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BIODIVERSITY OF ENDOPHYTIC BACTERIA IN TWO DIFFERENT MEDICINAL PLANTS AND EVALUATION OF THEIR ENZYMATIC POTENTIAL.

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ABSTRACT
Endophytes are the microorganisms that live in intercellular spaces of host tissues without causing any harm to their host. In the present study twenty three endophytic bacteria were isolated from leaves of two different locally available medicinal plants. Out of twenty three isolated colonies, physiologically and morphologically different ten endophytic bacteria were screened for their different enzyme production ability. Selected ten bacterial strains have different gram positive and gram negative in nature. Eight out of ten endophytes were showed strong ability to produce asparginase enzyme, which is used as an antitumor drug, two endophytic strains showed amylase and three strains showed lipase producing ability. Enzyme production is one of the important ability of endophytes. The results of present study depicts that the biodiversity of endophytic bacteria in medicinal plants and their potential to produce industrially and pharmaceutically important enzymes.

KEY WORDS: Biodiversity, Endophytes, Enzymes, Medicinal Plants.

INTRODUCTION

Microbes that colonize and live into the internal tissues of plants without giving any harmful effects on their hosts were known as endophytes. Endophytes were mentioned first time by Bray in 19th century. Endophytes have been found in nearly all plant families (Jalgaonwala and Mohite, 2010). Individual plants may be host to one or more endophytes and many endophytes may colonize certain hosts, suggesting that there may be many undiscovered endophyte species (Selim *et al* 2011).

Most of medicinal plants are known as a harbor for endophytes and it is believed that these microorganisms are related to the production of medicinal drug and are able to produce bioactive compounds similar to their hosts. Therefore, due to the possibility of production of useful medicinal compounds by endophytic microorganism with different biotechnological potentials such as antitumor agents (*Pestalotiopsis microspore*, taxol), antifungal compound (*Cryptoriopsis criptocandina*, quercine), plant growth promoting factors, toxins and enzymes which provide numerous opportunities to discover new products with economic importance about endophytes. Enzyme production is one of the important ability of endophytes. During fungal infection, a range of hydrolytic enzymes are excreted which help them to promote host colonization. A set of specific enzymes mostly composed of proteases and carbohydrates are secreted by wide range of bacteria, fungi, yeasts, actinomycetes, algae and plants are found to be producers of enzymes. Yet, microbes are regarded to be better resources of producing enzymes due to their easier culturing, extraction and purification of enzymes (Masumi and Mirzaei, 2014). Among the widely used and important enzymes, we can mention asparaginase enzyme which is the first enzyme with antitumor activity studied comprehensively in human diseases. Interest in Asparaginase has grown continuously since this enzyme was found to have antitumor activity (Joshi *et al.*, 2016). This enzyme affects asparagine and hydrolyzes it into L-aspartic acid and ammonia (Kamble *et al.*, 2012). The potential of finding new drugs which may effective agent for treating newly developing diseases of human being have great importance in field of medicine (Joshi *et al.*, 2014).

Amylase is another widely used enzyme in industry, able to break starch molecules, with a wide application in food industry, fermentation and pharmaceutical industry. Therefore bacterial amylase can be useful in food and

pharmaceutical industries (Souza *et al*., 2010). The studies on producing amylase, especially in developing countries, are increasing due to ubiquity and simple food needs of this microorganism (Jain *et al.*, 2012). The present research work focused on isolation and characterization of endophytes from medicinal plants and evaluation of their potential to produce industrially and pharmaceutically important enzymes.

MATERIALS AND METHODS

Collection of plant material

The plant leaves of *Catharanthus rosus* and *Custard apple* were collected from the Dayanand College campus, Solapur and brought to the laboratory. The leaves were washed by tap water to remove dust particles followed by 70% ethanol and 1% $HgCl_2$. The leaves were kept 3 minutes in 1% $HgCl_2$ for surface sterilization.

Isolation of endophytic bacteria

The surface sterilized plant leaves were crushed in sterile distilled water and the resulting sample was used for isolation of endophytic bacteria. The sample was streaked on sterile nutrient agar plates and incubated at 37^0C overnight.

Characterization of isolated endophytes

Well distinct isolated colonies with different phenotypic characters were selected for colony characterization and Gram staining. These colonies were sub cultured on nutrient agar slants and preserve for further study.

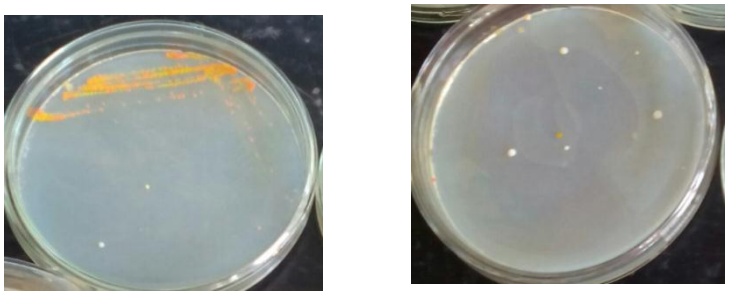
Enzyme production assay of endophytes

The isolated endophytic bacteria were screened for their different enzyme production ability. The isolates were inoculated on specific substrate containing agar plates for evaluation of their enzymatic potential. According to the method of Joshi *et al.*, 2014 nutrient agar with aspargin and phenol red was used for asparginase production assay, nutrient agar with starch was used for amylase assay and nutrient agar with egg yolk was used for lipase assay.

RESULTS AND DISCUSSION

Isolation of endophytic bacteria

Twenty three colonies from leaves of *Catharanthus rosus* and *Custard apple* were isolated on nutrient agar after overnight incubation (Figure 1).



