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Brevibacillus laterosporus strain BPM3, a potential biocontrol agent isolated from a natural hot water spring of Assam, India

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Abstract

A bacterial strain designated as BPM3 isolated from mud of a natural hot water spring of Nambar Wild Life Sanctuary, Assam, India, strongly inhibited growth of phytopathogenic fungi (*Fusarium oxysporum* f. sp. *ciceri*, *F. semitectum*, *Magnaporthe grisea* and *Rhizoctonia oryzae*) and gram-positive bacterium (*Staphylococcus aureus*). The maximum growth and antagonistic activity was recorded at 30 °C, pH 8.5 when starch and peptone were amended as carbon and nitrogen sources, respectively. In greenhouse experiment, this bacterium (BPM3) suppressed blast disease of rice by 30—67% and protected the weight loss by 35—56.5%. The maximum disease protection (67%) and weight loss protection (56.5%) were recorded when the bacterium was applied before 2 days of the pathogen inoculation. Antifungal and antibacterial compounds were isolated from the bacterium which also inhibited the growth of these targeted pathogens. The compounds were purified and on spectroscopic analysis of a purified fraction having R_f 0.22 which showed strong antifungal and antibacterial activity indicated the presence of C—H, carbonyl group, dimethyl group, $-CH_2$ and methyl group. The bacterium was characterized by morphological, biochemical and molecular approaches and confirmed that the strain BPM3 is *Brevibacillus laterosporus*.

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1. Introduction

Microbial diversity is an unseen international resource that deserves greater attention. North—Eastern India is best known for its rich biodiversity and its un-tapped bioresources have been identified as the Indo-Burma Mega Hot Spot (Myers et al. 2000). Very little has been done on exploration of these vast genetic resources for gainful purposes in the field of industrial, agricultural and pharmaceutical sectors. Hot water springs are manifestations of geological activity and represent extreme environment. Borpung, a natural hot water spring of Nambar Reserve Forest, Assam, India is located within Indo-Burma Mega Hot Spot. The temperature of Borpung was 45—47 °C in summer season (April to September) whereas it was found up to 43 °C during winter (October to March). A number of novel bacterial strains have already been identified from Borpung (Saha et al. 2005a,b; Saha and Chakrabarti 2006a,b).

Rice is the world's third largest crop after maize and wheat. More than 90% of the world's rice is grown and consumed in Asia. Rice blast disease caused by *Magnaporthe grisea* Barr (anamorph *Pyricularia grisea* Sacc., synonym *M. grisea* Cav.) is distributed in about 85 countries. Yield loss due to rice blast can be as high as 50% when the disease occurs in epidemic proportions (Babujee and Gnanamanickam 2000). It is one of the major production constraints particularly in tropical Asia where rice farmers generally have less access to chemical fungicides and often cannot afford the same economically (Gnanamanickam and Mew 1992). Therefore, selection of antagonistic microorganisms to biological control is considered an alternative practice (Nantawanit et al. 2010). Interaction between biocontrol agents and pathogens has been studied extensively in rice (Zarandi et al. 2009) and application of biocontrol agents to protect some commercially important crops has also been reported (de Vasconcellos and Cardoso 2009; Anand et al. 2010).

Biocontrol involves one or more natural processes, e.g. antibiosis, parasitism, competition that are influenced by different environmental factors, both abiotic and biotic (Noaman et al. 2004; Moita et al. 2005; Someya 2008).

Brevibacillus was established as new genera arising from the reclassification of the *Bacillus brevis* group of species (Shida et al. 1996). The pathogenicity potential of *B. laterosporus* against insects has been earlier demonstrated (Ruiu et al. 2008). *B. laterosporus* has the potential to be used as a biological control agent in comparison with strains of *Bacillus thuringiensis* and *B. sphaericus*, demonstrates a very wide spectrum of biological activities (Oliveira et al. 2004). Although there are many reports on *B. laterosporus* as biocontrol agent for insect and nematode control (Oliveira et al. 2004; Tian et al. 2007); however, there are very few reports on *B. laterosporus* as biocontrol agent for phytopathogens (Zhou et al. 2006). Therefore, the present study aims to (i) isolate bacteria from natural hot water spring, (ii) screen for biocontrol potentiality against pathogens and (iii) identify biocontrol potential strain.

