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Enzymatic Potential of Bacteria Isolated From the Rhizosphere of *Aegle Marmelos* (Bael Tree)

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Abstract:

Rhizosphere is the area of intense microbiological activity. The population of microorganisms differs qualitatively and quantitatively. Problem- Rhizospheric bacteria produce various hydrolytic enzymes for conversion of complex substances into simple ones and also enhance plant growth. It is necessary to study potential of rhizospheric bacteria. Method -In the present study two samples from the rhizosphere of *Aegle marmelos* trees were collected and twenty one isolates were obtained. Characterization of twenty one isolates was carried out by morphological and cultural characters. Further the isolates were studied for their enzymatic potential. The results showed amylase and caseinase activity in 61.89% isolates. Phosphate solubilization potential in 52 .37% isolates 47.61 % isolates showed catalase and 38.09 % oxidase activity. Only 19.04 % isolates were urease positive. From the study it is concluded that rhizospheric bacteria show enzymatic potential for various enzymes and promote plant growth.

Keywords: Rhizosphere, A*egle marmelos,* enzymes

1.0 Introduction:

Rhizosphere is the area of intense microbiological activity. The population of microorganisms differs qualitatively and quantitatively. (Atlas & Bartha 1987) The population of microorganisms in the rhizosphere also differs from one plant to another. It is influenced by root exudates. The rhizosphere region is a highly favorable habitat for the proliferation and metabolism of numerous types of microorganisms (Alexander 1977). It contains varied number of bacteria as important organisms. Rhizospheric bacteria produce various hydrolytic enzymes and convert complex carbon sources like starch, cellulose to simple one. They enhance the plant growth by producing the phytohormones like indole acetic acid, by phosphate solubilization or by nitrogen fixation.

The rhizosphere of medicinal plant may harbor some interesting organisms. *Aegle marmelos* (Bael) tree is found throughout India and other continents in dry forest as well as cultivated. It belongs to family Rutaceae. The roots are sweet, astringent and febrifuge. They are useful in diarrhea, dysentery, dyspenia, seminal weakness and stomachalgia. Plant properties affect rhizosphere population through root exudates(P.Shipkar 2010). Rhizosphere population varies in structure and species composition depending on root zones. Many researchers used extracts of *Aegle marmelos* on human pathogens. But there were few reports about rhizosphere bacteria of the medicinal plants like *Aegle marmelos.* As roots of *Aegle marmelos* are used as drug, the rhizosphere of *Aegle marmelos* may harbor. The present study was carried out to study enzymatic potential. In the present study bacteria were isolated from the rhizospheric soil of *Aegle marmelos* and the isolates obtained were studied for their enzymatic potential.

2.0 Materials and Methods:

2.1 Materials:

1) Soil sample - Soil samples from rhizosphere region from medicinal plants *Aegle marmelos*
2) Starch agar
3) Milk agar
4) Pikovskaya's agar

2.2 Methods:

2.2.1 Collection of Sample: For the present study rhizosphere soil samples from *Aegle marmelos* trees from the college campus were collected. These samples were obtained at a depth of 6 to 10 cm. in rhizosphere regions. The soil samples were allowed to air dry at room temperature.

2.2.2 Isolation of Bacteria: Bacteria were isolated from samples by serial dilution technique using nutrient agar. The different colonies were selected and cultural character were studied and transferred on nutrient agar slants. Total twenty one different isolates were obtained from two soil samples of two plants from two locations. Isolates were characterized on the basis of morphological and cultural characters. Gram staining, motility and endospore staining of isolates was performed. All isolates were studied for different enzymatic activities like catalase, oxidase, hydrolytic enzyme production and phosphate solubilization activity.
Amylase activity :- Isolates were spot inoculated on sterile starch agar plates separately (peptone - 1gm, NaCl - 0.5gm, Yeast extract – 0.3 gm, starch - 2 gm, agar – 2.5gm, D/W – 100 ml, pH – 7.2) and incubated at 28 ^{0} c for 24 hours. After incubation amylase activity was examined by adding dilute iodine solution and observed for zone of clearance around the colony.

2.2.3 Caseinase Activity: Isolates were spot inoculated on sterile milk agar plates separately (nutrient agar 90ml + 10 ml skimmed milk) and incubated at 28 ^{0} c for 24 hours. After incubation caseinase activity was examined by observing zone of clearance around the colony.

2.2.4 Phosphate Solubilization: Pikovskaya's agar plates (glucose - 10 gm, Ca_3 $(po_4)_2$- 5gm, $(NH_4)_2So_4$ - 0.5gm, Kcl- 0.2gm, $MgSo_4$, 7 H_2O -0.1gm, MnSo4- 0.006gm, FeSo4- 0.006gm, Yeast extract – 0.5 gm, agar- 15gm, D/W -1 L, pH – 7.2) were spot inoculated with bacterial isolates separately and incubated at 28 ^{0} c for five days. The bacterial colonies forming clear zones were considered as phosphate solubilizing .

2.2.5 Catalase and Oxidase Activity:-
Catalase activity of all isolates was studied using hydrogen peroxide and oxidase test was performed using oxidase reagents.

2.2.5 Urease Activity:
Urease activity of isolates was studied using Christencen's urea agar slants by inoculating and incubating the slants at 28 ^{0} c for 24 hours. After incubation the slants were observed for colour change from yellow to magenta red.

