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Anti-quorum sensing and antibiofilm activities of *Blastobotrys parvus* PPR3 against *Pseudomonas aeruginosa* PAO1

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ABSTRACT

The bacterial cell communication also termed as Quorum sensing (QS) system was involved in the expression of several virulence traits during Pseudomonas infection. The attenuating of this bacterial cell communication system is an attractive approach for the management of bacterial infections without the complication of resistance development. In this respect, the marine environment has gained significant attention due to its biodiversity and as a source of novel bioactive compounds. The present study aimed to screening effective QS inhibitors from marine associated fungal species for QS inhibitors. Twelve morphologically distinct fungal isolates were isolated from the wood of *Avicennia marina* from marine ecosystem. The anti-QS potential of fungal crude extract from was investigated in biosensor strain and test bacterium, *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1, respectively. Promising anti-QS activity was observed in the crude extract of one of the fungal isolate and identified by molecular characterization using internal transcribed spacer (ITS) region as *Blastobotrys parvus* PPR3. The anti-virulence and antibiofilm effects of ethyl acetate fractions from PPR3 against *P. aeruginosa* PAO1 were evaluated. The fungal metabolites responsible for the anti-QS activity of fungal crude extract was identified using gas chromatography-mass spectrometry (GC-MS). Furthermore, molecular docking studies were performed to understand the interaction of bioactive compounds with as receptors of *P. aeruginosa* PAO1. The crude extract of PPR3 showed reduction in different virulence traits of *P. aeruginosa* PAO1 such as production of pyocyanin, elastase, protease, chitinase, swimming and swarming motility, biofilm formation, exopolysaccharide production and alginate production at different sub-MIC concentrations. Interaction of bioactive metabolites with LasR and RhlR receptors of *P. aeruginosa* PAO1 was reported. The findings of the present study suggested that metabolites of *B. parvus* PPR3 interfere with QS system of *P. aeruginosa* PAO1 and alters the production of virulence factors.

1. Introduction

Controlling bacterial infections is still a challenging problem in the 21st century due to the emergence of multidrug resistance bacteria. Invention of the first antibiotic "Penicillin" was considered as a milestone for the development of different antibiotics. As a result, several antibiotics including penicillin are used in the antibiotic therapy for effective eradiation of bacterial infections. Herein, bacteria developed resistance to these antibiotics owing to selective pressure which elevated the risk of infections [1]. Hence, in recent years antimicrobial drug research is focused on alternative targets that will not impact the antibiotic resistance. In this context the term anti-pathogenic drug gains significant attention among researchers that works selectively on the virulence factors production without affecting the growth of bacteria. This strongly enables chance to control the bacterial infections without developing any resistance to the drug [2]. In certain bacteria, a complex bacterial cell communication process called as quorum sensing (QS) mediates the production and secretion of virulence traits. In general, QS is a regulatory mechanism that promotes the pathogen to establish the infection, expression of virulence factors, biofilm formation and resistance development [3]. In a recent study, prevalence of drug resistance and production of virulence factors in clinical isolates of *P. aeruginosa* were studied and the role of QS was established [4]. Furthermore QS also plays crucial role in biofilm formation, a major

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clinical complication in the treatment of bacterial infections. Biofilm formation was closely interconnected biological event with QS system of the bacteria. QS acts as an coordinating switch to a biofilm lifestyle that coordinates the bacterial population for maturation and disruption of biofilm [5]. The interruption of QS was widely accepted method to discover anti-pathogenic drugs. Since QS system of the pathogenic bacteria plays significant role in the expression of most of the virulent traits, inhibition of QS event could be an effective method to control the bacterial infections [6,7]. For instance, the virulence potential of the *P. aeruginosa* is strictly under the regulation of QS. Therefore, as compared to bactericidal or bacteriostatic methods, QS inhibition found to be an effective approach to attenuate the virulence factors of *P. aeruginosa* [8,9].

Pseudomonas infection in cystic fibrosis (CF) patients is one of the major cause of morbidity. CF is a complication in which lungs will progressively deteriorate due to the inflammatory response to chronic endobronchial *P. aeruginosa* infection. The development of biofilm facilitates the bacteria to tolerate the antimicrobial treatment as well as the action of the host defense system. To evade the host defense and establish the infection, *P. aeruginosa* utilize QS system that play a vital role in the production and secretion of virulence traits including lytic enzymes such as elastase, protease and exoenzyme S and non-enzymatic compound like exotoxin A, pyocyanin, rhamnolipid and lipopolysaccharides [10]. *P. aeruginosa* relies on four well-defined QS systems namely LasI-LasR, RhlI-RhlR, PQS-MvfR and inter-cellular communication signal (IQS). Herein, LasI involves in the production of signaling molecule, *N*-(3-oxododecanoyl) homoserine lactone ($3OC_{12}$-HSL) also known as auto-inducer and transcriptional regulatory protein LasR responds to this auto-inducer. The complex of LasR and $3OC_{12}$-HSL activates the transcription of many genes including RhlI-RhlR system. Likewise, RhlI produces *N*-butanoyl-L-homoserine lactone (C_4-HSL) that bind to its cognate transcriptional receptor RhlR, which directs the expression of several genes [11]. The QS system in the bacteria activates when the signal molecules reach threshold concentration and further binds to the cognate transcriptional regulatory protein. This complex initiates the expression of specific genes that involves in the production of virulence traits, along with the *N*-acyl homoserine lactone synthase [10]. In PQS-MvfR system, 2-heptyl-3-hydroxy-4(1H) quinolone (PQS) and its precursors binds to the transcription regulator MvfR and regulate the transcription of downstream targets [12]. The fourth QS system, IQS was recently reported which involved in the sensing of the environmental stress factors by using QS network. The signal molecule in PQS was chemically identified as 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde [13]. QS plays major role in bacterial pathogenesis, hence the development of antiQS molecules can be alternative to combat *P. aeruginosa* infection. There are several ways to interrupt QS associated cell communication system including competitive inhibition of signaling molecules, signal binding by introducing compounds which could bind with the signaling molecules that change the chemical structure of the autoinducers, degradation of the autoinducers [14]. Furanones are reported as effective QS antagonists that work by competitive inhibition mechanism [15].

Several natural and synthetic QS antagonists have been reported to block the QS regulated gene expression [16]. In the past few years, exploring the natural compounds are gaining greater attention as therapeutic as the synthetic therapeutic compounds possess adverse effect [17]. In the quest for natural QS antagonists, metabolites from plants, fungi and bacteria were reported to attenuate QS system in *P. aeruginosa* [18]. As an adaptive evolution, several eukaryotic species including plants and fungi synthesize metabolites that can interfere with QS in order to overcome the bacterial infections. *Centella asiatica* is a traditional medicinal plant used in several therapeutic applications, reported for antiQS activity [19]. Similarly, the compound 6-gingerol from extract of ginger was reported as effective anti-QS molecule [20]. Nevertheless, fungi also produce QS inhibitors like, Polyhydroxyanthraquinones are molecules reported from *Penicillium restrictum* that act as effective anti-QS agents [21]. Still marine environment remains unexplored by the researchers due to the lack of knowledge to maintain marine environment in the laboratory. Hence, numerous marine species and their metabolites are less exploited. Marine fungi are notable source of bioactive compounds with therapeutic activities such as antibacterial, antiviral, antifungal and antioxidant. The extracts of *Sarocladium, Fusarium, Epicoccum* and *Khuskia* isolated from marine source were reported for anti-QS activity against *P. aeruginosa* [22]. Naik and co-worker identified cinnamic acid and proline-derived dipeptides (proline-glycine) from marine *Streptomyces* species and studied for anti-QS activity against *P. aeruginosa* [23].

The aim of this investigation was to screen and isolate the marine fungi from wood samples and to evaluate their antiQS activity. The effect of fungal isolates on the production of virulence factors and biofilm formation in *P. aeruginosa* was evaluated. Further the crude extract was investigated for the presence of bioactive compounds and the antiQS activity of these compounds was established by molecular docking studies.

2. Materials and methods

2.1. Isolation, screening and identification of fungal isolate

The wood samples (*Avicennia marina*) were collected from coastal regions of Mutthupet village, Cuddalore district, Tamilnadu, India. The collected samples were tranported to the laboratory and persevered at 4 °C. The dry wood samples were surface sterilized with 70% of ethanol for 10 s to prevent the growth of air contaminants and further rinsed with sterile seawater to dilute the ethanol concentration on the sample. Afterwards, samples were made into small pieces using sterile blade and plated on the malt extract agar plates. The culture plates were incubated at room temperature (28 ± 2 °C) for 21 days. The culture plates were monitored for the development of fungal growth at every 2 days of incubation. Fungal colonies with distinct colony characters were isolated and transferred onto new culture media plates supplemented with artificial seawater [24].

