Botanicals as quorum quenching molecules and their effect in plant growth promoting traits in *Pantoea*, *Paenibacillus,* and *Rhizobium*

.

ABSTRACT

Quorum sensing is a cell-to-cell communication mechanism that was reported in *Rhizobium mayense* S11R1, *Pantoea dispersa* YBB19B, and *Paenibacillus illionoisensis* YBB20 by the presence of N-acyl homoserine lactones. This study explored possible mechanisms for expressing plant growth-promoting traits meditated through the quorum-sensing molecules. To prove the above hypothesis, a quorum quenching approach was carried out to study the regulation of plant growth-promoting traits of three isolates using botanicals like *Curcuma longa* (Turmeric) and *Andrographis paniculata* (Nilavembu) extracts (500, 1000, and 1500 ppm). At 1500 ppm, indole acetic acid production was completely inhibited in all three isolates. Extracellular Polymeric Substances (EPS) production was significantly reduced in *Pantoea dispersa* YBB19B (24 µg/mL) when treated with nilavembu extract. Biofilm-forming ability was weakened to a greater extent in *Pantoea dispersa* YBB19B and *Paenibacillus illionoisensis* YBB20 when treated with nilavembu extract. Hence, it is concluded that plant growth promotion traits in the selected bacteria are mediated through quorum sensing.

Keywords: Quorum sensing, cell-to-cell communication, quorum quenching, nilavembu, turmeric, plant growth promoting traits.

1. INTRODUCTION

Quorum sensing (QS), a cell-to-cell communication mechanism based on generating and detecting autoinducer or QS molecules, was first identified as a mode of communication among bacterial populations. This process allows bacteria to keep track of the cell density and plan behavioral changes [1]. Gram-negative bacteria often rely on producing autoinducers such as cyclodipeptides and N-acyl homoserine lactones (AHL). Numerous QS-regulated genes, such as those involved in virulence factors, biofilm formation, chemotaxis, and many others, are activated or deactivated by the perception of QS molecules in bacteria [2]. One of the most significant and investigated classes of QS compounds is AHL. This group's molecules comprise a homoserine lactone ring and an acyl side chain with four to eighteen carbons. A hydroxyl or ketone group may be substituted for the hydrogen at the C-3 position of the acyl chain to change its length [3][4]. The lactone ring, amide group, and fatty acid chain length work together to establish the specificity of the cell-to-cell contact and recognition of the AHL by its corresponding receptor [5]. A mechanism that involves disrupting these AHL molecules is called quorum quenching [6]. These molecules may be chemicals like salicylic acid [7] and gallic acid [8], enzymes like lactonase, acylase, and oxidoreductase [9], physical parameters like pH and temperature [10] botanicals like neem leaf extract [11] and turmeric extract. These quorum-quenching molecules inhibit IAA production [12], extracellular polymeric substances (EPS) synthesis, and biofilm formation [13] and also adversely affect their symbiotic relationship with their eukaryotic hosts [14]. No evidence exists that quorum sensing promotes plant growth in rhizobial and nonrhizobial endophytes. This study demonstrated how the botanicals, turmeric, and nilavembu extracts act as quorum-quenching molecules and affect plant growth-promoting traits in RE and NRE.

2. MATERIAL AND METHODS

2.1 Bacterial strains and culture conditions:

Rhizobial and non-rhizobial endophytes isolated from groundnut nodules [15] were used in this study. Rhizobial endophyte *Rhizobium mayense* S11R1 and nonrhizobial endophytes *Pantoea dispersa* YBB19B and *Paenibacillus illionoisensis* YBB20 were grown in Yeast Extract Mannitol Agar medium at 30 ± 2 °C, respectively.

2.2 Screening of rhizobial and non-rhizobial endophytes at different concentrations of quorum quenching molecules:

The rhizobial and nonrhizobial endophytes screened for tolerance levels of botanicals viz., nilavembu extract and turmeric extracts at 500, 1000, and 1500 ppm. These bacterial isolates were grown in their broth and kept for 24 h at 30 °C in 120 rpm shaking condition. Regarding optical density, growth was measured in a spectrophotometer (M/s. Shimadzu, Japan) at 660nm.

