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SCREENING FOR SIDEROPHORE AND AMMONIA PRODUCTION FROM RHIZOSPHERE ISOLATES OF ORYZA SATIVA

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ABSTRACT

The Rhizosphere is biologically and chemically highly diverse, complex and dynamic interaction occurs between plant roots, soil (micro) biota and the physicochemical conditions of the soil. The autotrophic plant partner is providing substrate and energy flow into the rhizosphere and gets in return essentials for its development and growth: nutrients, minerals and water. Heterotrophic soil biota usually are limited in the supply of carbon and energy and thus a complex sequence of responses are initiated, which in due course also influence the plants. In the present study the microorganisms were isolated from the rhizosphere soil of rice from nine different locations in and around Bangalore to understand the diversity of the microorganisms, interaction and to determine the siderophore activity and production of ammonia of the isolates. A total of 116 micro organisms were isolated of which 110 were bacterial isolates and 6 Actinomycetes. 53 bacterial isolates found to be morphologically different were used for the current study. 6 isolates out of the 53 microorganisms showed positive results for the production of siderophore by forming orange yellow halo zones on CAS agar media plate. R-19 isolate showed the highest zone and was considered as the highest producer of siderophore. Whereas 36 isolates showed positive for ammonia production out of which 6 isolates showed maximum production and 12 isolates with fairly good production.

KEYWORDS: Rhizosphere soil, siderophore, Rice, CAS, Actinomycetes.

INTRODUCTION

Plants have a active defence mechanism against the biotic stress mainly the pathogens and parasites of various scales ranging from microscopic viruses to phytophagous insects. The plants have mainly two types of defence mechanism like Systemic acquired resistance (SAR) and induced systemic resistance (ISR) that are preconditioned based on the prior infection or the treatment against the pathogens or the parasites. The PGPR show a systemic resistance in plant against both root and foliar pathogens by suppressing the disease by antagonistic effect thus the rhizosphere micro flora plays an important role for the growth of the plant by acclimatization against most of the stress [1]. Rhizobacteria-mediated induced systemic resistance (ISR) is dependent on jasmonic acid (JA) and ethylene (ET) signalling in the plant. The microorganisms requires nutrition for their growth mainly the carbon and nitrogen source which acts as a primary source apart from this they require micronutrients as well like the iron in order to survive and multiply. The microorganisms have developed several strategies to obtain iron from the environment for their growth and multiplication [2, 3, 4]. The iron plays an important role in the establishment of the infection by the bacteria on to the plants and animals as well [5, 6, 7].

The iron complex is taken up by the microorganisms via the cognate-specific receptor and a transport pathway [8] releasing siderophore in the surrounding. The importance of iron molecule for their growth is reflected as the microorganism have developed many mechanisms for the intake of iron, which include a membrane-bound chelator [9], reduction of Fe chelates [10,11], and an unknown mechanism in *Serratia marcescens* [12]. Thus screening of the microorganisms associated with the rice rhizosphere for the production of siderophore was carried out in the present study.

Rhizobacteria shows multiple traits for plant growth promotion and disease suppression. The predominant trait is IAA production, Ammonia production, phosphate solubilization followed by Antagonism. The soil accumulates inorganic Nitrogen, in the form of ammonia, onto C skeletons for the production of amino acids and this is one of the most important biochemical processes in plants. In an actively growing plant, N is taken up as nitrate and to a lesser extent as ammonia. Nitrogen is present in the atmosphere; it is often a limiting nutrient for plant growth as only some plants are capable of using this N source. In such plants, the atmospheric dinitrogen is converted to ammonium by a nitrogenise activity present in rhizobia bacteria that establish a symbiotic interaction by forming nodules.

MATERIALS AND METHODOLOGY

Sample Collection

Soil samples were collected from the rhizosphere of rice plants growing at different sites at rural areas of Bengaluru, India. Intact root system was dug out and the rhizospheric soil samples were carefully taken in plastic bags and stored at 4°C for further investigation. A Total of nine soil samples were collected for the isolation of rhizosphere bacterial isolates.

