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ENZYME ANALYSIS OF ENDOPHYTIC NEW *STREPTOMYCES* SP.VIJI10 ISOLATED FROM VELAMEN ROOTS OF ORCHID PLANT *VANDA SPATHULATA* (L.) SPRENG

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ABSTRACT

The present study deals with enzyme analysis of endophytic new *Streptomyces* sp.viji10 isolated from the velamen roots of *Vanda spathulata* is an epiphytic orchid plant. The plant root samples were collected from Alagar kovil Hills, Madurai district, Tamilnadu state, India. An endophytic *Streptomyces* sp was isolated by using NA (Nutrient Agar) medium; it was then identified by 16S rRNA gene sequencing analysis. The endophytic actinomycete culture to produce different enzymes was analyzed. The endophytic actinomycete culture has the ability to produce cellulase enzyme (except amylase, laccase, lipase and protease). The result of the study suggested that endophytic *Streptomyces* sp associated with velamen roots of *Vanda spathulata* plant are potential source of cellulase enzyme production.

Keywords: Enzyme analysis, *Streptomyces* sp.viji10, *Vanda spathulata,* Velamen roots

INTRODUCTION

Orchids are one of the largest families of flowering plants worldwide, with over 35,000 species in some 750 genera. The highest number of orchid species occurs in the tropical zone, and diversity decreases with increasing distance from the equator (Brundrett et al., 2001).

Actinomycetes are important soil microorganisms and are best known for their ability to produce antibiotics. Evidence indicates that actinomycetes are quantitatively and qualitatively important in the rhizospere, where they influence plant growth and protect plant roots against invasion by root pathogenic fungi (Crawford et al., 1993). Some actinomycetes are endophytes known to colonize the interior of healthy plants. Frankia strains, symbionts of actinorhizal plants, can induce N_2 fixing root nodules on certain nonleguminous plants and were identified as actinomycetes in 1964 (Benson and Silvester, 1993). These are mostly found living as saprophytes in the soil, but lately some species have been described in plant tissue and the rhizosphere of plant roots (Alam et al., 2010). It is now apparent that these filamentous bacteria also occur in living tissues of certain higher plants as endophytes and may serve as sources of novel bioactive compounds, as majority of them are untapped (Zin et al., 2007).

Endophytic Streptomycetes were isolated from surface -sterilized roots of 28 plant species in northwestern Italy (Sardi et al., 1992). The *Actinomycetes* are Gram positive bacteria having high G+C (>55%) content in their DNA. Actinomycetes were originally considered to be an intermediate group between bacteria and fungi but now are recognized as prokaryotic organisms. The majority of actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. Several species of *Streptomyces* genus produces bioactive molecules like antibiotics, pigments and many extracellular enzymes as glucose isomerase, amylase, cellulases and proteases (Claudia and Gabriela, 2001).

Rhizosphere is an admirable region which provides shelter to many types of living organisms. The organisms survives in the rhizosphere region gets enriched by plant root exudates. Rhizosphere microorganisms opportunistically enter into plant roots utilizing wounds and natural openings. Such entry of microorganisms is aided by the production of lytic enzymes. However the lytic enzymes produced by these microorganisms might also contribute to more efficient penetration and colonization. Such endophytic microorganisms are indigenous to most plant species and

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colonizing the plant tissue. The endophytic microorganisms occupy a relatively unexplored site in microorganism isolation so they can represent a new source in obtaining more enzymes with different potentialities (Rajesh Kannan et al., 2012). The aim of the present study deals with enzyme analysis of endophytic new *Streptomyces* sp. isolated from velamen roots of *Vanda spathulata*.

MATERIALS AND METHODS

Collection of samples

An orchid plant of *Vanda spathulata* velamen root samples were collected from Alagar Kovil Hills, Madurai District, Tamil Nadu state, India. The root samples were collected during the month of January 2013 at day time. The sample was utilized within 6 "h" of collection.