2. Materials and methods

2.1. Sample collection and isolation of bacteria

Mud samples from the natural host water spring were collected aseptically on March/2007 and temperature of the hot water spring was recorded. The samples were kept in a cold room until use. One gram of the mud was suspended in 100 ml of physiological water (NaCl 9 g/l) then incubated in an orbital shaker incubator at 28 °C with shaking at 200 rpm for 30 min. Mixtures were allowed to settle, and serial dilutions up to 10^{-4} were prepared using sterile distilled water (SDW) and agitated with a vortex at 200 rpm. Isolation of bacteria from this mixture was done with serial dilution technique in nutrient agar (g/l, peptone 5 g, beef extract 3 g, sodium chloride 5 g, agar 15 g; pH 7; HiMedia, Mumbai, India) (Johnson and Curl 1972). Purification of bacteria was done by repeated streaking and single colony culture by incubating at 28 ± 2 °C temperature (Orskov 1922). A total 150 numbers of bacterial isolates were obtained and checked for purity by plating and microscopic examination, maintained at 4 °C until use.

2.2. Microbial pathogens

Fusarium oxysporum f. sp. *ciceri* (FocRs9), *F. semitectum* (FsNJ9), *M. grisea*, *Rhizoctonia solani* f. sp. *oryzae* (RsNJ10) and *Brevibacillus laterosporus* RLBL19 were taken from the Culture Bank of Biotechnology Division, North-East Institute of Science Technology, Jorhat, Assam, India and *Staphylococcus aureus* (MTCC737), *Escherichia coli* (MTCC739), *Pseudomonas aeruginosa* (MTCC741) and *Klebsiella pseumoniae* (MTCC109) were obtained from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India. Fungal and bacterial pathogens were maintained on potato

dextrose agar (PDA) medium and nutrient agar (NA) medium, respectively.

2.3. *In vitro* antibiosis

The antagonistic activities of all the bacterial isolates were screened against the pathogens by dual inoculation technique (Bordoloi et al. 2001). Control plates consisted of the mycelial plugs of test fungal pathogen or bacterial pathogen only with 10 replications. Zone of inhibition was recorded after 5 days of incubation at $28 \pm 1\,^{\circ}C$ for fungal pathogens and after 2 days of incubation for bacterial pathogens.

The antagonistic activity was also studied with extracellular metabolites. Extracellular metabolites were extracted as described earlier (Bordoloi et al. 2001). Bacterial isolates were individually inoculated in 100 ml of nutrient broth in conical flask and incubated in a shaker incubator (200 rpm) at $30 \pm 1\,^{\circ}C$ for 48 h. After 48 h of incubation the whole broth from these flasks were subjected to solvent extraction process. Equal volume of ethyl acetate (1:1) were added to the culture broth and shaken vigorously for 15 min in a separating funnel and used for screening of antimicrobial activity by agar well diffusion method (Schillinger and Lucke 1989). Zone of inhibition was recorded after 2 days of incubation for bacteria and 5 days for fungal pathogens. Only one bacterial strain, BPM3 showed strong antagonist activity against fungal pathogen and gram-positive bacteria, so this isolate was used for further study. Highest growth inhibition was recorded with *M. grisea in vitro* study, so further experiment was conducted with this fungal pathogen only.

2.4. Optimum physiological parameters for maximum growth and antagonistic activity of BPM3

2.4.1. Effect of temperature

The strain BPM3 was inoculated into nutrient broth and grown in various ranges of temperatures from 10 to 55 °C for 24 h. Bacterial growth was monitored by measuring optical density (OD) of the culture in a spectrophotometer at a wavelength of 620 nm (Patton et al. 2006).

2.4.2. Effect of pH

To observe pH effect on growth, the bacterium was inoculated into nutrient broth where various pH ranges were adjusted from 4 to 9 and OD was recorded after 24 h of incubation at 30 °C.

2.4.3. Effect of carbon and nitrogen source

Various carbon (glucose, dextrose, glycerol, lactose, mannitol, sucrose and starch) sources at a concentration of $7.5\,g\,l^{-l}$ and nitrogen (beef extract, peptone, yeast extract, $(NH_4)_2SO_4$ and NH_4Cl) sources at a concentration of $2\,g\,l^{-1}$ were added to modified mineral-based medium (MM) containing (per liter) 2.39 g KH_2PO_4, 5.65 g K_2HPO_4, 1 g $MgSO_4{\cdot}7H_2O$ (Saddiqui and Shaukat 2004) and 1 ml trace salts stock solution (0.1 g $FeSO_4{\cdot}7H_2O$, 0.1 g $MnCl_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$ per 100 ml SDW) (Soliveri et al. 1987). A loopful of bacterial culture (2×10^8 CFU) was inoculated and dry weight of the bacterial cell was measured after 48 h of incubation at 30 °C.