Isolation of Bacteria from Rhizospheric Soils

The microbial populations in the above collected nine samples were isolated and quantitatively enumerated by standard serial dilution method. Five test tubes were taken, one with 10 ml sterile distilled water and other with 9ml sterile distilled water.1 gm of each collected rhizosphere soil samples was taken in 10ml of sterile distilled water to get 10^{-0} dilution.Serial dilutions was carried out by transferring 1ml of the 10^{-0} dilution to the 2^{nd} tube which contains 9ml of sterile water to get 10^{-1} dilution.Dilutions were made from upto 10^{-5}.These dilutions were used for the isolation of micro-organisms by spread plate technique. 100µl of each dilution (10^{-1} to 10^{-5}) of all samples was taken and spread onto nutrient agar media using a sterile L-rod by standard spread plate technique.Inoculated plates were incubated at 37ºC for 24-48 hours.Isolated colonies were later analysed for their colony, morphological and Gram's characteristics.

Maintenance of Pure Cultures

The pure cultures of the isolated microorganisms were maintained by culturing individual colonies on Nutrient agar slants for 24-48 hours at 37ºC after which they were maintained at 4ºC until the screening for siderophore was carried out.

Siderophore Production

The 53 Bacterial isolates were assayed for siderophores production on the Chrome azurol S agar medium (CAS media) described by [13]. Chrome azurol S agar media was prepared and sterilized at 121ºC for 15minutes and the plates were poured with the medium. After solidification the plates were inoculated with the test microorganisms then the plates were incubated at room temperature for 48-72h for the growth of the bacterial colonies. The development of Yellow-orange halo around the colonies indicated the production of siderophore by the organisms and was considered to be positive.

Ammonia Production

Freshly grown cultures were inoculated in peptone water and incubated for 48-72 hours. Nessler's reagent (0.5ml) was added to each tube. Development of yellow to brown colour indicated positive test for ammonia.

RESULTS AND DISCUSSION

A total of 116 microorganisms were isolated in which 110 were found to be bacteria and 6 actinomycetes. 51.81% of the bacterial isolates were Gram positive bacilli, 42.72% Gram Positive cocci and 0.054% Gram negative bacilli. 53 isolates among the 116 were selected for further analysis of siderophore production Fig-1 and Fig-2.

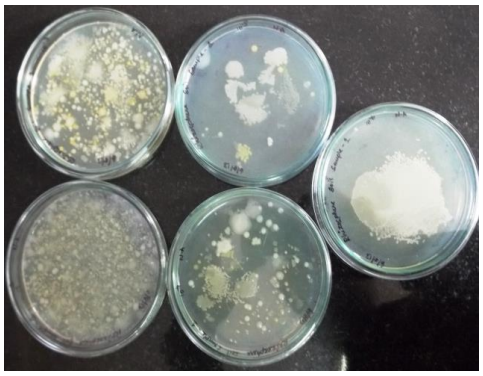

Figure 1: isolation of microorganisms from rhizosphere soil of rice.

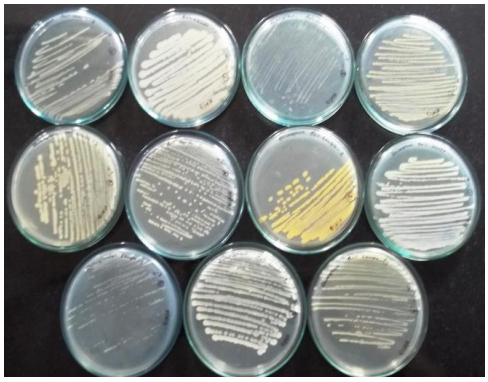

Figure 2: Pure culture maintenance on the plates.

Siderophore Assay

Siderophore are the compounds present in the microorganisms, which help them intake micronutrients like iron for their growth and development. Thus, the screening for the siderophore production was carried out for all the 53 isolates on CAS media. The appearance of yellow orange halo around the colonies on the CAS media plates indicate the production of siderophore by the isolates and indicate that the isolate can chelate the iron from the environment for their growth. Six isolates namely R-13, R-19, R-21, R-23, R-47 and R-48

showed halo zones indicating that they produced siderophore. The siderophore was maximally produced by R-19 isolate as the halo zone was larger when compared to the other isolates producing siderophore Fig-3.

