***Original Research Article***

# Isolation and screening of quorum quenching rhizobacterial isolates from the experimental farms of Gandhi krishi vigyana Kendra, Bengaluru, Karnataka, India



**Abstract**

A group of synergistic bacteria that nestles on the root surface and provide a benefitting response to the plants are the rhizobacteria. The rhizobacteria benefit the plants by promoting growth and acts as biocontrol agents. Antibiosis, competition, synthesis of cell wall degrading enzymes, and eliciting induced systemic resistance are the mechanisms of biocontrol exhibited by rhizobacteria. Quorum quenching (QQ) is a new mechanism of biocontrol of pathogens whose virulence is induced by population density dependant chemical signaling. Efficient quorum quenching rhizobacteria isolated from the crop rhizospheres can be used as potential inoculums to control phytopathogens. Soft rot is one pernicious plant and storage disease affecting almost all vegetable crops. Hence, the present study was conducted to isolate rhizobacteria from the rhizospheres of six crops Rice (*Oryza sativa*), Maize (*Zea mays*), Soyabean (*Glycine max* Mer.), Amaranthus (*Amaranthus viridis*), French Beans (*Phaseolus vulgaris*), Broad bean (*Vicia faba*) from the environs of GKVK. A total number of 96 rhizobacterial cultures were isolated from experimental fields of GKVK. The isolated cultures were screened for their quorum quenching ability by soft agar overlay assay and twenty-four out

**Comment [IM1]:** Use the same criteria when mentioning the scientific name of the plants, including or not the name of the botanist who described the species.

of ninety-six cultures were affirmative quorum quenchers. Proportionately, 25% of the total rhizobacterial isolates were quorum quenchers. The isolates were characterized morphologically and biochemically for their characterization and a discussion of the obtained results are deliberately discussed.

**Keywords:** Quorum sensing, Rhizosphere, Biocontrol, *Pectobacterium caratovorum* pv. *caratovorum, Chromobacterium violaceum*.



## 1. Introduction

For the antecedent century research scientists, any enhancement in crop yield and wellness was highly confided to plant parameters. But later researches revealed an ulterior entity that resides in and out of the plant ultimately deciding the competence of each plant, the microbes. The microorganisms are so vital for any issue regarding yield or sustainability, hence the phytomicrobiome is studied rather than the plant alone. The plant serves as a host for plenteous microbiota which influences the plant positively, negatively or neutrally. Any study of the phytomicrobiome will lead to a deep look into the rhizosphere region. The rhizosphere is the interface between two enormous natural systems, the soil and the plant. The rhizosphere nestles an unique population of microorganisms which defines the plant system stability and productivity (MacNear Jr, 2013). The rhizosphere is the hotspot for microbial activities where the microbial population is $10^{11}$ - $10^{12}$ cells per gram of soil which is 10 to 100 times higher than that in the bulk soil (Weller and Thomashow, 1994). Moreover in bulk soil 80% cells and 50% of the operational taxonomic units OTU's are inactive (Harkes et *al*., 2019). Though rhizosphere soil inhabits bacteria, fungi, protozoa,algae, actinomycetes, the bacteria are highly studied owing to the sizable benefits rendered by them to the phyto-metasystems. The rhizobacteria supply nutrients to the plant, stimulate plant growth and suppress the phytopathogens through their

**Comment [IM2]:** On italic

biocontrol activities (Kundan *et al*., 2015). The rhizobacteria impart biocontrol through localized antagonism or by the development of systemic resistance in the plant system inducing resistance to the spatially separated pathogens (Beneduzi *et al*., 2012). Signals provided by plant growth-promoting rhizobacteria to the plant root elate the resistance response even at the proximal parts of the plant. Several studies reported that the plant growth-promoting rhizobacteria mediated the promotion of plant health via induced systemic resistance (Pietrse *et al*., 2014). The rhizobacteria mediated response puts a selective pressure on the pathogen impelling them to spot mechanisms to overcome the host immune responses. Hence certain pathogens evolved to quorum sense. Quorum sensing (QS) is the communication between the bacterial cells. It is the population density-dependent expression of certain phenotypic characters. The virulence factor production by the pathogenic bacteria by the attainment of sufficient cell density provides time for the pathogen to overcome host defense responses (Deep *et al*., 2011). In soft rot causing pathogens like PCc the mode of pathogenesis is production of plant cell wall degrading enzymes (PCWDE). A less pathogenic population will produce less PCWDE which will escalate the plant defense response antagonizing the pathogen. The pathogen quorum sense to attain a sufficient population before which the transcription of genes PCWDE are not triggered. The sufficient population to overcome the host defense system serves as the optimum population for the induction of PCWDE thus preventing the morality of the pathogenic bacteria (Pollumaa *et al*., 2012). The quorum sensing thus evolved as a new mechanism of self-protection by pathogens. The traditional methods of control were challenged to break the competent barrier put forward by the pathogens. Bactericides and microbial pesticides were applied for the control of the pathogens but the attempts were short-lived due to the residual effects of the chemicals and resistance developed by the pathogens (Himel *et al*., 2017). There was a need for contemporary solutions. A new method of biocontrol, Quorum quenching came into existence. Quorum quenching is the interference of QS signals by blockage or interference of signal synthesis or by degradation of the signals.The phenomenon of QQ is more prevalent in the soil, with about 10% of the total culturable bacteria recovered from several bulk soils and rhizospheres harbor the ability to quorum quench (Dessaux *et al*., 2011). The QQ rhizospheric bacteria degrade quorum-sensing signal molecules, N-acyl-homoserine lactones (AHLs) secreted by the QS pathogens curtailing the achievement of the population density needed for the expression of virulence genes, decreasing the symptoms of the related infection.