The influence of temperature and pH for antagonistic activity were observed by dual culture technique on nutrient agar (NA) medium and influence of carbon and nitrogen source were studied on MM as mentioned earlier.

2.4.4. Greenhouse experiment for biocontrol activity

Local rice field soil was collected and autoclaved 3× (1 h, 121 °C) at 12 h intervals and 5 kg of sterilized soil was taken per earthen pot (diameter 230 mm). Seeds of local rice cultivar *Oryza sativa* L. (cv. mashuri) highly susceptible to blast disease were surface sterilized with 2% sodium hypochlorite for 30 s, then rinsed with SDW and dried in sterilized air stream. The seeds were sown in the above earthen pots (10 seed per pot). 25 days old seedling (5—6 leaves-stages) were separated in six groups as follows: (i) pathogen alone (2×10^2 spore/ml, 5 ml/pot), (ii) BPM3 alone (10^8 CFU/ml, 5 ml/pot), (iii) simultaneous inoculation of pathogen plus BPM3, (iv) pre-inoculation of BPM3 and then pathogen was inoculated after 2 days, (v) post inoculation of BPM3 after 2 days of pathogen inoculation and (vi) control (SDW treatment). For the preparation of bacterial suspension, the BPM3 was grown in nutrient broth in a shaker incubator at 30 °C and after 48 h of incubation at 200 rpm the cell concentrations were adjusted at 10^8 CFU/ml by mixing SDW where necessary. Spore suspension of the pathogen was prepared by adding SDW to Petri dishes of well-grown culture of the pathogen. For inoculation, a conidial suspension of *M. grisea* (2×10^2 conidia ml^{-1}) with Triton X-100 (250 ppm) was sprayed on the treated rice plants (Zarandi et al. 2009). Experiment was carried out as complete randomized block design (CRD) with 10 replications. Percentage of diseased severity was as described by Kim et al. (2005). The rice seedling were desoiled and washed to record the fresh weights of rice plant per pot with 10 repli-

cations (Zarandi et al. 2009). Whole experiments were repeated twice.

The significance differences in protective activity were determined by analysis of variance (ANOVA) by Students' *t*-test. The significance level for all analysis was $P = 0.05$.

2.4.5. Isolation, purification and partial characterization of antifungal and antibacterial compounds from BPM3

Antimicrobial compounds were extracted from the BPM3 as described earlier (Bordoloi et al. 2001). Purification was done through silica gel thin layer chromatography (TLC) using hexane + ethyl acetate (5:3) as solvent. Spots were developed by iodine vapour and observed under UV light (254 nm). Three distinct fractions were collected separately by TLC (R_f value 0.14, 0.22 and 0.98) and used to observe for antimicrobial activity (Saikia et al. 2004). The fraction having R_f value 0.22 exhibited strong antimicrobial activity *in vitro* was further purified by HPLC. The HPLC system used was Water (Millipore) 510 pump, 680 gradient controller with a 746 data module. The separation was performed in reverse phase by injecting 10 μl of sample in a Nucleosil C18 column, using an isocratic system of water—acetonitrile (60:40). The detection's wavelength was fixed at 250 nm. The purified compound was analysed by infrared spectroscopy (PerkinElmer 237B) with $CHCl_3$ as solvent, and nuclear magnetic resonance (NMR) spectroscopy (60 MHz and 300 MHz; both carbon and hydrogen NMR; with tetra methyl silane (TMS) as reference).

2.5. Identification of the strain BPM3

2.5.1. Phenotypic characterization

Morphological characterization was done on the basis of shape and size of the bacterium, and spore formation which was observed under compound microscope (ZEISS, Axioskop); biochemical characters, viz., gram stain, hydrolysis of casein gelatine, and tyrosine, assimilation of glucose, D-alanine, D-fructose, glycerol, DL-lactate, maltose, D-mannitol, sucrose and D-trehalose were observed (Bergey's Manual of Determinative Bacteriology, 1989).