All the fungal isolates were cultured in malt extract broth prepared with artificial seawater, at 28 ± 2 °C for 21 days in orbital shaker (120 rpm). After the incubation time culture broth (200 ml) was subjected to centrifugation to remove the mycelium. The obtained supernatant was added with equal amount of ethyl acetate (1:1 v/v ratio) and incubated at in room temperature for 12 h in a resting condition. Ethyl acetate layer, which contain fungal metabolites was isolated using aseparatory funnel. The obtained ethyl acetate was evaporated using a rotary evaporator and crude extract was harvested and preserved at cold temperature. The crude fungal extracts were diluted using dimethyl sulfoxide (DMSO) to prepare the stock solution of 2 mg mL^{-1} and screened for antiQS potential [25]. The extraction of fungal crude was performed for three times independently and studied for consistent antiQS activity.

The fungal crude extracts were screened for their anti-QS activity using biosensor strain, *Chromobacterium violaceum* (MTCC 2656) purchased from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh, India and against test bacterium, *P. aeruginosa* PAO1, a gift of Dr. E. Peter Greenberg (Department of Microbiology, University of Washington). Both the bacterial strains were cultured in 5 ml of Luria-Bertani (LB) broth separately and incubated at 37 °C for 24 h. The optical density of bacterial suspensions was adjusted to 0.5 McFarland Standard (1.5×10^8 CFU mL^{-1}). Hundred microliters of bacterial suspension was inoculated with 10 ml of soft agar and overlay on pre-prepared LB agar plate. After solidification, wells were prepared on the agar plate employing sterile 8 mm well borer and filled with crude fungal extracts at a final concentration of 250 and 500 μg mL^{-1}. All the plates were incubated at 37 °C for 24 h and clear zones around the wells was observed. Absence of pigment production around the well was considered as preliminary anti-QS effect attribute of fungal extract

[26]. To ensure reproducibility of antiQS activity of crude extract, the experiments were repeated three times independently.

The isolated fungi were partially identified through light and phase contrast microscopic observations [25]. The promising fungal isolate PPR3 was further identified by sequencing the Internal transcribed spacer (ITS) region sequencing. Herein, fungal culture was sequenced in Macrogen Inc., Seoul, South Korea by amplifying ITS region using ITS1 (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGA TATGC 3') as forward and reverse primers respectively. The resulted sequences were compared with DNA sequence in NCBI GenBank (http://www.ncbi.nlm.nih.gov.blast) to identify the closely related fungi. The obtained gene sequence was deposited to the NCBI GenBank database. The gene sequence were aligned with CLUSTAL_W. Phylogenetic tree was constructed using maximum likelihood method in MEGA 5.0 software [27].

2.2. Minimum inhibitory concentration (MIC) of fungal extract

The MIC of fungal crude extract against *P. aeruginosa* PAO1 was determined by employing the method described by Kalia et al. [28]. Briefly, an active culture of *P. aeruginosa* PAO1 was grown in LB medium for 18 h and mixed with two fold dilution of crude extract with a maximum concentration of 1000 μg mL^{-1} in microtiter plate. The lowest concentration of crude extract to inhibit the visible growth of the bacterium was considered as MIC. Based on the MIC value obtained, two subMIC concentration were selected to study the antiQS effect on *P. aeruginosa.*

2.3. Violacein inhibition assay

The effect of fungal crude extract on violacein production by *C. violaceum* was analyzed as discribed previously by Kalia et al. [28]. Aliquots of 100 μl of *C. violaceum* culture was added to the test tubes containing 2 ml of LB broth and incubated with varying concentrations of fungal crude extract (0.25 x MIC and 0.5 x MIC). The control was maintained without the fungal crude extract. The tubes were incubated at 37 °C for overnight and centrifuged at 10,000 rpm for 10 min. The cell pellets were collected and suspended into sterile DMSO, vortexed for 2 min and centrifuged at 10,000 rpm for 10 min. The obtained supernatant was subjected to spectrophotometric analysis at 590 nm to quantify the solubilized violacein. The cultures treated with DMSO were considered as negative control.

2.4. Growth curve analysis

Five hundred microliters of active culture of *P. aeruginosa* PAO1 was cultures in 250 ml conical flask containing LB broth supplemented with 0.25 x MIC and 0.5 x MIC concentration of fungal extract. The conical flask was incubated in a rotatory shaker (180 rpm) at 37 °C. The density of cells in respective time was measured in UV–visible spectrophotometer at 600 nm at every 1-h interval up to 24 h. The doubling times were calculated for each growth curve experiments using the formula

$$Doubling\ Time = \frac{ln2}{k} = \frac{0.693}{k}$$

where k is growth rate constant is determined from the slope of the exponential trendline [29].

2.5. Effect of fungal extract on virulent traits of P. aeruginosa PAO1

2.5.1. Inhibition of pyocyanin

The effect of crude extract on pyocyanin production was investigated using modified method of Ugurlu et al. (2016) [30]. Briefly, the test pathogen was treated with varying concentrations (0.25 x MIC and 0.5 x MIC) of fungal crude extract and incubated at 37 °C for 18 h. The cells were removed by centrifugation at 10,000 rpm for 10 min. The pyocyanin in the supernatant was extracted into chloroform by mixing supernatant with chloroform (3:5 ratio). The separated chloroform layer that contains pyocyanin was acidified with 0.2 N HCl that lead to the formation of pink-red colour solution which was measured spectrophotometerically at 520 nm.

2.5.2. Las A protease assay

Las A protease activity of cell free supernatant of test pathogen grown with or without supplementation of fungal crude extract was observed by azocasein degrading assay as described by Musthafa et al. (2011) [31]. Briefly, 150 μl culture supernatant of *P. aeruginosa* PAO1 broth extract with and without treatment of fungal crude extract (0.25 x MIC and 0.5 x MIC) was mixed with 1 ml of 0.3% azocasein in 0.05 M Tris-HCl and 0.5 mM $CaCl_2$ (pH 7.5) and incubated at 37 °C for 15 min. After the incubation, the degradation process was arrested by introducing trichloroacetic acid (10%, 0.5 ml) and subjected for centrifugation at 10,000 rpm for 10 min. The supernatant obtained was measured at 400 nm in a UV–visible spectrophotometer.

2.5.3. LasB elastase activity

LasB elastolytic activity was experimented by adopting the method illustrated by Husain *el al.* (2013) [32]. Herein, the cell free supernatant of *P. aeruginosa* PAO1 treated and untreated with fungal crude extract (0.25 x MIC and 0.5 x MIC) was added with ECR buffer (100 mM Tris, 1 mM $CaCl_2$, pH 7.5) in 1:9 (v/v) ratio containing 20 mg of elastin Congo red (ECR) and incubated in shaking condition at 37 °C for 3 h. After incubation, the suspension was subjected to centrifugation to remove the insoluble ECR and absorbance of the supernatant was measured at 495 nm. Cell free supernatant without sample was used as negative control.

2.5.4. Staphylolytic activity

Staphylolytic activity was assessed by observing the efficacy of cell free supernatant to lyse boiled *S. aureus* cells (MCC 2408) obtained fronMicrobial Culture Collection, National Centre for Microbial Resource, Pune, India [33]. Hundred microliter aliquot of *P. aeruginosa* culture supernatant with or without treatment of fungal extracts (0.25 x MIC and 0.5 x MIC) was added to 900 μl of boiled *S. aureus* culture suspension. The OD_{600} was measured after 0 and 60 min of incubation. Activity was represented as percentage reduction of protein release after treatment to that of untreated sample.

2.5.5. Chitinase inhibition

Chitinase inhibition was investigated according to Husain et al. (2017) [34]. Briefly, the test pathogen *P. aeruginosa* was cultured in LB broth with and without amendment of fungal extract (0.25 x MIC and 0.5 x MIC) at 37 °C for 18 h. Two volumes of filter sterilized culture supernatant was added with one volume 0.1 M sodium citrate buffer (pH 4.8) containing chitin azure (0.5 mg mL^{-1}). The mixtures were incubated in a shaking condition at 37 °C for 1 week. The absorbance of the test samples was measured at 570 nm.

2.6. Anti-swimming and swarming motility

Anti-swimming and swarming motility of *P. aeruginosa* were measured as stated previously by Packiavathy et al. (2012) [35]. In swimming motility assay, active *P. aeruginosa* culture was inoculated at the centre of the agar plate comprising of 1% tryptone, 0.5% NaCl and 0.3% of agar with 500 μg mL^{-1} of fungal crude extract. For swarming assay, the inoculation procedure was followed as same as anti-swimming study on medium formulated with 1% peptone, 0.5% 0f NaCl, 0.5% agar and 0.5% filter sterilized glucose with 500 μg mL^{-1} of fungal extract. The plates supplemented with DMSO were used as negative control. The plates were incubated at 37 °C in upright position for 24 h.