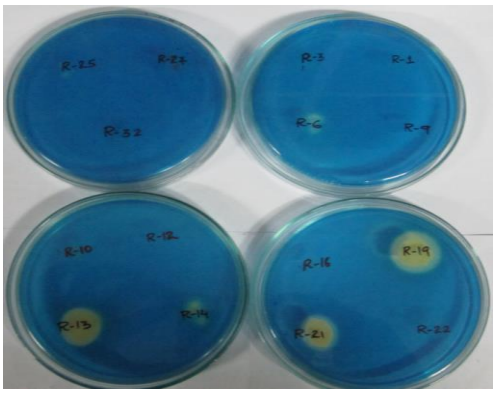

Figure-3: Production of Siderophore on CAS media Plates.

Ammonia Production

Of the 53 isolates 36 isolates showed positive for ammonia production out of which 6 isolates showed the maximum production (R-4, R-13, R-19, R-25, R-40 and R-42) and 12 isolates with fairly good production and other isolates were positive with low production of ammonia. 17 isolates were negative for ammonia production Fig-4.

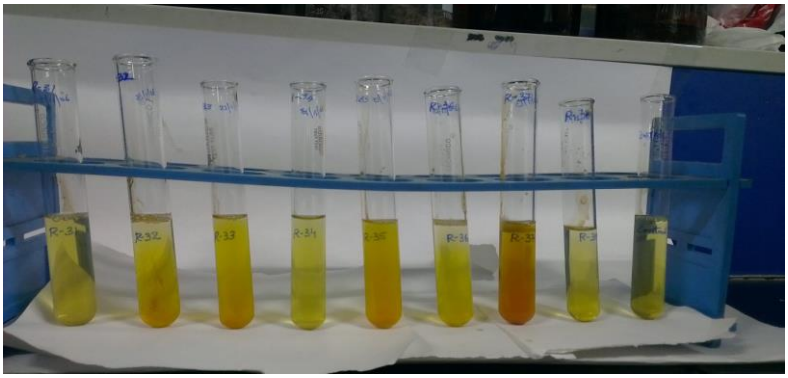

Figure-4: Ammonia production.

DISCUSSION

In the present study microorganisms were isolated from rhizosphere soil of rice and a total of 116 microorganisms were isolated from 9 different soil samples, similarly [14] evaluated the rice rhizosphere from Espinal, Tolima, Colombia, and they found 69 bacteria; among which 51% were *Pseudomonas* sp., mainly of species *P. putida, P. aeruginosa, P. fluorescens* and *P. citchori*; 26% were of genus *Azotobacter* sp., species, *A. vinelandii, A. chroococcum* and *A. nigrificans;* 21% were enterobacteria of genus *Klebsiella* sp., *Serratia* sp., *Enterobacter* sp. [15] isolated the microorganisms *Bradyrhizobium japonicum* and found that strain 61A152

showed positive for siderophore and identified that the activity was due to citric acid. This was the first report of citric acid release in response to iron stress by a rhizosphere bacterium. Azotobacter chroococcum has already been reported to release citric acid, but not in response to iron limitation.

Some investigations have also proved that ferric citrate can also serve as an iron source for a number of bacterial species [16, 17, 18, 19], and especially in *Escherichia coli* it has been proved that they require the products of the fecABCDE genes and functional tonB and exbB genes for transport of ferric citrate into the cells [20]. These genes are negatively regulated by iron, as are the other *E. coli* genes involved in siderophore production and transport [21]. On the basis of these criteria, ferric citrate is considered to be a siderophore, although its chelating ability is not as high as those of other siderophore compounds [22].

Several investigations by [23, 24] observed phosphate solubilization and ammonia production by Bacillus spp. which were isolated from cereal vegetable and other crops.[25] has been reported that the PGPR isolates (PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2) were successfully isolated and characterized from rice rhizosphere soils and evaluate their PGPR activities such as production of IAA, siderophore, ammonium and solubilization of phosphate, and antagonistic activity against phytopathogenic fungi such as ***Fusarium oxysporum***, *Rhizoctonia solani* and *Sclerotium rolfsii*.

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