A handful of researches have been conducted to study the quorum quenching activity of bacteria isolated from several plant rhizospheres.Quorum quenching abilities of several bacteria were reported earlier, the most common being the *Bacillus* spp. The quorum quenching abilities of several isolated *Bacillus* spp. are evaluated for their biocontrol traits against *Pectobacterium caratovorum* subsp. *Caratovorum* causing soft rot and is proved to be potent QS inhibitors under laboratory conditions (Garge and Nerurkar, 2017). The biocontrol potential of QQ bacteria against soft rot *Dickeya dadantii* in orchids was studied by Kohiri *et al*. (2017) and they isolated several species of *Bacillus* and for the first time reported QQ in *Brevibacillus brevis*. The characterization of AHL lactonase producing bacteria from the agricultural lands of Indonesia was done and several species of *Bacillus* were successfully isolated and reported (Fitriyah *et al*., 2015) The biological control agents such as *Pseudomonas aeruginosa* 2apa, *Pseudomonas flourescens*, *Serratia marcesens* and *Azospirillium amazonense* were tested for their QQ activity against the soil-borne phytopathogen *Ralstonia solanacearum.* The cell-free lysate of *Pseudomonas aeruginosa* 2apa had high AHL acylase activity inhibiting the pathogen *R.solanacearum* (Jayanna and Umesha, 2017). Hence, it was attempted to isolate bacteria from the rhizosphere of six crops from the experimental farms of Gandhi Krishi Vingyan Kendra, College of Agriculture, University of Agricultural Sciences, Bangalore and to screen them to study the phenomenon of quorum quenching.

## 2. Materials and methods

### 2.1. Collection of soil samples and isolation of bacteria from agricultural crop rhizospheres

Soil samples were collected from the rhizosphere of the associated crops Rice (*Oryza sativa*), Maize (*Zea mays*), Finger millet (*Eleusine coracana* L.), Amaranthus (*Amaranthus viridis*), French Beans (*Phaseolus vulgaris*), Broad bean (*Vicia faba*). The samples were excavated as whole plants along with soil as a 20X30 depth lump, transferred to polythene bags, labeled and transported to lab for isolation (Barillot *et al*., 2013). The soil tightly adhering to the roots and the soil surrounding the root were removed cautiously by manual shaking under sterile conditions. Isolations were usually done within 24 hours of sampling. One gram of the collected soil was added to 100 ml sterile water blank, mixed well, and serially diluted in water blanks to get $10^{-6}$ dilution. From the $10^{-6}$ dilution, one ml is pipetted out, plated on nutrient agar medium, and incubated at 28±2°C till the appearance of the bacterial colonies. Discrete colonies were picked up and maintained on NA slants for further study.

**Comment [IM3]:** Use the same criteria when mentioning the scientific name of the plants, including or not the name of the botanist who described the species.

### 2.2. Screening the isolates for quorum quenching

The isolated rhizobacteria were screened for the production of Acyl homoserine lactonase enzyme by soft agar overlay technique, the method adopted by Maclean *et al*., 2004 but the bioindicator strain used in the study, *Chromobacterium violaceum* ATCC 12472 was replaced by the strain *Chromobacterium violaceum* RU9. Overnight grown rhizobacterial cells were harvested by centrifugation at 8,000 rpm for 5mins. The collected pellets were washed (thrice) in phosphate buffer solution (pH 6.8, PBS, Sigma Co.) and then diluted to $10^8$ CFU (Colony

Forming Units)/mL with 0.5 McFarland standard and the cell suspensions were used as an inoculum. The inoculums were spot inoculated at the centre of the LB agar (full strength, 2%) plates. The plates were incubated at 30°C until growth appeared. Following the growth, the plates were overlaid with Luria Bertani (LB) soft agar (LB full strength with 0.5% (w/v) agar) containing $10^6$ CFU/ml of *Chromobacterium violaceum* (CV RU9). Uninoculated dual layered plate served as a control. A positive quorum quenching bacteria would be indicated by the loss of pigmentation around the test bacterium.

### 2.3. Morphological characterization of the QQ isolates

The morphological characteristics of the AHL degrading isolates like cell shape, colonial morphology, staining behavior were studied. The results of the biochemical tests were compared with the standard description given in *Bergey's Manual of Determinative Bacteriology* (Bergey and John, 1994).

### 2.4. Biochemical characterization of the QQ isolates

#### 2.4.1. Catalase Test

Twenty-four hour old, well-isolated test bacterial colonies were collected using a sterile inoculation loop and placed onto a microscope slide on which 3% hydrogen peroxide was poured using a dropper.The slides were observed for the formation ofbubbles. Positive reactions were evident by immediate effervescence.

**Comment [IM4]:** Include space.

#### 2.4.2. Indole production test

The test samples were inoculated in 5 ml tryptone broth and Incubate at 35°C for 24 hours. After incubation, five drops of Kovac's reagent was added directly to the tube. Formation of pink to red color (cherry red ring) at the top of the medium within seconds of reagent addition. An indole negative culture remains yellow or cloudy.