2.3 Plant Growth Promoting Traits:

2.3.1 IAA production:

The rhizobial and nonrhizobial endophytes were evaluated for IAA production at different levels of botanicals, viz., nilavembu extract and turmeric extract at 500, 1000, and 1500 ppm. These bacterial isolates were grown in liquid YEM medium added with 0.1% tryptophan at shaking condition (120 rpm) at 30 ± 2 °C for 48 hours. After incubation, the broths were centrifuged at 14,000 rpm for 10 minutes. The supernatant was collected. 2 mL of Salkowski reagent (1 ml of 0.5 M $FeCl_3$ dissolved in 50 ml of 35% perchloric acid) and 50 µL of 0.1 mM orthophosphoric acid were added to the 500 µL of the supernatant and incubated in the dark for 30 minutes. The IAA production was confirmed by the appearance of pink to red after incubation. The color intensity was measured at 530 nm [16] spectrophotometrically (M/s. Shimadzu, Japan). IAA was used to generate a standard curve and expressed in µg/mL.

2.3.2 Extracellular polymeric substances (EPS) production:

EPS production was examined at different concentrations of botanicals viz., nilavembu extract and turmeric extracts at 500, 1000, and ppm in RE and NRE in 30 ml of liquid YEM medium in shaking condition of 120 rpm at 30 °C for 24 hours. The ethanol precipitation method was followed to extract the EPS. The culture broth was centrifuged at 6000 rpm for 10 minutes. The supernatant was carefully transferred into fresh tubes, and equal 96% cold ethanol (v/v) was added. They were allowed to precipitate at °C in overnight. Then, the samples were centrifuged at 6000 rpm for 20 minutes. Finally, the pellets obtained were dissolved in distilled water. 1 mL of EPS extract was transferred to fresh tubes, 1 ml of 5% phenol solution, and 5 mL of pure sulphuric acid were added [17] and incubated at room temperature for 30 minutes. Then, brown color intensity was measured spectrophotometrically at 492 nm. Standard graphs were plotted with D-glucose concentration from 0 to 100 g /mL, and the concentration of total EPS content was determined [18].

2.3.3 Estimation of biofilm formation:

RE and NRE were grown in different levels of botanicals, viz., nilavembu extract and turmeric extract, at 500, 1000, and 1500 ppm for biofilm formation assay. To 150 µL of liquid YEM medium, they were supplemented with botanicals at 500, 1000, and 1500 ppm in 96 healthy microtiter plates. 10 µL of 24-hour culture ($1x10^8$cfu/mL) of RE and NRE was inoculated and incubated for two days. After incubation, the microtiter plates were washed with sterile distilled water and dried. 150 µL of 1% crystal violet solution is added to the dried plate and kept for 45 minutes. Then, plates were again rinsed with distilled water 2-3 times. The purple ring formation on the edge of the wells indicates the biofilm formation. 200 µL of 95% ethanol was added to the wells, and the purple color intensity was measured spectrophotometrically at 590 nm [19].

2.4 Statistical analysis:

All the experiments were carried out in triplicates, and the data was presented with mean and standard error. The experimental data were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test using SPSS software to identify the significant difference between the treatments at the 5% level. Wherever statistical significance was observed, the critical difference was worked out at a 5% probability level, and the values were furnished in respective tables.

3. RESULTS

3.1 Ability to grow in different levels of quorum quenching molecules:

Bacterial culture growth was greatly retarded with increasing concentrations of botanicals as quorum quenching molecules. The results revealed that at higher concentrations, these molecules were highly toxic to bacterial isolates tested in this study.

3.2 Plant Growth Promoting Traits:

3.2.1 IAA Production:

At 1500 ppm of both turmeric and nilavembu extract, *Rhizobium mayense* S11R1, *Pantoea dispersa* YBB19B, and *Paenibacillus illionoisensis* YBB20 were unable to produce indole acetic acid. Maximum production of indole acetic acid was reported in *Paenibacillus illionoisensis* YBB20 (47 µg/mL), followed by *Rhizobium mayense* S11R1 (41 µg/mL) and *Pantoea dispersa* YBB19B (36 µg/mL) without inhibitors. At 1000 ppm of turmeric extract, maximum production was recorded in *Paenibacillus illionoisensis* YBB20 (0.6 µg/mL), followed by *Rhizobium mayense* S11R1 (0.4 µg/mL) and at 1000 ppm of nilavembu extract *Paenibacillus illionoisensis* YBB20 recorded the maximum IAA (3 µg/mL).

