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RICE ENDOPHYTES: A POTENTIAL SOURCE OF PHYTOHORMONES AND ANTIMICROBIALS

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Abstract – Endophytes are endosymbiotic species often a bacterium or a fungus that lives within a plant for at least a part of its life cycle without causing an apparent disease. Endophytes are known to enhance host growth, nutrient acquisition and increases plants' resistance to pathogens. In the given study, we isolated endophytic bacteria from locally grown rice plant root, stem and leaves. 23 strains of bacteria were isolated. The bacterial population showed a high level of growth hormone production namely, Auxin and Gibberellin to the levels of 349 μg/mL and 240 μg/mL respectively. 19 strains could solubilize phosphate and 3 could fix atmospheric nitrogen. The bacterial population even showed antimicrobial activity against human pathogenic strains such as *Vibrio cholerae, Pseudomonas aeruginosa, Burkholderia cepacia, Acinetobacter baumannii, Klebsiella sp.* and *Escherichia coli*. From the isolated bacterial population, 5 showed antimicrobial activity against *Pseudomonas aeruginosa*, 3 against *Klebsiella sp.* and *Acenatobacter baumanii*, 7 against *Vibrio cholerae*, 9 against *Burkholdaria cepacia* and 1 against *E. coli.* Thus, the study suggests that these microbes have huge potential to synthesize of numerous novel compounds that can be exploited in pharmaceutical, agricultural and other industries.

INTRODUCTION

Endophytes are microorganisms that live inside the living plant tissues for at least part of their life without causing any apparent disease symptoms in the host (Wilson, 1995). Endophytes are treated as endosymbiont. To represent these types of microorganisms, De Bary, (1886) coined the term endophyte.

Endophytes enter inside plants primarily through the roots and the aerial portions of plants, such as leaves, flowers, stems and cotyledons (Kobayashi and Palumbo, 2000). They are localized at the point of entry and can spread in the whole host plant body. After entering the host, they reside within cells or the intercellular spaces or in the vascular (tissue) system (Patriquin and Dobereiner, 1978). After gaining residence in the plant tissues, the endophytes are known to produce a diverse range of natural products which could be consistent and successful source of drugs and agricultural supplies. Therefore, the natural products from endophytic microbes do have a great potential not only in pharmaceutical industry but agrochemical and biotechnology industries also (Findlay and Bethelezi, 1997). Recently, much attention has been devoted to studies on the beneficial association of rhizobia and cereals, since these bacteria were found to be natural endophytes of important cereal crops and promoted their growth with an increase in grain yield at harvest while reducing their dependence on chemical fertilizer inputs, independent of root nodulation and biological N_2-fixation (Yanni *et al*., 2011; Yanni *et al*., 1997). Rhizobia are now known to develop endophytic associations with roots of domesticated and wild rice (Biswas *et al*., 2000), maize (Gutierrez-Zamora *et al*., 2001), wheat (Lupwayi *et al*., 2004; Sabry *et al*., 1997), barley, canola (Hilali *et al*., 2001; Noel *et al*., 1996), lettuce (Noel *et al*., 1996), and *Arabidopsis thaliana* (Stone *et al*., 2001). Notably, the production of nitrogen fertilizers depends on the use of non-renewable resources such as oil, gas, or coal (Stoltzfus *et al*., 1997). In recent years, the use of bio-inoculants composed of diazotrophic bacteria as an alternative to nitrogen fertilizers (Welbaum *et al*., 2004) has

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emerged as a promising approach. Nitrogen-fixing bacteria belonging to PGPB (Plant Growth Promoting Bacteria) can fix atmospheric nitrogen and supply it to plants. Plant growth-promoting rhizobacteria genera: *Bacillus* (Idriss *et al*., 2002), *Enterobacter* (Gupta *et al*., 1998) and *Corynebacterium* (El-Banana and Winkelmann, 1988) have been reported to benefit plants by enhancing plant growth and improving plant health through various direct and indirect mechanisms. PGPB are commonly used as inoculants for improving the growth and yield of agricultural crops and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. These bacteria significantly affect plant growth by increasing nutrient uptake, producing biologically active phytohormones and suppressing pathogens by producing antibiotics, siderophores, and fungal cell wall-lysing enzymes (Arora *et al*., 2001; Persello-Cartieaux *et al*., 2003).