2.7. Effect of fungal crude extract on biofilm of P. aeruginosa PAO1

2.7.1. Microtitre plate assay (MTP)

Crystal violet staining assay was performed using modified method of Kalia et al. (2015) [28]. Briefly, 1 μL of *P. aeruginosa* culture was added in each well of 96-well microtiter plate filled with 100 μl of LB broth supplemented with sub-MIC doses of crude extract (250 μg mL^{-1} and 500 μg mL^{-1}). A control well was maintained without the addition of the sample. The plates were incubated at 37 °C for 24 h. Broth from the wells was discarded after incubation and the wells were rinsed with sterile PBS to remove the unadhered bacterial cells and stained with 100 μl of 0.4% crystal violet. The stain was discarded and the wells were rinsed with sterile PBS to remove excess stain before adding DMSO to solubilize the cell bound crystal violet. The optical density was measured spectrophotometrically at 590 nm.

2.7.2. Rhamnolipid quantification

The bacterial culture was grown in LB broth with or without fungal crude extract (0.25 x MIC and 0.5 x MIC) and incubated at 37 °C for 24 h. Rhamnolipids were extracted from culture supernatant by ethyl acetate evaporation method. The extracted rhamnolipid was dissolved in chloroform and the amount of rhamnolipid produced by *P. aeruginosa* was estimated by employing the modified method of Pinzon and Ju (2009) [36]. Briefly, 200 μl of 0.025% of freshly prepared methylene blue solution was added to 2 ml of rhamnolipid suspension. The reaction mixture was vigorously mixed by vortexing for 5 min and incubated at room temperature for 15 min. After incubation the chloroform layer was transferred into new tubes containing 500 μl of 0.2 N HCl and mixed well, then left at room temperature for 10 min for phase separation. Finally, 200 μl of the acidic phase holding a portion of the complex methylene blue was measured spectrophotometrically at 638 nm by using 0.2 N HCl as blank.

2.7.3. Congo red agar assay

Phenotypic changes and colony morphologies were studied using Congo red agar method reported by Lee el al. (2016) [37]. The Congo red agar was composed of 37 g L^{-1} of brain-heart infusion broth, 36 g L^{-1} of sucrose, 15 g L^{-1} of agar and 0.8 g L^{-1} of Congo red. The test pathogen was inoculated on Congo red agar supplemented with or without fugal extract (250 μg mL^{-1} and 500 μg mL^{-1}) and incubated at 37 °C for 48 h.

2.7.4. Extraction and quantification of EPS

Production of EPS by *P. aeruginosa* after the treatment with crude extract was estimated by modified method described by Rajesh el al. (2016) [38]. Briefly, the culture was grown with or without fungal cured extract (250 μg mL^{-1} and 500 μg mL^{-1}) in LB broth at 37 °C for 18 h. After incubation, the culture suspension was centrifuged at 10,000 rpm for 10 min and the resultant cell pellet was resuspended in high salt buffer. These test suspensions were subjected for centrifugation process for 15 min at 10,000 rpm. The released EPS was collected from supernatant by adding 95% ethanol. The collected precipitate was dissolved in milli-Q water and equal volume of ice-cold phenol (5%) was added. Concentrated H_2SO_4 was mixed with the reaction mixture at 2:5 ratio for the development of red colour. The amount of EPS produced was quantified by measuring the absorbance spectrophotometrically at 490 nm.

2.7.5. Alginate quantification assay

Alginate production was estimated according to the method described by Gopu el al. (2015) [39]. Briefly, 600 μl of boric acid-H_2SO_4 solution in the ratio 4:1 was slowly mixed to 70 μl of bacterial supernatant treated and untreated with crude extract under ice bath. The test bacteria was cultured with or without fungal extract \in LB broth at 37 °C for 18 h. The mixture was mixed well and incubated at ice both for 1 min. Approximately 20 μl of 0.2% of carbozole suspended in ethanol was added to the above solution and vortexed for 30 Seconds. The mixture was then incubated at 55 °C for 30 min. Alginate production was quantified spectrophotometrically by measuring the absorbance at 530 nm.

Table 1
Preliminary screening of crude extract obtained from different fungal isolates against QS systems in biosensor strain and test pathogen.

Sample Code	Zone of inhibition (mm)			
	Chromobacterium violaceum		*Pseudomonas aeruginosa* PAO1	
	250 μg mL^{-1}	500 μg mL^{-1}	250 μg mL^{-1}	500 μg mL^{-1}
DM28	10.67 ± 1.52	13.33 ± 1.15	10.67 ± 0.57	12.33 ± 1.15
DM27	8.33 ± 0.57	11.00 ± 1.00	8.33 ± 0.57	11.67 ± 1.52
DM29	10.67 ± 0.57	12.33 ± 1.15	10.00 ± 1.00	12.00 ± 1.00
MP1	8.66 ± 0.57	12.33 ± 0.57	8.33 ± 0.57	12.33 ± 2.08
MA	8.33 ± 0.57	12.33 ± 1.15	9.00 ± 1.00	13.67 ± 1.52
DM2	9.00 ± 1.00	10.33 ± 0.57	8.33 ± 0.57	12.00 ± 1.73
PPR3	23.67 ± 1.52	27.33 ± 0.57	19.00 ± 1.00	22.00 ± 1.00
MPS	10.00 ± 1.00	13.33 ± 1.15	11.00 ± 1.00	12.00 ± 1.00
DM30	8.33 ± 0.57	13.33 ± 0.57	8.33 ± 0.57	13.00 ± 1.00
PM2	8.66 ± 1.15	12.00 ± 1.00	8.33 ± 0.57	11.00 ± 1.00
PM5	8.33 ± 0.57	13.00 ± 1.00	8.66 ± 1.15	11.67 ± 1.52
PM7	8.66 ± 1.15	8.33 ± 0.57	8.33 ± 0.57	8.33 ± 0.57

2.7.6. MATH assay

Cell surface hydrophobicity of *P. aeruginosa* was observed by following the MATH assay describes by Sayem et al. (2011) [40] with slight modification. Briefly, bacteria was grown in LB broth supplemented with or without fungal extract (250 μg mL^{-1} and 500 μg mL^{-1}) at 37 °C for 18 h. Cell pellet was obtained by centrifugation, washed twice with sterile PBS and resuspended in 0.85% of NaCl solution. The initial OD_{600} was recorded and further 0.25 ml of toluene was added to 3 ml of cell suspension, mixed well for 2 min. The toluene phase was collected, and absorbance was recorded at 600 nm. The cell surface hydrophobicity was calculated using following formula

$$\text{Hydrophobicity \%} = [1\text{-}(OD_{600}\ \text{initial}\ /OD_{600}\ \text{final})]\ \text{x}\quad 100$$

2.7.7. Microscopic analysis of bacterial biofilms

Light microscopy and confocal laser scanning microscopy are used to observe the inhibition of bacterial biofilm formation. For both microscopic methods the slides were prepared by following the method described by Packiavathy et al. (2012) [35]. Briefly, 1% of overnight bacterial culture was added to 1 ml of LB broth containing cover glass, along with or without sub-MIC concentration of fungal crude extract (500 μg mL^{-1}). After 18 h of incubation at 37 °C, the cover glasses were rinsed thrice with sterile PBS to remove the planktonic cells. For the light microscopic observation cover glasses were stained with 0.2% of crystal violet solution whereas 1% of acridine orange was used for confocal laser scanning microscopic analysis (40X).

2.8. Gas chromatography-mass spectrometric (GC-MS) analysis

The bioactive compounds present in the fungal crude extract were identified using GC-MS analysis. GC-MS analysis was carried out on the gas chromatograph (model Clarus 680) with an ion-trap mass spectrometer (model clarus 600 EI) equipped with Elite-5MS column (30 m in length × 0.25 mm in thickness of film). An electron ionization system was operated in electron impact mode with an ionization energy of 70 eV for the detection of bioactive compounds. Helium gas was utilized as an carrier gas with constant flow of 1 ml min^{-1}. The initial temperature was set as 260 °C during the chromatographic run with an increasing rate of 10 °C min^{-1}. One microliter of fungal extract diluted present in ethyl acetate was injected. By using GC-MS NIST (2008) library the bioactive compounds in the fungal extract were identified