**2.4.3. Citrate utilization test**

Simmons citrate agar slants were prepared and the bacterial cultures were streaked onto the slants. The slants were incubated at 37 °C for 7 days and observed for color change. Growth with color change from green to intense blue indicates citrate positive cultures. An uninoculated slant serves as a control.

**2.4.4. Ammonia production test**

To deduct the production of ammonia, the screened rhizobacterial strains were grown in 10ml peptone broth and incubated at 30°C for 48 hrs. After incubation, about 0.5ml of Nessler's reagent was added to the bacterial suspension. Positive ammonia production was indicated by a change in color of the suspension to brown.

**2.4.5. Hydrogen sulfide production test**

The screened rhizobacterial isolates were stab inoculated into test tubes containing SIM (Sulfide, indole, motility) agar (Pancreatic digest of casein: 20.0 gm, Peptic digest of animal tissue: 6.1 gm, Agar: 3.5 gm, Ferrous ammonium sulfate: 0.2 gm, Sodium thiosulphate: 0.2 gm, pH 7.3 ± 0.2 at 25°C). The inoculated tubes were incubated at 37°C at 48hrs. The tubes were observed for the development of black precipitate on the medium.

**2.4.6. Amylase production test**

Starch agar media (Peptic digest of animal tissue 5.000, Sodium chloride 5.000, Yeast extract 1.500, Beef extract 1.500 Starch(soluble), 2.000 Agar 15.000 Final pH ( at 25°C) 7 was prepared, sterilized and poured in sterile Petri plates. The screened organisms were spot inoculated on the plates and incubated for 48 hours at 37°C. Post incubation, the plates were flooded with iodine solution with a dropper for about 1 min. Excess iodine solution was

**Comment [IM5]:** Include space.

discarded and the plates were observed for the appearance of a clear zone around the colonies indicating breakdown of starch by the bacteria.

**2.4.7. Gelatinase production test**

To test the production of enzyme gelatinase, 24-hour old rhizobacteria were stab inoculated on nutrient gelatin tubes. Incubated the tubes at 35-37°C for up to 14 days in ambient air. The gelatin tubes were removed from the incubator daily and placed at 4°C checked for liquefaction. Liquefaction of the media results positive for gelatinase production. An un-inoculated tube that remains solid at 4°C serves as a control. Liquefaction is determined only after the control has gelled.

**2.4.8. Casein hydrolysis**

Skim milk agar (Skim milk powder 28.0gm/L, Tryptone 5.0gm/L, Yeast extract 2.50gm/L, Dextrose (Glucose) 1.0gm/L, Agar 15.0gm/L, Final pH (at 25°C) plates were prepared and the test cultures were inoculated on the media. The plates were incubated at 37°C for 48hrs. The milk agar plates were examined for the presence or absence of a zone of proteolysis surrounding the bacterial test organisms.

**2.4.9. Urease test**

The media used for the test is Christensen's Urea Agar (Urea- 20.0 gm, Sodium Chloride- 5.0 gm, Monopotassium Phosphate - 2.0 gm, Peptone - 1.0 gm, Dextrose - 1.0 gm, Phenol Red - 0.012 gm, Agar - 15.0 gm, Distilled water -1l, Final pH 6.7 at 25°C. The urea agar plates were spot inoculated with the screened test rhizobacteria and incubated at 35°C in ambient air for 48 hrs for the development of pink color on the plates. The positive cultures turn the media pink due to the change in pH and for the negative cultures, the media remains yellow.

**3. Results**

The rhizospheric soil samples were collected from various cropped fields of Gandhi Krishi Vingyan Kendra, College of Agriculture, University of Agricultural Sciences, Bangalore. The crop from which the samples were taken, stage of the crop growth, the season of sample collection, and sampling location coordinates are given in Table.1. The soil samples were serially diluted and plated on NA media. The total bacterial count was enumerated (Table.2.). The collected soil samples had rich microflora as all the samples had a confined population of approximately $10^8$ cells. The maize (*Zea mays*) rhizosphere accounted for the highest bacterial count of $2.52x10^8$ colony forming units per gram of soil and finger millet (*Eleusine coracana* L.) had a low bacterial count of $8.9x10^7$ CFU/ gm of soil among the six samples analyzed. From the total colonies, morphologically distinct separate colonies were isolated and purified to screen them for the quorum quenching activities. A total of 96 cultures were isolated from six crop rhizospheres, which were at different growth stages. The isolates obtained were screened for their quorum quenching potential.

The ninety-six bacterial cultures isolated were screened for QQ potential by soft agar overlay technique with *Chromobacterium violaceum* RU9 as bioindicator. The cultures positive for AHL ase production were identified by the loss of pigmentation of CV RU9 overlaid on the test cultures (Fig.1.). Of the total 96 cultures, 24 cultures resulted positive for AHLase enzyme production. This gives a figure that 25% of the isolated rhizobacteria showed the ability to quorum quench. The total bacteria colonies counted on the Petriplates were considered as the total culturable bacteria and were compared to the number of quorum quenching isolates obtained after the final screening. As calculated, the QQ population in the rhizospheres were as follows Rice-2.5%, Maize-1.5%, Finger millet-3.4%, Broad bean-7%, Amaranthus-3.1%, French bean-2.0%. Any rich soil has about a 10% QQ population. Considering this fact when

**Comment [IM6]:** Check punctuation.

**Comment [IM7]:** Use the same criteria when mentioning the scientific name of the plants, including or not the name of the botanist who described the species.