Surface sterilization and Isolation of endophytic actinomycetes

The samples were washed in running tap water for 10 minutes to remove soil particles and adhered debris. The root samples were cut longitudinally before inoculation. Then the root samples were washed thoroughly with sterile distilled water and air dried under laminar air chamber. Samples were immersed in 10 mL distilled water for 3 minutes; then the samples were placed in 1 % aqueous solution of $HgCl_2$ for 3 minutes. Later the samples were rinsed in distilled water for 1 minute. The cut surface of the root samples were placed on the medium surface. The growth of *Streptomyces* sp. colonies from the surface of the root samples on the Nutrient agar medium was observed. The individual colonies pure cultured on nutrient agar plates and were incubated at room temperature (28 ± 2°C) for 5-20 days.

16S rRNA Analysis

The active strain identified as *Streptomyces* sp.viji10 isolated from velamen roots of *Vanda spathulata* was carried out by using 16S rRNA partial gene sequencing and phylogenetic tree analysis.

Screening for enzymes

Extra cellular enzymes assay were conducted to investigate the production of enzymes by the endophytic *Streptomyces* sp. It was assessed by digestion of suspended or dissolved substrate in agar plates after inoculation with 3 mm mycelia plugs and incubation for 3-5 days at 37 ℃. The clear zone was used as a measurement of the amount of enzyme production with modifications (Kavya Deepthil et al., 2012).

Amylase

Amylase enzyme activity was assessed by growing the endophytic *Streptomyces* sp. on glucose yeast extract peptone agar medium (GYP) (glucose-1g, yeast extract-0.1g, peptone-0.5g, agar 16g, distilled water 1000mL and pH 6) containing 1% soluble starch. After 5 days incubation, the plates with actinomycete colony were flooded with1% iodine in 2% potassium iodide. The appearance of clear zone surrounding the colony was considered positive for amylase enzyme.

Cellulase

Yeast extract peptone agar medium supplemented with Na-carboxymethyl cellulase was used. The *Streptomyces* sp. was cultured and kept for incubation. After incubation, the plates were flooded with 2 % aqueous Congo red and destained with 1 M NaCl for 15 minutes. The clear zone around the colony indicated the cellulase activity.

Laccase

Glucose yeast extract peptone agar (GYP) (glucose-1g, yeast extract-0.1g, peptone-0.5g, agar 16g, distilled water1000 mL and pH 6) medium amended with 1-napthol, 0.005% was prepared and *Streptomyces* sp. culture was inoculated and kept for incubation. On oxidation of 1-napthol by laccase, the medium changes from clear to blue.

Lipase

The *Streptomyces* sp. culture was grown on peptone agar (peptone-10.g, sodium chloride-5g, agar-14g, distilled water 1000 mL and pH 6) medium supplemented with Tween 20. A clear zone around the colony indicated the presence of lipase enzyme.

Protease

The *Streptomyces* sp. culture was grown on Glucose yeast extract peptone (GYP) (glucose-1g, yeast extract 0.1g, peptone 0.5g, agar 16, distilled water1000mL and pH 6) agar medium (glucose-1g, yeast extract 0.1g, peptone 0.5g, agar 16 g, distilled water 1000mL and pH 6) amended with 0.4% phenol and adjusted with pH 6. After 3 days of incubation, plates were

flooded with saturated aqueous ammonium sulphate. The undigested phenols were precipitated with ammonium sulphate and digested area around the colony appeared as a clear zone.

RESULTS & DISCUSSION

Vanda spathulata is an orchid belongs to the family Orchidaceae. It grows on tall and big tree (epiphytic plant), deriving moisture and minerals through its velamen roots hold on the substratum (the tree) (Fig 1).