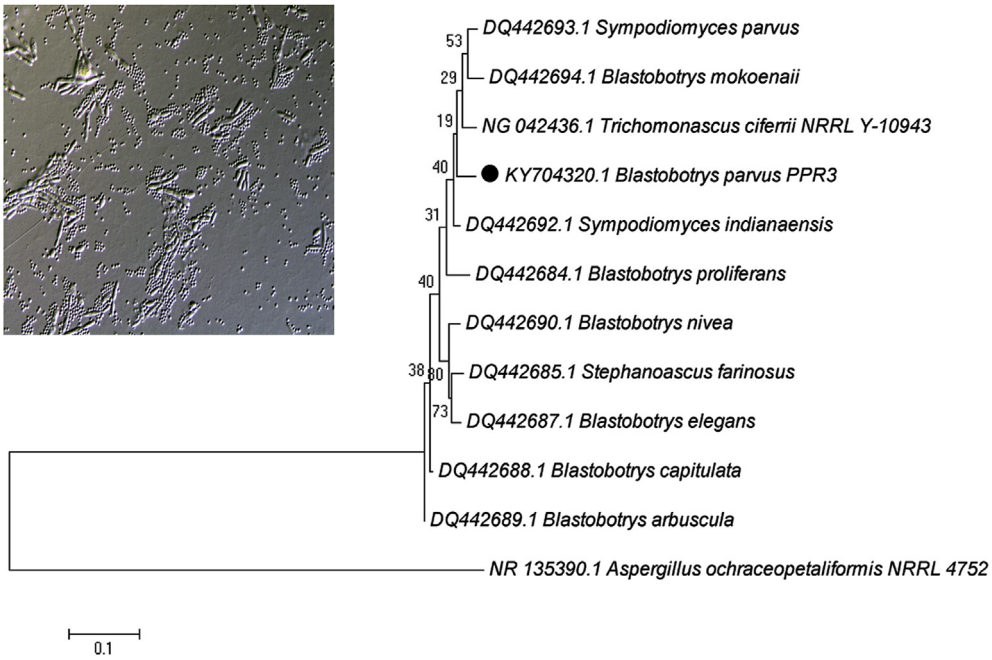

Fig. 1. Phylogenetic analysis of marine fungal isolate PPR3 ITS gene sequence with the most closely related strains from the NCBI GenBank database. The fungal strain mark with dot (●) was the isolate reported in this study. The phase contrast micrograph represents the morphology of the fungal isolate PPR3 (40X).

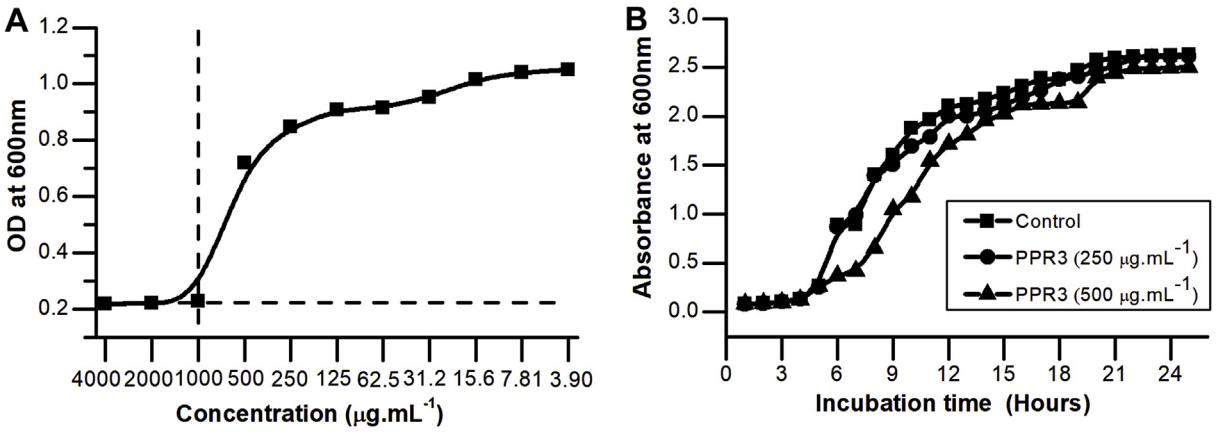

Fig. 2. (A) Minimum inhibitory concentration of fungal crude extract on *P. aeruginosa* PAO1; (B) Growth curves of *P. aeruginosa* PAO1 culture treated with 0.25 x MIC and 0.5 x MIC concentration of fungal crude extract and control.

[41].

2.9. In silico docking analysis

2.9.1. Receptor modelling and validation

The three dimensional structure of LasR ligand binding domain was extracted from protein data bank ID: 2UV0. As there is no experimental structure available for RhlR, protein sequence was obtained from on-line protein database Uniprot ID:P54292.1 and the three dimensional structure was modeled in ROBETTA on-line webserver. The generated protein structure files were evaluated by RAMPAGE webserver (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) [42]. The verified 3D structure of (http://nihserver.mbi.ucla.edu/Verify3D/) was used to validate the stereo chemical characteristics of generated protein using protein preparation wizard, were bond orders violations were resolved.

2.9.2. Molecular docking

Schrodinger Maestro software version 11.5 (Schrodinger, LLC, New York, NY, 2018) was used for molecular docking studies to understand the interaction of metabolites identified in crude extract against two major QS receptor proteins such as LasR and RhlR. The signaling molecules of *P. aeruginosa* PAO1 such as 3-oxo-C_{12}-HSL and C_4-HSL and well known anti-QS compounds such as biacelein and furanone C30 were used as reference molecules and positive control to their respective receptors proteins (LasR and RhlR). New polar hydrogen's were incorporated to the protein, the water molecules beyond 5 Å were deleted and finally, OPLS-2005 (optimized potentials for liquid simulations) force field was used for optimization of receptor protein. The Glide (version 7.8) in maestro software was used to generate the receptor grids. LasR, docking grid site was setup around its natural auto inducer (3-oxo-C_{12}-HSL) binding site whereas RhlR grid, was setup surrounding to the active site residue Trp-68 of its natural autoinducer (C_4-HSL) [43]. The three dimensional structure files of all fungal metabolites identified in GC-MS analysis were downloaded from PubChem database and LigPrep module of Maestro software version 11.5 was used for ligand preparation. All possible states (pH 7.0) i. e tautomeric and ionized states were generated for the ligands, finally created ligands were minimized using OPLS-2005 force field. Glide (version 7.8)

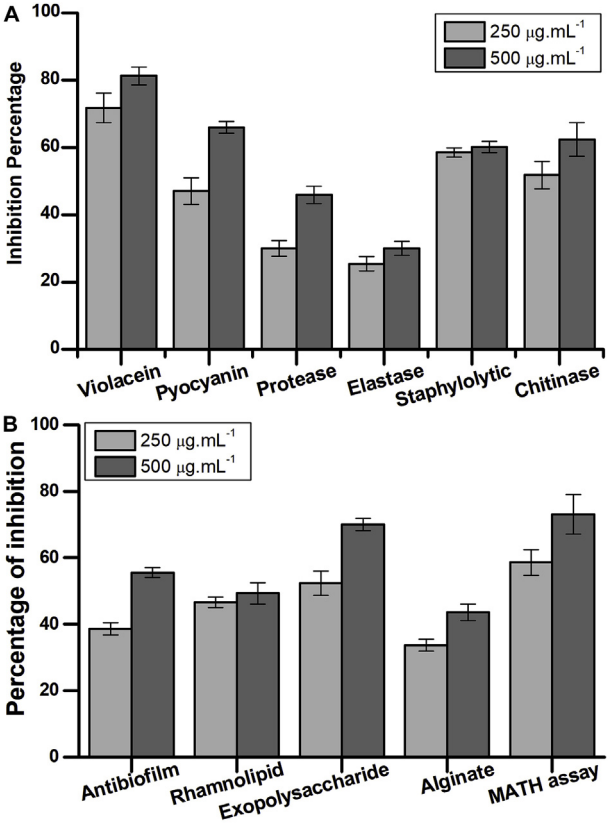

Fig. 3. Effect of crude extract on (A) violacein production in *C. violaceum* and virulent traits of *P. aeruginosa* PAO1. (B) Effect of sub-MIC of crude extract on biofilm attributes of *P. aeruginosa* PAO1.

in Maestro suite was used to dock prepared ligands with their respective receptor grids with enabled Extra Precision mode [44]. ProteinsPlus web server (https://proteins.plus/) and Chimera version 1.6.2 are used for further analysis.

2.10. Statistical analysis

All the experiments were performed independently in triplicate and the obtained data was represented as mean and standard deviation.

3. Results

3.1. Isolation, screening and identification

Twelve fungal colonies with morphologically distinct features were isolated and screened for their ability to inhibit quorum-sensing mechanism in both biosensor strain and test pathogen such as *C. violaceum* and *P. aeruginosa* PAO1 respectively. Among the fungal species PPR3 showed significant anti-QS activity against both the test bacterial strains (Table 1) and selected for further studies.

Molecular identification of fungal isolate PPR3 showed 99% similarity to different *Blastobotrys* sp. mainly with *Blastobotrys parvus*. Internal transcribed spacer (ITS) sequence of PPR3 was submitted to GenBank (NCBI) with the name *Blastobotrys parvus* PPR3 under the accession number KY704320.1. The phylogenetic tree of *B. parvus* PPR3 was constructed with MEGA 5.04 software, using maximum likelihood method with 1000 bootstrap replicates (Fig. 1).

3.2. Determination of MIC

MIC was determined for the crude extract of *B. parvus* PPR3 against *P. aeruginosa* PAO1. The MIC of fungal extract against *P. aeruginosa* PAO1 was determined as 1000 µg mL^{-1}, and further experiments were performed at sub-lethal concentrations such as 250 (0.25 x MIC) and 500 (0.5 x MIC) µg mL^{-1} (Fig. 2).

