

## Quorum Sensing and Biofilm Inhibition by Lactonase Producing *Bacillus amyloliquefaciens* SBF1 Strain Isolated from Date Palm Rhizosphere of Saudi Arabia

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Indiscriminate use of antibiotics has led to development of drug resistance among infection causing bacteria rendering the current antibiotic therapy ineffective. This has compelled the scientific community across the globe to look for new drug targets. Since quorum sensing plays a key in the expression of virulence genes and biofilm formation it can be a potential target for curing infections caused by pathogens. Therefore, keeping in mind the ubiquitous and diverse nature of quorum quenching bacteria, date palm rhizospheric soil of Saudi Arabia was selected to screen for quorum quenching bacteria. In the present study, SBF1 strain demonstrated promising inhibition of violacein *Chromobacterium violaceum* 12472 biosensor strain and was selected for anti-virulence assay. Using 16S rDNA analysis the strain SBF1 was identified as *Bacillus amyloliquefaciens* (GenBank Accession Number: KC494392). Culture extract of SBF1 demonstrated dose-dependent (0.75–6 mg/ml) reduction against the QS regulated violacein production in *C. violaceum* (45–79%) and LasB elastase (4.9–63.8%), total protease (34.3–84.4%) and pyocyanin (32.7–63.7%) in PAO1. The extract also decreased the swarming migration of CV12472 and PAO1 significantly. Biofilm forming ability of PAO1 was also significantly impaired after treatment with SBF1 extract. Ring opening assay suggests that the anti-QS activity is due to the production of lactonase enzyme. Findings of the present study demonstrate the anti-QS and antibiofilm potential of SBF1 extract. Quorum quenching ability of SBF1 can be exploited to treat bacterial infections, to prevent food spoilage and in bioremediation.

**Keywords:** *Bacillus amyloliquefaciens*, biofilm, date palm, lactonase, quorum sensing, virulence.

Quorum sensing (QS) can be defined as density dependent signaling system that helps the coordinated regulation of gene expression by the

production, release, and recognition of signal molecules called autoinducers<sup>1</sup>. One of the most well studied QS signal molecules (autoinducer) are *N*-acylhomoserine lactones (AHL)<sup>2</sup>. AHLs are highly conserved molecules having the common homoserine lactone moiety but differ in the length

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and structure of the acyl side chain<sup>3</sup>. Synthesis of AHL molecules involves LuxI synthase that uses S-adenosylmethionine and fatty acid biosynthesis intermediate as substrates. The generated AHL molecules bind to LuxR receptor protein followed by subsequent regulation of downstream gene expressions. Each receptor protein is highly selective for its cognate AHL signal molecule<sup>4</sup>. The complex of signaling molecules and receptor proteins trigger the expression of specific genes responsible for various phenotypes including violacein pigment in *Chromobacterium violaceum* (CviI/R), virulence factors production and biofilm formation in *Pseudomonas aeruginosa* (LasI/R). QS also regulates certain bacterial behaviours that have great economic impacts like food spoilage, aquaculture, water purification, ship industry, etc.<sup>5</sup>.

QS plays a key role in bacterial pathogenesis by coordinating the expression of virulence genes that are responsible for the invasion and colonization of the pathogenic bacteria in higher organisms<sup>6</sup>. Ability of the pathogenic strains of *P. aeruginosa* to form biofilms leads to reduced susceptibility of the pathogen towards antibiotics and causes severe chronic infections. Since virulence factors like elastase, protease, motility and biofilm formation in *P. aeruginosa* are regulated by quorum sensing, thus the discovery of anti-QS compounds can be of great interest in the treatment of biofilm-associated chronic infections<sup>7</sup>.