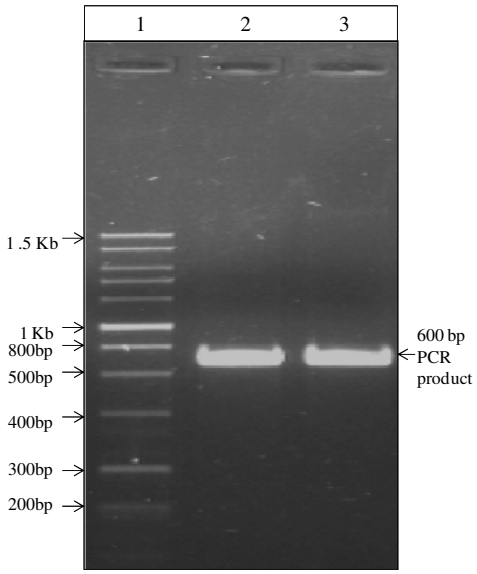
16S rRNA analysis

Endophytic new *Streptomyces* sp. viji10 isolated from velamen roots of *Vanda spathulata* plant. It is a gram positive, filamentous bacterium which produced well developed hyphae with branches and this type of genus belongs to the family Streptomycetaceae. Mostly endophytic actinomycetes are symbiotic association with their host. Endophytic new *Streptomyces* sp.viji10 was isolated by using NA (Nutrient Agar) medium. It was then identified by PCR amplification, 16S rRNA gene sequencing and phylogenetic tree analysis. This gene sequence was deposited in Genbank at NCBI, EMBL and DDBJ Accession number:

KF312214 (Fig 2, 3, 4). Similar result was found in the endophytic actinomycetes isolated from surface-sterilized root of healthy wheat plant, belonging to *Streptomyces*, *Microbispora*, *Micromonospora*, and *Nicardia* (Coombs and Franco, 2003). The total of 246 strains of actinomycetes isolated from plant origin belonging to *Streptomyces* (97 strains), *Microbispora* (57 strains), *Micromonospora* (18 strains), *Actinomonodura* (4 strains), *Nicardia* (23 strains) (Okazaki, 2003).

Fig. 1: An Epiphytic orchid plant *Vanda spathulata* (L.) Spreng.

