Short Communication(NS-1)

ISOLATION OF OXALOTROPHIC PHOSPHATE SOLUBILIZING RHIZOBACTERIA AND THEIR SCREENING FOR PLANT GROWTH PROMOTING TRAITS

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ABSTRACT

Rhizospheric bacteria are known to be effective for plant growth by direct and indirect mechanisms. Oxalate metabolizing bacteria have the great interest for the phytopathogenic fungal inhibition and kidney stone management purposes. A total of 27 oxalotrophic phosphate solubilizing bacteria were isolated from rhizosphere soil of oxalogenic plant. These isolates were studied for their plant growth promoting factors like indole acetic acid production , cell wall degrading enzyme activities, cellulase, chitinase and proteolytic enzyme. The results showed that rhizospheric oxalate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture.

Key Words : Oxalotrophic bacteria, Rhizobacteria, Plant growth promotion, Isolation, Enzyme

INTRODUCTION

Oxalic acid is prime end product present in plant cells either as a free acid, as sodium and potassium oxalate or may most commonly calcium oxalate insoluble salts. Many times it can be precipitated as an insoluble salt, most commonly calcium oxalate. Due to death and decay of plants, oxalate is released into the soil, can become toxic and interfere with plant growth[1]. Therefore, microbes involved in the oxalate degradation play important role in the biological carbon cycle. So various species of aerobic microorganisms are known to be oxalotrophic bacteria[2, 3]

The oxalate-degrading bacteria are closely related with plant roots that are rich in oxalic acid. Where presence of oxalate is a prime carbon source for these bacteria in the rhizosphere[4]. The *Spinacia oleracea* (Spinach) plant is one such example of oxalogenic plant[5] hence, the rhizospheric soil of spinach was used to isolate oxalotrophic bacteria.

Plant Growth Promoting Rhizobacteria (PGPR) are very small portion of rhizobacteria (2-5%) that promote the plant growth[6]. PGPR use different mechanisms of action for improvement of plant growth and health. These mechanisms are acting sequentially or simultaneously at different stages of plant growth. The growth of plant is due to uptake of solubilized phosphate or converted N_2 products or phytohormone like indole -3- acetic acid are some of the examples of mechanisms of direct influence on plant growth. The present study was designed for isolated oxalotrophic microbes to study their plant growth promoting phenotypic traits in vitro.

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AIMS AND OBJECTIVES

Isolation of oxalotrophic phosphate solublising rhizobacteria from rhizospheric soil samples of *Spinacia oleracea* (Spinach) and study their different traits important for plant growth.

MATERIAL AND METHODS

Isolation and screening

The rhizosphere soil samples of spinach were

collected and transferred under aseptic conditions to laboratory. The soil samples were enriched and isolation of bacteria on basal media containing potassium oxalate[2]. Bacterial colonies were considered to be OxaloTtrophic Bacteria (OTB).

Phenotypic traits

Phenotypic traits for the OTB isolates were done by using standard test methods as described in laboratory exercises in Microbiology[7]. Gram nature, catalase, oxidase, H_2S production and citrate utilization .

Assay for phosphate solubilization

OTB isolates were spot inoculated on modified Pikovaskaya's agar plates for phosphate solubilization detection[8]. Bacterial colonies forming halo zones were considered to be phosphate solubilizers.

Assay for IAA production

All OTB isolates were subjected to qualitative analysis for the production of IAA[9]. Bacteria producing IAA were identified by the formation of characteristic red halo around the colony on filter paper disc. The paper discs after treatment with Salkowaski's reagent were observed under UV light.

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 respectively according to Cattelan et al[10]. The agar plates were prepared and spot inoculated with test organism and incubated at 30^0C for 5 days. Development of halo zone around the colony was considered as positive for cell wall degrading enzyme production.

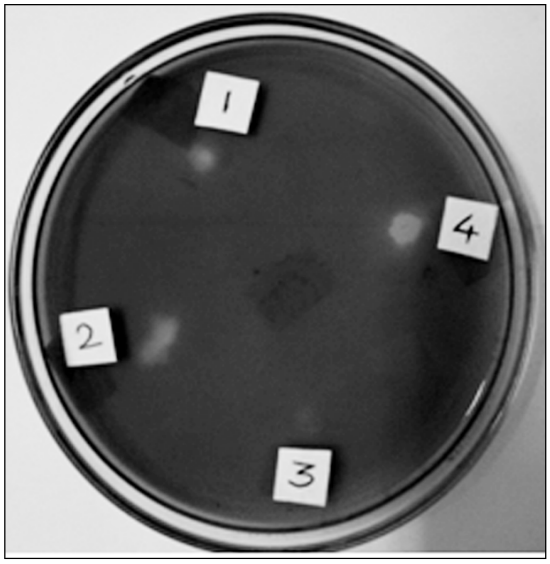

Fig. 1 : Isolates showing the cellulase test positive on CMC agar plate.