The potential for microbiological degradation of quorum sensing is important for several reasons and since the signal AHL-mediated QS mechanisms are widespread and highly conserved in many pathogenic bacteria, they can be attractive targets for novel anti-infective therapies<sup>8</sup>. Quorum quenching (QQ) is term given to the interference of QS by molecules produced by prokaryotes and eukaryotes. This QQ mechanism plays an important role in microbe-microbe and pathogen-host interactions. Over the last decade, workers all over the world have documented a wide range of AHL degrading microbes. Halogenated furanones from marine red macroalgae *Delisea pulchra* were the first reported quenchers of quorum sensing<sup>9, 10</sup>. Since then, N-acyl-homoserine lactone acylase from *Ralstonia* sp. XJ12B and *Bacillus pumilus*<sup>11, 12</sup>, N-acyl homoserine lactonases from *Bacillus* species, *B.*

*thuringiensis*, *B. subtilis* and *B. cereus*<sup>13-16</sup> and culture extracts of *Bacillus* and *Paenibacillus* spp.<sup>17, 18</sup> have been reported for their quorum quenching activity.

Soil is a major source of bacteria that synthesize a wide range of compounds with wide range of biological activities<sup>19</sup>. Consequently, we have also explored a novel rhizospheric environment (date palm) in search of quorum quenching bacterial communities. The date palm (family Arecaceae) is the primary fruit crop of the Middle East. Saudi Arabia is one of the major date producing countries in the world. The tree is valued mainly for its fruits (date) as well as for its ornamental value across Saudi Arabia<sup>20</sup>. Considering the importance of date plants in Saudi Arabia an investigation, targeting rhizospheric bacteria as sources of diverse quorum-quenching agents against *Chromobacterium violaceum* (CV 12472 and CVO26) and *Pseudomonas aeruginosa* PAO1 was planned. In the present study, *Bacillus amyloliquefaceins* SBF1 demonstrated a significant decrease in the production of QS controlled violacein pigment in biosensor strains CV 12472 and CVO26 strains. Furthermore, the SBF1 strain showed inhibition of QS-controlled virulence factors in *P. aeruginosa* PAO1. The study for the first time demonstrates the quorum quenching potential of *Bacillus amyloliquefaceins* SBF1 isolated from rhizospheric soil of date palm in Saudi Arabia.

## MATERIALS AND METHODS

### Bacterial strains and growth conditions

*C. violaceum* 12472 is a wild-type strain producing QS regulated purple colored pigment, violacein. It produces and responds to cognate C<sub>4</sub> and C<sub>6</sub> Acyl homoserine lactone (AHL) molecules. *Chromobacterium violaceum* CVO26 is a Tn5 mutant strain, produces the purple pigment violacein upon induction with externally added short-chain autoinducers<sup>21</sup>. *P. aeruginosa* PAO1 is pathogenic bacteria and many of its virulence factors and traits are QS controlled. All strains were maintained on Luria Bertani or LB broth (15.0 g tryptone, 0.5% yeast extract, 0.5% NaCl) solidified with 1.5% agar (Hi-media). *C. violaceum* 12472, *C. violaceum* CVO26 and *P. aeruginosa* (PAO1)

strains were cultivated at 28 °C and 37 °C respectively.

#### **Bacterial screening for quorum quenching (QQ) activity**

A total of 52 bacteria isolated from the rhizospheric soil of date palm trees were screened for their anti-QS activity against *Chromobacterium violaceum* CV12472 biosensor strain. To screen for bacterial that quenched AHL-mediated violacein production, the bacterial isolates were spotted on to the centre of the LB agar plate and incubated overnight at an appropriate temperature (all our isolates grew at 30°C). Following overnight incubation the test organisms were overlaid with 5 ml LB soft agar (0.5% w/v agar) cooled to 45 °C containing 10<sup>6</sup> CFU/ml of the indicator organisms *C. violaceum* ATCC 12472<sup>22</sup>. A positive QQ result was indicated by the lack of pigmentation of the indicator strain. *C. violaceum* 12472 was used as negative control as it produces cognates C<sub>6</sub> AHL and therefore would not inhibit its own QS.

