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Full Length Article

Antagonistic Potential of N-acyl-homoserine Lactone Degrading *Bacillus* Species for Controlling *Pectobacterium* Based Infections in Potato

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Abstract

Interference with quorum sensing (QS) naturally through quorum quenching is an established bio-control approach. In the present study, quorum quenching strategy was employed against *Pectobacterium atrosepticum* causing blackleg disease of potato. N-acyl-homoserine lactone (NAHL) degrading bacteria were isolated from potato rhizosphere using serial dilutions on different growth mediums and their ability to degrade NAHLs was evaluated using *Chromobacterium violaceum* (CV026) and *Agrobacterium tumefaciens* (NTLR-4) biosensor strains. Six rhizospheric isolates, capable of degrading NAHLs extracted from *Pectobacterium atrosepticum* were molecularly identified as belonging to genus, *Bacillus*. NAHL degradation ability of all these 6 *Bacillus* strains was also assessed using plate streak and thin layer chromatography assays. Resultantly, these strains remarkably degraded both short and long chain synthetic NAHLs. Furthermore, these *Bacillus* species also acted as potential bio-control agents when co-inoculated under quorum quenching tuber assay and have shown effective results in reducing QS-regulated soft rot tuber maceration in potatoes. Overall, all six *Bacillus* strains showed substantial capability in controlling *Pectobacterium* based infections through quenching of the NAHL signals; however, *Bacillus cereus* SSB1 was determined as the most efficient quencher strain. This work highlights a promising strategy for the bio-control and prevention of infectious plant diseases through quenching of the QS signals. © 2019 Friends Science Publishers

Keywords: *Bacillus cereus*; Biosensor; N-acyl-homoserine lactone; Quorum quenching

Introduction

Modern trends in microbiological sciences have changed the view that bacteria are mostly isolated cells. They are now considered as more social and interactive micro-organisms based on their cell density. This bacterial ability to perceive and generate concentrated response to the cell density is named as quorum sensing (QS) (Whiteley *et al.,* 2017). QS relies upon successful production, accumulation and perception of the particular signal molecule. N-acyl-homoserine lactones (NAHLs) are the most common QS signalling molecules in Gram negative bacteria (Gotz-Rosch *et al.,* 2015). *Pectobacterium* species are one of the most important plant pathogens responsible for rotting diseases, such as carrot or melon soft rot, or potato blackleg and soft rot diseases (Sarfraz *et al.,* 2018). Pathogenicity in *Pectobacterium* relies essentially upon the biosynthesis of plant cell wall degradation enzymes (PCWDEs) like pectate lyases, pectin methylesterases and pectin lyases etc. All these enzymes are the real weapons for pectinolytic bacteria belonging to *Enterobacteriaceae* family and are regulated by NAHLs based QS process (Remy *et al.,* 2018). Quorum sensing molecules (NAHLs) can be studied using different modified bacterial strains including *Chromobacterium violaceum* (CV026) and *Agrobacterium tumefaciens* (NTLR4) (Torres *et al.,* 2018). CV026 is a modified wild type strain that is unable to synthesize its specific NAHLs but still able to produce the violet pigment termed as "violacein" in response to exogenous NAHL molecules (Williams *et al.,* 2007). Therefore, use of different biosensors in different combinations allows the determination of wide range of NAHLs (Morohoshi *et al.,* 2008). Quorum quenching (QQ) is a phenomenon in which the signals produced by the pathogenic QS bacteria are quenched, inhibited or degraded (Helman and Chernin, 2015). QQ does not allow the QS pathogenic bacteria to sense the concentration of their population and to establish their pathogenicity or virulence functions (Torres *et al.,* 2018). Quorum quenching can be operated at any level of the QS regulatory process, *i.e.,* signal synthesis, stability and perception, all aiming at nullifying the effect of bacterial density (Rehman and Leiknes, 2018). Such strategies have been shown to control QS dependant pathogenicity of various plant pathogens like *P. carotovorum*. This could be done through degradation of QS

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signal molecules *i.e.*, NAHLs through NAHL-degrading bacteria, eco-engineered bacterial populations and/or plants expressing NAHL lactonases (Dong *et al*., 2007; Faure and Dessaux, 2007). Since QS plays a central role in controlling virulence and pathogenicity in *Pectobacterium*, QQ gives options for developing control strategies against it (Lin *et al*., 2003). A wide range of QQ bacteria belonging to α, β and γ-*proteobacteria* demonstrate the ability to degrade NAHL molecules (Kang *et al*., 2004; Jafra *et al*., 2006). Similarly, both high and low G+C% Gram-positive bacteria like *Rhodococcus*, *Arthrobacter*, *Streptomyces* (Uroz *et al*., 2003; Park *et al*., 2006) and *Bacillus* spp. inactivate the NAHL signals (D'Angelo-Picard *et al*., 2005; Faure and Dessaux, 2007). Briefly, successful attempts involved the disruption of QS signals by NAHL degrading bacteria, eco-engineered bacterial consortia or plants expressing NAHL lactonases (Faure and Dessaux, 2007). Effective attempts involved the identification of *Bacillus* species that could disarm the *Pectobacterium* by degrading its NAHL signals thereby protecting the potato tubers from infection (Dong *et al*., 2007). NAHL dissimilating bacteria also consist of *Comamonas* spp., *Ochrobactrum* and *Rhodococcus* strains (Jafra *et al*., 2006; Uroz *et al*., 2006). These results are encouraging because NAHL degrading bacteria are quite frequent in soil and plant environments (5-10% of the cultivable bacteria) (D'Angelo-Picard *et al*., 2005) and can serve as a huge reservoir for desirable functions and/or genes.