Fig. 2: PCR Amplification



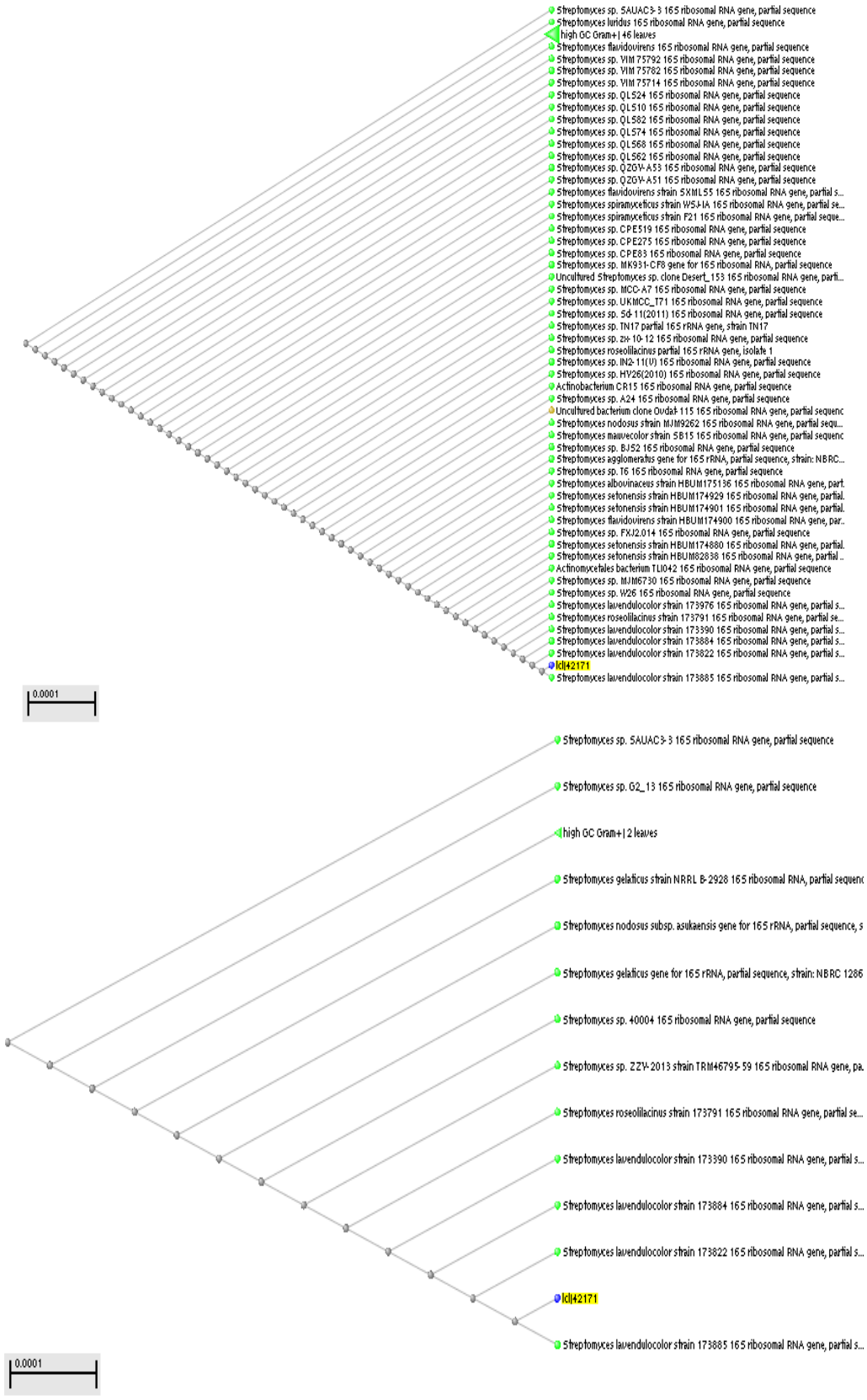


Fig. 3: Phylogenetic tree analysis

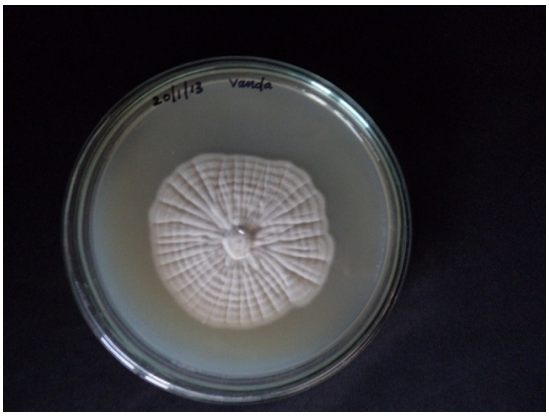


Fig. 4: New *Streptomyces* sp.viji10

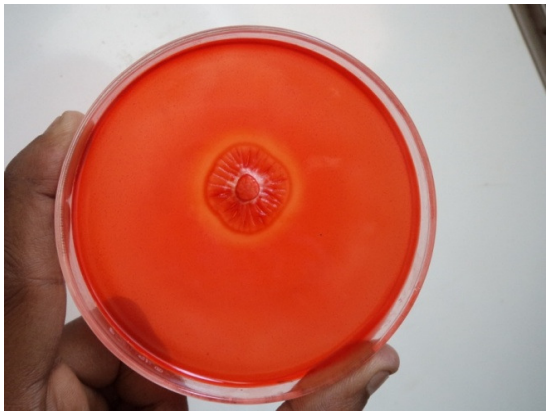


Fig. 5: New *Streptomyces* sp.viji10 produce Cellulase enzyme

Cellulase enzyme activity

For cellulase screening, the isolate were grown on yeast extract peptone agar medium supplemented with Na-carboxymethyl cellulose. The endophytic *Streptomyces* sp. was cultured and kept in incubation for 4-5 days. After incubation, the plates were flooded with 2 % aqueous Congo red and destained with 1 M NaCl for 15 minutes. Here plate yellow color clear zone around the colony indicating cellulase production. But endophytic *Streptomyces* sp.viji10 did not show any amylase, laccase, lipase and protease activity (Fig 3). Our results similar to the endophytic *Streptomyces* strains were screened for chitinase production and inhibition of fungi and oomycete phytopathogens. Most strains produced a high concentration of chitinase and inhibited the phytopathogens (Quecine et al., 2008). Purified a bioactive compound from endophytic *Streptomyces violaceusniger* that showed a strong antagonism towards various wood-rotting fungi, and chitinase enzymes were associated with this inhibition. In general, the higher chitinase activity was correlated with higher fungal inhibition. For this reason, chitinolytic *Streptomyces* strains are a likely choice as potential biological control agents (Shekhar et al., 2006). The *Streptomyces* sp. from coringa mangrove soils for enzyme production and antimicrobial activity (Kavya Deepthil et al., 2012).

CONCLUSION

Endophytic actinomycetes inhabit tissues of a wide variety of native and cultured crop plants. The endophytic presence of some actinomycetes may play important role in plant development and health because of their role in nutrient assimilation and in secondary metabolite production. This study presented the cellulase production of the endophytic new *Streptomyces* sp.viji10. The reported enzyme may have wide industrial application.

REFERENCES

Alam MT, Merlo ME, Takano E and Breitling R, 2010. Genome based phylogenetic analysis of *Streptomyces* and its relatives. Mol. Phylogenet. Evol, 54: 763-77.