#### **Bacterial characterization**

Among bacterial strains screened, the strain SBF1 showing highest quorum quenching activity was selected for further characterization. The strain SBF1 was identified by the morphological, physiological and biochemical tests which included Gram reaction, citrate utilization test, indole production test, methyl red test, nitrate reduction, Voges Proskauer, catalase test, carbohydrates (dextrose, mannitol and sucrose) utilization test, starch hydrolysis, and gelatin liquefaction test. These tests were performed following the standard methods outlined in Bergey's Manual of Determinative Bacteriology.

#### **16S rDNA based identification**

The sequencing of 16S rDNA of the strain SBF1 was done commercially by DNA Sequencing Service, Macrogen, Inc., Seoul, South Korea using universal primers, 518F (5' CCAGCAGCCGC GGTAATACG3') and 800R (5'TACCAGGGT ATCTAATCC3'). Later, nucleotide sequence data was deposited in the Gen-Bank sequence database.

The online program BLAST was used to find out the related sequences with known taxonomic information in the databank at NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST>) to accurately identify the strain SBF1.

#### **Quantitative estimation of violacein**

Extent of violacein production by *C. violaceum* (CV12472) in presence of bacterial extract was studied by extracting violacein and quantifying photometrically using method described by Husain and Ahmad<sup>23</sup> with little modifications. Briefly, 50 µl of freshly grown culture was inoculated in LB broth with or without culture extract and incubated at 28 °C till complete pigmentation was achieved in untreated culture (approx 24 h). The treated and untreated cultures were incubated at room temperature after lysis with 10% SDS. Further, 900 µl of water saturated butanol was added to cell lysate, vortexed for 5s and centrifuged at 13,000 x g for 5 min. The butanol phase containing the violacein was collected, and absorbance was read at 585 nm in Spectronic 20 D+.

#### **Qualitative analysis of Quorum quenching using CVO26**

Anti-QS activity of *B. amyloliquefaciens* culture extract using CVO26 was assayed by agar well diffusion method in the presence of synthetic C6-HSL. Briefly, LB agar plates were spread with 0.1 ml of freshly grown cultures and 8-mm diameter wells were cut and loaded with varying concentrations of the culture extract (.75-6 mg/ml).

#### **Elastase assay**

The elastolytic activity of *Pseudomonas aeruginosa* suspension in the presence and absence of SBF1 extract was determined with the elastin Congo red (ECR; Sigma.) assay<sup>17</sup>. A 100 µL aliquot of PAO1 supernatant (treated and untreated) of 16-h culture was added to 900 µl of ECR buffer (100 mM Tris, 1 mM CaCl<sub>2</sub>, pH 7.5) containing 20 mg of ECR and then incubated with shaking at 37 °C for 3 h. Insoluble ECR was removed by centrifugation, and the absorption of the supernatant was measured at 495 nm. LB medium was used as a negative control.

#### **Total proteolytic activity**

*Pseudomonas aeruginosa* PAO1 was grown overnight at the given temperature in LB-broth with or without SBF1 extract. Cells were removed from the medium by centrifugation, and 50-µl aliquots of supernatant were taken for assay; 500 µl of 0.25% (wt/vol) azocasein (Sigma-Aldrich Ltd. St. Louis, MO USA) in 0.1 M sodium citrate (pH 6) was added to each supernatant aliquot to be tested and incubated at 37°C for 2 h. The

protease reaction was stopped, and protein was precipitated, by the addition of 550  $\mu$ l of ice-cold 10% (wt/vol) trichloroacetic acid (TCA) followed by incubation on ice for 15 min. Azodye released by the action of proteases in supernatant aliquots was determined at OD<sub>366</sub> after the removal of precipitated protein by centrifugation<sup>12</sup>.

#### **Pyocyanin quantification**

The pyocyanin assay according the method described by Husain et al.<sup>24</sup>. Briefly, 5-ml supernatant (with or without culture extract) from stationary-phase culture of PAO1 (16 h) in LB broth was mixed with 3 ml of chloroform. The pyocyanin from the chloroform phase was then extracted into 1 ml of 0.2 N HCl, giving it a pink to deep red color, indicating the presence of pyocyanin. The absorbance was measured at 520 nm. Concentration, expressed as micrograms of pyocyanin produced per mL of culture supernatant was determined by multiplying the optical density at 520 nm by 17.072.