2.5.2. Molecular characterization

Genomic DNA was extracted by using WizardR Genomic DNA purification kit (Promega). The DNA purity and quantity were checked by spectrophotometer at 260 and 280 nm. PCR amplification of 16S rDNA was done with bacterial universal primer fD1 (5′-GAGTTTGATCCTGGCTCA-3′) and Rp2 (5′-CGGCTACCTTGTTACGACTT-3′) as described earlier (Weisburg et al. 1991). The PCR products were purified by using a QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions and sequenced the 16S rDNA region (1.5 kb) with the same primers. DNA sequence was determined by fluorescent terminators (Big Dye, Applied Biosystems) and run in an Applied Biosystems ABI prism automated DNA sequencer (3130 ×l). The 16S rRNA gene sequences of bacteria used in the phylogenetic analysis were retrieved from NCBI-GenBank. Phylogenetic and pair wise evolutionary distances were analysed by using a software MEGA version 2.1 (Kumar et al. 2004). A phylogenetic tree was inferred by the Neighbour Joining method (Saitou and Nie 1987). Tree topologies were evaluated by bootstrap analysis (Felsenstein 1985) based on resamplings of 1000 times of the neighbour joining (NJ) data set.

2.5.3. DNA G + C content

DNA was enzymically degraded into nucleosides and the G + C content of the purified DNA was determined by high performance liquid chromatography (Mesbah et al. 1989).

3. Results

Total 115 bacteria were isolated from the hot water spring where temperature was recorded 42 °C during sample collection. Only one isolate, BPM3 strongly inhibited the growth phytopathogenic fungi, *F. oxysporum* f. sp. *ciceri*, *F. semetactum*, *M. grisea* and *R. solani*, and gram-positive bacterium, *S. aureus* in dual culture technique. Among the fungal pathogens, maximum zone of inhibition (29 mm) was recorded in *M. grisea* followed by *F. semitectum* (25 mm), *R. solani* (24 mm) and *F. o.* f. sp. *ciceri* (22 mm) (Fig. 1a). In *S. aureus* growth inhibition was recorded 17 mm (Fig. 1a), however, no growth inhibition was observed against gram negative bacteria, *viz.*, *E. coli*, *P. aeruginosa* and *K. pseumoniae* which were tested in this study. Similarly, extracellular metabolite extracted from the bacterium was also significantly inhibiting growth of the fungal pathogens and gram-positive bacterium. Three distinct fractions were collected separately by TLC, the fraction B (R_f value 0.22) showed maximum antifungal and antibacterial activity (Fig. 1b) followed by the fraction A (R_f value 0.14) and the fraction C (R_f value 0.98). The fraction B recorded highest growth inhibition zone with *M. grisea* (25 mm) followed by *F. semitectum* (23 mm; Fig. 1b), *R. solani* (21 mm) and *F. oxysporum* (20 mm). Similarly, this fraction recorded growth inhibition zone (20 mm) with *S. aureus* (Fig. 1b).

