbers from three ponds), *Bacillus* sp. were detected (PI-1, PII-2 and PIII-3) in all shrimps and later it was confirmed as *B. cereus* WPD. The challenged results revealed that, the bacterial micro biota PI-1, PII-2 and PIII-3 from the three ponds were highly virulent and effectively killed the tested shrimp and artemia within 3 days. Gram staining in the muscle sample also confirmed that, there was Gram positive rod shaped bacteria in the striated muscle WPD infected *L. vannamei*. Based on the results, the bacterial micro biota of PI-1, PII-2 and PIII-3 from the three ponds were confirmed as the real culprit for the infection. The earlier work by Wang et al. (2000) also evidenced that, the bacterial white spot syndrome (BWSS) disease in *P. monodon* caused by *Bacillus subtilis* was confirmed by microscopical and PCR studies.

In the present study, PI-1, PII-2 and PIII-3 had the ability of having higher proteolytic, hemolytic, gelatinase and lipase activity. This suggests the bacterium has the ability to lyse the cuticle of shrimp constituting protein, chitin, calcium carbonate and lipid (Branson, 1993; Dennell, 1960). The carapace of the white patch disease affected *L. vannamei* had the white opaque patches and in later stage, white patches changed into black spot or splinter and roughness. *B. cereus* is an important enterotoxigenic food borne pathogen isolated from fish samples and had hbla gene responsible for toxigenicity (Das et al., 2009). *B. subtilis* has been reported to excrete enzymes, mainly protease,

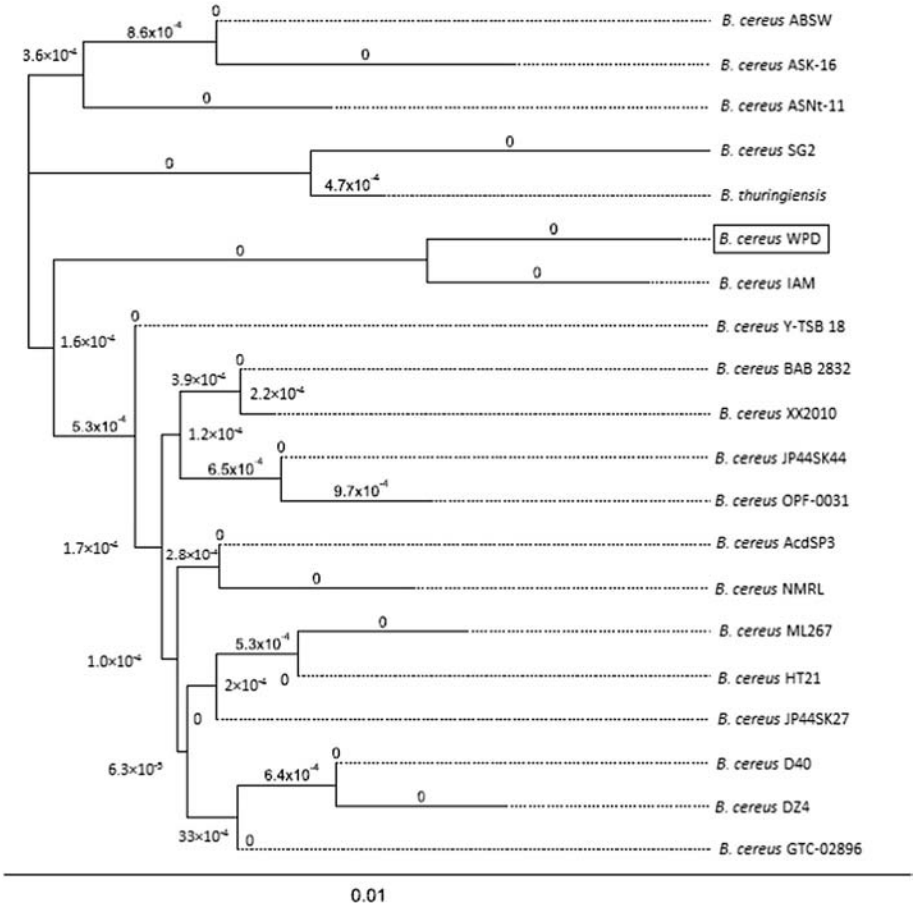

Fig. 3. Phylogenetic tree analysis of *Bacillus cereus* WPD based on 16S rRNA gene sequence data compared with other *Bacillus cereus* in the database.

amylase, glucanase and lipase (Formigoni et al., 1997; Shady, 1997). Hendriksen et al. (2006) demonstrated that the abundance of *B. cereus* in the soil sediments carries a potentially functional hlyII gene and expresses the virulence factors which are optimal at the temperature of their natural host. In our study, the disease outbreak may be caused by adverse environmental factors. Concerning the adverse environmental conditions, the hly gene may be switched on and express the virulence factors. Andreeva et al. (2007) supported that, optimum temperature for HlyII expression and action is between 15 and 28 °C and could use this weapon in the crustacean gut (Sineva et al. 2009).

Based on the phenotypic and genomic level identification the causative pathogen (PI-1, PII-2 and PIII-3) was confirmed as *Bacillus cereus* WPD and the strain was deposited in the NCBI database (GenBank: KF673474.1). Based on the identification and molecular characterization, *B. cereus* is a group of ubiquitous, rod shaped endospore-forming bacteria (De Jonghe et al., 2008) capable of proliferating in a wide range of environments including soils and clays, sediment, dust, mineral water, processed foods, etc. (Goepfert et al., 1972; Johnson, 1984; Norris et al., 1981). The intestines of insects were suggested as a habitat for *B. cereus* when spore forming bacteria, later identified as *B. cereus*, were isolated from guts of different soil-dwelling arthropod species (Margulis et al., 1998). Based on the Gram staining in the tissue, virulence factors and phenotypic and genomic level identification, the causative bacterial pathogen responsible for the white patch disease in *L. vannamei* was confirmed as *B. cereus* WPD.

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