International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Report of Endophytes in Neem Tree of Bhopal

Roopali Jain[1], Adarsh Lalit[2]

[1, 2]Mansaroval Global University, Sehore
[2]Email: *adarsh.xcell[at]gmail.com*

Abstract: ***Neem leaf is a perfect tissue for habitat of endophytes. This can helps in elongation as well as in cell division, to form root hair and to make short root length. This is also helps to increase the nutrient absorption capability of plant. Total six actinomycetes were isolated from neem plant leaves of three different locations of Bhopal viz. Chetak Bridge, BHMG (Barkheda Haat and Market Ground), BHEL Ground.***

Keyword: Neem, Bhopal, Endophytes, Genomic DNA, Biochemical, Morphological

1. Introduction

Neem tree belongs to family Meliaceae and widely found in abundance in India, Bangladesh, Pakistan and Nepal. Taxonomic position of *Azadirachta indica* (neem) is following (Girish, 2008). Microbes residing within plant tissues like in leaves, roots or stems tissue are endophytes (Oldroyd *et al.*, 2011; Turner *et al.*, 2013; Andreote *et al.*, 2014). Different endophytes have several roles as per their interaction of plant species. Some microbes show pathogenic interaction with plants (Andreote *et al.*, 2014). Some are playing a key role in growth of plant, maintain their health and development, while, some are neutral (Mendes *et al.*, 2013; Philippot *et al.*, 2013). Non - pathogenic endophytes which are associated to plants without causing any disease were first reported by De Bary in1866. This study was conducted to classify the endophytic actinomycetes associated with leaf of neem plants.

2. Materials and Methods

Four healthy plants of *Azadirachta indica* A. Juss (Neem) were identified in different locations of Bhopal. Leaves of each plant were collected in a sealed sterile bag and brought to laboratory for this study. Collected healthy leaves of neem were surface sterilized by following the method of Strobel *et al.*, 1996 with some modification. Morphological characteristics viz. colony growth, presence or absence of aerial mycelium, colony color, presence of wrinkles and furrows, pigment production etc. in reference to Barnett, 1992 by lactophenol blue stains under microscope with 40X resolution were performed on all isolated endophytes. Functional characterization was done on the basis of certain assays like Amylase (Peterson and Bridge, 1994), Cellulase (Teather and Wood, 1982), Pectinase (Peterson and Bridge, 1994), Xylanase (Pointing, 1999), Siderophore (Schwyn and Neilands, 1987), Lipase activity (Sierra, 1957), Protease activity (Shakeri *et al.*, 2007). Genomic DNA of antagonistic endophytic actinomycetes was extracted and amplification of 16S rDNA region using appropriate primers was performed. BLAST was done and analysis on the basis of result obtained (Sai *et al.*, 1987).

3. Results and Discussion

Thousands of microbes are associated to a single plant which is categorized as epiphytes and or endophytes. Different endophytes have several roles as per their interaction of plant species. Some microbes show pathogenic interaction with plants (Andreote *et al.*, 2014). Some are playing a key role in growth of plant, maintain their health and development, while, some are neutral (Mendes *et al.*, 2013; Philippot *et al.*, 2013). Actinomycetes are belongs to phylum actinobacteria which have mycelium and form spores. This is looks like a fungus (Chaudhary *et al.*, 2013; Barka *et al.*, 2016). Actinomycetes are sometimes considered as transitional forms between the fungi and bacteria but they are counted in bacteria (Barka *et al.*, 2016). Actinomycetes are producing many phytochemical bioactive compounds, reported their wide medicinal importance (Gayathri and Muralikrishnan, 2013; Singh and Dubey, 2015: Gouda *et al.*, 2016). Total six unidentified actinomycetes were isolated from neem plant leaves of three different locations of Bhopal viz. Chetak Bridge, BHMG (Barkheda Haat and Market Ground), BHEL Ground. Samples were coded as A for Chetak Bridge, B for BHMG and C for BHEL Ground. They were identified by following their morphological, qualitative enzyme activities, quantitative enzyme activities and molecular characteristics and reported the isolates were species of *Cladosporium*, *Nigrospora oryze*, *Streptomyces*, *Acremonium*, *Fusarium* and *Curvularia*. Total 63 colonies were isolated in this study and did that pure. Out of which 3, 3 and 4 colonies were identified as *Cladosporium* species in samples of Chetak Bridge, BHMG and BHEL Ground respectively.3, 4 and 4 colonies were identified as *Nigrospora oryzae* species in samples of Chetak Bridge, BHMG and BHEL Ground respectively.2, 4 and 4 colonies were identified as *Streptomyces* species in samples of Chetak Bridge, BHMG and BHEL Ground respectively.3, 4 and 4 colonies were identified as *Acremonium* species in samples of Chetak Bridge, BHMG and BHEL Ground.3, 4 and 3 colonies were identified as *Fusarium* species in samples of Chetak Bridge, BHMG and BHEL Ground respectively.3, 4 and 4 colonies were identified as *Curvularia* species in samples of Chetak Bridge, BHMG and BHEL Ground respectively. In sum isolates of *Streptomyces* species was least reported in Chetak Bridge samples. *Cladosporium* species was least reported in BHMG samples. Isolates of *Fusarium* species was least reported in BHEL Ground samples. Average colony frequency was reported 95.37% with colony frequency of *Cladosporium* species in collected samples of Chetak Bridge, BHMG and BHEL Ground were 100, 75 and 100 % respectively; Colony frequency of *Nigrospora oryzae*