On the applied side, this work aimed at evaluating whether NAHL degrading QQ bacteria could be used practically to fight plant pathogen, from the well-documented *Pectobacterium* case studies. The value of such studies and strategies lies in the facts that QQ bacteria will help to minimize the use of agrochemicals such as pesticides application. To fulfil these needs, research methodology and experimental approach were designed to explore the potential of identified rhizospheric *Bacillus* strains that degrade QS signals. Isolates presenting this ability were obtained and tested as potential control agents directed against *Pectobacterium* induced disease in potatoes. Several strains, isolated for their ability to degrade NAHLs were able to reduce the appearance of disease symptoms and some could reasonably reduce the rotting of the tubers caused by pathogenic *Pectobacterium*.

Materials and Methods

Bacterial Strains, Media and Culture Conditions

Pectobacterium atrosepticum isolated on nutrient agar medium (beef extract 3 g, peptone 5 g, glucose 2.5 g and agar 15 g) was confirmed through biochemical tests on specific crystal violet pectate (CVP) medium followed by molecular identification. Luria Bertani (LB) media (10 g tryptone, 5 g yeast extract and 10 g NaCl per litre) supplemented with 2.5 m*M* L^{-1} $CaCl_2 \times 2H_2O$ and 2.5 m*M* L^{-1} $MgSO_4 \times 7H_2O$ (LB/MC) containing 50 μg gentamycin $mL^{-1}$was used for the growth of biosensor strain *Agrobacterium tumefaciens* NTLR4. Another biosensor strain *Chromobacterium violaceum* CV026 was also grown on LB medium supplemented with kanamycin (50 μg L^{-1}) at 30℃ (Torres *et al*., 2018). All QQ assays were performed in LB medium buffered to pH 7 for avoiding NAHL degradation (Yates *et al*., 2002). All the culture mediums were autoclaved at 121℃ and 103.4 kPa for 20 min.

Isolation of Rhizospheric Bacteria

Serial dilution method was used to isolate bacteria from the rhizospheric samples of potato. For this purpose, 1 g of rhizospheric sample (root and soil) was mixed in 10 mL of distilled sterilized water in a conical flask. Stirrer was used to completely uniform rhizosphere sample in the sterilized water for at least 20 min. This homogenized mixture was then poured through 4 mm Whattmann filter paper and dilutions were made up to 10^{-6}. From the diluted mixture, 100 μL from each of 10^{-5} and 10^{-6} test tubes was overlaid on LB medium (Zamani *et al*., 2013). The plates were incubated at 27 ± 2℃ till the presence of bacterial colonies. Morphologically different colonies were observed, which were selected and streaked on fresh LB plates to get pure, single colonies followed by preservation in 20% glycerol and placed at -80℃ freezer till further evaluations.

NAHL Production by *P. atrosepticum*

P. atrosepticum strain SS15 submitted at NCBI (GenBank accession number: MK392513) was selected and evaluated for its NAHL signal production before proceeding towards NAHL extraction and degradation assays. *P. atrosepticum* and biosensor strain CV026 were streaked on the same Petri plate in "T" shape but with a distance of 10 mm on LB medium. Change in the colour of CV026 from creamy yellow to violet after incubation of 24 h at 28℃, showed the violaceum production by CV026 as well as production of NAHL signals by *P. atrosepticum* (Fig. 1).

Extraction of NAHLs from *P. atrosepticum*

P. atrosepticum was grown on nutrient broth medium by shaking at 200 rpm for 16-18 h at 28℃. Five millilitre culture of this strain was centrifuged at 7500 rpm at 4℃ for 10 min and supernatant was transferred to a new vial. This bacterial supernatant was centrifuged at 10,000 rpm with the same volume of absolute ethyl acetate twice and 5 mg anhydrous sodium sulphate was added in the newly collected supernatant, filtered and evaporated until dried in a rotating evaporator. Six hundred microliters of ethyl acetate was added again and placed in the evaporator until dried. In the end, 50 μL of ethyl acetate was added and resultant solution was preserved and placed at ‗20℃ for evaluation of NAHL degrading strains.

Detection of Extracted NAHLs

NAHLs extracted from *P. atrosepticum* was detected by spotting 10 μL of NAHL molecules, 10mm apart from the streaked line of biosensor *Chromobacterium violaceum* CV026 on LB medium. Plates were incubated for 24-48 h at 28℃. Transformation of CV026 colony colour from creamy to violet indicated the production of violet pigment in CV026.