3.0 Results and Discussion:

The organisms isolated were found to be Gram positive cocci, Gram positive spore forming rods, Gram positive non spore forming rods and Gram negative rods. The enzymatic potential of isolates showed Catalase in 47.61 %. Oxidase in 38.09% Amylase and Caseinase in 61.89 %, Urease in 19.04% and Phosphate solubilization potential in 52.37 % isolates. Isolate AMH9 showed enzyme activity for all 6 enzymes while AMH6 showed no activity for all enzymes. AMH10 showed only urease activity. AM1, AM3, AM7, AM8 showed moderate enzyme activity, remaining all isolates showed least enzyme activity.

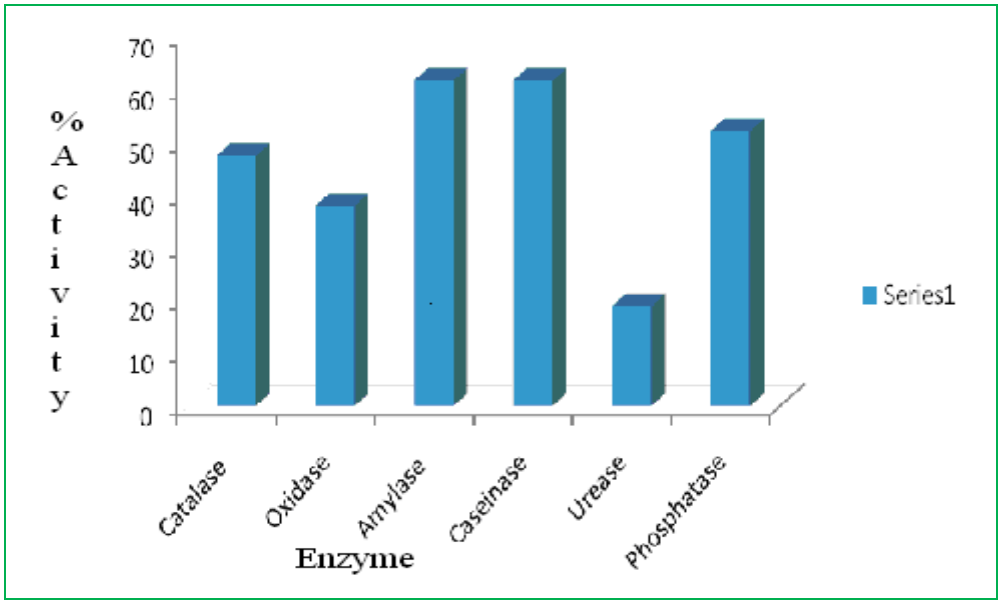

Figure 1: Percentage activity of 21 bacterial isolates.

Table 1: Enzymatic activity of isolates

Enzyme						
Isolate	**Catalase**	**Oxidase**	**Amylase**	**Caseinase**	**Urease**	**Phosphatase**
AM 1	+	-	+	+	-	+
AM 2	-	+	+	+	-	-
AM3	+	+	+	-	+	+
AM 4	+	-	-	-	-	+
AM5	-	-	+	+	-	+
AM6	-	-	+	+	-	+
AM7	+	+	-	-	+	+
AM8	+	-	-	+	-	+
AM9	-	+	+	-	-	+
AM10	+	-	-	-	-	-
AM11	-	+	-	+	-	+
AMH1	-	-	+	+	-	-
AMH2	-	-	+	+	-	+
AMH3	-	-	+	+	-	-
AMH4	-	-	+	+	-	-
AMH5	+	-	-	+	-	-
AMH6	-	-	-	-	-	-
AMH7	+	+	+	-	-	-
AMH8	+	+	+	+	-	-
AMH9	+	+	+	+	+	+
AMH10	-	-	-	-	+	-

Table 2: Number of isolates showing enzyme activity

Total No of isolates	No of isolates					
	catalase positive	oxidase positive	amylase positive	caseinase positive	urease positive	phosphatase positive
21	10	8	13	13	4	11

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Ahmad *et al*.,(2008) were found that 80% phosphate solubilizing bacteria were *Bacillus*, in free living rhizosphere bacteria while studying their PGP activities. Chaiharn *et al*., obtained 220 rhizobacteria capable of solubilizing tri calcium phosphate from rhizosphere soil in Thailand and also concluded that 5% isolates showed protease activity and 6%& isolates cellulase activity. Tamilarasi *et al*., (2006) studied diversity of microorganisms in rhizosphere of different medicinal plants and studied their hydrolytic enzymatic production abilities and found that 91.26 % isolates were amylase positive, 90.32% isolates were urease positive, 73.6% isolates produce caseinase, 39% isolates exhibited gelatinase activity, only 3% produce cellulase and 70.96 % isolates exhibited phosphate solubilizing activity. Waseem safdar *et al*.,(2011) studied phosphate solubilizing activity of bacteria and fungi from rhizosphere of different medicinal plants and obtained highest phosphate solubilizing fungi associated with *Aloe vera.*

Comparative to these studies we found that wide range of enzymes which are of bacterial origin present in the rhizosphere catalyze the breakdown of organic materials. The presence of these enzymes will help the breakdown of complex nutrients into simpler form and thus make available to plants and increase plant growth. Phosphate solubilizers solubilize inorganic phosphate which is unavailable to plants by producing phosphatase enzyme and make it available to plant and help in plant growth promotion. From the results it is concluded that enzymatic potential of rhizospheric bacteria helps in plant growth promotion.

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