**Comment [IM8]:** Include space.

deliberated, the quorum quenching phenomenon is observed upto 7% of the total culturable bacterial colonies, 1.5% being the least as observed in Maize (*Zea mays*) rhizosphere and 7% being the highest percent of quorum quenching bacteria isolated from the Broad beans (*Lablab purpureus*) rhizosphere. The total culturable bacteria obtained on the culture plates, the total bacterial isolates obtained, the number of isolates positive for quorum quenching and their relative percentage as proportioned with total culturable bacteria are given in table.2. The comparisions reveal that the QQ behavior in the crop rhizosphere of GKVK is within 10%.

**Comment [IM9]:** Check punctuation.

The selected isolates were characterized morphologically and biochemically for the identification. The isolates were microscopically observed for their cell shape and gram staining (Table 4). The bacterial cells were either rods or cocci with most bacteria are rod shaped indicating they are bacilli. Eighteen isolates were bacilli and six isolates were cocci. Gram staining revealed that 13 isolates BRR05, BRR09, BMR17, BMR29, RAR37, RAR39, BBR57, BBR61, BAR70, BAR75, BAR79, BFR83 and BFR86 were Gram positive and the remaining 11 isolates, BRR06, BMR22, BMR32, RAR38, BBR46, BBR51, BBR58, BBR60, BBR62, BAR77 and BAR78 were Gram negative. The bacterial colonies were observed on plates for their morphological characters with colonies color ranging from white to dull white. Orange and yellow colored colonies were also observed. The colonies had either circular or irregular form one colony, BAR 75 showed rhizoid form. The bacterial colonies were flat, raised, umbonate, cratriform and convex in elevation with most bacterial colonies having flat and raised elevation. Entire or undulate in margins, maximum colonies had smooth and glistening surface. Some colonies had rough and wrinkled surfaces. The biochemical properties of the cultures were studied (Table 5). Out of the 24 isolates, 17 isolates were positive for citrate production test, 16 were positive for Ammonia production, showing their ability to reduce sulphur containing amino acids. Only 2 isolates among the 24 isolates had the ability to convert tryptophan to indole. The catalase test proved that most cultures were Aerobic which is evident by immediate active effervescence upon addition of 15% $H_2O_2$. Only 5 cultures were negative indicating that they were facultative anaerobes.

Amylase production, casein hydrolysis, gelatin hydrolysis, urease production by the bacterial isolates were also studied as a part of biochemical characterization. Nine bacterial isolates produced enzyme amylase as evident from the starch degradation. The isolates BRR05, BMR17, BMR29, RAR37, RAR39, BBR57, BBR58 produced a clear zone on starch agar differential media when flooded with iodine solution. Sixteen isolates were positive for the proteolytic activity, casein hydrolysis. Among the twenty four bacterial isolates, thirteen were positive for the gelatin liquefaction by production of enzyme gelatinase. These bacterial isolates liquefied the gelatin substrate provided whereas the gelatinase negative cultures does not degrade gelatin to liquefaction. Enzyme urease production by the bacterial isolates was tested, in which the urease positive isolates broke down the supplemented urea to ammonia and $CO_2$ which changed the colour of the inoculated media to pink. Sixteen isolates were positive for urease enzyme production. *Bergey's Manual of Determinative Bacteriology* was referred to have a basic idea of the isolate in question.

**4. Discussion**

Quorum quenching is a novel concept in the forum of biocontrol (Chen *et al*., 2013). The phenomenon of quorum quenching can be used as a tool for the control of the phytopathogens in whose virulence factor expression is a resultant of quorum sensing. Numerous physiological functions of certain bacteria are determined by their population density. The population size of many bacteria depends on the diffusible sensory signals called 'auto inducers' produced by them.

Once the signals are produced, the bacteria quorate and induces the expression of certain genes corresponding to several physiological functions. This quorating property renders the bacteria to behave as a single unit, Quorum sensing (Fray, 2002). The reversal of quorum sensing by blocking the sensory signals, thus the population density reducing the virulence of the pathogen is quorum quenching (Garge and Nerurkar, 2017). The phenomenon of QQ is more prominent in the soil ecosystem. Hence, isolation of QQ rhizobacteria and studying their efficacy may provide a solution for the control of several phytopathogenic infections for which chemical methods of control are the only resort owing to the environmental and human health degradation. Understanding this prodigy, we attempted to isolate rhizobacteria from agricultural crops and examined the isolates for their QQ ability by the production of QQ enzymes.  The rhizospheric soil samples were collected and general isolation of rhizobacteria was carried out. The total colony forming units when analyzed, the samples had a high microbial population of $10^8$ cells per gram of soil. The rhizosphere effect outlines the fact that, 6-21% of the carbon fixed by the plant is secreted by the root hence rhizosphere has 10 to 100 fold higher microbial population (Lugtenberg, 2015). The total culturable bacteria on the plates were observed, colony counts were noted and distinct colonies were isolated yielding to a total of ninety six bacterial isolates.Nordstedt and Jones, 2020isolated a total of 1,056 bacteria from the rhizosphere of five green house ornamentals coleus, petunia, geranium, vinca, and zinnia plants. In a study by Zhao *et al*, (2018) a total of 860 strains of bacteria were isolated from rhizosphere soil collected from locations in China. The total isolates in our study when screened for quorum quenching ability, twenty four cultures were positive for signal (Acyl Homoserine Lactone) degrading enzyme based quorum quenching. About 10% of the total culturable bacteria recovered from several bulk soils and rhizospheres harbor the ability to quorum quench (Dessaux *et al*., 2011, d'Angelo-