Screening of Rhizospheric Bacterial Isolates Degrading NAHLs

Rhizospheric bacterial isolates preserved as glycerol stocks were grown to get single, pure colonies and then inoculated into 5 mL LB medium containing 5 mg L^{-1} of extracted NAHLs from *P. atrosepticum* strain SS15, placed on rotary shaker for 18-20 h at 28℃. These bacterial liquid cultures were then centrifuged at 10,000 rpm for 10 min and supernatant was transferred to a new tube. Fifty microliters of this supernatant was then added into 96 well plate containing 500 µL of fresh biosensor CV026 and NTLR4 (with X-gal) diluted liquid cultures. Plates were placed at 28℃ for 2 days with slight shaking and a control experiment involved the inoculation without test bacteria. Absence or presence of violet and/or blue colour indicated the NAHL degrading and non-degrading bacteria, respectively.

Molecular Identification of NAHL Degrading Bacterial Strains Through 16S rRNA

All the 16 isolates that showed NAHL degradation in screening assay were identified by amplification of universal 16S rRNA primer pair, pA (5´-AGAGTTTGATCCTGGCTCAG) and pH (5´-AGGAGGTGATCCAGCCGCA) (Casale *et al.*, 2011). Almost 1500 bp of the ITS region was amplified using these primers. PCR was performed in a master volume of 26 µL having 10× PCR buffer, 1 μM of each dNTPs, 25 mM $MgCl_2$, 1 μM primers, 2 μL of DNA lyses template and 1 U of Taq DNA Polymerase. Following temperature cycles were set: an initial denaturation of 5 min at 94℃, 35 cycles of 1 min denaturation at 94℃, 30 s of annealing at 56℃ and 1 min 30 s extension at 72℃ followed by a final extension of 5 min at 72℃. The amplified PCR product was evaluated by electrophoresis on 1% agarose gel stained with ethidium bromide followed by visualization under Gel Doc system (Sambrook *et al*., 2001). Phylogenetic analyses were also performed using Mega7 software (Kumar *et al*., 2016). Maximum likelihood method was used in order to locate the relationship and closeness with other related species that were used as reference sequences from NCBI database (Tamura and Nei, 1993).

NAHL Degradation using Plate Streak Assay

NAHL degradation assay was performed on plates containing LB medium by streaking CV026 on one side of Petri plate and 25 μL of NAHL were spotted on the other side. In between, test bacteria were streaked with equal distance of 10 mm (Mahmoudi *et al*., 2011). Change in the colour of CV026 from creamy to violet indicated that test bacteria did not degrade the NAHLs whereas on the other hand if biosensor CV026 remains creamy it means test bacteria have the ability to degrade the NAHLs. In case of control, CV026 and NAHLs were spotted on LB plates without test bacteria.

NAHL Degradation Ability of *Bacillus* spp. Using TLC Plates

Six *Bacillus* spp. (test bacteria isolated from potato rhizosphere) identified on molecular basis were grown in 100 mL LB broth medium at 28℃ for 18 h (Table 1). Twenty millilitre liquid culture of test bacteria was then added into 180 mL LB medium appended with NAHL extracted from *P. atrosepticum* (5 mg L^{-1}) and ammonium sulphate (1 g L^{-1}). Cultures were incubated at 27℃ for 3 days, with shaking. Then the bacterial cultures having test strains and NAHLs inoculated were marked on the soft LB medium having biosensors already grown on the 18-reverse phase TLC plates. TLC analysis of NAHL was performed according to Elasri *et al*. (2001). Absence or presence of spots indicated the degradation or non-degradation of NAHLs by test bacteria. NAHLs were used at different concentrations in LB media to determine a broad range of degradation.

Ability of *Bacillus* Species to Inhibit Different NAHLs

Ninety-six well plates were poured with two films of LB inoculated medium. The first coating contained 70 µL of LB media having different NAHLs including Oxo-C6HSL, oxo-C8HSL, C6-HSL, C8-HSL and NAHLs extracted from *P. atrosepticum* SS15 strain poured independently in each well. The second consisted of 150 μL cell suspension of each 6 test strains (*Bacillus* spp.) individually, with three replications for each treatment in semisolid LB media. Thirty microliter of each of the biosensor strains CV026 and NTLR4 grown in liquid LB medium were poured to each well individually at the concentration of 4×10^7 CFU mL^{-1}. Negative controls were maintained, one by not adding test strains and other with test strain cells exchanged by a control *Bacillus* strain that was not found to degrade any NAHL molecules. Incubations were done at 28℃ for 24-48 h. Data was taken with visual inspection for the production or degradation of violacein or blue colour by using different NAHLs (Uroz *et al*., 2005).

