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Short Communication

Isolation and characterization of plant growth promoting bacteria from wheat-sugarcane cropping system

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An increasing demand for low-input agriculture has resulted in a greater interest in soil microorganisms which are able to enhance plant nutrition and health, and to improve soil quality. Microorganisms present in rhizosphere can be beneficial to plant by producing plant growth-promoting substances such as indole acetic acid (IAA),or being antagonist to pathogens by siderophore and HCN production or by increasing the availability of nutrients such as nitrogen, phosphorus, iron and zinc. Soil microorganisms not only have the capability to produce compounds that possess plant growth promoting activity which leads to high yield but also inhibit phytopathogens. Among the soil microorganisms, bacteria and fungi have received considerable attention as plant growth-promoters and biocontrol agents. Plant growth-promoting and antagonistic potential of *Pseudomonas fluorescens*, *Trichoderma harzianum*, *Penicillium citrinum*and *Bacillus* spp. have been reported by several researchers (Haiyambo *et al.*, 2015). Actinomycetes represent a high proportion of the soil microbial biomass and have the capacity to produce a wide variety of antibiotics and extracellular enzymes. Several strains of actinomycetes have also been found to be possessing plant growth promoting potential and also able to protect plants against various diseases. Actinomycetes are important producers of bioactive compounds such as chitinase, -1,3-glucanase and various antifungal substances. Actinomycetes also known produce extracellular active compounds such as IAA, phosphate solubilizing substances and intracellular siderophores, which induce germination of seeds and their growth in several crops including wheat (Jog *et al.,* 2014). Among actinomycetes, *Streptomyces* spp. has been investigated predominantly, mainly because of their dominance and the ease of isolation and their ample capacity for production of secondary metabolites, such as antibiotics and extracellular enzymes (Gopalakrishnan *et al.*, 2013). Hence, the present study was planned to screen out the plant growth promoting activity in *Streptomyces sp*.

The soil samples were collected from wheat-sugarcane system and P3 were isolated from the soil sample collected from drought area field of ICAR-Indian Institute of Sugarcane Research, Lucknow. Isolation was carried out by employing serial dilution plate technique and incubated at 37°C for 24-48 hours. The morphological characteristics were also studied.

The genomic DNA was extracted from 2ml of overnight culture. The Genomic DNA was confirmed by running the sample on the 0.8% agarose gel electrophoresis and examined under UV-Trans illuminator (Mauti*et al.*, 2013). The 16S Forward Primer:5'-CMGSCVTDACACAWGCHAGYC-3' and 16s Reverse Primer: 5'-GGCGSMTGWGTNCAAGSV-3' was used for amplification of 16S rDNA sequence.

The isolated Streptomyces sp. was first screened for phosphate solubilization on a selective media i.e.

Pikovskaya's agar medium (PAM) and Production of siderophore by antagonists is assayed by plate assay using Chrome Azural S (CAS) and tertiary complex CAS / Fe hexadecyltrimethyl ammonium bromide served as an indicator. Development of yellow–orange halo around the growth was considered as positive for siderophore production (Tokala et al., 2002).The IAA and ammonia production was estimated according to Yadav et al., 2010. HCN production was tested by the method of Bakker and Schippers (1987). The culture was checked for their Zn solubilizing ability based on halo formation on solid basal medium supplemented with 0.1% ZnO according to the method of Sarvanan et al., (2003).

Morphological and molecular characterization of Streptomyces sp.: In particular, the group Streptomyces shows dry, smooth or hairy colonies with airborne mycelium of different colour. The colonies also produced soluble pigments of various colours. However, these phenotypic characteristics depend on the composition of the culture medium. The colonies of Streptomyces sp. in the present study was found to be white colonies which is firmly adhered to the solid growth medium and have aerial mycelium, with no coloured pigmentation. The emergence of new powerful tools of systematic bacteriology has changed proteobacterial classification over the last few years. The identification of bacteria at the species level can be confirmed by molecular methods and use of 16srRNA sequencing.