3.3. Growth curve

The effect of fungal crude extract on the growth of *P. aeruginosa* PAO1 at sub-lethal concentrations was evaluated. The results emphasized that the sub-lethal concentrations (250 & 500 µg mL^{-1}) did not showed any significant effect on the growth curve of the test bacterium (Fig. 2). This result was further confirmed by doubling time analysis where control, bacteria treated with 250 µg mL^{-1} and 500 µg mL^{-1} of crude extract showed 27.30, 27.85 and 29.62 min as doubling time, respectively.

3.4. Violacein production

Effect of sub-MIC concentrations of fungal crude extract on the production of violacein was quantification estimated. Violacein production was decreased with increase in the concentration of crude extract. Violacein production was inhibited by 71.8 ± 4.42% at a concentration of 250 µg mL^{-1}. Significant reduction in pigment production i. e 81.31 ± 2.62% was observed with increased dose of crude extract (500 µg mL^{-1}) (Fig. 3a).

3.5. Effect of fungal extract on virulent traits of P. aeruginosa PAO1

3.5.1. Production of pyocyanin

Pyocyanin production in *P. aeruginosa* is regulated by QS system. The effect of fungal crude extract on production of pyocyanin by *P. aeruginosa* PAO1 was investigated. A significant reduction in pyocyanin production was observed at different sub-lethal concentrations. At a concentration of 250 µg mL^{-1} of fungal crude extract 47.05 ± 3.93% reduction in pyocyanin production was observed, whereas 66.03 ± 1.72% reduction was observed when treated with 500 µg mL^{-1} of crude extract (Fig. 3a).

3.5.2. LasA protease assay

Proteases are one among several hydrolytic enzymes produced by *P. aeruginosa* that involve in the virulence. The expression of this enzyme is controlled by QS system. Experiments were conducted to evaluate the effect of crude extract in the secretion of protease enzyme by PAO1. The results reveled that a considerable reduction in the production of LasA protease was observed in the test bacterium in a dose dependent manner when grown in the presence of different concentrations of fungal crude extract (Fig. 3a). The experimental results showed that 30.05 ± 2.32 and 45.94 ± 2.55% reduction in protease production was observed when treated with 250 and 500 µg mL^{-1} of crude extract, respectively.

3.5.3. LasB elastase activity

Elastase enzyme also plays an important role in the pathogenicity of *P. aeruginosa* PAO1. The test result showed reduction in the production of elastase enzyme when treated with fungal extract. An inhibition percentage of 25.4 ± 2.16% was recorded when test bacterium was treated with a concentration of 250 µg mL^{-1} and 30.01 ± 2.05% inhibition was observed at a concentration of 500 µg mL^{-1}, when compared to control (Fig. 3a).

3.5.4. Inhibition of staphylolytic potential

A considerable reduction in staphylolytic activity compared to that of control was observed when test pathogen was grown in presence of fungal extract (Fig. 3a). Where 58.55 ± 1.38% and 60.16 ± 1.63% inhibition of staphylolytic activity was recorded when treated with of 250 and 500 µg mL^{-1} of fungal extract, respectively.

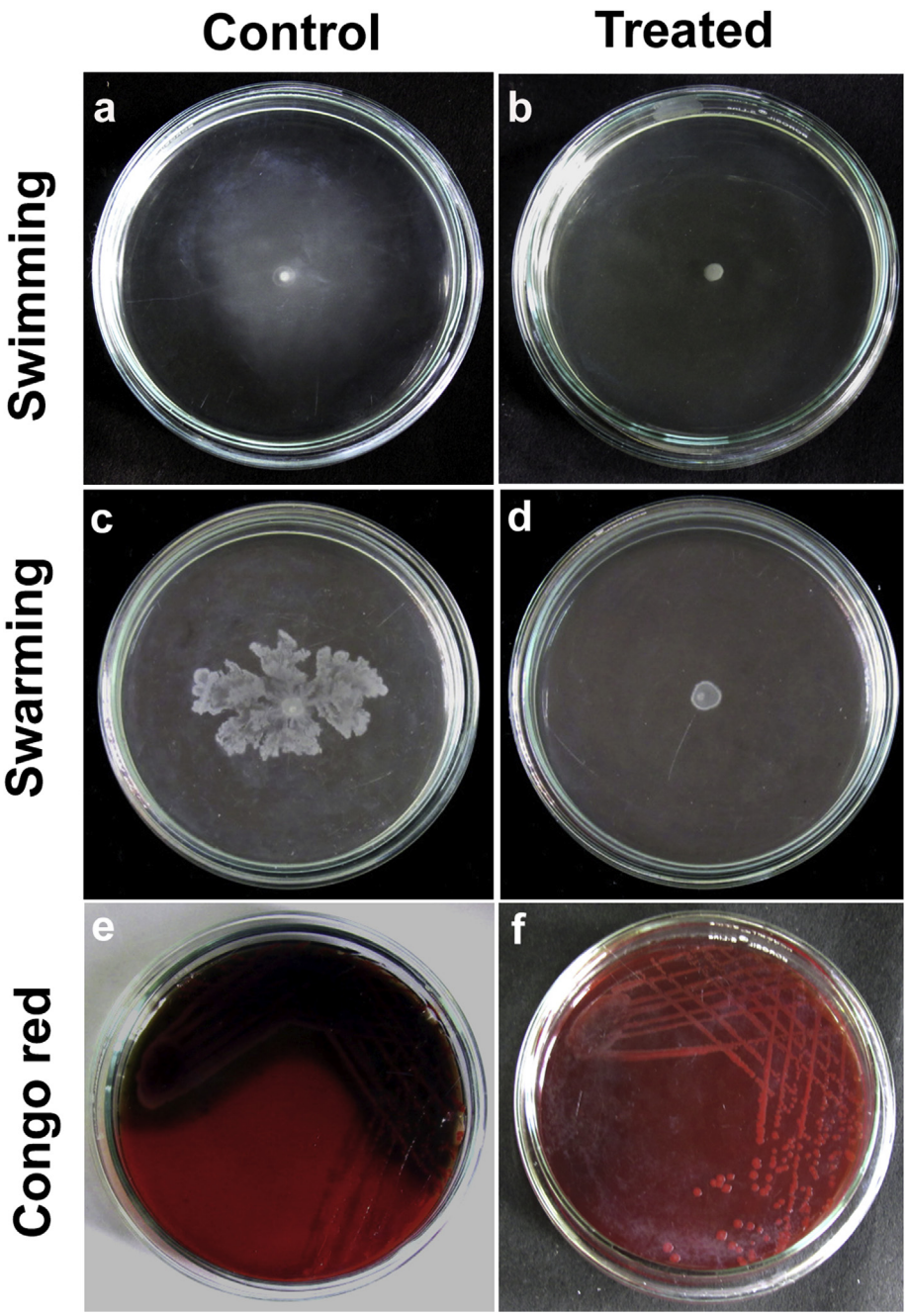

Fig. 4. Inhibition of swimming motility of *P. aeruginosa* PAO1 by fungal extract (a) control, (b) treated with of fungal crude extract; Reduction in swarming motility in the test pathogen by extract of PPR3 (c) control, (d) treatment; Congo red agar plates showed the potential of the fungal extract in the production of EPS (e) control plate inoculated with *P. aeruginosa* PAO1 without sample, (f) treated plate seeded with *P. aeruginosa* PAO1 with fungal extract at 0.5 x MIC concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5.5. Chitinase inhibition

Chitinase enzyme is also a widely known virulence trait of *P. aeruginosa* and the production of this enzyme was controlled by QS system. The efficacy of fungal crude extract on the production of chitinase enzyme by test bacterium was evaluated. The chitinase activity was inhibited by 62.39 ± 4.95% when *P. aeruginosa* treated with 500 μg mL^{-1} of fungal extract. However, the lower concentration (250 μg mL^{-1}) of crude extract showed moderate reduction in enzyme activity (Fig. 3a).

3.6. Anti-swimming and swarming motility

In the QS dependent biofilm formation, flagella are responsible for the swimming and swarming motility. Effect of fungal extract on the motility of *P. aeruginosa* PAO1 was observed. Fungal extract exhibited notable reduction in the swimming and swarming motility of test bacterium (Fig. 4).