3.2.2 EPS Production:

Increasing turmeric and nilavembu extract concentrations significantly reduced Exo Polymeric Substance production. The maximum production of EPS was recorded in *Paenibacillus illionoisensis* YBB20 (91 µg/mL), followed by *Pantoea dispersa* YBB19B (82 µg/mL) and *Rhizobium mayense* S11R1 (68 µg/mL) under normal conditions. At 1500 ppm, the maximum production was recorded in *Paenibacillus illionoisensis* YBB20 (32 µg/mL), followed by *Rhizobium mayense* S11R1 (27 µg/mL) and *Pantoea dispersa* YBB19B (24 µg/mL).

3.2.3 Biofilm formation:

Under normal conditions, *Pantoea dispersa* YBB19B exhibited more robust biofilm formation and showed moderate biofilm formation at 1500 ppm concentration of both extracts. At 1500 ppm, all three isolates showed weaker or harmful biofilm-forming ability.

Table 1: Growth of rhizobial and non-rhizobial endophytes under different concentrations of botanicals

Concentration of Botanicals (ppm)	*Pantoea dispersa* YBB19B	*Paenibacillus illionoisensis* YBB20	*Rhizobium mayense* S11R1
Without inhibitor	1.503±0.022^{a}	0.822±0.005^{f}	0.915±0.019^{c}
Turmeric extract 500	0.669±0.016^{f}	0.248±0.004^{j}	0.61±0.006^{g}
1000	0.596±0.002^{g}	0.213±0.004^{k}	0.435±0.006^{i}
1500	0.416±0.005^{i}	0.113±0.001^{n}	0.24±0.001^{j}
Nilavembu extract 500	1.061±0.004^{b}	0.178±0.002^{l}	0.885±0.021^{d}
1000	0.538±0.003^{h}	0.168±0.001^{l}	0.413±0.002^{i}
1500	0.426±0.006^{i}	0.14±0.002^{m}	0.086±0.002^{n}

Values are mean ± standard error with three replicates. Means followed by the same letter do not differ by DMRT at a 5% probability.

Table 2: IAA Production of the rhizobial and non-rhizobial endophytes under different concentrations of botanicals

Concentration of Botanicals (ppm)	*Pantoea dispersa* YBB19B	*Paenibacillus illionoisensis* YBB20	*Rhizobium mayense* S11R1
Without inhibitor	36.892±0.001	47.96±0.012	41.086±0.01
Turmeric extract 500	36.892±0.007	21.408±0.001	15.064±0.004
1000	ND	0.626±0.007	0.440±0.001
1500	ND	ND	ND
Nilavembu extract 500	1.193±0.001	19.688±0.001	27.752±0.007
1000	ND	3.666±0.001	ND
1500	ND	ND	ND

Values are mean ± *standard error with three replicates. Means followed by the same letter do not differ by DMRT at a 5% probability.*

Table 3: EPS production of the rhizobial and non-rhizobial endophytes under different concentrations of botanicals

Concentration of Botanicals (ppm)	*Pantoea dispersa* YBB19B	*Paenibacillus illionoisensis* YBB20	*Rhizobium mayense* S11R1
Without inhibitor	82.02±0.011^{b}	91.42±0.004^{a}	68.22±0.018^{d}
Turmeric extract 500	62.92±0.007^{e}	75.72±0.008^{c}	47.72±0.005^{g}
1000	56.02±0.015^{f}	66.32±0.013^{d}	40.32±0.005^{hi}
1500	47.02±0.004^{g}	47.52±0.011^{g}	38.82±0.007^{ij}
Nilavembu extract 500	75.82±0.007^{c}	45.92±0.005^{g}	41.02±0.006^{hi}
1000	27.92±0.001^{l}	41.82±0.004^{h}	36.62±0.002^{j}
1500	24.42±0.001^{m}	32.62±0.001^{k}	27.12±0.005^{l}

Table 4: Biofilm formation of the rhizobial and non-rhizobial endophytes under different concentrations of botanicals

Concentration of Botanicals (ppm)	*Pantoea dispersa* YBB19B	*Paenibacillus illionoisensis* YBB20	*Rhizobium mayense* S11R1
Without inhibitor	Strong	Moderate	Moderate
Turmeric extract 500	Moderate	Moderate	Moderate
1000	Moderate	Moderate	Moderate
1500	Weak	Weak	Weak

Nilavembu extract 500	Moderate	Weak	Moderate
1000	Weak	Weak	Moderate
1500	Negative	Negative	Weak