The use of 16S rRNA gene sequencing to study bacterial phylogeny and taxonomy has been by far the most common genetic marker used primarily because: i) its presence in all bacteria, often existing as a multigene family, ii) the function of the 16S rRNA gene is stable over much of evolutionary time, suggesting that sequence changes can be an accurate measure of the lapse of time, iii) the 16S rRNA gene (1,500bps) is large enough for information purposes. The gene sequence of 16S rRNA can mark evolutionary distance and relatedness of organisms. Thus comparison of 16S rRNA gene sequences has emerged as a preferred genetic technique for bacterial identification. 16S rRNA gene sequence analysis can be used to identify poorly described, rarely isolated or phenotypically aberrant strains and can be routinely used for identification of bacteria leading to the recognition of novel bacteria and non-culturable bacteria (Mauti *et al.*, 2013). The obtained aligned sequence data comprises of 1217bp and it showed that P3 isolate was found to be most similar to *Streptomyces sp. R8-11* (Sequence ID: dbj|AB841019.1) and the next closest homologue was found to be *Streptomyces coeruleorubidus strainKSRO2*1(Sequence ID: gb|JF682781.1).Homology tree based on sequence alignment of 16S rDNA of the P3 isolate was also made (Figure 1).

Phylogenetic analysis and elucidation of rRNA Secondary Structure: In order to understand the significance in predicting the stability of chemical or biological molecules or entities of Streptomyces coeruleorubidus, RNA secondary structure prediction has been performed. The 16S RNA gene sequence obtained was used to deduce the secondary structure of RNA using ViennaRNA Web Services (Figure 2). The free energy of secondary structure rRNA of Streptomyces coeruleorubidus was -492.30 kcal/

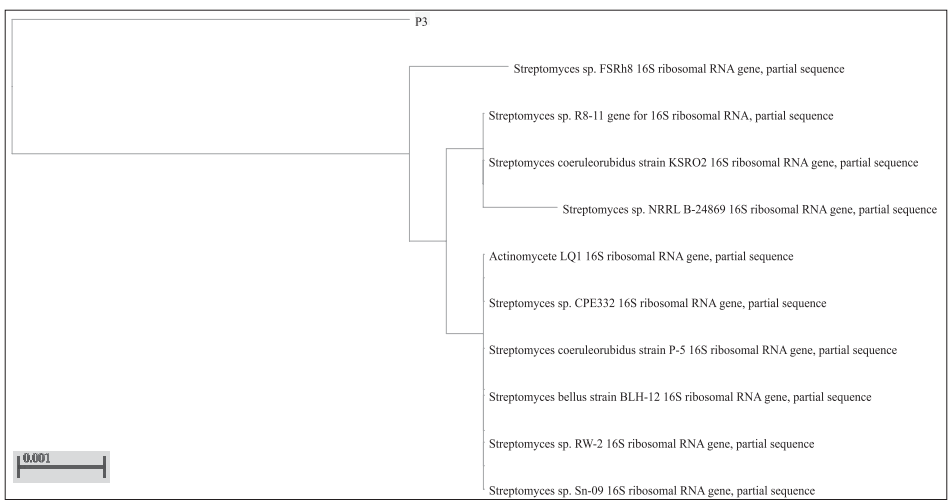

Fig. 1 Phylogenetic tree of *Streptomyces sp.*

mol. Mountain Plot was also drawn, this representation helps depict the hierarchical organization of RNA secondary structure, as nested helices translate into stacking mountains, easing the visual segmentation into domain. Here the sequence is drawn linearly, but this representation also presents, at each position i, the number of base-pairs nesting the position, i.e. involving bases respectively before and after i. In this setting, helices give rise to mountains while terminal loops translate into peaks.