#### **Swarming motility assay**

The method described by Vattem et al.<sup>25</sup> was used in this assay with slight modification. Different concentrations of bacterial extract were mixed with 0.5% LB agar separately and were poured into plates, point inoculated with PAO1 and CV12472 and incubated at 37°C for 48 h. The extent of swarming was determined by measuring the diameter of swarm and compared with control.

#### **Biofilm inhibition assay**

The effect of SBF1 extract on biofilm formation of PAO1 was measured using the microtiter plate assay<sup>26</sup>. Briefly, overnight cultures of PAO1 were resuspended in fresh LB medium in the presence and the absence of culture extract. After 24-h incubation at 30 °C, the biofilms in the microtiter plates were visualized by staining with a crystal violet solution. The plates were rinsed to remove planktonic cells, and the surface-attached cells were then quantified by solubilizing the dye in ethanol and measuring the absorbance at OD<sub>470</sub>.

#### **Identification of AHL degrading enzyme**

To find out the AHL degradation enzyme, supernatant of untreated and treated PAO1 was acidified with 10 mM HCl to bring down the pH to 2 and incubated for 48 h at 4 °C as described by Yates et al.<sup>27</sup>. The acidified mixture was spotted on to the TLC plates and revealed using biosensor CVO26. After overnight incubation, production of

violacein by CVO26 confirmed that the degradation activity was AHL lactonase.

## **RESULTS**

In the present investigation a total of 52 Gram positive *Bacilli* isolated from the rhizospheric soil of date palm tree were screened for their anti-quorum sensing activity using the CV12472 test system. Eleven isolates (21%) demonstrated varying levels of pigment inhibition (quorum quenching). The QQ activity of the soil isolates were characterized as low (1+), moderate (2+) and high (3+) depending upon the zone of pigment inhibition. Isolate SBF1 demonstrated highest pigment inhibition activity and this isolate was selected for further studies (Figure 1).

#### **Characterization and molecular identification of the Strains SBF1**

The strains were characterized both by biochemical and molecular methods. The strain SBF1 was characterized and identified by using standard morphological, physiological and biochemical tests. The characteristics of the strain SBF1 are described in Table 1. On the basis of these features, SBF1 was tentatively identified as *Bacillus* sp. To further confirm the identity of the strain 16S rDNA sequence analysis of this strain was performed. 16S rDNA of the strain was found to be approximately 1000 bp in size. The sequences of 16S rDNA of this strain were submitted to Gen-Bank (Gen-Bank accession number KC494392). A similarity search was performed by using the BLAST program that indicated the strain SBF1 shared a close relationship with the DNA sequence of *Bacillus amyloliquefaciens* CJ20 (16S: 98% similarity with the reference strain JQ936678) in NCBI database. Similar high values confirmed the strain SBF1 to be *Bacillus amyloliquefaciens*. Phylogenetic analysis supported the conclusion that SBF1 is a strain of *Bacillus amyloliquefaciens* (Figure 2). Hence, SBF1 was named *Bacillus amyloliquefaciens* SBF1.

#### **Violacein quantification assay**

The quorum quenching activity of the *Bacillus amyloliquefaciens* SBF1 strain was determined by the analysis of violacein production in CV12472. The results of the violacein quantification assay is depicted in the Table 2. The extract of SBF1 exhibited concentration dependent

decrease in the production of violacein and significant reduction was recorded at all tested concentrations. Violacein production dropped by up to 45, 67, 74 and 79% at 0.75, 1.5, 3 and 6 mg/ml, respectively. Viable cell count performed on LB agar plates at 24 h incubation showed no significant difference in the number of colony forming units (CFU) between the treated and untreated *C.*

**Table 1.** Morphological and biochemical characteristics of *Bacillus amyloliquefaciens* SBF1 strain

Characteristics	Strain SBF1
Morphological	
Gram reaction	+ve
Cell shape	Rod
Biochemical	
Indole	-
Methyl red	-
Voges Proskauer	+
Citrate utilization	+
Catalase	+
Oxidase	+
Nitrate reduction	+
Carbohydrate utilization	
Glucose	+
Fructose	+
Sucrose	+
Mannitol	+
Hydrolysis	
Starch	+
Gelatin	+

+ indicates positive and – indicates negative reactions

*violaceum* strain (Table 2). This confirms that the decreased production of violacein by extract is not due to the reduction in number of the bacteria.