Evaluation of NAHL Degrading *Bacillus* Strains on Potato Tubers

All the 6 isolates that degrade NAHLs under different assays were selected for bio protection screening and quorum quenching tuber assay. Healthy potato tubers (cv. Santee) were washed; surface sterilized using 2% sodium

Table 1: Antagonistic potential of NAHL degrading *Bacillus* strains against *Pectobacterium* based infection in potato tubers

Sr. No	Code	16S rRNA Identification	(GenBank) Accession number	QQ ability in reducing tuber maceration	Oxo-C6HSL	Oxo-C8HSL	C6HSL	C8HSL	*Pba* NAHLs
1	SSB1	*Bacillus cearus*	MK346197	++++	+	++	+++	++++	++++
2	SSB2	*Bacillus* spp.	MK346192	+++	-	+	++	++	+++
3	SSB3	*Bacillus* spp.	MK346193	++	-	+	++	++	+++
4	SSB79	*Bacillus cearus*	MK346194	+++	++	++	++	+++	+++
5	SSB80	*Bacillus cearus*	MK346195	+++	++	++	+++	+++	+++
6	SSB81	*Bacillus cearus*	MK346196	+++	+	++	+++	+++	+++

Oxo-C6-HSL, N-(3-oxo-hexanoyl)-L-homoserine lactone; oxo-C8-HSL, N-(3-oxo-octanoyl)-L-homoserine lactone; C6-HSL, N-hexanoyl-L-homoserine lactone; and C8-HSL, N-octanoyl-L-homoserine lactone; *Pba* NAHL, *P. atrosepticum* SS15 (*Pba*), NAHL

[a] Degradation ability of QQ bacteria against different NAHLs under TLC plate assays reflected by a decrease of violet or blue spots produced by Biosensor strains Cv026 and NTLR4: –, no decrease; +/–, slight decrease; +, moderate decrease; ++, strong decrease; +++, absence of violet or blue spots.[b] Effect of QQ bacterial strains in controlling potato tissue maceration against *Pba* (SS15) under tuber assay: +, weak; ++, moderate; +++, strong; ++++, very strong

hypochlorite for 10 min and rinsed with sterile water followed by air drying at room temperature. Tubers with uniform size were placed in sterile square Petri plates placed in boxes. Isolates to be inoculated were grown at 28°C in LB medium for 16-18 h, centrifuged and washed twice with 0.8% NaCl solution. Ten microliter of washed bacterial culture was set to OD1 by diluting with 0.8% NaCl. Pathogenic strain *P. atrosepticum* was inoculated alone and with test bacteria with 1:1. All the test bacteria were also inoculated alone as positive controls and to access that they could induce any type of symptoms on potato tubers. Five replications were used for each treatment and boxes were positioned at 24℃ for 36 h in an incubator with humidity (above 80% moisture). For negative control, tubers were inoculated with 0.8% NaCl solution (Dong *et al*., 2007). After three days of inoculation, tubers were cut from the centre and results were noted by visual observation (Zamani *et al.,* 2013).

Results

Isolation and Screening of Rhizosphere Bacteria Degrading NAHLs

Overall, 36 bacterial isolates were recovered from potato rhizosphere using two growth mediums including NA and LB. Among 36, 16 isolates induced a disappearance of the violet or blue colour due to the degradation of NAHLs after 24 h cultivation in LB medium containing extracted NAHLs as a sole carbon source and CV026 and NTLR4 as biosensor strains. Negative control was maintained by spotting LB medium containing only NAHLs without test bacteria, which showed complete appearance of violet and blue colour produced by the biosensor strains (Table 1).

Molecular Identification and Phylogenetic Analyses of NAHL Degrading Bacteria

Results of BLAST searches revealed that the nucleotide sequences of 6 bacterial isolates were identical to *Bacillus* spp. (4 *Bacillus cereus* and 2 *Bacillus* spp.), 3 belonged to *Pseudomonas* spp., 2 belonged to each *Variovorax* and *Arthrobacter* spp. and 1 each for *Rhodococcus erythropolis*, *Commomonas* and *Delftia* species. However, 6 isolates belonging to *Bacillus* spp. were selected for further assays to evaluate their antagonistic potential. Phylogenetic analyses done using Maximum likelihood method through MEGA version 7.0 clearly showed the relationship and closeness of our *Bacillus* isolates with other related *Bacillus* spp. used as reference sequences from NCBI database (Fig. 1).

Detection of NAHLs Produced and Extracted from *P. atrosepticum*

NAHLs production in *P. atrosepticum* was evaluated using the biosensor plate streak assay. Extracted NAHLs from *P. atrosepticum* were also detected similarly. In both of the assays, change of CV026 colour from creamy to violet indicated the production of violacein by CV026 due to the production of NAHLs by *P. atrosepticum* (Fig. 2).

NAHL Degradation Ability of Test Bacteria

After 4 days, all the 6 *Bacillus* strains prompted complete disappearance of NAHLs, thus ensuring their degradation activity. Negative control was retained by spotting LB medium containing only NAHLs without test bacteria and it showed complete appearance of violet colour produced by CV026 and blue colour by *A. tumefaciens* NTLR4 (Fig. 3 and 4). Moreover, all these test strains were also evaluated for their NAHL degradation ability using synthetic NAHLs (Oxo-C6HSL, oxo-C8HSL, C6-HSL and C8-HSL) in a 96 well plate assay. The investigation of the degradative activity of these strains showed a wide but gradual degradation range (Table 1).