Endophytes also stimulate the production of some secondary metabolites which creates a "barrier effect", where the local endophytes outcompete and prevent pathogenic organisms from taking hold, which are of huge potential to screen novel, highly active and low toxic antimicrobial compounds and act to inhibit potent human pathogens also (Benhamou *et al*., 2000).

The Endophytic Bacteria appears to be a potential source of novel antibiotics (Christina *et al*., 2013). It is well known fact that until now the soil bacteria have been the source for most of the antibiotics. Now the endophytic bacteria seem to be a promising alternative source of novel antibiotics.

The aim of the present study is to isolate bacterial endophytes from *Oryza sativa* and to determine their potential for the production of phytohormones and antimicrobials. Moreover their potential as biofertilizer was also exploited.

MATERIALS AND METHODS

Sample collection

Samples were obtained from Rice plant (*Oryza sativa*) collected from local farming grounds of Lauhati, North 24 parganas, West Bengal. The roots, stems and leaves of the collected plants were taken aseptically to the laboratory and kept refrigerated until use.

Isolation of Endophytes

Surface sterilization of the root, stem and leaves was done with tap water, tween 20, sodium hypochlorite, 70% alcohol and sterile distilled water.

Slurry was prepared in isotonic saline water and then plated in Luria Bertani agar and incubated at 37 °C for 2 days.

Gibberellin production assay

Gibberellic acid was estimated by Borrow *et al*. (1955). The isolates were grown in Nutrient broth for 4 days then were centrifuged at and the supernatant was used for extraction of Gibberellin. pH of the supernatant was adjusted to 2.8 by 1N HCL and to 1.5mL supernatant 0.2ml of zinc acetate solution and 0.2mL of potassium ferrrocyanide solution was added and centrifuged, 0.5mL of supernatant was then added to 0.5 mL of 30% HCL and the mixture was then incubated at 27 °C. Absorbance was measured at 250nm in UV-Vis spectrophotometer and compared to a standard curve.

Auxin production assay

Auxin was estimated by Gordon *et al*., (1951). The isolates were made to grow in IAA production media for 10 days and then centrifuged and the supernatant was used for IAA production. 1 part of the supernatant was added to 2 parts of Salkowsky reagent and incubated for 30 minutes to observe red colour. Absorbance was measured at 530nm and compared to a standard curve for quantification.

Phosphate solubilization

The isolates were streaked onto plates containing the Pikovskaya's agar medium and were incubated for 7 days at 28 °C. They were observed for forming clear zones around the streaked area, which was considered as the positive result.

Nitrogen fixation

In order to screen the bacterial isolates for nitrogen fixation ability, they were made to grow on slants of glucose nitrogen free mineral media. Slants were prepared of the mentioned agar and isolates were streaked onto it and incubated at 28° C for 7 days. Growth of the isolates indicate N_2 fixing ability.

Antimicrobial assay

Isolated bacterial strains were cultured in Luria Bertani for 5 days then were centrifuged and the filtrate was used for the assay. The assay was done by agar well diffusion method on Luria Agar plates containing test pathogenic organisms namely, *Vibrio*

cholerae, Pseudomonas aeruginosa, Burkholderia cepacia, Acenatobacter baumanii, Klebsiella sp. and *Escherichia coli.*

RESULTS AND DISCUSSION

In the present study, 23 bacterial isolates were obtained from the root, stem and leaf samples of the rice plant collected from local farming grounds of Lauhati, North 24 parganas, West Bengal. There was a wide variety in appearance of the colonies but majority of them were colourless and small to moderate in size. Majority of the isolates were rod shaped and gram positive in nature.