Further, the effect of different concentrations of the culture extract on violacein production was also studied using agar well method in CVO26. A representative result is shown in Figure 3. Nearly all C6-HSL was degraded after incubating with SBF1 extract indicating AHL degradation.

#### Effect on QS-regulated virulence factors of PAO1

A significant decrease in LasB elastase activity was observed in the culture supernatant of PAO1 treated with *B. amyloliquefaciens* SBF1 extract. Maximum of 63.8% inhibition was observed at the highest tested concentration (6 mg/ml). Inhibition was also significant (49.6%) at 3 mg/ml concentration while at lower concentrations the decrease in elastase activity was statistically insignificant (Table 3).

Significant dose-dependent reduction of azo-casein degrading proteolytic activity was observed at all tested concentrations. The findings of the present investigation showed 34.3%, 53.6%, 73.1% and 84.4% decrease in total protease production when treated with 0.75, 1.5, 3 and 6 mg/ml concentrations, respectively (Table 3).

To assess the effect of the culture extract on the pyocyanin production, the PAO1 cells were cultivated in the presence and absence of SBF1 extract. Culture extract (0.5-6 mg/ml) treated PAO1 supernatants demonstrated significant decrease in pyocyanin production ranging from 32.7–63.7% as compared to untreated control.

#### Swarming inhibition assay

Efficiency of SBF1 extract was also tested on the swarming motility of CV12472 and PAO1.

**Table 2.** Concentration-dependent inhibition of violacein by SBF1 in CV12472

Concentration of extract (mg/ml)	OD of violacein at 585 nm	Reduction in absorbance of violacein (%)	Cell viability (log CFU ml <sup>-1</sup> at 10 <sup>5</sup> dilution)
Control	0.185±0.003		8.50
0.75	0.101±0.007	45	8.50
1.5	0.067±0.005	67	8.49
3	0.048±0.006	74	8.49
6	0.039±0.005	79	8.49

The data represents mean values of three independent experiments. \*, significance at  $p \leq 0.05$ , \*\*, significance at  $p \leq 0.005$

The addition of SBF1 supernatant showed a dose dependent decrease in the swarming migration of CV12472. The maximum inhibition of 67.7% in swarm was recorded at 6 mg/ml extract concentration (Fig 4A, B). Significant reduction of 35.4% and 45.2% was also recorded at 1.5 and 3 mg/ml concentration, respectively. Similar dose dependent decrease in swarming motility was observed in SBF1 treated *P. aeruginosa* PAO1. The maximum reduction (70.8%) in swarm diameter was recorded at highest tested concentration (6 mg/ml). At 3 mg/ml extract concentration statistically significant decrease (52%) was recorded while at lower concentrations insignificant reduction was observed (Table 4, Figure 4 C, D).

#### Biofilm inhibition assay

Since biofilm formation in *P. aeruginosa* is QS dependent, therefore SBF1 extract was used

to study its effect on biofilm formed by the pathogen PAO1. Microtiter plate quantitative assay showed that treatment with 0.75, 1.5, 3 and 6 mg/ml of SBF1 extracts resulted in dose dependent reduction in biofilm formation in the order of 14.8%, 35.7%, 51.5% and 60.6%, respectively. Biofilm formation was significantly impaired at 3 and 6 mg/ml concentration of the SBF1 extract only (Figure 5).

