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SCREENING OF RHIZOBACTERIA FOR THEIR PLANT GROWTH PROMOTING ACTIVITIES

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ABSTRACT

Rhizospheric bacteria are known to influence plant growth by direct and indirect mechanisms. A total of 220 phosphate solubilizing bacteria were isolated from different rhizosphere soil in Northern part of Thailand. These isolates were screened for their plant growth promoting factors like production of ammonia, siderophore and cell wall degrading enzyme activities; cellulase, chitinase and proteolytic enzyme. More than 64% of the isolates produced ammonia and 23% produced siderophore on chrome azurole S agar plates. Moreover, test isolates produced cell wall degrading enzyme; cellulose (6%), chitinase (6%) and proteolytic enzyme (5%) on agar plate method. The results show that rhizospheric phosphate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture.

KEYWORD: rhizospheric bacteria, plant growth promoting trails, phosphate solubilizing bacteria

1. INTRODUCTION

Rhizobacteria are bacteria that colonize plant roots. Plant growth promoting rhizobacteria (PGPR) are very small portion of rhizobacteria (2–5%) that promote the growth [1]. PGPR use one or more direct mechanism of action to improve plant growth and health. These mechanisms may be sequentially or active simultaneously at different stages of plant growth. Improvement of plant uptake by phosphate–solubilization or N_2 fixation and phytohormone production like indole -3-acetic acid are examples of mechanisms of direct influence on plant growth. Biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzyme, hydrogen cyanide and siderophore or through competition for nutrient and space can significantly improve plant health and promote growth by increasing of seedling emergence, vigor and yield [1].

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Phosphate solubilizing bacteria (PSB) are the group of common PGPR in rhizosphere. Secretion of organic acids and phosphatases to solubilize insoluble phosphate to soluble forms are common in this group [2]. Although several phosphate solubilizing bacteria occur in soil, their numbers are not adequate to compete with other bacteria commonly established in the rhizosphere [3]. Moreover, the population of inorganic P-solubilizing microorganism is very low, less than 10^2 cfu $g^{-1}$of soil. Therefore the number of PSM is more important in the rhizosphere than in non - rhizosphere soil [4].

Exportation of soil microbial diversity for PGPR having combination of PSB activities and adaptation to particular soil environment are necessary. The present study was designed to screen effective rhizospheric bacteria with plant growth promoting activities and successful application.

2. MATERIALS AND METHODS

2.1 Isolation and screening

The rhizosphere soil samples were collected and transferred under aseptic conditions to laboratory. Zero point one milliliter of the soil samples was spread on modified Pikovskaya agar plates for phosphate solubilization assay as described by Nautiyal [5]. Bacterial colonies forming halo zones were considered to be phosphate solubilizers.

2.2 Assay for NH_3 production

PSB isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated into 10 ml peptone water in each tube and incubated for 48 h at 30°C. Nessler' s reagent (0.5 ml) was added to each tube. Development of brown to yellow colour was a positive test for ammonia production [6].

2.3 Siderophore production

PSB isolates were assayed for siderophore production on the chrome azurole S agar (CAS) described by Clark and Bavoil [7]. Chrome azurole S agar plates were prepared and spot inoculated with test organism and incubated at 30° C for 5 days. Development of yellow - orange halo around the colony was considered as positive for siderophore production.

2.4 Cell wall degrading enzyme production

Protease activity (casein degradation) was determined from clear zone in skimmed milk agar. Colonies were screened for chitinolytic and cellulase activity by plating on chitin agar and CMC agar according to Cattelan *et al*. [8]. The agar plates were prepared and spot inoculated with test organism and incubated at 30 °C for 5 days. Development of halo zone around the colony was considered as positive for cell wall degrading enzyme production.

3. RESULTS AND DISCUSSION

Detection and estimation of the phosphate solubilization ability of microorganisms have been possible using agar plate method. Phosphate solubilizer produce clear zone around the bacterial colonies on media containing insoluble mineral phosphate such as tricalcium phosphate or hydroxypatile. Two hundred and twenty rhizobacteria capable of solubilizing tricalcium phosphate were isolated from rhizospheric soil in northern part of Thailand. Phosphate solubilization was

most frequently encountered in rhizobacteria. The production of ammonia was a common trait in these bacteria. More than 64% of the isolates produced ammonia.

Another important trail of PGPR is the production of siderophore. One hundred and eighty three isolates (83 %) could grow on CAS agar and fifty isolates (23 %) were considered as siderophore producers because orange haloes were formed around the colonies (Figure 1). These isolates need further investigation.