**Comment [IM10]:** Control spacing.

**Comment [IM11]:** Include space.

**Comment [IM12]:** Include space.

Picard *et al*., 2004, Reimmann *et al*., 2002, Dong *et al*., 2000). Biosensor based detection of quorum quenching activity is the most common way to screen microbes quantitatively and qualitatively. The biosensor strain *C. violaceum* CV026, which produces a purple pigment violacein in response to C6-AHLs on a solid plate assay was used to screen about 71 bacterial isolates for their quorum quenching activity (Rehman and Leiknes, 2018). The QS signal interference by AHL degrading bacterial strains isolated from soil samples collected from a power plant, Hebei province, China by enrichment technique was evaluated for quorum quenching efficiency aand biocontrol property. *Ochrobactrum intermedium* D-2 a AHL degrading isolate was found to be highly efficient in quorum quenching and effectively attenuating maceration by soft rot pathogen *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) on radish and potato. Every bacterial isolate obtained carry its own characteristic physiological and morphological properties. Hence, the preliminary observation of the morphological characters and studying the biochemical properties is the initial step for identification of the bacteria. Chu *et al*., 2010, isolated 200 strains of quorum quenching bacteria from a silver prussian carp for the control of pathogen *Aeromonas hydrophilia. Bacillus* spp. QSI-1 had a strong interference with violacein pigment production by *Chromobacterium violaceum.* QSI-1 was characterized at the biochemical and morphology levels. Results of biochemical tests for strain QSI-1showed that the isolate QSI-1 was aerobic, spore-forming, Gram-positive, rod-shaped, motile; resulted positive for oxidase, catalase and indole production, urease, citrate utilization and starch hydrolysis; but unable to hydrolyse casein. Based on these biochemical properties, the strain shows close resemblance to *Bacillus* spp.

**5. Conclusion**

Rhizobacteria isolated from six crop rhizospheres yielded 24 isolates with AHLase degradation potential based QQ. The isolated cultures will be screened for their biocontrol efficiency *in vitro* and *in vivo*. The inoculation of the isolated and screened efficient AHL degrading bacterial isolates will interfere with the microbial QS system which induces virulence in most soil pathogens. Quorum quenching is evolving as a new method of biocontrol. The method can be considered as a weapon in hand to combat several QS induced phytopathogenesis. QQ gains its importance because it serves as a preventivebiocontrol measure which is better than the curative chemical methods where the development of resistance is a serious aftermath. Quorum quenching rhizobacteria inoculation is a welcoming alternative to combat bacterial diseases overcoming the negativities of chemical control on the environment and human health thus paving way for sustainable agriculture.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.



**References**

Comment [IM13]: Use the same criteria in bibliographic citations.

Barillot, C.D., Sarde, C.O., Bert, V., Tarnaud, E. and Cochet, N., 2013. A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. *Annals of microbiology*, *63*(2), pp.471-476.

Beneduzi, A., Ambrosini, A. and Passaglia, L.M., 2012. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and molecular biology*, *35*(4), pp.1044-1051.

Bergey, D.H. and GH, J., 1994. Bergey's manual of determinative biology. *Chapter*, *4*, pp.181-186.

CHEN, F., GAO, Y., CHEN, X., YU, Z. AND LI, X., 2013, Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. *Int. J. Mol. Sci.,* **14**: 17477-17500.

Chu, W., Lu, F., Zhu, W. and Kang, C., 2011. Isolation and characterization of new potential probiotic bacteria based on quorum- sensing system. *Journal of applied microbiology*, *110*(1), pp.202-208.

d'Angelo-Picard, C., Faure, D., Carlier, A., Uroz, S., Raffoux, A., Fray, R., and Dessaux, Y. (2004) Bacterial populations in the rhizosphere of tobacco plants producing the quorum sensing signals hexanoyl-homoserine lactone and 3-oxo-hexanoyl-homoserine lactone. FEMS Microbiol Ecol 51: 19–29.

Deep, A., Chaudhary, U. and Gupta, V., 2011. Quorum sensing and bacterial pathogenicity: from molecules to disease. *Journal of laboratory physicians*, *3*(1), p.4.

Dessaux, Y., Chapelle, E. and Faure, D., 2011. Quorum sensing and quorum quenching in soil ecosystems. *Biocommunication in Soil Microorganisms*, pp.339-367.

DINA, F., ARIS, T.W. AND IMAN, R., 2008, Characterization of bacteria producing acyl homoserine lactone (AHL) lactonase from agricultural lands.*Adv. Environ. Biol.*, **9(8):** 140-148.

**Comment [IM14]:** See previous comment.

**Comment [IM15]:** Idem to the previous comment.

**Comment [IM16]:** Unify the criteria for bibliographic citations. Surname in capital letters ?.

**Comment [IM17]:** Include space.

Dong, Y.H., Xu, J.L., Li, X.Z., and Zhang, L.H. (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. Proc Natl Acad Sci USA 97: 3526–3531.

Fitriyah, D., Wahyudi, A.T. and Rusmana, I., 2015. Characterization of bacteria producing acyl homoserine lactone (AHL) lactonase from agricultural lands. *Advances in Environmental Biology*, *9*(8), pp.140-149.