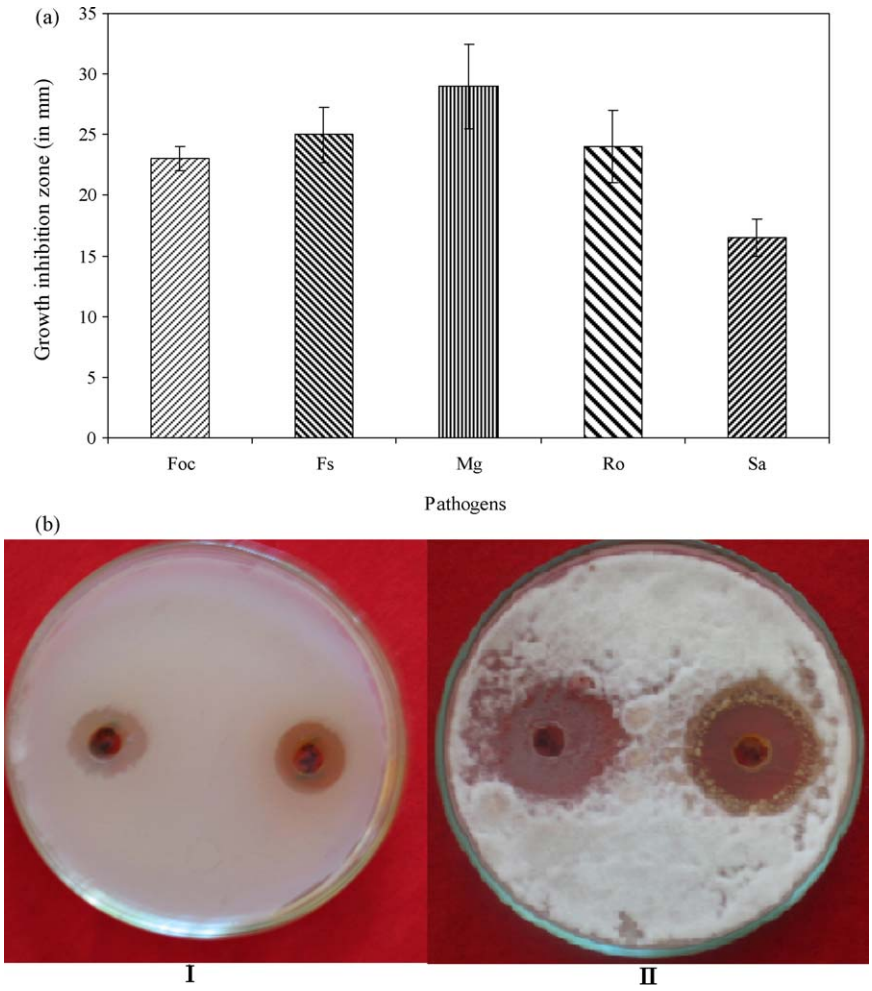

Fig. 1. (a) Antagonistic activity of BPM3 against pathogens, Foc: *Fusarium oxysporum* f. sp. *ciceri*; Fs: *F. semitectum*; Mg: *Magnaporthe grisea*: Ro: *Rhizoctonia oryzae*; Sa: *Staphylococcus aureus*. (b) Bioassay results of TLC purified compound (fraction B, R_f value 0.22) isolated from BPM3; Plate I and II inhibition zones produced with *Staphylococcus aureus* and *Fusarium semitectum*, respectively.

Maximum growth (0.3 OD) and inhibition zone (28.5 mm) was recorded at the incubation temperature 30 °C (±1) followed by 32 °C (25.3 mm) and 28 °C (24 mm) (Fig. 2a). Lowest growth and inhibition zone was observed at low temperature (15 °C) as well as at high temperature (50 °C). No growth and antagonistic activity was recorded at ≤10 and ≥55 °C.

The growth and antagonistic activities of BPM3 were also found to be influenced by pH of the medium (Fig. 2b). The inhibition of the test fungus *M. grisea* was greater (27 mm zone of inhibition) at alkaline pH than at acidic. The maximum growth and antagonistic activity was observed at pH 8.5 in the medium; with any increase or decrease from this pH, the growth and antagonistic activity also reduced. No growth and antagonistic activity was observed below pH 5.0.

The results show that carbon and nitrogen sources also influence on growth and antagonistic activity exhibited by BPM3 against *M. grisea* and significantly enhanced cell dry weight and zone of inhibition was recorded in comparison to control (Fig. 3a and b). Out of six carbon sources, the bacterium produced highest cell dry weight (1.7 mg) and lowest (0.5 mg) in presence of starch and sucrose, respectively. Starch was also the best carbon source (25 mm zone of inhibition) followed by dextrose (21.7 mm) for antagonistic activity of the bacterium (Fig. 3a). Fig. 3b reveals that peptones followed by $(NH_4)_2SO_4$ were the most suitable nitrogen source for growth and antagonistic activity of BPM3 as zone of inhibition were recorded 24 and 21 mm, respectively, whereas the other tested nitrogen sources gave comparatively lower levels of growth and antagonistic activity of the bacterium.

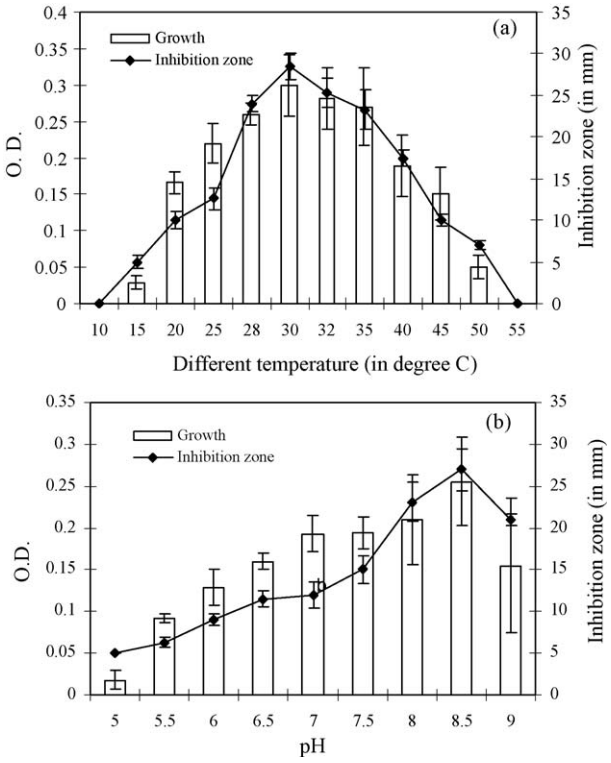

Fig. 2. Effect of physiological parameters: (a) temperature and (b) pH on growth and antagonistic activity of BPM3 against *Magnaporthe grisea*.

