ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF ENDOPHYTIC FUNGI WHICH ISOLATED FROM *SANSEVIERIA ROXBURGHIANA* PLANT

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KEYWORDS

Endophytes, endophytic fungi, *Sansevieria roxburghiana,* secondary metabolites, antagonistic activity.

ABSTRACT

Endophytic microorganisms are lives in inside of the plant tissues without causing any infections or diseases in the plant. It may be transferred from environment to plant and plant bodies by some natural actions. A plant associated fungi are rich biodiversity group of microorganisms that lives in plant with asymptomatic conditions. It promotes plant growth by production of secondary metabolites. It also enhances the plant resistant nature against of insects, pest, biotic, abiotic stresses and against of some bacterial plant pathogens. The endophytic fungi can synthesis medically important components that involved in cancer treatment, diabetic and some diseases which caused by multidrug resistant bacteria. In this study, endophytic fungi which isolated from *Sansevieria roxburghiana* plant. A single potential fungal strain selected based on the antagonistic activity, used it for mass cultivation and identify the potential antimicrobial activity against of pathogenic bacteria.

Introduction

Endophytic fungi are group of fungal communities that colonized in the intracellular and inter cellular tissues of the host. Moreover, these endophytic fungi do not cause any infections or diseases in the host plant. In recent years, the endophytic research has been increased over a period of time. Those research progresses are reported that, biodiversity of endophytes and its wide range of distribution in nature, multitype interaction with host microbes and host plants. Another remarkable mile stone was, the host associated fungal communities has been produce many secondary metabolites that support to induce plant growth and make protection of plants from the pathogens, insects and pests. The secondary metabolites have multidimensional interaction in the plants and its associated communities. The biosynthesis of secondary metabolites from the endophytes, support to manage many complications in the treatment process.

Plants or perennial types of plants are colonized by both bacteria and fungi as endophytic microorganisms (Demain, 2014). Those are lives with plant cycles and interfere with plant metabolism without causing of physical damages or morphological changes (Gouda et al., 2016). According to behaviors of endophytes, it can be categorized as facultative microbes or obligate microbes. Specifically, these endophytic microbes are making a colonization on plants in certain stages of plant cycle and associated with plant either outside or inside of the plants (Abreu-Tarazi et al., 2010). The endophytic microbes are entering into the plant on certain cycle but it will be stayed on it at entire life cycle of the plant. Those endophytic are proliferate in to the plant by vertical transmission or any other natural actions and the strains are using the plant productions for their own survival (Gouda et al., 2016 and Hardeim et al., 2008).

Endophytes are having such a unique alteration because they provide lot of cytotoxic components as well as anticancer components (Uzma et al., 2018). It has antibacterial substance, to promote plant growth (Poreda et al., 2021), it acts as biological controlling agent, it provides

resistant to both biotic and abiotic stress (Poreda et al., 2020; Cui et al., 2021, Poveda and Baptista 2021).

Sansevieria roxburghiana

It is stemless and evergreen perennial plant. It producing succulent, rigid leaves develops up to 75cm or more 25mm of wide angle. Leaves are strap shaped or narrowly lanceolate with subulate point. The leaf surface is smooth with sharp edges (eol.org). Fiber like material collected from the leaves not in root. In raceme, spik like flowering grows in 30-75 cm long (MBG, RHS 1992). This plant herbaceous perennial plant having stem and root stock. It mostly occurs in Eastern Coastal region of India. It also found in Sri Lanka, Indonasia, Myanmar, Trophical Africa and Bangladesh (Saravanan et al., 2015).

MATERIALS METHOD

Collection of plant sample

Fresh and healthy plant samples are collected from Yercaud hills located in Salem district, Tamilnadu state, India. The collected plant samples were placed in sterilized zip lock cover and transported in to laboratory for the isolation endophytic fungi.

Isolation of endophytic fungi

The plant's root shoot material separated and washed with tap water for removal of soil and dust. Again, it washed with distilled water. Then the material washed with 70% ethanol for 5 minutes for removal of contaminants in the surface of the plant. Then it transferred to tube which contain 3% sodium hypochloride for surface sterilization then it washed with double distilled water several times (Yousefi et al., 2018). Potato Dextrose Agar (PDA) media was used for the isolation process and it added with Gentamycin for the avoiding of unwanted microbial growth of bacteria in the plates. The sterilized plant material cut into small pieces in size of 5X5 mm and inoculated in to PDA for the isolation of endophytes and the inoculated plates are incubated in room temperature.

Morphological identification

The fungal endophytes were identified by macroscopic and microscopic observation. Lactophenol cotton blue staining method were used to identify the fungal structure which are isolated as endophytic fungi.