FRAY, R.G., 2002, Altering plant-microbe interaction through artificially manipulating bacterial quorum sensing. *Ann. Bot.*, **89**: 245-253.

GARGE, S.S. AND NERURKAR, A.S., 2017, Evaluation of quorum quenching *Bacillus* spp. for their biocontrol traits against *Pectobacteriumcarotovorum*subsp. *carotovorum*causing soft rot. *Biocatal. Agric. Biotechnol.*, **9**: 48-57.

Harkes, P., Suleiman, A.K.A., van den Elsen, S.J.J. *et al.* Conventional and organic soil management as divergent drivers of resident and active fractions of major soil food web constituents. *Sci Rep.*, **9,** 13521 (2019).

Himel, R.M., Khan, A.A. and Reza, M.E., 2017. Effect of chemicals against bacterial soft rot of fruits. *Journal of Bioscience and Agriculture Research*, *13*(01), pp.1087-1091.

JAYANNA, S. K. AND UMESHA, S., 2017, Quorum quenching activity of rhizosphere bacteria against *Ralstonia solanacearum*. *Rhizosphere*, **4**: 22-24.

Khoiri, S., Damayanti, T. A., & Giyanto. (2017). Identification of quorum quenching bacteria and its biocontrol potential against soft rot disease bacteria, *Dickeya dadantii*. *AGRIVITA Journal of Agricultural Science*, *39*(1), 45-55.

Kundan, R., Pant, G., Jadon, N. and Agrawal, P.K., 2015. Plant growth promoting rhizobacteria: mechanism and current prospective. *J FertilPestic*, *6*(2), p.9.



Comment [IM18]: Unify the criteria for bibliographic citations. Year in parentheses?

Comment [IM19]: :

Comment [IM20]: Unify the criteria for bibliographic citations. Surname in capital letters ?.

Comment [IM21]: Include space.

Comment [IM22]: Unify the criteria for bibliographic citations.

Comment [IM23]: Unify the criteria for bibliographic citations. Surname in capital letters ?.

Comment [IM24]: Unify the criteria for bibliographic citations. Year in parentheses?

Lugtenberg, B., 2015. Life of microbes in the rhizosphere. In *Principles of plant-microbe interactions* (pp. 7-15). Springer, Cham.

McLean, R.J., Pierson III, L.S. and Fuqua, C., 2004. A simple screening protocol for the identification of quorum signal antagonists. *Journal of microbiological methods*, *58*(3), pp.351-360.

McNear Jr., D. H. (2013) The Rhizosphere - Roots, Soil and Everything In Between. Nature Education Knowledge 4(3):1

Nordstedt, N.P. and Jones, M.L., 2020. Isolation of Rhizosphere Bacteria That Improve Quality and Water Stress Tolerance in Greenhouse Ornamentals. *Frontiers in Plant Science*, *11*, p.826.

Pieterse, C.M., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C. and Bakker, P.A., 2014. Induced systemic resistance by beneficial microbes. *Annual review of phytopathology*, *52*.

POLLUMAA, L., ALAMAE, T. AND MAE, A., 2012, Quorum sensing and expression of virulence in *Pectobacteri*a. *Sensors*, ***12*(3)**: 3327-3349.

Rehman, Z.U. and Leiknes, T., 2018. Quorum-quenching bacteria isolated from Red Sea sediments reduce biofilm formation by *Pseudomonas aeruginosa*. *Frontiers in microbiology*, *9*, p.1354.

Reimmann, C., Ginet, N., Michel, L., Keel, C., Michaux, P., Krishnapillai, V., et al. (2002) Genetically programmed autoinducer destruction reduces virulence gene expression and swarming motility in *Pseudomonas aeruginosa* PAO 1. Microbiology 148: 923–932.

Vukanti, R.V., 2020. Structure and Function of Rhizobiome. In *Plant Microbe Symbiosis* (pp. 241-261). Springer, Cham.

**Comment [IM25]:** :

**Comment [IM26]:** :

**Comment [IM27]:** Unify the criteria for bibliographic citations. Year in parentheses?

**Comment [IM28]:** Unify the criteria for bibliographic citations.

**Comment [IM29]:** Unify the criteria for bibliographic citations.

**Comment [IM30]:** :

Weller, M. and Thomashow, L.S., 1994. Molecular ecology of rhizosphere microorganisms, biotechnology and release of GMOs. *Current challenges in introducing beneficial microorganisms into the rhizosphere. VCH, Weinheim, Germany*, pp.1-18.

Zhao, D., Zhao, H., Zhao, D., Zhu, X., Wang, Y., Duan, Y., Xuan, Y. and Chen, L., 2018. Isolation and identification of bacteria from rhizosphere soil and their effect on plant growth promotion and root-knot nematode disease. *Biological control*, *119*, pp.12-19.