**Biofilm formation -OD590nm; Strong->0.3; Moderate-0.2-0.29; Weak-0.1-0.19; Negative -<0.1*

4. DISCUSSION:

It was reported that these three isolates have quorum sensing ability and synthesize the autoinducers. The quorum sensing compounds produced by *Pantoea dispersa* YBB19B were C6, C7, C8 and 3-oxo-C14 HSL, *Paenibacillus illionoisensis* YBB20 were C6, C7, 3-hydroxy-C8 and 3-oxo-C14 HSL and *Rhizobium mayense* S11R1 were C6, C7 and 3-hydroxy-C8 HSL (Unpublished data). These autoinducers will induce plant growth-promoting traits like indole acetic acid [20], extracellular polymeric substances [21], and biofilm formation [22]. Indole acetic acid belongs to the auxin class of phytohormone that promotes root length, cell elongation, and cell division. In *Agrobacterium tumefaciens* AttM lactonase enzyme, a quorum quenching molecule that degrades N-acyl homoserine lactones and significantly reduces the indole acetic acid production [12]. Similarly, in our study, the higher concentration of the botanicals significantly reduced the IAA production at 1500 ppm. Extracellular polymeric substances produced by the plant growth-promoting rhizobacteria help them escape abiotic stresses like drought, salinity, heavy metal pollution, and biotic stresses [23] and protect them from toxic substances [24]. During waste water treatment, a bacterial strain was discovered with quorum quenching ability, significantly reducing the extracellular polymeric substances by 37% [25]. In this study, 1500 ppm of the botanicals reduced the EPS production in *Pantoea dispersa* YBB19B (24 µg/mL). Biofilm formation is essential for plant growth, promoting rhizobacteria in the colonization of roots, nitrogen fixation, phosphorus solubilization, indole acetic acid-like substance, and organic acid production [26]. Quorum-quenching enzymes like lactonase, acylase, and oxidoreductase could disrupt the N-acyl homoserine lactones, thereby preventing biofilm formation [13]. In this study, biofilm formation was significantly weakened by increasing the concentration of quorum-quenching botanicals.

CONCLUSION

In this study, it is understood that the turmeric and nilavembu extracts, which act as quorum quenching molecules, disturbed the quorum sensing systems, thereby inhibiting the plant growth-promoting traits when compared with normal conditions of the plant growth-promoting rhizobacteria (PGPR). Hence, it is concluded that in this study's selected RE and NRE, plant growth promotion is mediated through quorum sensing.

REFERENCES

[1] S.Wu, J.Liu, C.Liu, A.Yang, and J.Qiao. Quorum sensing for population-level control of bacteria and potential therapeutic applications. Cellular and Molecular Life Sciences. 2020: 77; 1319-1343.

[2] I. Bellezza, M. J. Peirce, and A. Minelli. Cyclic dipeptides: from bugs to brain. Trends Mol. Med. 2014:20:551–558.

[3] N. A. Whitehead, A. M. L. Barnard, H. Slater, N. J. L. Simpson, and G. P. C. Salmond. Quorum-sensing in Gram-negative bacteria. FEMS Microbiol. 2001:25; 365–404.

[4] M. M. Marketon, M. R. Gronquist, A. Eberhard, and J. E. González. Characterization of the *Sinorhizobium meliloti* sinR/sinI locus and the production of novel N-acyl homoserine lactones. J. Bacteriol. 2002:184;5686–5695.

[5] M. E. A. Churchill and L. Chen. Structural basis of acyl-homoserine lactone-dependent signaling. Chem. Rev. 2011:111;68–85.

[6] Y.-H. Dong, A. R. Gusti, Q. Zhang, J.-L. Xu, and L.-H. Zhang. Identification of quorum-quenching N-acyl homoserine lactonases from *Bacillus* species. Appl. Environ. Microbiol.2002:68;1754–1759.

[7] A. Delago, A. Mandabi, and M. M. Meijler. Natural quorum sensing inhibitors–small molecules, big messages. Isr. J. Chem. 2016:56;310–320.

[8] Nazzaro, F, Fratianni, F, and Coppola, R. Quorum sensing and phytochemicals. *International journal of molecular sciences*., 2013:*14*;12607-12619.

[9] S. Fetzner. Quorum quenching enzymes. J. Biotechnol.2015:201;2–14.