(a) (b)

Figure 1. (a) Endophytes isolated from leaves of *Catharanthus rosus*
(b) Endophytes isolated from leaves of *Custard apple.*

Characterization of isolated endophytic bacteria

Phenotypically different 10 endophytes were selected for colony characterization (Table 1) and grams staining. These 10 colonies showed different morphological characters and Grams nature (Figure 2).

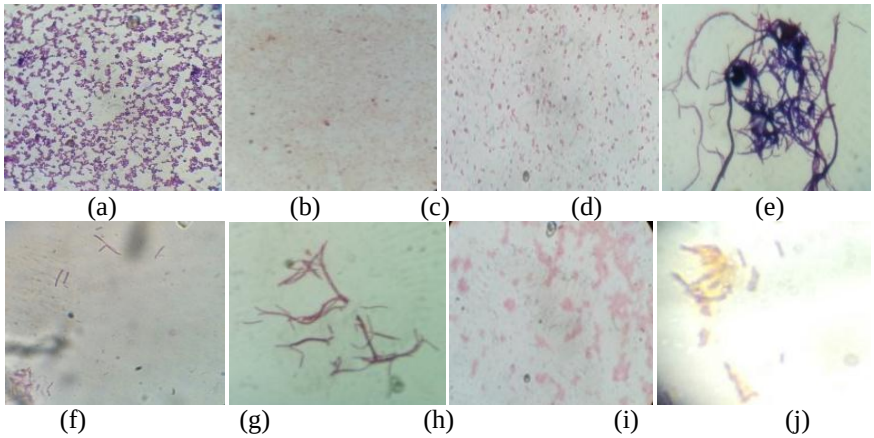


Figure 2: (a) to (j) Gram staining photographs of isolated bacterial endophytes.

Colony characterization

Table 1: Colony characters of selected bacterial endophytes.

Isolates	Size	Shape	Colour	Opacity	Consistency	Grams nature
1	0.5mm	Circular	White	Opaque	Mucoid	Gram positive cocci
2	0.5-1mm	Irregular	White	Opaque	Smooth	Gram negative rods
3	1mm	Irregular	White	Opaque	Mucoid	Gram negative cocci
4	1-2mm	Circular	Orange	Opaque	Mucoid	Gram positive rods
5	0.5-1mm	Circular	Yellow	Opaque	Slimy	Gram negative cocci
6	2mm	Irregular	White	Opaque	Slimy	Gram negative rods
7	0.5-1mm	Circular	White	Opaque	Smooth	Gram negative rods
8	1mm	Circular	Off white	Opaque	Mucoid	Gram negative cocci
9	0.5-1mm	Circular	Orange	Opaque	Smooth	Gram positive rods
10	1mm	Circular	Orange	Opaque	Mucoid	Gram positive rods

Enzyme production assay of endophytic bacteria

Morphologically different ten strains were selected for enzyme assays. All the ten strains were inoculated on aspargin containing nutrient agar plates with phenol red as an indicator dye. After overnight incubation the bacterial strains which have ability to produce asparaginase utilized substrate aspargin and turns pink in colour whereas the strains those were unable to produce asparaginase remains yellow in colour due to the indicator dye (Fig 4). Phenol red in acidic condition showed yellow and in alkaline condition indicate pink colour. The results of our study showed similarity to the Masumi and Mirzaei, 2014, they were screened the endophytic fungi for asparginase production using phenol red as an indicator. Eight strains out of selected ten strains showed strong ability to produce asparginase. Strain number 2 and 7 has potential to utilize starch with amylase producing ability (Figure 4) and strain number 2 was capable to produce the lipase enzyme (Figure 5). Strain number two has ability to produce all three tested enzymes.

Figure 3: Asparginase production by endophytic bacteria.

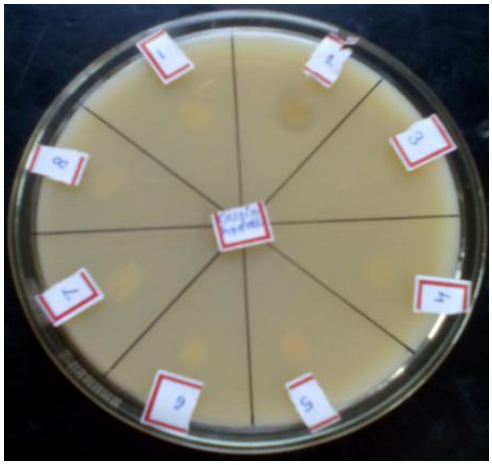


Figure 4: Amylase production by endophytic bacteria.

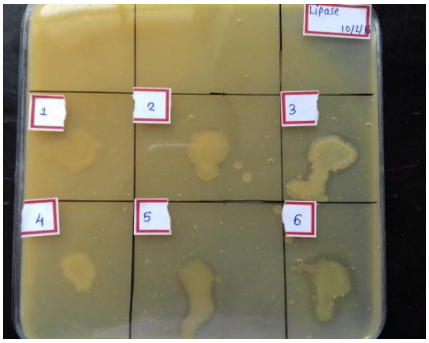


Figure 5: Lipase production by endophytic bacteria.

CONCLUSION

The most significant findings from the current study are the degree of variability of enzyme production by the endophytic bacteria isolated from selected medicinal plants. The present study has shown excellent enzymes producing ability of endophytes. This is a promising indication for the further purification and standardization of different enzymes which may have pharmaceutical and medicinal importance. Identification of the isolated endophytic bacteria by the 16S rRNA sequencing is the further milestones of this study.

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