**Comment [IM31]:** :

**Comment [IM32]:** :



## Tables

**Table 1.** Crop, climatic, geographic data of the rhizospheric soil samples

| Sl. No. | Crop | Crop Growth Stage | Season of sample collection | Sampling Location Coordinates |
|---|---|---|---|---|
| 1. | Rice (*Oryza sativa*) | Tillering Stage | Late fall | 13.05’09.5”N77$^{o}$34’02.4”E |
| 2. | Maize (*Zea mays*) | Vegetative stage | Winter | 13.04’41.9”N77$^{o}$34’12.1”E |
| 3. | *Finger millet* (*Eleusine coracana* L.) | Maturity Stage | Winter | 13.05’02.1”N77$^{o}$34’23.6”E |
| 4. | Broad beans (*Lablab purpureus*) | Flowering stage | Winter | 13.08’60.0”N77$^{o}$57’20.1”E |
| 5. | Amaranthus (*Amaranthus viridis*) | Vegetative stage | Summer | 13.05’09.1”N77$^{o}$34’02.7”E |
| 6. | French Beans (*Phaseolus vulgaris*) | Vegetative stage | Summer | 13.04’41.6”N77$^{o}$34’12.5”E |

**Table 2.** Tabulation of enumeration of total bacterial population, total number of QQ isolates and proportional comparison of total bacterial population to quorum quencher’s population.

| Sl. No. | Sample | Total bacterial count (CFU/g of soil) | Total no of bacterial isolates obtained | No of QQ isolates | % of QQ isolates obtained |
|---|---|---|---|---|---|
| 1. | Rice (*Oryza sativa*) | $1.23x10^{8}$ | 11 | 3 | $2.5^{d}$ |
| 2. | Maize (*Zea mays*) | $2.52x10^{8}$ | 21 | 4 | $1.5^{f}$ |
| 3. | *Finger millet* (*Eleusine coracana* L.) | $8.90x10^{7}$ | 13 | 3 | $3.4^{b}$ |
| 4. | Broad beans (*Lablab purpureus*) | $1.01x10^{8}$ | 16 | 7 | $7.0^{a}$ |
| 5. | Amaranthus (*Amaranthus viridis*) | $1.60x10^{8}$ | 17 | 5 | $3.1^{c}$ |
| 6. | French Beans (*Phaseolus vulgaris*) | $9.60x10^{7}$ | 19 | 2 | $2.0^{e}$ |
| | | | **96** | **24** | **25** |

(The percentage values were calculated for 100% and were converted to 10% considering the maximum limit of QQ population in the soil ecosystem).

**Comment [IM33]:** Mention what the letters indicate.

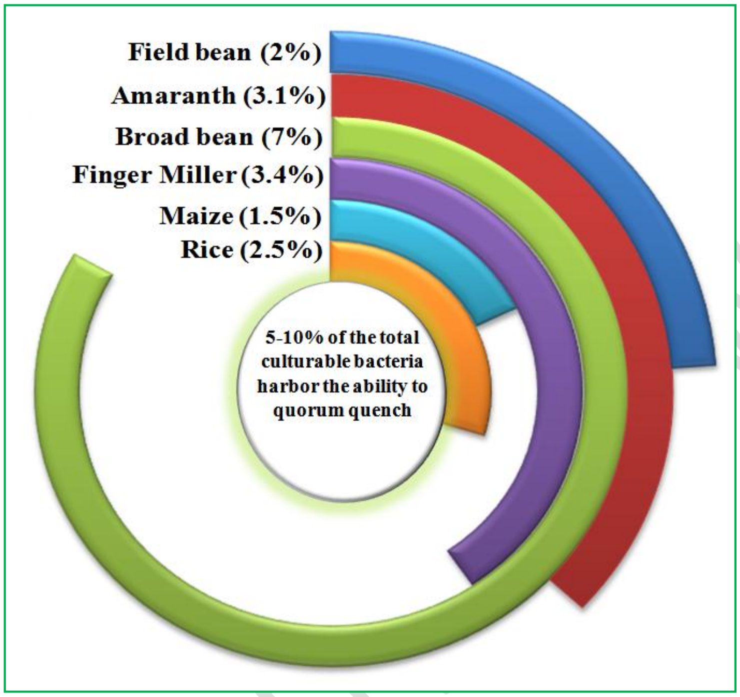


**Fig 1.** Percentage of quorum quenching isolates obtained from the rhizosphere soil samples.

**Table 3.** Bacterial isolates positive for quorum quenching.

| Sl. No. | Isolate Code | AHLase Activity |
|---|---|---|
| 1. | **BRR05** | + |
| 2. | **BRR06** | ++ |
| 3. | **BRR09** | +++ |
| 4. | **BMR17** | +++ |
| 5. | **BMR22** | ++ |
| 6. | **BMR29** | ++ |
| 7. | **BMR32** | +++ |
| 8. | **RAR37** | ++ |
| 9. | **RAR38** | ++ |
| 10. | **RAR39** | + |
| 11. | **BBR46** | ++ |
| 12. | **BBR51** | +++ |
| 13. | **BBR57** | ++ |
| 14. | **BBR58** | +++ |
| 15. | **BBR60** | +++ |
| 16. | **BBR61** | +++ |
| 17. | **BBR62** | +++ |
| 18. | **BAR70** | +++ |
| 19. | **BAR75** | ++ |
| 20. | **BAR77** | +++ |

| | | |
|---|---|---|
| 21. | **BAR78** | +++ |
| 22. | **BAR79** | +++ |
| 23. | **BFR83** | +++ |
| 24. | **BFR86** | +++ |

(Scoring based on the zone diameters in mm + - diameters of zone <1.225, ++ - diameters of zone <2.45, +++- diameters of zone >2.45).