Bio-control of *P. atrosepticum* by NAHL Degrading Bacteria

Co-inoculations of *P. atrosepticum* strain with NAHL degrading *Bacillus* strains delivered substantial decrease in tissue maceration as compared to the pathogen alone (Positive control). Interestingly, the co-inoculation of *P. atrosepticum* and *Bacillus* strain SSB3 partially inhibited the

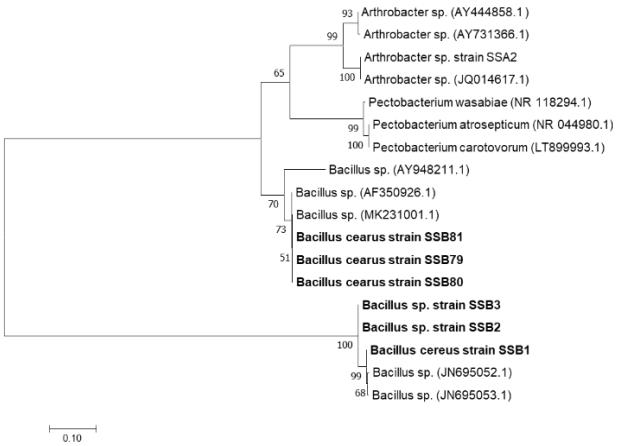


Fig. 1: Phylogenetic analyses using 16S rRNA sequence of all 6 *Bacillus* strains

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-5571.25) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 18 nucleotide sequences. There were a total of 1783 positions in the final dataset. *Pectobacterium* and *Arthrobacter* spp. were used as out-groups. Evolutionary analyses were conducted in MEGA7

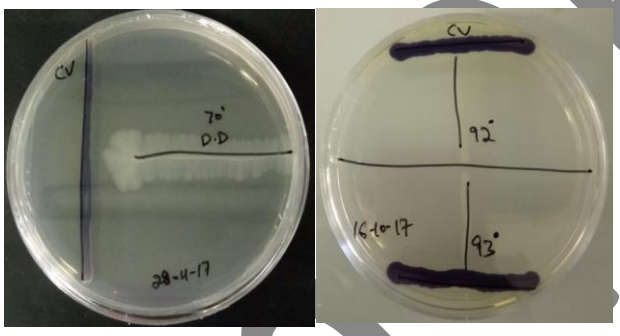


Fig. 2: Change of CV026 colour to violet

pathogenic strain from macerating the tissues. Whereas, all other 5 *Bacillus* strains, showed good quenching potential by completely reducing the maceration as compared to tubers that were co-inoculated with other test bacteria and pathogen alone (Table 1). This assay was repeated three times with equivalent results. In case of positive control, tubers inoculated with *P. atrosepticum* alone resulted in complete maceration. Whereas, in case of negative controls, inoculations with *Bacillus* strain alone and 0.8% NaCl results in no maceration (Fig. 5).

Discussion

This study was aimed at isolating and identifying NAHL degrading bacteria from potato rhizosphere against *Pectobacterium*. A number of studies relevant to this have been conducted and a group of NAHL degrading bacteria

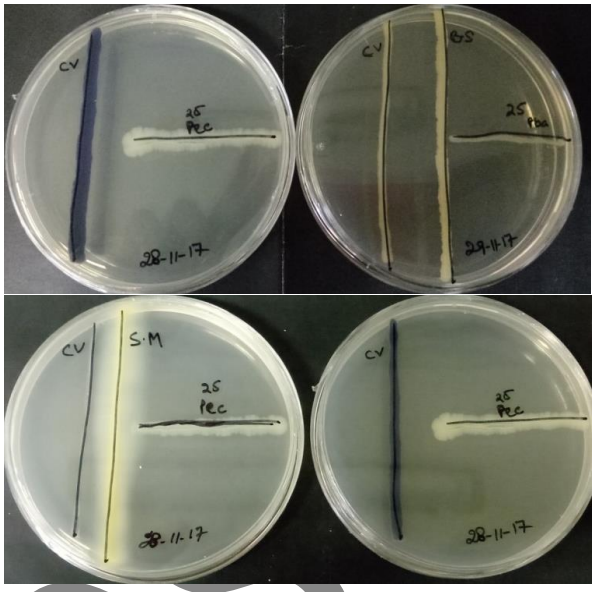


Fig. 3: Test bacteria degraded NAHLs in plate streak assay

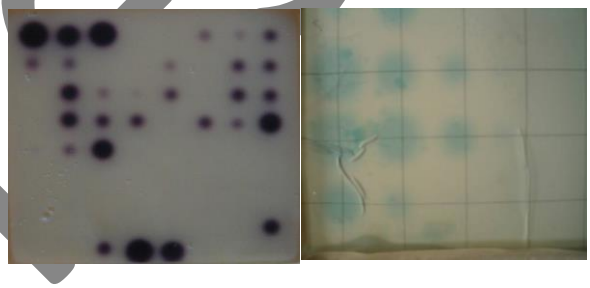
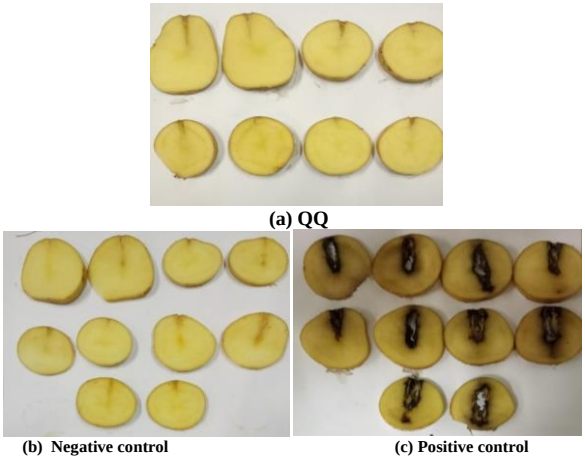