Benson DR & Silvester WB, 1993. Biology of Frankia strains actinomycete symbionts of actinorhizal plant. Microbiological Reviews, 57: 293–319.

Brundrett M, Sivasithamparam K, Ramasamy M, Krauss S, Taylor R, Bunn E, Hicks A, Karim NA, Debeljak N, Mursidawati S, Dixon B, Batty A, Bower C, Brown A, 2001. Orchid conservation techniques manual, first international orchid conservation congress-training course. Plant Science, King Park & Botanic Garden, Perth.

Claudia Popa, Gabriela Bahrim, 2001. Streptomyces Tyrosinase: Production and

practical applications. Innovative Romanian Food Biotechnology, Vol.8: 1-7.

Coombs JT, Franco CMM, 2003. Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl. Environ. Microbial. 69: 5603-5608.

Crawford DL, Lynch JM, Whipps JM & Ousley MA, 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Applied and Environmental Microbiology, 59: 3899–3905.

Kavya Deepthil M, Solomon Sudhakar M and Nagalakshmi Devamma M, 2012. Isolation and screening of Streptomyces sp from coringa mangrove soils for enzyme production and antimicrobial activity. International Journal of Pharmaceutical, Chemical and Biological Sciences, 2(1): 110-116.

Okazaki T, (2003). Studies on actinomycetes isolated from plant leaves. In selective isolation of Rare Actinomycetes. National Library of Australia. 102-121.

Quecine MC, Araujo WL, Marcon J, Gai CS, Azevedo JL, izzirani-Kleiner AA, 2008. Chitinolytic activity of endophytic *Streptomyces* and potential for biocontrol. Letters in Applied Microbiology 47: 486-491.

Rajesh Kannan V, Sumathi CS, Balasubramanian V, Ramesh N and Rajesh P, 2012. Exploration of Defense Mechanisms on Endophytic Microbial Isolates from Selected Traditional Medicinal Plants. Int J Med Res, 1(6): 315-320.

Sardi P, Saracchi M, Quaroni S, Petrolini B, Borgonovi G.E & Merli S, 1992. Isolation of endophytic Streptomyces strains from surface-sterilized roots. Applied and Environmental Microbiology, 58: 2691–2693.

Shekhar, N., Bhattacharya D, Kumar D and Gupta, RK, 2006. Biocontrol of wood-rotting fungi with *Streptomyces violaceusniger* XL2. Can J Microbiol, 52: 805–808.

Zin NM, Sarmin NIM, Ghadin N, Basri DF, Sidik NM, Hess WM., Strobel GA, 2007. Bioactive endophytic *streptomycetes* from the Malay Peninsula. FEMS Microbiology Letters: 274: 83-88.