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Hydrolytic enzyme and plant growth promotion activity of fungal and bacterial endophytes of tomato

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Abstract
Endophytes are plant associated microorganisms that live inside plant tissues without causing any harm to plants. Studies on microorganisms from plant species are recently becoming more frequent, since these fungi and bacteria have been studied for biological control and production of compounds with pharmacological properties. An attempt was made to study the efficacy of the endophytic microorganisms isolated from tomato in showing hydrolytic enzyme and plant growth promotion activity under *in vitro* condition and results revealed that, among nine fungal and eight bacterial isolates, TRBBA-23, SBHKA-2 and LBDRA-5 showed positive reaction for all the tests (amylase, protease, lipase, siderophore production and phosphate solubilisation). Isolates RFBBE-19, SFDDE-12 and LFDLA-9 showed positive reaction for all the tests except for phosphate solubilisation.

Keywords: Endophytes, hydrolytic enzyme activity and plant growth promotion

Introduction
Endophytes are plant associated microorganisms that live inside plant tissues without causing any harm to plants. Studies on microorganisms from plant species are recently becoming more frequent, since these fungi and bacteria have been studied for biological control and production of compounds with pharmacological properties. They are different from phytopathogenic microorganisms because they are not detrimental, do not cause diseases to plants and are distinct from epiphytic microorganisms which live on the surface of plant organs and tissues (Hallmann *et al*., 1997) [6]. Endophytes are sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo, 2000) [8].
The potential mechanisms of endophytes inhibition of plant pathogen are by several means, including direct effects such as direct inhibition of plant pathogens, antibiotic production, secretion of lytic enzymes and indirect effects *viz.,* induction of plant resistance, stimulation of plant secondary metabolites and promotion of plant growth and physiology (Fukang *et al.*, 2010) [5]. Therefore, an attempt was made to study the efficacy of the endophytic microorganisms isolated from tomato in showing hydrolytic enzyme and plant growth promotion activity under *in vitro* condition.

Material and methods
A total of 66 fungal endophytes and 45 bacterial endophytes were isolated from apparently healthy plant parts (root, stem and leaf) and evaluated against *Sclerotium rolfsii* and *Rhizoctonia solani* and *Fusarium Solani.* The effective nine fungal and eight bacterial endophytes were selected and evaluated for their hydrolytic enzyme activity and plant growth promotion activity under *in vitro* condition.

Hydrolytic enzyme activity
Hydrolytic enzyme activity of endophytes were analyzed for production of three enzymes *i.e.*, protease, amylase and lipase by plate method as described by Allu *et al.* (2014) [2].
Proteolytic activity of the bacterial endophytes was studied in a medium containing skimmed milk. Zone of precipitation of paracasein around the colonies in the next two days were taken as evidence of proteolytic activity. For fungal endophytes, the medium used to detect proteolytic enzyme activity contained gelatin as the protein substrate. This medium consisted of potato dextrose agar plus 0.4 per cent gelatin at pH 6. An 8 per cent solution of gelatin in water was sterilized separately and added to the potato dextrose agar at the rate of 5 ml per 100

ml of medium. After incubation, plates were flooded with an aqueous saturated solution of ammonium sulfate which precipitates protein. A clear zone around colonies indicated the presence of protease.

The presence of amylolytic activity was determined using starch agar medium. After inoculation of endophytes and incubation at five days, the plates were flooded with one per cent IKI (Ludol's solution) solution. The clear zone formation around the growing colony was considered as positive.

The lipase activity of endophytes was determined by using tributyrin agar medium. 100 ml medium contained peptone 0.5 g, yeast extracts 0.3 g, agar 2 g, distilled water 100 ml and it is supplemented with 0.1 per cent tributyrin. Presence of opaque halo zone around the colonies was considered as positive.

Plant growth promotion activity

Phosphate solubilisation

Solubilisation of tri-calcium phosphate was detected in Pikovaskayas agar with bromo phenol blue at a concentration of 0.003 per cent. Each endophytic isolate was streaked on the surface of Pikovskaya agar medium and phosphate solubilizing activity was estimated after one to five days of incubation at room temperature. Phosphate solubilisation activity was determined by the development of the clear zone around colony.

Siderophore production

Siderophore production was tested qualitatively using Chrome Azurol S medium (CAS-medium) as described by Husen (2003) [7].