Antagonistic activity

The potential fungal strains were selected based on the antagonistic activity. Nutrient broth was prepared and pathogenic bacteria were inoculated. *Staphylococcus aureus* and *E.coli* were used for this antagonistic activity, those bacterial pathogens were collected from BP Tech Clinical Laboratory, Dharmapuri Tamilnadu. Muller Hinton agar was prepared and inoculated with pathogenic bacteria. The antagonistic activity was confirmed by agar well diffusion method (Nedialkova and Naidenova 2005), each isolated fungi was cultivated in PDA and 5-6 mm size of fungal blocks were taken from the cultivated plate then placed on the pathogenic bacteria inoculated plates. Later, the inoculated plates were incubated at $37^{O}C$ for 24 hours. The antagonistic activity was measured with transparent ruler in mm (millimeter). The zone of inhibition was taken in two different timing like 24^{th} hour of inhibition and 48^{th} hour of inhibition from the incubated timing. The strong potential isolate will be selected and used to further analysis.

Mass cultivation of fungi

The selected fungal strain used to identify its antimicrobial activity. 500 ml of Potato Dextrose Broth (PDB) was prepared and sterilized in autoclave. The sterilized broth was inoculated with selected endophytic strains and incubated in room temperature up to 15 days with shaker for microbial development. After the development of fungal strains, the fungal strains have been removed and the broth solution forwarded to another process.

Microbial extract

The collected sample was mixed together with equal volume of ethyl acetate then poured in separating funnel for the collection of microbial extract. The separation process may take up to three days. There are three layers has been formed, the middle and top of the layer combinedly collected. Collected material was placed in fume wood for evaporation of ethyl acetate and collect the secondary metabolites in crude form.

Antimicrobial activity of Microbial extract

Well diffusion method was performed for the identification of antimicrobial activity of collected microbial extract. *Staphylococcus aureus, Streptococcus pyogens, E.coli, Klebsiella sp.,* and *Pseudomonas sp.,* has been used for this study. The pathogenic bacterial strains are isolated from diabetic wound sample. The bacterial strains were inoculated into peptone water and incubated at 37^{O}C for 18 hours. MHA plates were prepared and inoculated with bacterial pathogens and 6 mm size well was created in the media. The well was filled with various concentration of microbial extract like 5µL, 10 µL, 15 µL and 20 µL of microbial extract with one control point. The processed plates were incubated at 37^{O}C for 24 hours. The zone of inhibition was measured with transparent ruler.

RESULT AND DISCUSSION

The collected plant material transferred to lab for the isolation endophytic bacteria from the plant sample.

Isolation and identification of endophytes

The collected plant material sterilized and inoculated for the isolation of endophytic fungi. There was no bacterial contamination because addition of gentamicin. There four different colors of colonies showed in the root segment inoculated plates and three different colors of colonies showed in the shoot segment inoculated plates. Developed fungal strains was subculture in another plates for purification process. The purified fungal strains observe under the microscope with lactophenol cotton blue staining. Based on the microscopic and macroscopic identification, six different strains identified in root segment and four different strains identified in shoot segment. Those strains were forwarded to antagonistic activity for identification potential strains.

Antagonistic activity

Pathogenic bacteria were inoculated in the nutrient broth and then inoculated in the MHA medium for antagonistic analysis. After the inoculation 5-6 mm size of fungal blocks were transferred and inoculated in MHA plates. The inoculated plates were incubated at 37^{O}C for 24 hours. **5 strains (EFR-3, EFR-4, EFS-2, EFS-5 and EFS-6) were produced 17 to 22 mm of** zone of inhibition and one strain(**EFS-1**) produced 24 mm of zone of inhibition. Rest of the strains were produced 12 mm and 14mm zone of inhibition, respectively.

Based on the observation, name of the endophytic fungal strain listed in below.

S.No	Segment	Isolate	Name of the isolate
1	Root	EFR -1	*Aspergillus flavus*
2		EFR -2	*Rhizopus sp.,*
3		EFR -3	*Fusarium sp.,*
4		EFR -4	*Beauveria sp.,*
5	Shoot	EFS -1	*Alternaria sp.,*
6		EFS -2	*Aspergillus niger*
7		EFS -3	*Rhizopus sp.,*
8		EFS -4	*Mucor sp.,*
9		EFS -5	*Colletotrichum sp.,*
10		EFS -6	*Pestalotia sp.,*

Table 1: Isolated fungi from *Sansevieria roxburghiana* plant

EFR-Endophytic Fungi from Root
EFS- Endophytic Fungi from Shoot

Mass cultivation

The maximum zone of inhibition produced by EFS-1 and it selected for mass cultivation process. The selected strain subculture in PDA plates, later it transferred to PDB medium. The inoculated flask was kept in room temperature on shaker for the mass cultivation. It was allowed up to 15 days for development of fungal strains and production of secondary metabolites.

Separation of microbial extract

Filtration method used for the removal of fungal cells and the liquid component mixed together with equal volume of ethyl acetate. The mixture of component has been poured in separating funnel and placed it for separation of secondary metabolites. The separated component has collected in container and placed in fume hood for the evaporation of excess of components.

