Original Research Article

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

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ABSTRACT

Quorum sensing is a cell-to-cell communication mechanism which was reported in *Rhizobium mayense* S11R1, *Pantoea dispersa* YBB19B and *Paenibacillus illionoisensis* YBB20 by the presence of N-acyl homoserine lactones. This study, we explored possible mechanisms for expression of plant growth promoting traits is meditated through the quorum sensing molecules. In order to prove 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 completed inhibited in all three isolates. Extracellular Polymeric Substances (EPS) production was greatly 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 is 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 the generation and detection of autoinducer or QS molecules, was first identified as a mode of communication among bacterial populations. This process gives bacteria the ability to keep track of the cell density and plan behavioral changes [1]. Gram-negative bacteria often rely on the production of autoinducers such 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 perception of QS molecules in bacteria [2]. One of the most significant and investigated classes of QS compounds is AHL. This group's molecules are made up of two moieties: 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 which involves in the disruption of these AHL molecules is referred as quorum quenching [6]. These molecules may be chemicals like salicylic acid [7], 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 inhibits IAA production [12], extracellular polymeric substances (EPS) synthesis and biofilm formation [13] and also adversely affect their symbiotic relationship with their eukaryotic hosts [14]. There is no such evidence is available that quorum sensing is responsible for plant growth promotion in rhizobial and non rhizobial endophytes. In this

study we have demonstrated that, how the botanicals, turmeric and nilavembu extracts act as quorum quenching molecules and their effect on 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 non rhizobial 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 non rhizobial endophytes screened for tolerance level of botanicals viz., nilavembu extract and turmeric extracts at 500, 1000, and 1500 ppm. These bacterial isolates were grown in their respective broth and kept in for 24 h at 30 °C in 120 rpm shaking condition. Growth was measured in spectrophotometer (M/s. Shimadzu, Japan) at 660nm in terms of optical density.

2.3 Plant growth promoting Traits:

2.3.1 IAA production:

The rhizobial and non rhizobial 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 48hours. After incubation, the broths were centrifuged at 14,000 rpm for 10 minutes and 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 ortho phosphoric acid were added to the 500 µL of the supernatant and incubated in dark for 30 minutes. The IAA production was confirmed by the appearance of pink to red after incubation. Then colour intensity was measured at 530 nm [16] by spectrophotometrically (M/s. Shimadzu, Japan). IAA was used to generate standard curve and expressed in µg/mL.

2.3.2 Extracellular polymeric substances (EPS) production:

EPS production was examined at different concentrations botanicals viz., nilavembu extract and turmeric extracts at 500, 1000, and1500 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. Supernatant was transferred into fresh tubes carefully and equal volume of 96% cold ethanol (v/v) was added. They were allowed to precipitate at 4°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, 1ml of 5% phenol solution, and 5 mL of pure sulphuric acid were added [17] and incubated in room temperature for 30 minutes. Then brown colour intensity was measured spectrophotometrically at 492 nm and standard graph 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 in different levels of botanicals viz., nilavembu extract and turmeric extract at 500, 1000, and1500 ppm for biofilm formation assay. To 150 µL of liquid YEM medium which was supplemented botanicals at 500, 1000, and 1500 ppm in 96 well microtiter plate. 10 µL of 24 hours old culture ($1x10^8$cfu/mL) of RE and NRE was inoculated and incubated for 2 days. After incubation, the microtiter plates were washed with sterile distilled water and dried. To the dried plate, 150 µL of 1% crystal violet solution is added and kept for 45 minutes. Then, plates were again rinsed with distilled water for 2-3 times. The purple ring formation in edge of the wells indicate the biofilm formation. 200 µL of 95% ethanol was added to the wells and the purple colour 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 for identifying 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:

Exo Polymeric Substance production was greatly reduced with increasing concentrations of turmeric and nilavembu extracts. The maximum production of EPS was recorded in *Paenibacillus illionoisensis* YBB20 (91 µg/mL) followed *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 condition, *Pantoea dispersa* YBB19B exhibited stronger biofilm formation and at 1500 ppm concentration of both the extracts, showed the moderate biofilm formation. At 1500 ppm all the three isolates shown weaker and or negative 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 3 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 3 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 concentration 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 concentration 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 the plant growth promoting traits like indole acetic acid [20], extracellular polymeric substances [21] and biofilm formation [22]. Indole acetic acid belongs to auxin class of phytohormone that promotes the root length, cell elongation and cell division. In *Agrobacterium tumefacieans* AttM lactonase enzyme, a quorum quenching molecule which degrades N-acyl homoserine lactones and greatly reduced the indole acetic acid production [12]. Similarly in our study at higher concentration of the botanicals greatly reduced the IAA production at 1500 ppm concentration. Extracellular polymeric substances produced by the plant growth promoting rhizobacteria helps them to escape from abiotic stresses like drought, salinity and heavy metal pollution, biotic stresses [23] and protects them from toxic substances [24]. A bacterial strain was discovered with quorum quenching ability which greatly reduced the extracellular polymeric substances by 37%, during waste water treatment [25]. In this study, at 1500 ppm of the botanicals reduced the EPS production in *Pantoea dispersa* YBB19B (24 µg/mL). Biofilm formation is important for plant growth promoting rhizobacteria in colonization of roots, nitrogen fixation, phosphorus solublization, indole acetic acid like substance and organic acid production [26]. Quorum quenching enzymes like lactonase, acylase and oxidoreductase were able to disrupt the N-acyl homoserine lactones thereby leads to the prevention of biofilm formation [13]. In this study, increasing concentration of quorum quenching botanicals, biofilm formation was greatly weakened.

CONCLUSION

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

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