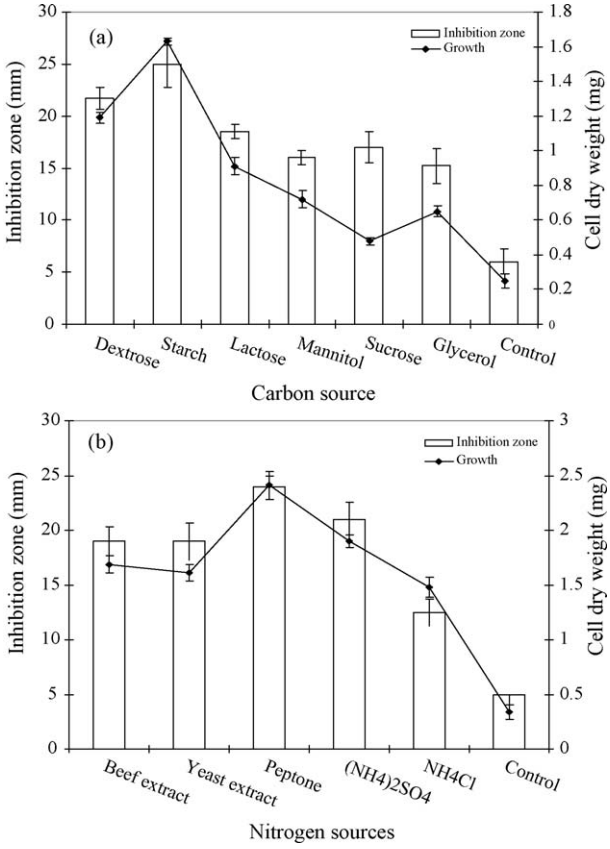

Fig. 3. Effect of physiological parameters: (a) carbon and (b) nitrogen source on growth and antagonistic activity of BPM3 against *Magnaporthe grisea*.

Inoculation of *M. grisea* in pot experiment with rice plant showed severe blast disease (58%) (Table 1). However, on application of BPM3 the disease severity was reduced significantly ($P = 0.05$). The result exhibits that the BPM3 protected rice plant from the blast disease by 30—67% compared to pathogen treated control. It was also observed that maximum protection (67%) was recorded when BPM3 was applied before 2 days of the pathogen inoculation followed by (58% protection) simultaneous inoculation of the pathogen and BPM3, then post application of BPM3 where 30% disease protection was recorded (Table 1).

Table 1. Effect of BPM3 on blast disease suppression and weight loss protection of rice during infection with *Magnapothe grisea* (Mg).

Treatment	Disease severity (%)	Plant weight (g)
Pathogen control (Mg)	58.0 ± 12.3	0.50 ± 0.07
Pathogen+BPM3	24.2 ± 8.7	0.92 ± 0.06
Pre-inoculation of BPM3	15.0 ± 2.7	1.15 ± 0.08
Post inoculation of BPM3	28.5 ± 4.2	0.77 ± 0.05
BPM3 inoculation	0.0	1.64 ± 0.20
Water treated plant	0.0	1.65 ± 0.23

Critical difference (C.D.) = 16.55 for disease severity and 0.26 for plant weight, computed at $P = 0.05$; ±S.D.

In this study we observed that 70% fresh weight was lost by rice plant for the pathogen *M. grisea* infection (Table 1). However, 56.5% recovery was recorded in pre-inoculation of the antagonist, BPM3 followed by (45.6% recovery) simultaneous inoculation of the pathogen and BPM3, then post inoculation of BPM3 (35% recovery).

The antimicrobial compound isolated from the BPM3 was soluble in methanol; however, it was insoluble in water. Three main fractions having R_f value 0.14, 0.22 and 0.98, exhibited on TLC. The fraction having R_f value 0.22, showed maximum antifungal and antibacterial activity *in vitro* (Fig. 1b), was further analysed by IR-spectroscopy and NMR-spectroscopy, 60 and 300 MHz (both carbon and hydrogen NMR). In IR-analysis, the bands at 2958, 2924 and 2853 cm^{-1} indicates C—H stretching as in alkane; the band 3410.2 cm^{-1} indicates either NH or —OH groups; the band 1670.8 cm^{-1} indicates the presence of α, β-unsaturated cyclic (may be six ring) or cyclic carbonyl group and band at 1452.6 or 1386.6 cm^{-1} for the presence of a dimethyl group $(CH_3)_2C<$.