#### Identification of AHL degrading enzyme

Activity of lactonase enzyme convert AHLs into their inactive open ring form. This cognate *N*-acyl homoserine derivative does not act as a QS signal molecule. Therefore to test the presence of lactonase enzyme in SBF1, procedure described in the material method section was adopted. Violacein production was observed when the biosensor CVO26 was tested with acidified AHL extracted from SBF1 treated *P. aeruginosa*

**Table 3.** Effect of *B. amyloliquefaciens* SBF1 extract on QS regulated virulence factors of *P. aeruginosa* PAO1

Concentration of extract (mg/ml)	Elastase activity <sup>a</sup>	Total protease <sup>b</sup>	Pyocyanin production <sup>c</sup>
Control	0.163±0.017	1.110±0.034	5.8±0.28
0.75	0.155±0.011 (4.9)	0.729±0.039 (34.3)*	3.9±0.30 (32.7)
1.5	0.121±0.009 (25.7)	0.515±0.018 (53.6)**	3.3±0.15 (43.1)*
3	0.082±0.005 (49.6)*	0.298±0.007 (73.1)***	2.6±0.25 (55.1)*
6	0.059±0.009 (63.8)**	0.176±0.015 (84.4)***	2.1±0.30 (63.7)*

<sup>a</sup>Elastase activity is expressed as the absorbance at OD<sub>495</sub>.

<sup>b</sup>Total protease activity is expressed as the absorbance at OD<sub>600</sub>.

<sup>c</sup>Pyocyanin concentrations were expressed as micrograms of pyocyanin produced per microgram of total protein.

The data represents mean values of three independent experiments. \*, significance at p ≤ 0.05, \*\*, significance at p ≤ 0.005, \*\*\* significance at p ≤ 0.001

Values in the parentheses indicate percent reduction over control

**Table 4.** Effect of SBF1 extract at sub-inhibitory concentrations on swarming motility of bacterial pathogens

Concentration of extract (mg/ml)	Diameter of the swarm (mm)	
	<i>C. violaceum</i> CV12472	<i>P. aeruginosa</i> PAO1
Control	31±2.8	48±3.5
0.75	25±1.2 (19.3)	45±2.4 (6.2)
1.5	20±1.2 (35.4)*	39±3.0 (18.7)
3	17±2.0 (45.1)*	23±1.6 (52)**
6	10±0.8 (67.7)**	14±1.2 (70.8)***

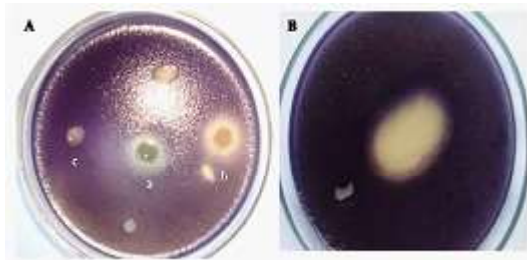
The data represents mean values of three independent experiments. \*, significance at p ≤ 0.05, \*\*, significance at p ≤ 0.005, \*\*\* significance at p ≤ 0.001 Values in the parentheses indicate percent reduction over control

PAO1, suggesting the presence of lactonase activity in the SBF1 (Figure 6). This is probably the first time that *B. amyloliquefaciens* has been reported for its AHL degrading lactonase activity.

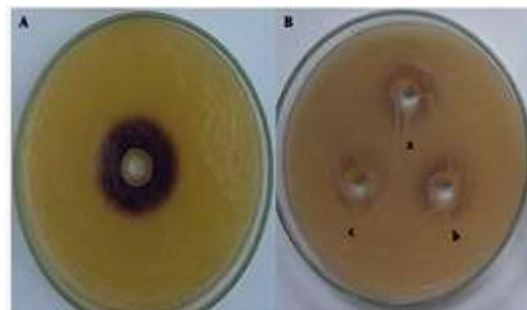
## DISCUSSION

The results of the present investigation suggest that the presence of quorum quenching bacteria from soil is more ubiquitous and diverse as for the first time QQ bacteria has been reported from date palm rhizosphere. It has been reported that about 5% of several hundred soil bacteria tested were able to inactivate AHLs (Dong et al. 2002). Recently it was also reported that *Bacillus cereus* isolated from the forest soil displayed rapid AHL degrading activity<sup>16</sup>. It has previously been