3.7. Effect of B. parvus PPR3 extract on P. aeruginosa biofilm

3.7.1. Biofilm inhibition

Formation of biofilm by *P. aeruginosa* PAO1 is a QS dependent phenomenon. An investigation was performed to observe antibiofilm efficacy of fungal crude extract against PAO1. Fig. 3b depicts the biofilm inhibition (38.53 ± 1.83% and 55.51 ± 1.55%) in PAO1 when treated with fungal crude extract at different sub-lethal concentrations

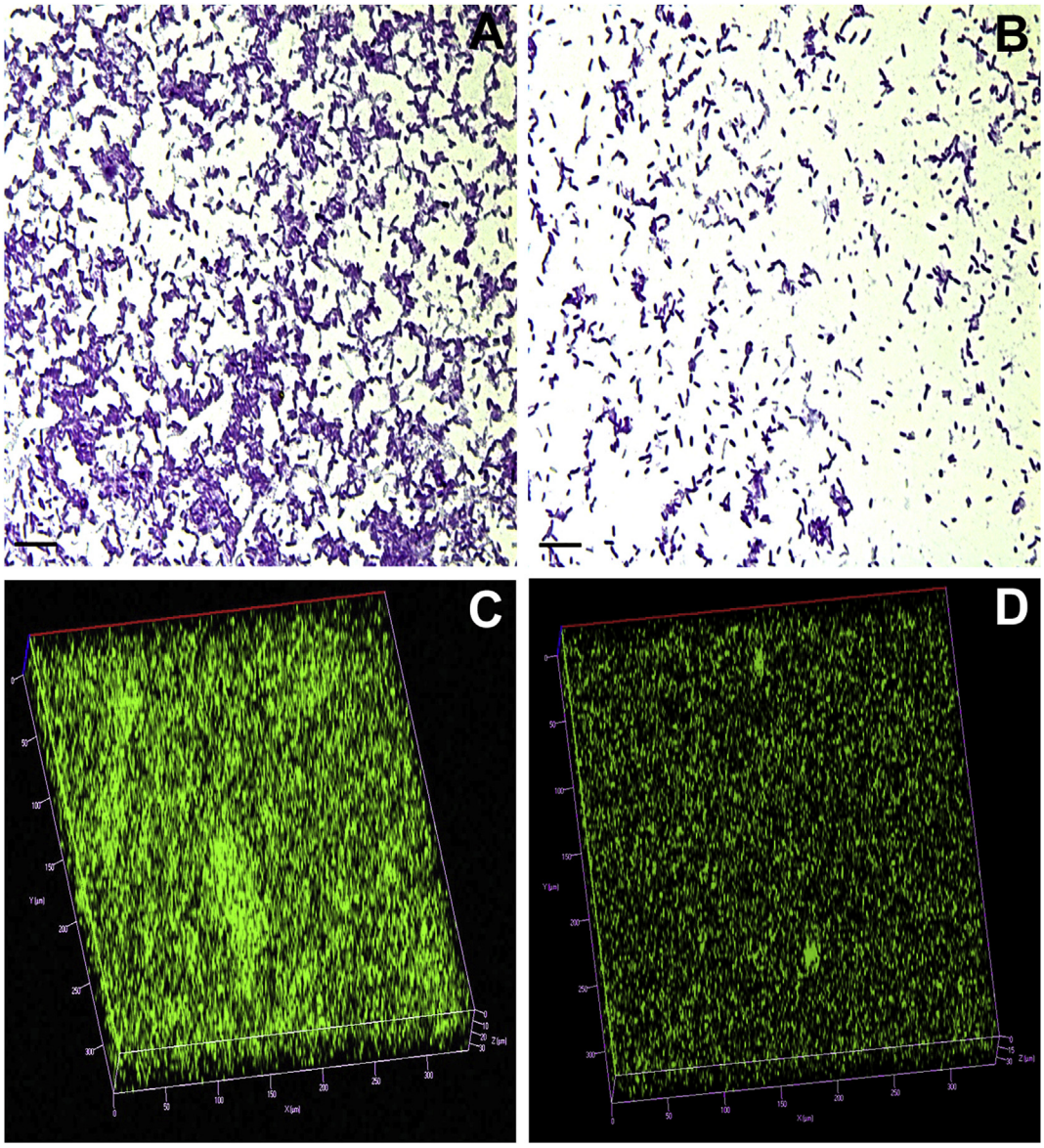

Fig. 5. Effect of fungal extract on biofilm of *P. aeruginosa* PAO1, when treated with or without fungal extract: light microscopic observation (A) control; (B) Treated, Confocal Laser Scanning Microscopy (CLSM) analysis (C) control; Treated (D).

Table 2
GC-MS literature of fungal metabolites in the crude extract of the PPR3.

Fungal metabolites	Retention time (min)	Molecular formula	Molecular weight (g/mol)	Peak area (%)
2-Phenylethanol	9.03	$C_8H_{10}O$	122.167	1.97
Phenylacetic acid	12.69	$C_8H_8O_2$	136.15	3.70
2-(4-Hydroxyphenyl) ethanol	17.20	$C_8H_{10}O_2$	138.166	2.14
2-Hydroxyphenylacetic acid	18.40	$C_8H_8O_3$	152.149	0.66
3-Hydroxybenzoic acid	19.23	$C_7H_6O_3$	138.122	0.48
2,4-DI-Tert-butylphenol	19.31	$C_{14}H_{22}O$	206.329	0.76
4-Hydroxyphenylacetic acid	20.28	$C_8H_8O_3$	152.149	2.28
Phenylalanyl-prolyl diketopiperazine	35.90	$C_{14}H_{16}N_2O_2$	244.294	1.38

(250 and 500 μg mL^{-1}).

3.7.2. Production of EPS, rhaminolipid and alginate

Virulence factors like EPS, rhaminolipid and alginate plays a major role in the biofilm development, which is regulated by QS system in the test bacterium. Experiments were conducted to determine the effect of fungal extract in the production of these virulence factors. The production of different virulence factors were inhibited significantly by fungal crude extract in a concentration dependent manner (Fig. 3b). The maximum inhibition of 70.04 ± 1.85%, 49.25 ± 3.21% and 43.57 ± 2.44% was observed for EPS, rhaminolipid and alginate, respectively in the bacterium treated with fungal crude extract at a concentration of 500 μg mL^{-1}.

3.7.3. Cell surface hydrophobicity

The measurement of cell surface hydrophobicity confirmed the inhibition of biofilm production, which is an important factor for the adherence of cells to the surface. The effect of fungal extract on the level of cell surface hydrophobicity of *P. aeruginosa* was investigated. The Sub-lethal concentration (500 μg mL^{-1}) of fungal extract showed 73.03 ± 5.94% reduction in cell surface hydrophobicity (Fig. 3b).

3.7.4. Congo red agar assay

Biofilm formation of *P. aeruginosa* was observed by culturing the

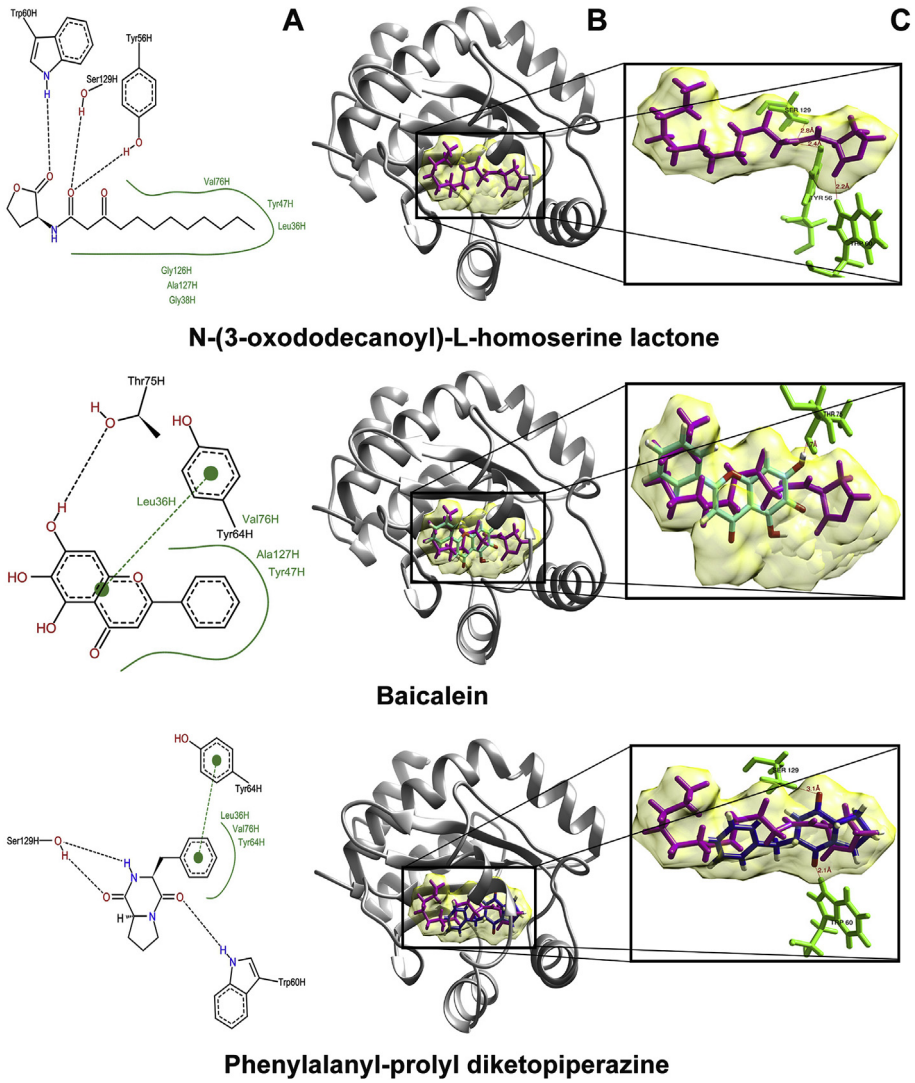

Fig. 6. Molecular docking analysis of the LasR protein with natural ligand, $3OC_{12}$-HSL, biacelein (Positive control) and Phenylalanyl-prolyl diketopiperazine (bioactive compound from *B. parvus* PPR3). (A) Left panel represent two dimensional views of ligand-receptor interactions; (B) middle panel represent full view of the ribbon structure of the LasR with ligands; (C) right panel represent enlarged view of docking confirmation between LasR receptor and ligands.

organism in Brain-Heart infusion agar medium supplemented with Congo red in the presence and absence of fungal crude extract at sub-lethal concentration (500 μg mL^{-1}). The agar plate incubated without fungal extract showed dry, crystalline black colonies that confirme the production of exopolysaccharides, which is major constituent of biofilm. Whereas the plates treated with fungal extract showed colonies with reduced EPS production, with less number of dry, crystalline black colonies (Fig. 4).