Fig. 4: TLC plate assays indicating presence or absence of NAHLs



(a) QQ **(b) Negative control** **(c) Positive control**

Fig. 5: Bio-control of *P. atrosepticum* through QQ tuber assay
(a) QQ results of co-inoculations of test strains with *P. atrosepticum*
(b) and **(c)** Negative and positive control, respectively

from potato and tobacco plants rhizosphere have also been identified (Torres *et al*., 2016, 2018). Therefore, plant rhizosphere has been considered as an important source for isolating and evaluating beneficial bacterial isolates as bio-

control agents (Faure and Dessaux, 2007; Ali *et al.*, 2017). Quorum quenching bacteria have been identified and isolated from rhizospheric bacterial populations in many systems (Tan *et al*., 2014) using the procedure explained by Jafra *et al.* (2006). Firstly, NAHL production was detected in *P. atrosepticum* SS15 using biosensor based assays and by extraction of NAHLs. Change in the colour of biosensor strain (CV026) from creamy to violet indicated the production of violacein by CV026 due to the production of QS signals (NAHLs) by *P. atrosepticum* (Reimmann *et al*., 2002). All the rhizospheric bacterial isolates (test strains) were then screened for their NAHL degradation activity by inoculating NAHLs, biosensor and test strains together in a 96 well plate biosensor based assay as explained by Uroz *et al*. (2003). Moreover, all the 16 NAHL degrading test strains screened were identified on molecular basis using 16S rRNA gene sequence analysis as designated in a couple of previous reports (Uroz *et al*., 2003; Jafra *et al*., 2006). Consequently, on the basis of BLAST searches, isolates that degraded NAHLs belong to various taxonomic classes, 6 out of 16 belonged to *Bacillus* spp., 3 belonged to *Pseudomonas* spp., 2 each for *Variovorax* and *Arthrobacter* and 1 each for *Rhodococcus erythropolis*, *Commomonas* and *Delftia* species. Some of the recent studies have also identified a somehow similar QQ bacteria mostly belonging to *Proteobacteria*, the low and high G+C Gram-positive bacteria (Tan *et al*., 2015; Saurav *et al*., 2016; Torres *et al*., 2016). Therefore, most of the NAHL degrading strains were identified as *Bacillus* spp. (about 38%), whereas all other identified species were isolated at considerably lesser occurrences (*Pseudomonas* 18%; *Arthrobacter* and *Streptomyces* each 12%; *Rhodococcus erythropolis, Commomonas* and *Delftia* each 6%*)*. Many of the previous studies have also suggested that *Bacillus, Pseudomonas, Arthrobacter, Streptomyces, Mesorhizobium* and *Rhodococcus* have better NAHL degradation properties (Dong *et al*., 2007; Cirou *et al*., 2010). Among 16 different isolates capable of degrading NAHLs, 6 isolates belonging to *Bacillus* spp. (4 *Bacillus cereus* and 2 *Bacillus* spp.) were selected to evaluate their antagonistic potential through a series of biosensor based quorum quenching assays. *Bacillus* spp. were selected for this study because of well-established antagonistic activity of this genus. For instance, *Bacillus* sp. 240B1 and *B. thuringiensis* were successfully tested for their NAHL degradation ability (Lee *et al*., 2002; Dong *et al*., 2007) Therefore, NAHL degradation ability of all the 6 *Bacillus* strains evaluated using plate streak and TLC plate assays was also confirmed, firstly by not allowing or inhibiting the NAHLs produced by *P. atrosepticum* to reach the biosensor strain CV026 and secondly by disappearance of the NAHLs used in the TLC plate assay as compared to the negative control which showed the appearance of violet and blue spots produced by the NAHLs. All these results are found to be very much parallel to those of Uroz *et al*. (2003). Overall, all of the 6 *Bacillus* strains analysed through a series of NAHL degradation assays were found to have a wide range of NAHL degradation spectrum (Oxo-C6HSL, oxo-C8HSL, C6-HSL and C8-HSL).

The last objective of this study was to explore the potential use of these 6 NAHL degrading *Bacillus* strains to antagonize *Pectobacterium* based QS-regulatory processes. For this purpose, quorum quenching tuber assay was performed using all 6 test strains to evaluate whether these strains could affect the pathogenicity of *P. atrosepticum* in tubers. Test strains were challenged with *P. atrosepticum* strain SS15 alone or co-inoculated with the candidate bio-control bacteria at 1:1. Thus, on the basis of QQ tuber assay, two observations were perceived. Firstly, one of the *Bacillus* strain SSB3, revealed a very partial bio-control activity whereas *Bacillus cereus* SSB1 was determined as the most efficient quencher strain categorized by reduction in maceration as compared to negative control. Secondly, all remaining, 4 *Bacillus* test strains exhibited good degradation or inhibition of the NAHLs with no maceration in tubers (Table 1). Moreover, the tubers inoculated with *P. atrosepticum* alone lead to complete maceration as compared to inoculations with *Bacillus* strains and 0.8% NaCl alone, which showed no maceration. However, in future, experiments may focus on more efforts to explore quorum quenching activity of SSB1 strain in order to study the ecological effects of using this strain as a bio-control agent, precisely.