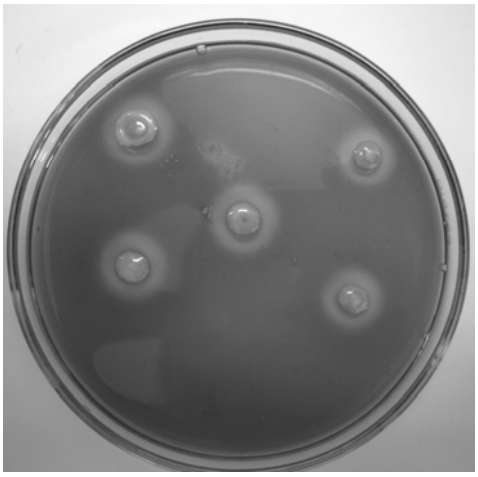


Figure 1 Siderophore production of bacterial isolates

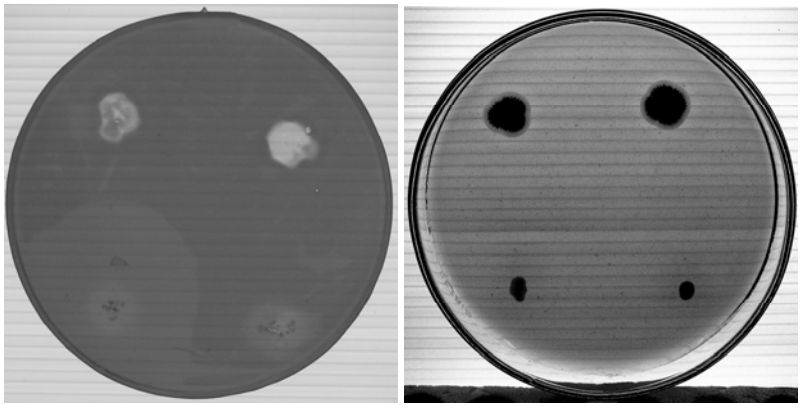
Iron is a limiting bioactive metal in soil and essential for the growth of soil microorganisms. The iron concentration in the soil is low (10^{-7}M) enough to limit the growth of soil microorganism (10^{-8} – 10^{-6} M) [9]. Rhizobacteria have to develop some strategies to acquire iron. The major strategy is the production and utilization of siderophores. The rhizobacteria that can produce siderophores could compete for iron with soil borne pathogens. Competition for iron is also a possible mechanism in agriculture to control the pathogenic fungi in the soil. Several studies have demonstrated the production of siderophore, other secondary metabolites and lytic enzyme production by rhizospheric bacteria were involved in the control mechanism against plant root pathogens including *Fusarium oxysporum* and *Rhizoctonia solani* [10].

Siderophore producing bacteria are good candidates for plant growth promotion, especially in neutral to alkaline soil. All of the P- solubilizing bacteria (220 isolates) have good phosphate prospects to improve plant growth, especially in the soil with large amount of precipitation.

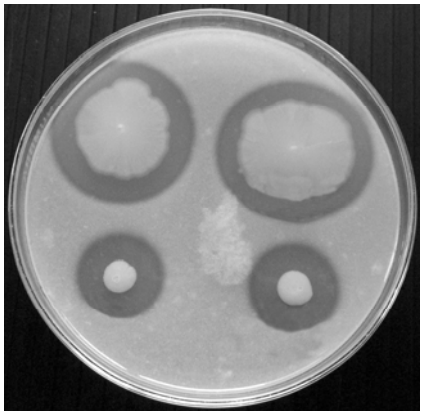
Production of fungal cell wall degrading enzymes was analysed because this is an important mechanism of fungal inhibition. Fourteen rhizosphere isolates (6%) were found to produce cellulase, a fungal cell wall degrading enzyme. Chitinase was detected in fifteen isolates (6%) and eleven isolates (5%) could produced halo zones on skim milk agar that showed protease activity (Table1 and Figure 2).

Table 1 Some selected cell wall degrading enzymes produced by bacterial isolates

Bacterial Isolates	Enzyme Type (Diameter of halo zone in cm^3)		
	Cellulase	**Chitinase**	**Protease**
A 103	-	0.48 ± 0.05	-
A 130	-	0.78 ± 0.05	1.03 ± 0.1
A142	-	0.48 ± 0.05	1.05 ± 0.1
B 1.3	0.88 ± 0.05	-	-
C 5	0.73 ± 0.10	0.48 ± 0.05	
CR 1.1	-	-	2.13 ± 0.1
D 3	1.00 ± 0.05	-	-
D 4.2	0.55 ± 0.05	-	-
D 4.11	1.00 ± 0.08	-	-
D 5.2	-	1.58 ± 0.05	-
G 1	0.53 ± 0.10	0.58 ± 0.05	-
G 5	0.75 ± 0.06	-	-
H 1	-	-	1.33 ± 0.05
J 2	-	0.58 ± 0.05	-
K 2.2 (3)	-	1.03 ± 0.05	-
MW 2.3	-	0.53 ± 0.05	-
MW 2.5	-	-	1.50 ± 0.05
MW 2.6	-	-	1.50 ± 0.05
P 3.14	-	0.58 ± 0.05	-
P 12	-	0.58 ± 0.05	-
PA 7.1	-	0.68 ± 0.05	-
SA 7	-	-	0.95 ± 0.06
SN 3.1	1.10 ± 0.05	-	-
SN 4.12		0.58 ± 0.05	-
SN 4.13	1.80 ± 0.08	-	-
SN 5.1	0.45 ± 0.06	-	-
SN 5.13	-	1.03 ± 0.05	-
SN 5.15	1.43 ± 0.10	-	-
SN 8.1	-	1.33 ± 0.05	-
SN 8.7	1.08 ± 0.05	-	-
SN 9.2	1.00 ± 0.08	-	-
T 3	-	-	1.08 ± 0.10
T 4	-	-	0.6 ± 0.10
T 11	0.73 ± 0.10	-	-
T 13	-	-	1.03 ± 0.05
TS 3	-	-	1.73 ± 0.10
TS 4	-	-	2.78 ± 0.05



(A) (B)



(C)

Figure 2 Cell-wall degrading enzyme: A = Cellulase production, B = Chitinase production, and C = Protease production

The ability of antagonistic bacteria to produce proteolytic enzyme, chitinase and cellulase was at low percentage and the composition of antagonistic mechanisms was specific for each isolate. Some of the tested isolates could exhibit more than two or three PGP trails, which may promote plant growth directly or indirectly or synergistically. The finding of multiple PGP activities among PGPR have been reported by some other workers [11].

4. CONCLUSIONS

From this present study, it can be concluded that rhizobacteria showed variation in their biocontrol characteristics. Further research should be carried out with such efficient PGPR isolates. These studies may be on antifungal metabolite, IAA production and their effect on plant growth.

5. ACKNOWLEGEMENTS

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