3.8. Microscopic analysis of biofilm inhibition

The light microscopic observation disclosed the poor development of test bacterium biofilm when treated with fungal crude extract than that of control slide (Fig. 5a and b). Further, the CLSM analysis displayed the loose biofilm architecture of *P. aeruginosa* treated with fungal crude extract when compare with control (Fig. 5c and d).

3.9. GC-MS analysis

Different bioactive compound present in the ethyl acetate extract of *B. parvus* PPR3 was identified by GC-MS analysis (Table 2). The major bioactive compounds identified in *B. parvus* PPR3 extract are 2-Phenylethanol, Phenylacetic acid, 2-(4-Hydroxyphenyl)ethanol, 2-Hydroxyphenylacetic acid, 2,4-Di-Tert-butylphenol and Phenylalanyl-prolyl diketopiperazine.

3.10. In silico docking analysis

3.10.1. Molecular modelling of RhlR

In the current study 3D structure of RhlR was modeled using the protein sequence from UniPort database (ID: P54292.1) in ROBETTA on-line webserver. The final quality of the modeled RhlR structure was validated using RAMPAGE, that provides knowledge on stereo chemistry qualities of constructed three dimensional model structures based on the inspection of psi/phi Ramachandran plot. The result displayed the presence of 99.6% of the amino acid residues in favored region, 0.4% amino acid residues are in the allowed region, no amino acid residues in outlier region with verified average 3D-1D score.

3.10.2. Molecular docking

Molecular docking results confirmed the interaction of bioactive compounds with LasR and RhlR receptor proteins. Natural ligands such as 3-oxo-C_{12}-HSL and C_4-HSL were used as reference in molecular docking analysis as ligand interacting domains of LasR and RhlR

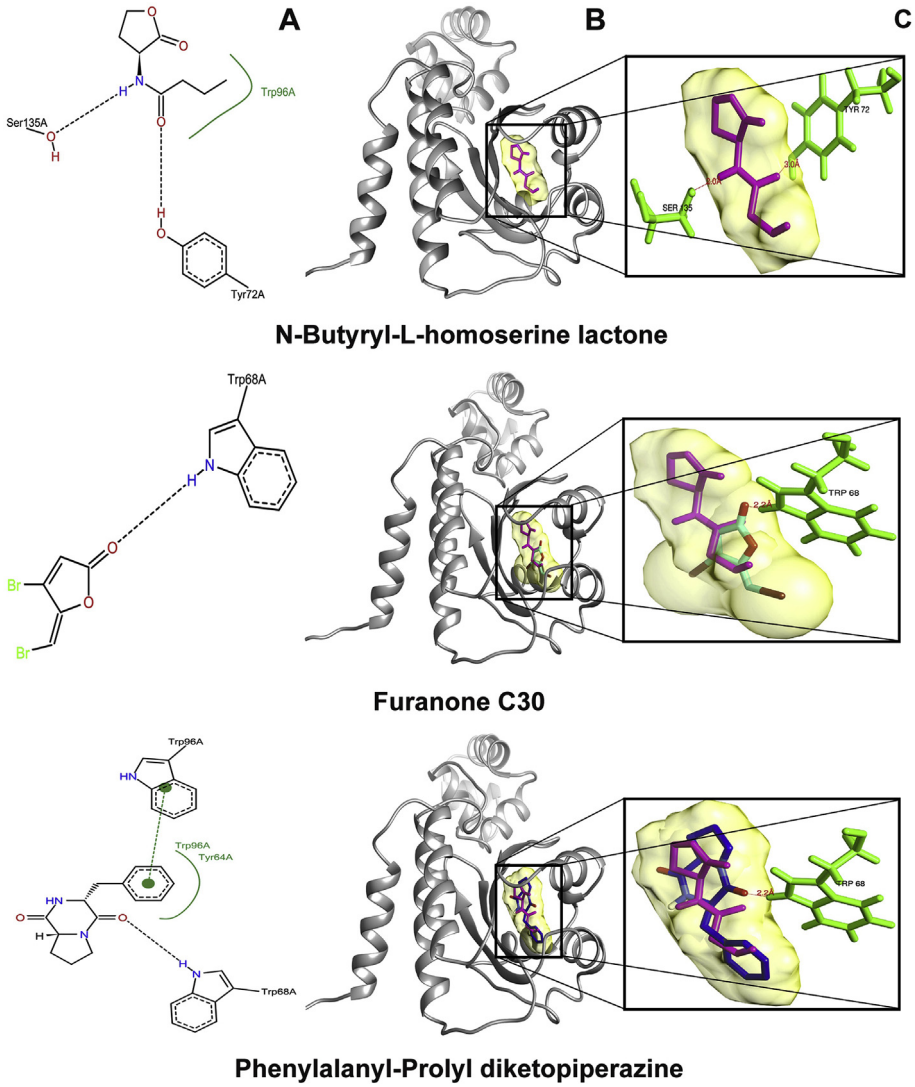

Fig. 7. Molecular docking analysis of the RhlR protein with natural ligand, C_4-HSL, furanone C30 (Positive control) and Phenylalanyl-prolyl diketopiperazine (bioactive compound from *B. parvus* PPR3). (A) Left panel represent two dimensional views of ligand-receptor interactions; (B) middle panel represent full view of the ribbon structure of the RhlR with ligands; (C) right panel represent enlarged view of docking confirmation between RhlR receptor and ligands.

respectively. The Molecular docking studies of different bioactive compounds identified in the ethyl acetate extract of *B. parvus* PPR3 depicted in Fig. 6. Phenylalanyl-prolyl diketopiperazine exhibits highest docking score of −6.972 kcal/mol with LasR and forms hydrogen bonds with residues Tyr56, Thr75 and Ser129. The docking score was comparatively equal to that of natural ligand (Fig. 6). The compound posses docking score of −6.96 kcal/mol with RhlR receptor and formed hydrogen bond with residue TRP-68, which is relatively higher than the natural ligand and positive control (Fig. 7). This result concluded that presence of different bioactive compounds in the crude extract of *B. parvus* potentiated the antiQS activity against *P. aeruginosa* (Table 3).

4. Discussion

Anti-QS efficacies of fungal extract against *P. aeruginosa* PAO1 was determined in this study and attenuation of QS dependent virulence traits such as pyocyanin, protease, elastase, chitinase and biofilm development was reported. CviIR-dependent QS circuit in *C. violaceum* is responsible for the expression of genes involved in the production of violacein pigment. The natural and synthetic chemical compounds that limit the production of violacein without creating any adverse effect on the growth of *C. violaceum* are widely accepted as promising anti-QS agents [45,46]. In the present study, notable degree of reduction in the CviIR mediated production of violacein in *C. violaceum* was observed when treated with fungal extract (Table 1). A similar inhibitory activity was observed by Martin-Rodriguez et al. (2014) where extract of aquatic fungi inhibited the production of violacein in a dose depended manner [22]. Based on preliminary results, anti-QS activity of PPR3 extract was confirmed against the test strains. The fungal isolate was identified as *Blastobotrys parvus* and deposited in GenBank (NCBI) as *Blastobotrys parvus* PPR3. The fungal species *B. parvus* frequently found in the plants that occasionally causes infection to the plant [47].

In *P. aeruginosa*, two separate but interrelated QS circuits, *las* and *rhl* are present as described elsewhere [48]. Las mediated quorum sensing mediated the synthesis and sensing of autoinducer, $3OC_{12}$-HSL which initiates the synthesis of various virulence factors such as elastase A, pyocyanin and alkaline protease [49]. Interestingly, the *las* system also involves in the activation of second QS circuit known as *rhl* which employ *N*-butyryl homoserine lactone (BHL) as signaling molecule that binds with RhlR receptor and activates expression of the certain virulence genes that encodes the synthesis of rhamnolipids and pyocyanin [50]. The transcriptomic analysis on *las*I and rhlI mutants disclosed that the regulons are on a continuum, with certain genes like LasA obey

Table 3
Molecular docking analysis of fungal metabolites with QS receptors of *P. aeruginosa* (LasR and RhlR).