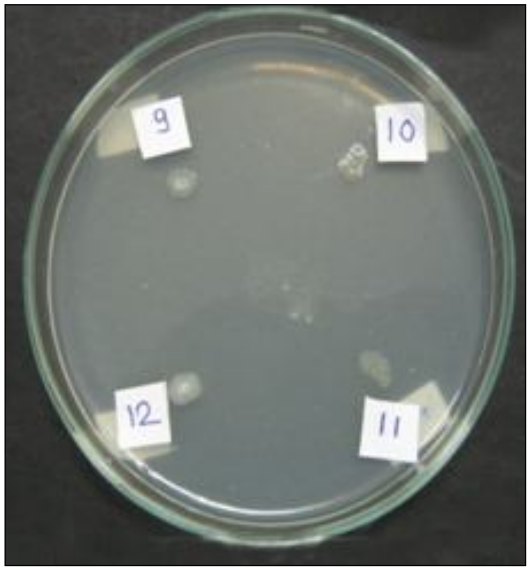

Fig. 2 : Isolates showing the growth in chitinase agar.

RESULTS AND DISCUSSION

These oxalotrophic microorganisms play very important role in biogeochemical carbon cycle[11] and oxalate utilizing enzymes[12]. Oxalotrophic isolates were screened for the phosphate solubilization ability using Pikovaskaya's agar medium plate method. Phosphate solublising bacteria were detected by clear zones around growth of colony. All twenty seven oxalotrophic rhizobacteria were capable of solubilizing tricalcium phosphate, catalase positive and utilizing citrate from rhizospheric soil **(Table 1)**. Phosphate solubilization is a frequently observed characteristic in rhizobacteria which was a common trait also observed in all these bacterial isolates. Another important trait of PGPR is the production of Indole Acetic Acid (IAA). Nine isolates (33.33 %) were considered as IAA producers with the help of color detection test on filter paper disc. The rhizobacteria that can produce oxalate degrading enzymes could compete with soil borne pathogens. Several studies have demonstrated the production of oxalate degrading enzymes, other

Table 1 : Characteristics of oxalotrophic bacterial isolates from soil samples.

Isolate code	Gram staining	Catalase	oxidase	H_2S production	Citrate solubilization	Phosphate production	IAA	Caseinase	Chitinase	Cellulase
01S	-ve rod	+	_	-	+	++	-	+	+	+
02S	-ve rod	+	-	-	+	+	-	+	-	+
03S	-ve rod	+	-	-	+	+	-	-	+	-
04S	-ve rod	+	-	+	+	+	+	+	+	+
05S	-ve rod	+	-	-	+	++	-	+	-	+
06S	-ve rod	+	-	+	+	+	+	-	+	-
07S	-ve rod	+	-	-	+	++	-	-	-	+
08S	-ve rod	+	-	+	+	+	+	-	-	-
09S	-ve rod	+	-	-	+	+	+	-	+	-
10S	-ve rod	+	-	-	+	+	+	-	-	+
11S	-ve rod	+	-	-	+	+	+	-	-	+
12S	-ve rod	+	-	-	+	++	+	-	-	+
13S	-ve rod	+	-	-	+	+	+	-	-	-
14S	-ve rod	+	-	-	+	+	-	-	-	-
15S	-ve rod	+	-	-	+	++	+	+	-	-
16S	-ve rod	+	-	-	+	++	-	-	-	-
17S	-ve rod	+	-	-	+	+	-	-	-	+
18S	-ve rod	+	-	-	+	+	-	-	-	+
19S	-ve rod	+	-	-	+	+	-	-	-	+
20S	-ve rod	+	-	-	+	+	-	-	-	-
21S	-ve rod	+	-	-	+	+	-	-	-	+
22S	-ve rod	+	-	-	+	+	-	-	+	+
23S	-ve rod	+	-	+	+	+	-	-	-	-
24S	-ve rod	+	-	+	+	+	-	-	+	+
25S	-ve rod	+	-	+	+	+	-	-	-	-
26S	-ve rod	+	-	+	+	+	-	-	-	-
27S	-ve rod	+	-	+	+	+	-	+	+	+

+ = Positive, - = Negative, ++ = More potent

secondary metabolites and lytic enzyme production by rhizospheric bacteria were involved in the control mechanism against plant root pathogens. e. g. *Rhizoctonia solani* is inhibited due to action of extracellular lytic enzymes and secondary metabolites produced by rhizospheric bacteria.[13] Study is required to detect same type of action by these oxalotrophic bacteria isolates. Out of twenty seven isolates only six isolates (22.22%) showed positive caseinase activity with skim milk agar plates. Productions of fungal cell wall degrading enzymes were studied[14]. A fungal cell wall degrading enzyme; cellulase production test was showed positive by fifteen (55.55%) rhizosphere isolates while chitinase was detected positive for eight isolates (29.62%).Many isolates from this study have exhibited more than two or three PGP traits which can help plant growth by various

mechanisms. Different PGP activities of so many PGPR have been reported by some other workers[15-16].

CONCLUSION

Basic information from this work can be proposed for future investigation of oxalate-degrading bacteria. By studying the production and mechanism of action of enzymes produced by these bacteria one can use it for medicinal purpose in hyperoxaluric patients. In further research the efficiency of PGPR isolates will be decided on the different qualitative and quantitative PGP products. Antifungal metabolite production, GA production, NH_3 production and their effect on plant growth along with molecular identification of isolates is needed for further studies.

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