Table 2. Biochemical characters of BPM3 and *Brevibacillus laterosporus* RLBL19.

Test	BPM3	RLBL19
Hydrolysis of		
Casein	+	+
Gelatine	+	+
Tyrosine	+	+
Starch	—	—
Assimilation of		
Glucose	+	+
Sucrose	—	—
Maltose	+	+
D-Mannitol	+	+
D-Fructose	+	ND
D-Trehalose	+	+
DL-Lactate	—	—
D-Alanine	—	—
Glycerol	+	ND
L-Aspartate	+	+

(+) Stands for positive reaction", (—) stands for negative reaction", (ND) stands for not determined".

NMR (60 MHz, $CDCl_3$ as solvent) showing ∂ 0.78—1.18(m) and 1.69—2.31(m) indicates the presence of $-CH_2$ groups (saturated); ∂ 1.20—1.26(S) indicates the presence of methyl group. In ^{13}C NMR (300 MHz), the peak at 30.09 also indicates the methyl group.

The cells of the strain BPM3 were rod-shaped, size (length 2.0—3.0 μM × width 0.8—1.0 μM), gram-positive, aerobic and spore-forming. Both BPM3 and the reference strain *B. laterosporus* RLBL19 were positive in hydrolysis of casein, gelatin and tyrosine; showed negative in starch hydrolysis (Table 2). The bacterium, BPM3 assimilated glucose, maltose, mannitol, fructose, trehalose, glycerol and L-aspartate, however, did not assimilate sucrose, lactate and alanine.

16S rRNA gene (1500 nucleotides) sequence of the strain BPM3 was aligned to generate a NJ phylogenetic tree (Fig. 4), it was confirmed that the strain is *B. laterosporus* (GenBank Accession Number: DQ371289) and nearest homologous *Paenibacillus larvae* (Accession No. AY530296). Sequence similarity of strain BPM3 to the type strain of *B. laterosporus* (DQ371289) and *B. laterosporus* (DQ122932.2) was 99%. Based on the 16S rRNA gene sequence similarity to *B. laterosporus*, we therefore refer to BPM3 as *B. laterosporus* strain BPM3 and the sequence was submitted to the NCBI Gene Bank, accession no. EU159585. The DNA G + C content of strain BPM3 was determined as 54 mol%.

4. Discussion

The results show that the purified compounds isolated from strain BPM3 strongly inhibited the growth of the test pathogens. Inhibition zones revealed the production of potent antifungal and antibacterial compounds by the strain BPM3. Zhou et al. (2006) had also reported on antifungal activity of *B. laterosporus* against phytopathogens, *viz. Cylindrocarpon didynum*, *C. destructans*, *M. grisea* and *R. solani*.

Different studies proved that temperature is one of the major conditions for affecting the growth rate of antagonist (Kok and Papert 2002). Our data also show that temperature is an important fac-

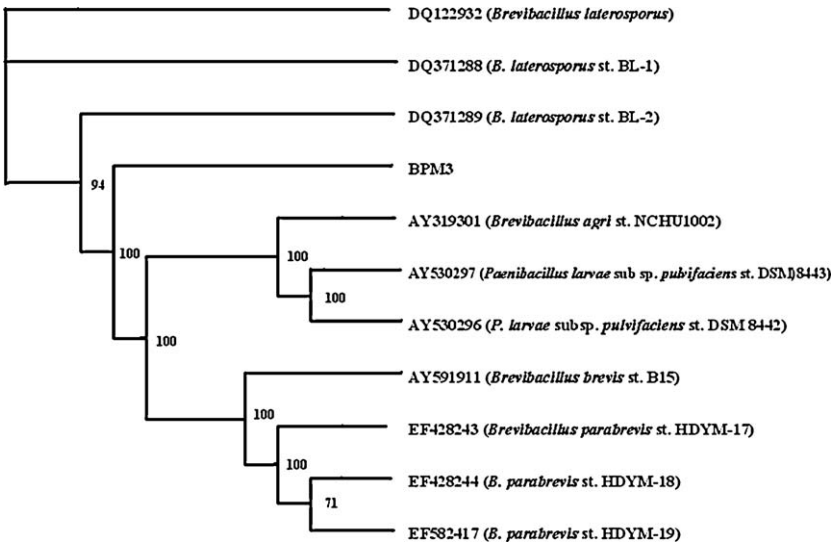

Fig. 4. Phylogenetic tree-based on the 16S rDNA sequence indicating the position of strain BPM3 (using Neighbour Joining method).