Compound Name	LasR				RhlR			
	Docking Score (Kcal/mol)	Glide Emodel Score	Hydrogen Bonding	Hydrophobic Interaction	Docking Score (Kcal/mol)	Glide Emodel Score	Hydrogen Bonding	Hydrophobic Interaction
N-(3-oxododecanoyl)-L-homoserine lactone[a] (lasR) *N*-Butyryl-L-homoserine lactone[a] (rhlR)	−6.928	−73.83	TYR56, TRP60, SER129, ASP73	TYR64, LEU36, LEU110, ALA105, PHE101, TYR93, PRO74, TRP88, CYS79, LEU125, LEU39, ALA50, LEU40, TYR47, ALA70, VAL76, ILE52	−5.47	−38.87	SER135	ALA83, PHE101, TRP96, TRP68, ALA111, LEU107, TRP108, TYR72, LEU116, LEU69, TYR64, VAL60, TYR45, ALA44, ILE84
Baicalein[b] (lasR) Furanone C30[b] (rhlR)	−4.93	−60.27	THR75	PHE101, LEU110, TRP60, TYR56, TYR64, LEU36, ILE52, ALA50, LEU39, LEU125, LEU40, CYS79, VAL76, ALA127	−4.53	−26.86	TRP68	ILE84, ALA83, TRP96, LEU107, ALA111, PHE101, TRP108, TYR72, LEU69, TYR64
Phenylalanyl-prolyl diketopiperazine	−6.972	−44.40	TRP60, THR75, SER129	LEU36, ILE52, TYR64, VAL76, ALA127, TYR56, ALA105, PHE101, TYR93, LEU110, TRP88	−6.96	−23.59	TRP68	ILE84, LEU116, ALA111, ALA83, LEU107, PHE101, TRP108, TYR64, TYR72, LEU69, VAL60, TYR45, ALA44
2-Phenylethanol	−6.02	−32.29	TYR56	ILE92, TRP88, TYR93, LEU110, ALA105, TRP60, TYR64, VAL76, LEU36	−5.20	−16.73	SER135	ALA44, LEU116, TYR64, TRP68, TRP108, LEU107, PHE101, ALA111, TRP96, ALA83, TYR72, ILE84
P-Hydroxyphenylacetic acid	−5.927	−30.58	TYR56, SER129, LEU110	LEU36, TRP60, TYR64, TRP88, VAL111, ALA105, PHE102, TYR93, PRO74, VAL76	−4.63	−23.95	ASP81	TYR45, ALA44, ILE84, ALA83, LEU107, TRP96, TRP68, LEU69, TYR72, VAL60
3-Hydroxybenzoic acid	−5.579	−38.25	TYR93, THR75, SER129, TYR56	LEU36, LEU110, ALA105, PHE102, ILE92, PRO74, PHE101	−4.98	−26.97	TRP68	TYR72, VAL60, LEU69, LEU134, ILE84, TYR45, VAL133, ALA44
2-(4-Hydroxyphenyl)ethanol	−5.55	−35.57	ASP73, SER129, LEU110	TRP88, TYR93, ALA105, TRP60, TYR56, LEU36, TYR64, VAL76	−4.79	−29.13	ASP81	ALA44, ALA83, PHE101, TRP96, ILE84, LEU107, TYR72, TRP68, LEU69, TYR45, VAL60
2,4-DI-Tert-butylphenol	−4.46	6.053	NO H BOND	LEU36, TYR64, TRP60, ILE92, PRO74, ALA105, PHE101, TYR93, TRP88, LEU110, PHE102, VAL76, ALA127	−3.14	12.58	NO H BOND	LEU134, VAL133, ALA44, LEU116, TYR45, ALA83, TRP96, ILE84, LEU107, PHE101, TRP68, LEU69, VAL60
Phenylacetic acid	−4.33	−27.34	TYR56, SER129	LEU36, TYR64, TRP60, LEU110, PHE101, TR93, ALA105, TRP88, VAL76	−4.37	−22.66	TRP68	VAL133, TYR45, LEU134, ALA44, TYR72, LEU69, TYR64, VAL60, ILE84
2-Hydroxyphenylacetic acid	−4.30	−33.97	TYR56, SER129	TRP88, TYR93, LEU110, ALA105, TRP60, TYR64, VAL76, LEU36	−5.48	−18.73	TRP68, ASP81	ILE84, LEU116, ALA111, TRP108, LEU107, PHE101, ALA83, PRO82, TRP96, TYR72, TYR64

[a] Natural Ligands.
[b] Positive Control.

extremely to $3OC_{12}$-HSL, while some with C_4HSL specificities as observed with gene *rhlAB*. Certain other genes responsible for virulence traits respond for both signaling systems [51]. Moreover, the pathogenicity of the *P. aeruginosa* after deleting both the signaling systems (Δ*lasIR*/Δ*rhlIR*) was analyzed in *in vivo* condition, in which the QS-deficient mutant showed complete attenuation of the virulence in mouse model as compared with the wild type [31]. Transformation of planktonic state of *P. aeruginosa* to biofilm condition, confers resistance to the antimicrobial agents which is a challenging task to treated the microbial infection [52]. It was well reported that development of biofilm in *P. aeruginosa* was directly associated with QS phenomenon. The mutant strains without QS genes failed to develop three dimensional architecture of the biofilm as that of wild type *P. aeruginosa* [53].

The lytic enzymes like protease and elastase are actively participate in the pathogenesis of *P. aeruginosa*, where they involve in the digestion of structural components at the infected site and facilitate the invassion of bacteria. In this study, the crude extract of the *B. parvus* PPR3 showed inhibition of lytic enzymes production like protease, LasB elastase, total protease and LasA protease in *P. aeruginosa* PAO1 as shown in Fig. 3a. Studies of Alasil et al. (2014) showed that the extract from *Paenibacillus* strain 139SI was effectively inhibited the QS controlled lytic enzymes production such as total protease, LasA protease and *las*B elastase. Pyocyanin a virulence trait in *P. aeruginosa* governed by QS system was considerably reduced with sub-MIC concentration of fungal crude extract. Pyocyanin is a tricyclic phenazine class of compound which contributes to the cytotoxicity to host cells by inducing oxidative stress [54]. Zhou et al. (2017) recent findings have evidenced the inhibition of the pyocyanin production in *P. aeruginosa* PAO1 when treated with crude extract of *Plectosphaerella cucumerina* [55].

Development of biofilm in *P. aeruginosa* was considered as an important attribute to pathogenicity [56]. Moreover, the maturation of the biofilm was directly governed by the QS system in *P. aeruginosa* PAO1. *B. parvus* PPR3 extract exhibited significant biofilm inhibition against *P. aeruginosa* PAO1. QS regulon controls the production of exopolymeric substance (EPS) and biofilm formation. The fungal metabolites of this study significantly inhibited the production of EPS. The antibiofilm potential of the crude extract of PPR3 was further confirmed with microscopic analysis. The results of microscopic analysis showed that *P. aeruginosa* PAO1 treated with fungal extract was failed to form mature biofilm when compared to the control. The results of antibiofilm activities of PPR3 extract in this study was in alignment with the results [31], where notable reduction in biofilm formation in *P. aeruginosa* treated with *Bacillus* sp. Strain SS4 extract was observed. *In silico* analysis was carried out to understand the interaction QS receptors (LasR and RhlR with bioactive compounds. Similar work was conducted using bioactive compound, diketopiperazine obtained from *Rheinheimera aquimaris* wherein, molecular docking results showed high binding affinity with docking score of −8.68 kcal/mol as compared to that of natural ligand (−6.86 kcal/mol) [57]. The possible mechanism of antiQS activity might be the interaction of bioactive metabolites present in the crude extract with the QS receptors and further preventing the binding of effector molecules to DNA and interfering with RNA polymerase [58].

Antiinfective drug discovery is of importance in the present context due to the emergence of multidrug resistance and biofilm forming pathogenic bacteria. Development of antiinfective drugs could be an alternative to prevent the bacterial infections unlike the use of antibiotics where development of resistance was observed. The present study provides insights into the isolation of bioactive compounds from less exploited marine fungi to attenuate quorum sensing and biofilm formation in *P. aeruginosa.*.

5. Conclusion

The present study revealed the antiQS potential of matine derived fungi and its effect on the production of virulence factors. The GC-MS result identified the bioactive compounds present in the fungal crude extract was identified by GC-MS analysis. The *in silico* molecular docking studies further confirmed the antiQS activity of fungal crude extract. The bioactive compounds identified in the crude extract of *B. parvus* PPR3 can be further studied to develop antiinfective agents.

Declaration of competing interest

The authors hereby declare no conflict of interest.

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