Gibberellin is an important phytohormone as it induces stem elongation, germination, and flowering. All of the isolates could produce appreciable amounts of Gibberellin ranging from 170-250 µg/mL. It was observed that isolates from the leaves were more potent for gibberellic acid production. The strains RR1-1, RR1-2, RLS-2, RLS-7 and RLS-9 could produce maximum amount of gibberellic acid, i.e., 250µg/mL. Figure 1 shows the amount gibberellin production by the isolates. Ambawade and Pathade (2013) estimated Gibberellic acid production from banana plant endophyte to the levels of 0.240 mg/mL .

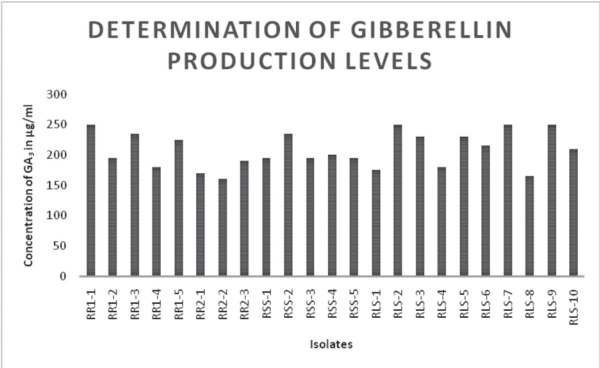

Fig. 1. Gibberellin production by each isolate

Auxin was estimated by the method of Gordon and Weber (1951). Auxins are known to stimulate both rapid (e.g. increases cell elongation) and long term (e.g. cell division and differentiation) responses in plants (Cleland, 1990). Diverse microorganisms including bacteria (Arshad and Frankenberger, 1998; Khalid *et al*., 2004), filamentous fungi (Kaldorf and Ludwig-Muller, 2000) and yeasts (El-Tarabily, 2004) are capable of producing physiologically active quantities of auxins and which have pronounced effects on plant growth and development. Auxin controls many important physiological processes including cell enlargement and division, tissue differentiation and responses to light (Frey-Klett *et al*., 2005). All the isolates produced auxin at appreciable levels of 190-349 µg/mL which was confirmed by production of red colouration of the supernatant. The strain RR2-3 (349 µg/mL) was the most potent Auxin producer followed by RR1-5 (345 µg/mL). Similar to Gibberellin, strains isolated from rice leaf could produce more Auxin compared to the other parts (Figure 2). The strains show higher values of auxin than reported by Khalid *et al*., 2004.

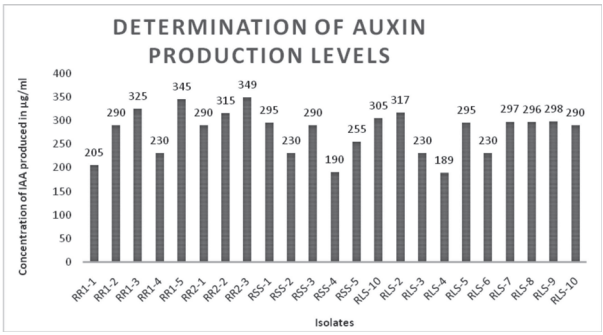

Fig. 2. Auxin production by each isolate

Releasing of insoluble and fixed form of phosphorous is an important aspect of increasing soil phosphorous availability (Rodriguez and Fraga, 1999). The use of phosphate solubilizing bacteria as inoculants simultaneously increase phosphorous uptake by the plant (Mehta and Nautiyal, 2001). Out of the 23 isolates 17 could solubilize phosphate by utilizing tricalcium phosphate by forming clear halo around the colony. RR2-1, and RLS-10 were most potent in solubilizing phosphate. Strains isolated from roots of rice plant could show more levels of phosphate solubilisation than those of leaf and stem (Table 1).