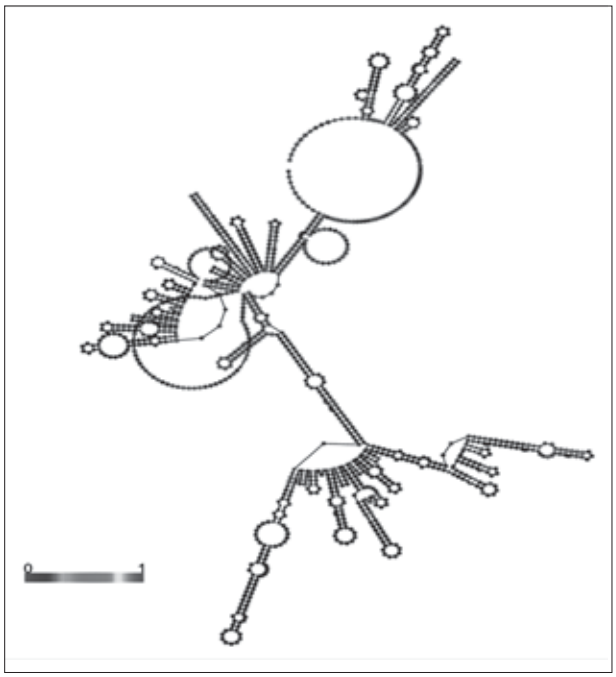

Fig. 2 Secondary structure of 16S rRNA of *Streptomyces sp*

In-vitro screening of Streptomyces sp. for its plant growth promoting activities: A group of rhizosphere bacteria that exert beneficial effect on plant growth is referred as Plant growth promoting rhizobacteria (PGPR). Plant growth promoting rhizobacteria are free living, soil-borne bacteria, which enhance the growth of the plant either directly or indirectly. The direct mechanism involve nitrogen fixation, phosphorus solubilisation, HCN production, production of phytohormone such as auxin, cytokinin and gibberellins and lowering of ethylene concentration,PGPR may induce plant growth promotion by direct or indirect modes of action. The result of the study showed that *Streptomyces sp*. was found positive for plant growth promoting activity (phosphate solubilisation, ammonia production, IAA production, siderophore production and HCN production. Phosphate solubilizing microorganisms convert the insoluble phosphates into soluble forms through the processes of acidification, chelation and exchange reactions. Therefore the application of P solubilizing microorganisms is a promising approach for plant growth (Vassilev*et al.*, 2006). In the study, *Streptomyces sp*. showed phosphate solubilising activity. The culture grown on Pikovskaya's agar medium showed the zone of clearing of 20 mm. The production of siderophores is a mechanism or strategy used by PGPR for rhizosphere colonization competence. The production of these siderophores has been linked to the disease suppression potential of PGPRs. The siderophores produced by PGPRs have a very strong affinity for ferric ions are secreted during growth under low iron conditions due to which ferric ions becomes unavailable for pathogens (Tokala *et al.*, 2002). Isolated *Streptomyces sp*. in the present study produce siderophores as yellow coloured 14 mm zone was found on basal medium supplemented with chrome azural S dye (CAS). PGPR can exhibits a variety of characteristics responsible of influencing plant growth. The common traits include production of plant growth regulators (auxin, gibberellins, ethylene etc). Indole Acetic Acid is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR (Yadav *et al.*, 2010). Isolated *Streptomyces sp*. when grown in basal medium containing 0.1% DL-tryptophan and its extracellular filtrate were then treated with orthophosphoric acid and Salkowski reagent which results into the pink colour which is the clear indication of the production of IAA by the culture. After quantification it was calculated that *Streptomyces sp*. produced 10.9 µl/ml of IAA. The accumulation of ammonia in soil may increase in pH creating alkaline condition of soil at pH 9-9.5. It suppresses the growth of certain fungi and nitrobacteria due to it potent inhibition effect. It also upset the microbial community and inhibits germination of spores of many fungi (Yadav *et al.*, 2010). The test carried out through Nessler's reagent showed that isolated culture has the potential to produce ammonia. HCN inhibits the electron transport thereby the energy supply to the cell is disrupted leading to the death of the organism. It inhibits proper functioning of enzymes and natural receptors by reversible mechanism of inhibition. It is also known to inhibit the action of cytochrome oxidase. HCN is produced by many rhizobacteria and is postulated to play a role in biological control of pathogens (Bakker and Schippers,

1987). Isolated culture in this study also showed HCN production which indicates that these are potent PGPRs. Zinc-solubilizing microorganisms can solubilize zinc from inorganic and organic pools of total soil zinc and can be utilized to increase zinc availability to plants(Sarvanan*et al.*, 2003). The *Streptomyces sp.*in the study is capable of solubilising zinc as clear zone size of 20 mm was observed on basal medium supplemented with 0.1% ZnO.

It is concluded that, Actinomycetes most commonly inhabit the rhizosphere and known to improve the availability of nutrients and minerals, synthesized plant growth regulators, and specially, they are capable of inhibiting phytopathogens. They have been studied for phosphate solubilization, siderophores production, and nitrogen fixation. Based on the present study, it can be concluded that the *Streptomyces sp*. isolate could be the potential candidate for the further study as agents or in the development of consortia to promote plant growth and control of plant diseases in wheat as well as in sugarcane. The elucidation of rRNA secondary structure of the strain is first time documented. To the best our knowledge, the plant growth promoting activity of *streptomyces coeruleorubidus* is also first time reported

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