Conclusion

This study specifies that *Bacillus* species have a significant potential as a bio-control agent against *Pectobacterium* based infections in potatoes and moreover in future, these strategies could be used as a feasible and eco-safe way of managing bacterial diseases of plants.

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References

Ali, M.A., M. Naveed, A. Mustafa and A. Abbas, 2017. The Good, the Bad and the Ugly of Rhizosphere Microbiome. *In: Probiotics and Plant Health*, pp: 253–290. Kumar, V., M. Kumar, R. Prasad and D.K. Choudhary (Eds.). Springer Publishers, Singapore

Casale, A.D., P.V. Flanagan, M.J. Larkin, C.C. Allen and L.A. Kulakov, 2011. Analysis of transduction in wastewater bacterial populations by targeting the phage-derived 16S rRNA gene sequences. *FEMS Microbiol. Ecol.*, 76: 100–108

Cirou, A., S. Uroz, E. Chapelle, X. Latour, N. Orange, D. Faure and Y. Dessaux, 2010. Quorum sensing as a target for novel bio-control strategies directed at *Pectobacterium. In*: *Recent Developments in Management of Plant Diseases*, pp: 121–131. Gisi, U., I. Chet and M.L. Gullino (Eds.). Springer Publishers, Dordrecht, The Netherlands

D'Angelo-Picard, C., D. Faure, I. Penot and Y. Dessaux, 2005. Diversity of N-acyl homoserine lactone producing and degrading bacteria in soil and tobacco rhizosphere. *Environ. Microbiol.*, 7: 1796–1808

Dong, Y.H., L.H. Wang and L.H. Zhang, 2007. Quorum-quenching microbial infections: mechanisms and implications. *Phil. Trans. Roy. Soc. Lond. B Biol. Sci*., 362: 1201–1211

Elasri, M., S. Delorme, P. Lemanceau, G. Stewart, B. Laue, E. Glickmann, P.M. Oger and Y. Dessaux, 2001. Acyl-Homoserine Lactone Production Is More Common among Plant-Associated *Pseudomonas* spp. than among Soil-borne *Pseudomonas* species. *Appl. Environ. Microbiol*., 67: 1198–1209

Faure, D. and Y. Dessaux, 2007. Novel bio-control strategies directed at *Pectobacterium carotovorum. Eur. J. Plant Pathol*., 119: 353–365

Gotz-Rosch, C., T. Sieper, A. Fekete, P. Schmitt-Kopplin, A. Hartmann and P. Schroder, 2015. Influence of bacterial N-acyl-homoserine lactones on growth parameters, pigments, antioxidative capacities and the xenobiotic phase II detoxification enzymes in barley and yam bean. *Front. Plant Sci*., 6: 1-13

Helman, Y. and L. Chernin, 2015. Silencing the mob: disrupting quorum sensing as a means to fight plant disease. *Mol. Plant Pathol.*, 16: 316–329

Jafra, S., J. Przysowa, R. Czajkowski, A. Michta, P. Garbeva and J.M.V.D. Wolf, 2006. Detection and characterization of bacteria from the potato rhizosphere degrading N-acyl-homoserine lactone. *Can. J. Microbiol.*, 52: 1006–1015

Kang, B.R., J.H. Lee, S.J. Ko, Y.H. Lee, J.S. Cha, B.H. Cho and Y.C. Kim, 2004. Degradation of acyl-homoserine lactone molecules by *Acinetobacter* spp. strain C1010. *Can. J. Microbiol.*, 50: 935–941

Kumar, S., G. Stecher and K. Tamura, 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*., 33: 1870–1874

Lee, S.J., S.Y. Park, J.J. Lee, D.Y. Yum, B.T. Koo and J.K. Lee, 2002. Genes encoding the n-acyl homoserine lactone-degrading enzyme are widespread in many subspecies of *Bacillus thuringiensis*. *Appl. Environ. Microbiol*., 68: 3919–3924

Lin, Y.H., J.L. Xu, J. Hu, L.H. Wang, S.L. Ong, J.R. Leadbetter and L.H. Zhang, 2003. Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Mol. Microbiol*., 47: 849–860

Mahmoudi, E., B.E.S. Tabatabaei and V. Venturi, 2011. Virulence attenuation of *Pectobacterium carotovorum* using N-acyl-homoserine lactone degrading bacteria isolated from potato rhizosphere. *Plant Pathol. J*., 27: 242–248

Morohoshi, T., M. Kato, K. Fukamachi, N. Kato and T. Ikeda, 2008. N-acyl homoserine lactone regulates violacein production in *Chromobacterium violaceum* type strain ATCC 12472. *FEMS Microbiol. Lett.*, 279: 124–130