**Table 4.** Morphological characteristics, Gram reaction and cell shape of the quorum quenching isolates

| Sl. No. | Isolate code | Colony | | | | | Gram Reaction | Cell Shape |
|---|---|---|---|---|---|---|---|---|
| | | **Colour** | **Form** | **Elevation** | **Margin** | **Surface** | | |
| 1. | **BRR05** | White | Circular | Raised | Entire | Smooth, glistening | Gram+ve | Rods |
| 2. | **BRR06** | White | Circular | Raised | Undulate | Smooth, glistening | Gram -ve | Rod |
| 3. | **BRR09** | White | Circular | Umbonate | Entire | Smooth, glistening | Gram+ve | Rod |
| 4. | **BMR17** | White | White | White | White | Smooth | Gram+ve | Rods |
| 5. | **BMR22** | white | Irregular | Raised | Entire | Smooth, glistening | Gram-ve | Rods |
| 6. | **BMR29** | Yellow | Circular | Flat | Entire | Rough | Gram+ve | Cocci |
| 7. | **BMR32** | Pale yellow | Circular | Raised | Entire | Smooth, glistening | Gram -ve | Rods |
| 8. | **RAR37** | White | Irregular | Umbonate | Entire | Wrinkled | Gram+ve | Rod |
| 9. | **RAR38** | Dull white | Circular | Umbonate | Entire | Rough | Gram -ve | Cocci |
| 10. | **RAR39** | White | Circular | Flat | Entire | Wrinkled | Gram+ve | Rod |
| 11. | **BBR46** | White | Circular | Umbonate | Entire | Rough | Gram -ve | Rod |
| 12. | **BBR51** | White | Irregular | Flat | Entire | Rough | Gram -ve | Rod |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13. | **BBR57** | White | Circular | Flat | Lobate | Smooth, Glistening | Gram+ve | Rods |
| 14. | **BBR58** | White | Circular | Raised | Undulate | Smooth, Glistening | Gram -ve | Cocci |
| 15. | **BBR60** | Yellow | Irregular | Raised | Entire | Smooth, Glistening | Gram -ve | Cocci |
| 16. | **BBR61** | White | Irregular | Cratriform | Entire | Smooth, Glistening | Gram +ve | Cocci |
| 17. | **BBR62** | Orange | Circular | Raised | Entire | Smooth, Glistening | Gram -ve | Rods |
| 18. | **BAR70** | Dull white | Circular | Convex | Entire | Smooth, Glistening | Gram+ve | Rod |
| 19. | **BAR75** | White | Rhizoid | Umbonate | Lobate | Wrinkled | Gram +ve | Rod |
| 20. | **BAR77** | White | Irregular | Raised | Undulate | Smooth, Glistening | Gram -ve | Rods |
| 21. | **BAR78** | Dull white | Circular | Flat | Entire | Smooth, Glistening | Gram -ve | Rods |
| 22. | **BAR79** | Yellow | Irregular | Raised | Undulate | Smooth, Glistening | Gram+ve | Rods |
| 23. | **BFR83** | White | Circular | Umbonate | Undulate | Smooth, Glistening | Gram +ve | Cocci |
| 24. | **BFR86** | White | Irregular | Flat | Undulate | Smooth, Glistening | Gram+ve | Rods |

**Table 5.** Biochemical characterization of the quorum quenching bacterial isolates

| Sl. No. | Isolate Code | Citrate Utilization Test | Ammonia Production Test | $H_2S$ Production | Indole Test | Catalase Test | Amylase Production Test | Casein Hydrolysis | Gelatin Hydrolysis | Urease Test |
|---|---|---|---|---|---|---|---|---|---|---|
| **1.** | **BRR05** | + | + | + | - | + | + | + | - | - |
| **2.** | **BRR06** | + | + | + | - | + | - | - | - | + |
| **3.** | **BRR09** | + | - | - | - | + | - | - | - | - |
| **4.** | **BMR17** | + | + | + | - | + | + | + | - | - |
| **5.** | **BMR22** | + | + | + | - | + | - | + | - | - |
| **6.** | **BMR29** | - | - | - | - | + | + | + | - | - |
| **7.** | **BMR32** | - | - | - | - | + | - | + | + | + |
| **8.** | **RAR37** | + | + | - | - | + | + | - | - | - |
| **9.** | **RAR38** | + | - | - | + | + | - | + | + | + |
| **10.** | **RAR39** | + | + | + | - | + | + | + | - | - |
| **11.** | **BBR46** | + | + | - | - | + | - | + | + | + |
| **12.** | **BBR51** | + | - | - | - | + | - | + | + | + |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **13.** | **BBR57** | + | + | + | - | + | + | + | - | - |
| **14.** | **BBR58** | + | + | + | - | - | + | + | + | + |
| **15.** | **BBR60** | + | + | + | - | - | - | + | + | + |
| **16.** | **BBR61** | - | + | + | - | + | - | + | + | + |
| **17.** | **BBR62** | - | + | - | - | - | - | + | + | + |
| **18.** | **BAR70** | - | - | - | - | + | - | - | - | - |
| **19.** | **BAR75** | - | + | + | - | - | - | + | - | - |
| **20.** | **BAR77** | + | - | - | - | - | - | - | - | + |
| **21.** | **BAR78** | - | - | - | - | + | - | + | + | + |
| **22.** | **BAR79** | + | + | + | - | + | + | + | - | - |
| **23.** | **BFR83** | + | + | - | - | + | - | + | + | + |
| **24.** | **BFR86** | + | + | + | - | + | + | + | - | - |