[10] L. Delalande, D. Faure, A. Raffoux, S. Uroz, C.D. Angelo-Picard, M. Elasri, A. Carlier, R. Berruyer, A. Petit, P. Williams, Y. Dessaux. N-hexanoyl-L-homoserine lactone, a mediator of bacterial quorum-sensing regulation, exhibits plant-dependent stability and may be inactivated by germinating *Lotus corniculatus* seedlings. FEMS Microbiol. Ecol.2005:52;13–20.

[11] R. Mishra, K. Gwalani, N. Nashikkar, and S. Bundale. Antibacterial and Anti-Quorum Sensing Studies of Extracellularly Synthesized Silver Nanoparticles from *Azadirachta indica* (Neem) Leaf Extract. Biosci. Biotechnol. Res. Asia. 2022:19;1065–1078.

[12] M. Boyer, R. Bally, S. Perrotto, C. Chaintreuil, and F. Wisniewski-Dyé. A quorum-quenching approach to identify quorum-sensing-regulated functions in *Azospirillum lipoferum*. Res. Microbiol.2008:159;699–708.

[13] E. Paluch, J. Rewak-Soroczyńska, I. Jędrusik, E. Mazurkiewicz, and K. Jermakow. Prevention of biofilm formation by quorum quenching. Appl. Microbiol. Biotechnol.2020:104;1871–1881.

[14] M.Gao, H. Chen, A. Eberhard, M. R. Gronquist, J. B. Robinson, M. Connolly, M.Teplitski, B. G. Rolfe, and W. D. Bauer. Effects of AiiA-mediated quorum quenching in *Sinorhizobium meliloti* on quorum-sensing signals, proteome patterns, and symbiotic interactions. Mol. plant-microbe Interact., 2007:20;843–856..

[15] R. Preyanga, R. Anandham , R. Krishnamoorthy , M. Senthilkumar , N.O. Gopal , A. Vellaikumar , S. Meena. Groundnut (*Arachis hypogaea*) nodule *Rhizobium* and passenger endophytic bacterial cultivable diversity and their impact on plant growth promotion," Rhizosphere, 2021:17;100309

[16] C. L. Patten and B. R. Glick. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. 2002:68;3795–3801.

[17] T. Masuko, A. Minami, N. Iwasaki, T. Majima, S.-I. Nishimura, and Y. C. Lee. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Anal. Biochem.2005:339;69–72.

[18] M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. t Rebers, and F. Smith. Colorimetric

method for determination of sugars and related substances. Anal. Chem.1956:28; 350–356.
[19] D. Djordjevic, M. Wiedmann, and L. A. McLandsborough. Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Appl. Environ. Microbiol. 2002:68;2950–2958.
[20] B. K. Jung ,J. C. Ibal, H. Q. Pham M.C. Kim, G.S. Park, S.J. Hong, H.W.Jo, C. E. Park, S.D. Choi, Y. Jung, S. B.Tagele, J.H. Shin.Quorum sensing system affects the plant growth promotion traits of *Serratia fonticola* GS2. Front. Microbiol.2020:11; 536865.
[21] B. J. Pellock, M. Teplitski, R. P. Boinay, W. D. Bauer, and G. C. Walker. A LuxR homolog controls production of symbiotically active extracellular polysaccharide II by *Sinorhizobium meliloti*. J. Bacteriol. 2002:184;5067–5076.
[22] A. Alagely, C. J. Krediet, K. B. Ritchie, and M. Teplitski. Signaling-mediated cross-talk modulates swarming and biofilm formation in a coral pathogen *Serratia marcescens*. ISME J.2011:5;1609–1620.
[23] R. J. L. Morcillo and M. Manzanera. The effects of plant-associated bacterial exopolysaccharides on plant abiotic stress tolerance. Metabolites. 2021:11;337.
[24] S. Miquel, R. Lagrafeuille, B. Souweine, and C. Forestier. Anti-biofilm activity as a health issue. Front. Microbiol.2016:7;592.
[25] X. Dong, R. Zhu, Y. Li, J. Qin, and S. Ge. Characterization of an indigenous quorum quenching bacterium and its effect on the SMP, EPS, and microbial community of sludge mixture during wastewater treatment. Int. Biodeterior. Biodegradation. 2020:152;104995.
[26] G. Seneviratne, M. Weerasekara, K. Seneviratne, J. S. Zavahir, M. L. Kecskés, and I. R. Kennedy. Importance of biofilm formation in plant growth promoting rhizobacterial action. Plant growth Heal. Promot. Bact. 2011;81–95.