Park, S.Y., B.J. Hwang, M.H. Shin, J.A. Kim, H.K. Kim and J.K. Lee, 2006. N-acyl homoserine lactonase producing *Rhodococcus* spp. with different AHL-degrading activities. *FEMS Microbiol. Lett.*, 261: 102–108

Rehman, Z.U. and T. Leiknes, 2018. Quorum-Quenching Bacteria Isolated From Red Sea Sediments Reduce Biofilm Formation by *Pseudomonas aeruginosa*. *Front. Microbiol*., 9: 1-13

Reimmann, C., L. Michel, H. Harms, K. Heurlier, P. Michaux, M. Zala, N. Ginet, D. Haas, G. Defago, C. Keel, K. Triandafillu and V. Krishnapillai, 2002. Genetically programmed autoinducer destruction reduces virulence gene expression and swarming motility in *Pseudomonas aeruginosa* PAO1. *Microbiology*, 148: 923–932

Remy, B., S. Mion, L. Plener, M. Elias, E. Chabrière and D. Daudé, 2018. Interference in Bacterial Quorum Sensing: A Biopharmaceutical Perspective. *Front. Pharmacol*., 9: 1-17

Sambrook, J., D.W. Russell and D.W. Russell, 2001. Molecular cloning: a laboratory manual (3-volume set). *Immunology*, 49: 895–909

Sarfraz, S., K. Riaz, S. Oulghazi, J. Cigna, S.T. Sahi, S.H. Khan and D. Faure, 2018. *Pectobacterium punjabense* sp. *nov*., isolated from blackleg symptoms of potato plants in Pakistan. *Intl. J. Syst. Evol. Microbiol.*, 68: 3551–3556

Saurav, K., R. Bar-Shalom, M. Haber, I. Burgsdorf, G. Oliviero, V. Costantino, D. Morgenstern and L. Steindler, 2016. In search of alternative antibiotic drugs: quorum-quenching activity in sponges and their bacterial isolates. *Front. Microbiol.*, 7: 1-18

Tamura, K. and M. Nei, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.*, 10: 512–526

Tan, C.H., K.S. Koh, C. Xie, J. Zhang, X.H. Tan, G.P. Lee, Y. Zhou, W.J. Ng, S.A. Rice and S. Kjelleberg, 2015. Community quorum sensing signalling and quenching: microbial granular biofilm assembly. *Npj Biof. Microbiom.*, 1: 1-19

Tan, H., J. West, J. Ramsay, R. Monson, J. Griffin, I. Toth and G. Salmond, 2014. Comprehensive overexpression analysis of cyclic-di-GMP signalling proteins in the phytopathogen *Pectobacterium atrosepticum* reveals diverse effects on motility and virulence phenotypes. *Microbiology*, 160: 1427–1439

Torres, M., J.C. Reina, J.C. Fuentes-Monteverde, G. Fernández, J. Rodríguez, C. Jiménez and I. Llamas, 2018. AHL-lactonase expression in three marine emerging pathogenic *Vibrio* spp. reduces virulence and mortality in brine shrimp (*Artemia salina*) and Manila clam (*Venerupis philippinarum*). *PLOS One.*, 13: e0195176

Torres, M., E. Rubio-Portillo, J. Antón, A.A. Ramos-Esplá, E. Quesada and I. Llamas, 2016. Selection of the N-acyl homoserine lactone-degrading bacterium *Alteromonas stellipolaris* PQQ-42 and of its potential for bio-control in aquaculture. *Front. Microbiol.*, 7: 1-13

Uroz, S., S.R. Chhabra, M. Camara, P. Williams, P. Oger and Y. Dessaux, 2005. N-Acylhomoserine lactone quorum-sensing molecules are modified and degraded by *Rhodococcus erythropolis* W2 by both amidolytic and novel oxidoreductase activities. *Microbiology*, 151: 3313–3322

Uroz, S., C. D'Angelo-Picard, A. Carlier, M. Elasri, C. Sicot, A. Petit, P. Oger, D. Faure and Y. Dessaux, 2003. Novel bacteria degrading N-acyl homoserine lactones and their use as quenchers of quorum-sensing-regulated functions of plant-pathogenic bacteria. *Microbiology*, 149: 1981–1989

Whiteley, M., S.P. Diggle and E.P. Greenberg, 2017. Progress in and promise of bacterial quorum sensing research. *Nature*, 551: 313-320

Williams, P., K. Winzer, W.C. Chan and M. Camara, 2007. Look who's talking: communication and quorum sensing in the bacterial world. *Phil. Trans. Roy. Soc. Lond. B Biol. Sci*., 362: 1119–1134

Yates, E.A., B. Philipp, C. Buckley, S. Atkinson, S.R. Chhabra, R.E. Sockett, M. Goldner, Y. Dessaux, M. Camara, H. Smith and P. Williams, 2002. N-Acylhomoserine Lactones Undergo Lactonolysis in a pH, Temperature, and Acyl Chain Length-Dependent Manner during Growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infect. Immun.*, 70: 5635–5646

Zamani, M., K. Behboudi and M. Ahmadzadeh, 2013. Quorum quenching by *Bacillus cereus* U92: a double-edged sword in biological control of plant diseases. *Biocontr. Sci. Technol.*, 23: 555–573

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