tor on growth and in determining the effect of *in vitro* antagonistic tests. The results showed that the strain, BPM3 was very less active against *M. grisea* at 15 and 50 °C. Production of antibiotics and bioactive metabolites by bacteria can be influenced by temperature as reported earlier in many reports (Lee and Magan 1999; Kok and Papert 2002). Several reports indicated that most bacteria are capable of synthesizing an antibiotic in media with pH ranging from 5.5 to 8.5 (Thongwai and Kunopakarn 2007). BPM3 also exhibited antagonistic activity against *M. grisea* at wide range of pH. The results indicate that carbon and nitrogen sources in the growth medium play an important role on growth and in the biosynthesis of the antimicrobial agents by the BPM3 *in vitro*. Earlier reports also observed that C and N-source amendments differentially influence medium during bacterial growth (Dekleva and Strohl 1987), which indirectly affects secondary metabolite production (Slininger and Shea-Wilbur 1995) and subsequently biocontrol activity (Meidute et al. 2008).

In this study we present a practical and an effective approach using the antagonistic bacterium, BPM3 to control blast pathogen of rice, an economically very important phytopathogen. The *in vitro* activity obtained in the laboratory experiment also strongly supported *in vivo* trail in pot plants as strong antifungal agent. In this study, plants inoculated with *M. grisea*, 2 days after BPM3 treatment suppressed the pathogen much more efficiently than the control. Plants respond to a variety of chemical stimuli produced by different soil- and plant-associated microbes and these stimuli can either induce or condition plant defenses through biochemical changes that increase resistance against subsequent infection by pathogens (Zhang and Reddy 2001; Baysal et al. 2008). The BPM3 strain observed in the present investigation showed antagonistic effect towards important phytopathogenic fungi like *M. grisea*, *R. oryzae*, *F. oxysporum* f. sp. *ciceri* and gram-positive bacterium. Hence, the strain has potential to be used as biocontrol agent.

Antimicrobial substances had already been purified from *B. laterosporus* (Ren et al. 2007). Tauramamide, a lipopeptide antibiotic produced in culture by *B. laterosporus* was also characterized (Desjardine et al. 2007). Romero et al. (2007a,b) identified three lipopeptide antibiotics having R_f values similar to fengycin ($R_f = 0.09$), iturin A ($R_f = 0.3$) and surfactin ($R_f = 0.7$) from antagonistic *Bacillus subtilis*. Lipopeptide antibiotics, surfactin with R_f 0.37 and 0.51 were also reported in *B. subtilis* (Mutaz et al. 2007). However, in this study we observed multiple fractions of antimicrobial compound in TLC with different R_f values, *viz.*, 0.14, 0.22 and 0.98. All the three fractions showed antifungal and antibacterial activity indicating that antimicrobial compound of the bacterium is mixture of three compounds. Fractions having R_f 0.22 showed maximum antimicrobial activity against the test pathogens. Spectroscopic analysis of this fraction indicated the presence of C—H, carbonyl group, dimethyl group, $-CH_2$ and methyl group.

In this study, we observed that the bacterial cells are rod-shaped, gram-positive, aerobic and spore-forming. The bacterium casein, gelatin and tyrosine, and negative in starch hydrolysis; it assimilated glucose, maltose, D-mannitol, D-fructose, D-trehalose, glycerol and L-aspartate; however, did not assimilate sucrose and DL-lactate. These findings are corroboratory to Logan et al. (2002).The bacterial strain BPM3 reported here exhibited a 54% G + C content. Based on 16S rDNA sequence homology revealed the taxonomic affiliation of strains, BPM3 as *B. laterosporus* which has 99% similarity with *B. laterosporus* (GenBank Accession Number: DQ371289).

This is the first report of isolation of a broad-spectrum antagonistic potential *B. laterosporus* from the natural hot water spring of Assam, India. The antagonistic bacterium is now under investigation for suitable formulation to use in fields, including a delivery system and shelf-life of the bacterium in formulations. In this study, we have given preliminary information of chemical composition of an antimicrobial compound; however, elucidation of complete structure of the compound is also under progress.

Overall, our result demonstrated that — (i) *B. laterosporus* strain BPM3 isolated from natural hot water spring, inhibited growth of the phytopathogenic fungi and gram-positive bacteria; (ii) pre-inoculation of the bacterial strain suppressed the blast disease of rice more efficiently and antimicrobial compounded isolated from the bacterium with R_f 0.22 indicated the presence of C—H, carbonyl group, dimethyl group, $-CH_2$ and methyl group.

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