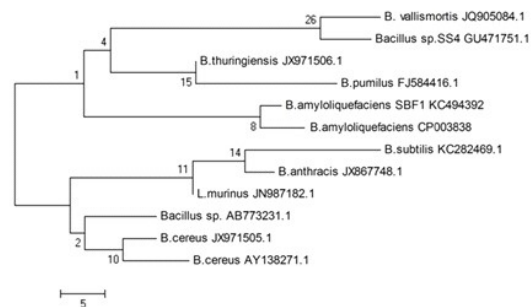
proven that in *C. violaceum*, the CviIR-dependent QS system coordinates the production of violacein pigment, and the compound, which has the ability to inhibit the violacein production without any antibacterial activity, is considered to be the promising quorum quencher<sup>28</sup>. Here we observed significant dose dependent inhibition of AHL regulated violacein production in *Chromobacterium violaceum* strains in liquid and agar well assay. Our results find support from the observations with *B. pumilus*<sup>12</sup> and *Bacillus* spp. SS4<sup>17</sup> which demonstrated significant reduction in



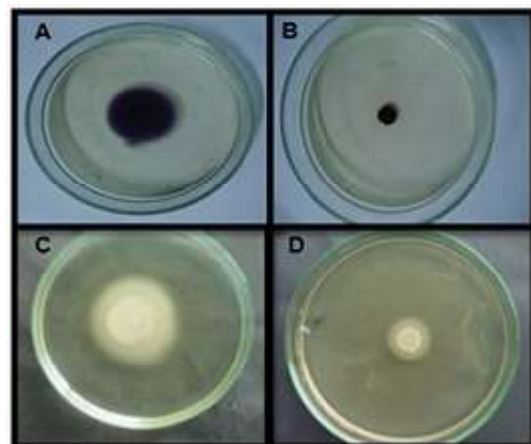
**Fig. 1.** (A) Screening of soil bacteria for anti-QS activity a. Positive control; b. isolate with positive anti-QS activity; c. isolate with no activity. (B) Anti-QS activity of SBF1 strain



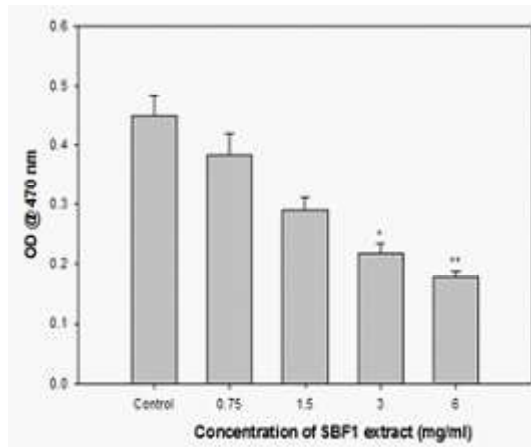
**Fig. 3.** Inhibition of violacein production in CVO26 by SBF1 (A) control, violacein synthesis by CVO26 in the presence of 10  $\mu$ M N-Hexanoyl-homoserine lactones (HHL) (B) (a) reduction in violacein synthesis in the presence of 1.5 mg/ml (b) 3 mg/ml (c) 6 mg/ml culture extract



**Fig. 2.** Phylogenetic tree constructed by neighbor-joining analysis based on the 16S rDNA sequences depicting the phylogenetic relationship of strain SBF1 with closely related taxa. Bar represents evolutionary distance as 0.01 change per nucleotide position. Bootstrap values (%) over 50% from 1,000 replications are shown



**Fig. 4.** Inhibition of swarming motility of test pathogens (A, C) untreated control of CV12472 and PAO1 (B, D) SBF1 (6 mg/ml) extract treat plates of CV12472 and PAO1



**Fig. 5.** Effect of SBF1 extract on biofilm formation of *P. aeruginosa* PAO1. The data represents mean values of three independent experiments. \*, significance at  $p < 0.05$ , \*\*



**Fig. 6.** TLC plate showing production of violacein after AHL acidification (ring closure) assay

violacein. There are numerous reports on the quorum quenching activity of *Bacillus* sp. but this is the first report on the *B. amyloliquefaciens*.