Antimicrobial activity of microbial extract

Staphylococcus aureus, Streptococcus pyogens, E.coli, Klebsiella sp., and *Pseudomonas sp.,* has been used for the identification of antimicrobial activity of separated microbial extract. Moreover, all pathogenic bacteria controlled by microbial extract. Specifically, 23mm size of zone of inhibition produced on *Staphylococcus aureus* inoculated plate. 24mm size of zone of inhibition produced on *E.coli* inoculated plate. 19 to 22 mm of zone of inhibition produced on bacterial pathogens inoculated plate.

Conclusion

Ten different types of endophytic fungal strains have been isolated from *Sansevieria roxburghiana* plant. Particularly, the EFS-1 has been produced more zone of inhibition when it compares to others. The microbial extract which collected from the EFS-1 showed potential level of antimicrobial activity. It works equal to commercially available antibiotics.

Figure 1: Plant samples were collected from Yercaud hills located in Salem district, Tamilnadu state, India.

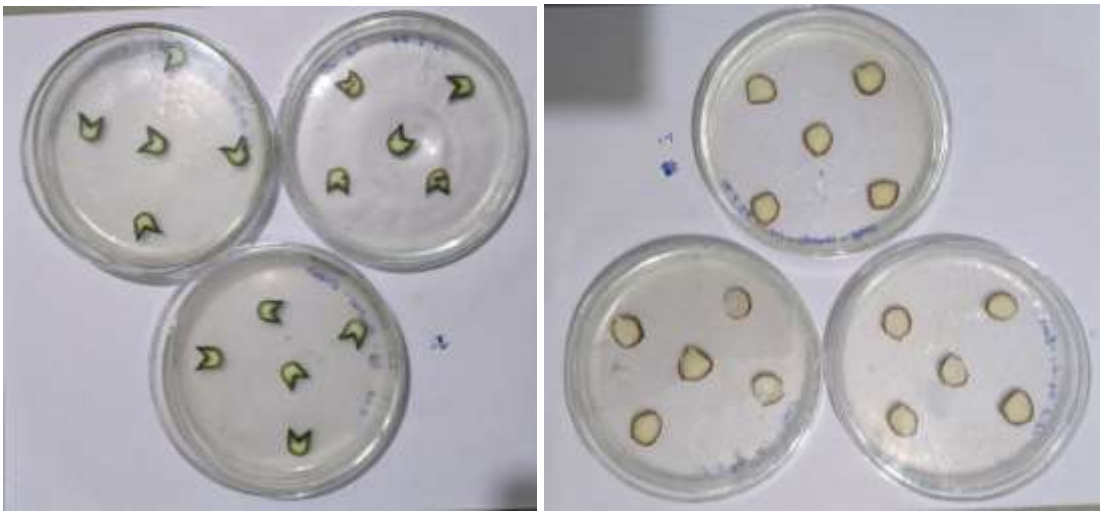

Figure 2: Shoot and root segment inoculated plates.

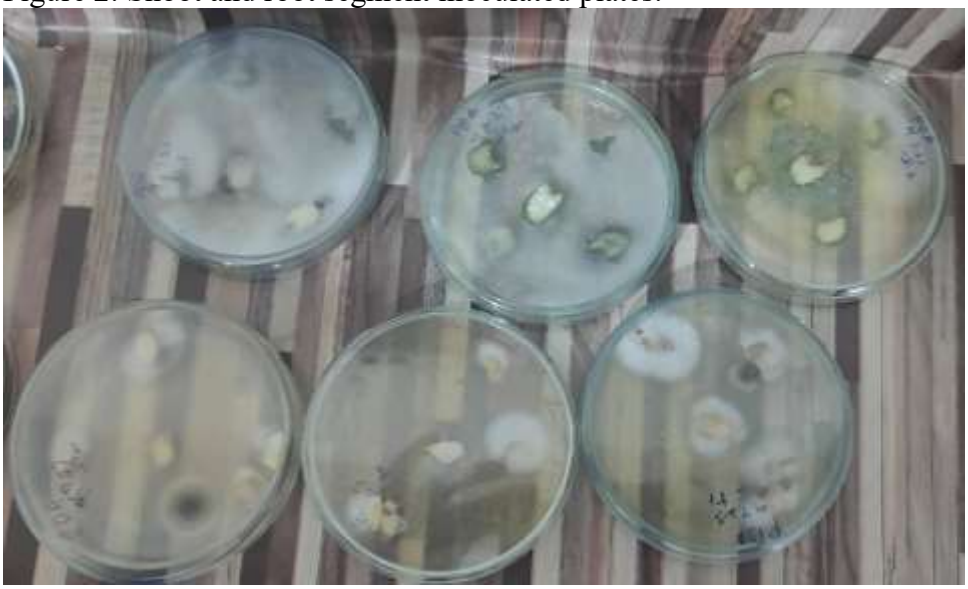

Figure 3: fungal strain developed in shoot and root segment inoculated plates

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