Table 1. Phosphate solubilization by each isolate

Isolate	Zone of solubilization
RSS-1	0.6cm
RSS-2	-
RSS-3	0.8cm
RSS-4	0.2cm
RSS-5	-
RR1-1	-
RR1-2	0.2cm
RR1-3	1cm
RR1-4	0.8cm
RR1-5	-

RR2-1	1cm
RR2-2	0.4cm
RR2-3	1cm
RLS-1	0.4cm
RLS-2	0.5cm
RLS-3	-
RLS-4	0.3cm
RLS-5	0.5cm
RLS-6	0.5cm
RLS-7	0.5cm
RLS-8	-
RLS-9	0.7cm
RLS-10	1cm

Nitrogen is the necessary element for plants as it is a primary constituent of nucleotides, proteins and chlorophyll (Robertson and Vitousek, 2009). Endophytic bacteria take dissolved nitrogen from sap flow and convert it into amines and ammonium nitrogen for the use of plant and thus can reduce chemical fertilizer application. Out of 23 isolates 3 could fix atmospheric nitrogen as indicated by the change of color of bromothymol blue containing glucose nitrogen free minimal medium from green to blue indicating ammonium has been produced. Ammonium is alkaline and increase pH of the medium which results in change of colour. Some of the isolates were acid producing which could be seen from the change of colour from greenish blue to yellow (Sulistiyani and Meliah, 2017).

On testing with pathogenic strains, the selected endophytic cultures showed a visible zone of inhibition confirming its antibacterial potential against the test organisms. 10 isolates were selected for screening for antimicrobial activity against the given test organisms. From the isolated bacterial population, 5 showed antimicrobial activity against *Pseudomonas aeruginosa*, 3 against *Klebsiella sp*. and *Acinetobacter baumanii*, 7 against *Vibrio cholerae,* 9 against *Burkholderiacepacia* and 1 against *E. coli* (Figure 3). RR1-1 had a widest spectrum of antibacterial activity as it could inhibit 5 out of 6 pathogenic strains. RR2-2, RSS-4 and RR2-3 also had a broad spectrum as they inhibited 4 out of 6 strains. RLS-8 and RLS-9 had narrower spectrum. Similar results were noted by (Vijaylakshmi *et al*., 2016) who also showed broad spectrum antimicrobial activity against both gram positive and negative pathogens by endophytes of medicinal plants.

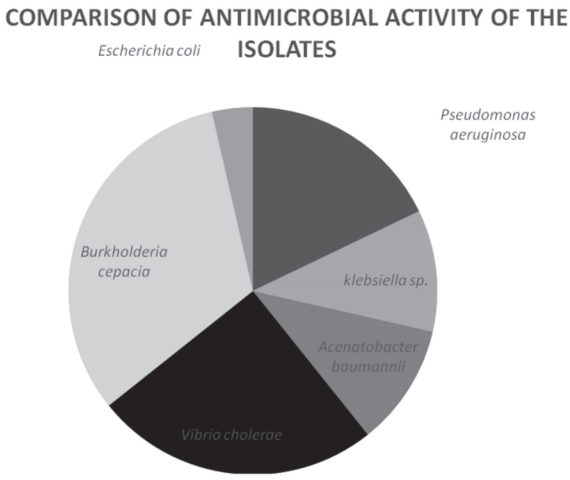

Fig. 3. Showing percentage of isolates resisting common human pathogens

CONCLUSION

After qualitative and quantitative study of the isolates it was observed that they could produce plant growth hormones such as Auxin and Gibberellin. They could even solubilize phosphate and fix atmospheric nitrogen. Thus, from this study it can be clearly concluded that the isolated endophytes can be beneficial to the host.

We have even studied the antimicrobial activity of the bacterial endophytes and they are seen to significantly resist common pathogenic organisms. Thus, it can be further concluded that they can be an attractive source of antibiotics. Due to the increase in resistance to antibiotics from common sources such as soil, plant endophyte being an unusual source can be an attractive alternative.

Moreover, the nitrogen fixing and phosphate solubilizing ability can render the endophytes to be a potential source of bio fertilizer.

Rice (*Oryza sativa*) is the most important cereal crop in the world feeding more than 50% of the world's population. Most importantly it is a staple food for most part of the Indian subcontinent. Thus, increasing its yield is very important in order to match the rise in consumption. This must be achieved without the mass use of chemical fertilizer and pesticides, which may cause environmental pollution and negatively influence human health. Thus, application of endophytic bacteria having beneficial characteristics to the cultivation of rice as well as other plants and to some extent humans is also crucial.

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