species in collected samples of Chetak Bridge, BHMG and BHEL Ground were reported 100%.66.67% Colony frequency of *Streptomyces* species was reported in samples of Chetak Bridge, whereas 100% Colony frequency of *Streptomyces* species was reported in samples of rest two sampling sites. Colony frequency of *Acremonium* species in collected samples of Chetak Bridge, BHMG and BHEL Ground were reported 100%.75% Colony frequency of *Streptomyces* species was reported in samples of BHEL Ground, whereas 100% Colony frequency of *Fusarium* species was reported in samples of rest two sampling sites. Colony frequency of *Acremonium* species in collected samples of Chetak Bridge, BHMG and BHEL Ground were reported 100%. Isolates of *Streptomyces* species was least reported in Chetak Bridge samples with 66.67% colony frequency. *Cladosporium* species was least reported in BHMG samples with 75% colony frequency. Isolates of *Fusarium* species was least reported in BHEL Ground samples with 75% colony frequency. This was studied as per method of Raunker's class and reported that all the isolates comes under frequency class of 'E' only.

4. Conclusions

Total six actinomycetes were isolated from neem plant leaves of three different locations of Bhopal viz. Chetak Bridge, BHMG (Barkheda Haat and Market Ground), BHEL Ground. The collected neem leaves sample bare the colonization of different microbial species. Total 63 colonies were isolated and did that pure comprising of *Cladosporium*, *Nigrospora oryze*, *Streptomyces*, *Acremonium*, *Fusarium* and *Curvularia*.

References

[1] Andreote FD, Gumiere T, Durrer A (2014) Exploring interactions of plant microbiomes. Sci. Agric.71: 5280539.
[2] Andreote FD, Gumiere T, Durrer A (2014) Exploring interactions of plant microbiomes. Sci. Agric.71: 5280539.
[3] Barka EA, Vatsa P, Sanchez L, Gaveau - Vaillant N, Jacquard C, Klenk HP (2016) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol. Mol. Biol. Rev.80: 1 - 43.
[4] Chaudhary HS, Soni B, Shrivastava AR, Shrivastava S (2013) Diversity and versatility of actinomycetes and its role in antibiotic production. Int. J. Pharm. Sci.3: S83 - S94.
[5] De Bary A (1866) Morphologie und Physiologie der Pilze, Flechten und Myxomyceten, Vol.2. Leipzig: Hofmeister's Handbook of Physiological Botany.
[6] Gayathri P, Muralikrishnan V (2013) Isolation and characterization of endophytic actinomycetes from mangrove plants for antimicrobial activity. Int. J. Curr. Microbiol. Appl. Sci.2: 78 - 79.
[7] Girish K, S BS (2008) Neem, "A green treasure, " Electronic Journal of Biology.4: 102 - 111.
[8] Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance. Front. Microbiol.7: 1538.
[9] Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev.37: 634 - 663.
[10] Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev.37: 634 - 663.
[11] Oldroyd GED, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume - rhizobial symbiosis. Annu. Rev. Genet.45: 119 - 144.
[12] Paterson, R. R. M., Bridge, P. D.1994. Biochemical techniques for filamentous fungi. CAB International, London, UK.
[13] Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol.11: 789 - 799.
[14] Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol.11: 789 - 799.
[15] Pointing, S. B., Buswell, J. A., Vrijmoed, L. L. P., Jones, E. B. G.1999a. Extracellular cellulytic enzyme profiles of ligninolous mangrove fungi. Mycological Research, 103: 6, 696 - 700.
[16] Schwyn, B., Neilands, J. B.1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem, 160: 47 - 56.
[17] Shakeri, J., Howard, A.2007. Proteolytic activity and antibiotic production by *Trichoderma harzianum* in relation to pathogenicity to insects. Foster enzyme and Microbial Technology, 40: 961 - 968.
[18] Sierra, G.1957. Asimple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fattysubstrates. Antonie Van Leewenhoek Ned. Tijdshr Springer, Berlin, Heidelberg, Germany
[19] Singh R, Dubey AK (2015) Endophytic actinomycetes as emerging source for therapeutic compounds. Indo Global J. Pharm. Sci.5: 106 - 116.
[20] Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews.67 (4): 491 - 502.
[21] Teather, RM., Wood, P. J.1982. Use of congored polysaccharide interaction in enumeration and characterization of cellulytic bacteria from bovinal rumen. Appl. Environ Microbiol, 43: 777 - 780.
[22] Turner TR, James EK, Poole PS (2013) The plant microbiome. Genome Biol.14: 209.