The LasIR-encoded protease and elastase play a key role in the pathogenesis of PAO1<sup>29</sup>. These enzymes degrade the structural components of the infected tissue and enhance the growth and invasiveness of the organism. In this investigation, the extract of SBF1 demonstrated a dose-dependent inhibition of total protease, elastase in PAO1, as shown in table 3. Our observations find support from the report on soil bacteria *Paenibacillus* strain 139SI that showed significant decrease in the activities of QS-controlled LasB elastase of *P. aeruginosa*. Similar inhibition of virulence factors in PAO1 by the culture of marine bacteria *B. pumilus*, *Bacillus* spp. SS4 and *Paenibacillus* spp. was reported by Nithya et al.<sup>12</sup>, Musthafa et al.<sup>17</sup> and Alasil et al.<sup>18</sup>, respectively. The rhl QS system in *P. aeruginosa* consisting of rhlI in conjunction with RhlR, activates expression of pyocyanin production<sup>30</sup>. Pyocyanin is an important virulence factor in pathogenesis of *P. aeruginosa* as some of its metabolite causes severe toxic effects by damaging the neutrophil-mediated host defense in patients with cystic fibrosis<sup>31</sup>. The extract of SBF1 demonstrated significant reduction in pyocyanin production indicating the extract is acting on the rhl system also.

QS-dependent flagellar and pili

dependent motility called swarming is considered as one of the virulence factors because it is involved in biofilm formation through mass translocation of cells, relying on expression of biosurfactant molecules. Expressions of this behaviors is mediated by AHL-dependent QS system<sup>32</sup>. Hence, any compound that inhibits swarming motility is expected to interfere with QS and its regulated biofilm formation. In the present study, the extract of SBF1 demonstrated concentration dependent reduction in swarming migration of *P. aeruginosa* PAO1 and *Chromobacterium violaceum* CV12472.

Biofilm formation is another important component of *P. aeruginosa* pathogenicity as it increases the survival capability of the bacteria by providing a physical barrier against the entry of antimicrobial agents, thereby developing resistance to antibiotics. It is well known that las system of *P. aeruginosa* QS circuit plays a crucial role in the biofilm maturation<sup>33</sup>. Therefore, any interference with the biofilm formation of the pathogen is a direct evidence of QS inhibition. The results of the present investigation revealed strong biofilm inhibitory potential of *Bacillus amyloliquefaciens* SBF1 strain against PAO1 biofilms in a concentration-dependent manner, as depicted in Figure 5.

The AHL acidification (ring closure) assay confirmed that the quorum quenching



activity of SBF1 is due to lactonase activity. This probably for the first time that AHL degrading lactonase activity is reported in *B. amyloliquefaciens* SBF1 strain. N-acyl homoserine lactonase enzyme from *Bacillus* species, *B. thuringiensis*, *B. mycoides*, *B. subtilis* and *B. cereus* have also been reported<sup>11, 13, 15, 16</sup>.

To conclude, the findings of this study brings to light the ubiquitous and diverse nature of soil bacteria involved in quorum quenching. The *B. amyloliquefaciens* SBF1 isolated from rhizospheric soil of date palm is reported to inhibit QS regulated functions in *C. violaceum* and *P. aeruginosa* biosensor strains. The results indicate that the lactonase enzyme is responsible for the QQ effect of the bacteria and the enzyme may have broad-spectrum effects. Quorum quenching ability of SBF1 can be exploited to treat bacterial infections, to prevent food spoilage and in bioremediation Further, biochemical and molecular investigations are needed to confirm the exact mechanism of this AHL degradation.

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