Preparation of CAS for the detection of siderophores

CAS medium is used to detect siderophore production by microorganisms. To prepare one litre of blue agar CAS medium, 60.5 mg dehydrated Chromo Azurol S (Himedia) was dissolved in 50 ml water and mixed with 10 ml of iron solution (1mM $FeCl_3.6H_2O$ in 10 mM HCl). While stirring, this solution was slowly added with 40 ml aqueous solution containing 72.9 mg Cetyl trimethyl ammonium bromide with continuous stirring and the final solution was autoclaved. King's B agar medium was prepared and pH of the medium was adjusted to 6.8 by addition of 50 per cent (w/w) sodium hydroxide (NaOH) solution and autoclaved. Cooled CAS dye was added along the glass wall with gentle agitation to achieve mixing without formation of foam. To each plate 20 ml of CAS agar dye was added. The plates were stored in a refrigerator (4 ºC) for 24 h before use. Each endophytic isolate was spotted on the surface of CAS medium and incubated at room temperature for 1 to 3 days. Siderophore production was indicated by orange halos around the colonies after the incubation for two to four days.

Results and discussion

The efficacious endophytes were evaluated for their hydrolytic enzyme activity and plant growth promotion activity under *in vitro* condition and among nine fungal and eight bacterial isolates, RFBBA-23, SBHKA-2 and LBHRA-5 showed positive reaction for all the tests. Isolates RFBBE-19, SFDDE-12 and LFDLA-9 showed positive reaction for all tests except for phosphate solubilisation (Table 1). These results are in agreement with the work of Amaresan *et al.* (2012) [4], Nandhini *et al.* (2012) [9] and Abdallah *et al.* (2016) [3], who have also evaluated tomato endophytes for their hydrolytic enzyme production and plant growth promotion activity under *in vitro* condition in different places.

Neilson and Sorenson (1999) [10] demonstrated lytic enzyme production by endophytes which acted as an antagonistic mechanism against some fungal pathogens like *R. solani*, *Pythium ultimum* and *Fusarium* spp and bacterial pathogen like *R. solanacearum*. Hydrolytic enzymes help the endophytes to enter the plant tissues. Plant cell wall and hydrolytic enzymes produced by microbes play important role in plant microbe interactions and intercellular colonization of the microbes in the plant roots. It has been suggested that hydrolytic enzymes might only be produced by endophytes during early invasive phase and not after residing in the plant tissues (Cho *et al.,* 2007) [1]. All the efficacious endophytes showed positive reaction for atleast one of the enzyme activity for the interaction.

Endophytic microorganisms can influence plant growth which differs among species and strains and there may be many mechanisms through which plant growth is promoted. Conceptually, researchers have speculated that plant growth promoting endophytes may influence plant growth either directly or indirectly. Direct promotion of plant growth occurs when either (i) the plant growth promoting endophytes enable the attaining of resources from the environment including potassium, nitrogen, phosphorus and iron; (ii) modify plant growth by providing or regulating various plant hormones including cytokinin, auxin or ethylene. Indirect promotion of plant growth by endophytes through production of metabolites, HCN and antibiotics against pathogenic bacteria and fungi (Rekha *et al.,* 2017) [11]. In the present study, the overall growth promotion behaviour of some isolates receives the support from the findings of the researchers in these aspects in different crops.

In plant growth promoting endophytic bacteria, iron in Fe^{3+}-siderophore complex on bacterial membrane is reduced to Fe^{2+} which is further released into the cell from the siderophore via a gating mechanism. Binding of the siderophore to a metal increases the soluble metal concentration. On the alleviation of high level of heavy metal contamination bacterial sideophores are released and plants assimilate iron from bacterial siderophores by means of different mechanisms, for instance, chelate and release of iron, the direct uptake of siderophore-Fe complexes, or by a ligand exchange reaction (Rekha *et al.,* 2017) [11]. Sharma *et al.,* (2003) [12] evaluated the role of the siderophore-producing *Pseudomonas* strain GRP3 on *Vigna radiate* for iron nutrition. After 45 days, the plants showed a decline in chlorotic symptoms and iron, chlorophyll a and chlorophyll b content increased in strain GRP3 inoculated plants compared to control. In the present investigation also many isolates have given positive reaction to siderophore production.

Table 1: Hydrolytic enzyme and plant growth promotion activity of effective fungal and bacterial endophytes

Endophyte	Hydrolytic enzyme activity			Plant growth promotion activity	
	Amylase	Protease	Lipase	Siderophore	Phosphate solubilization
Effective fungal endophytes					
RFHKM-9	+	-	-	-	-
RFBBE-19	+	+	+	+	-
RFDUN-22	+	+	-	-	-
RFBBA-23	+	+	+	+	+
SFDOF-11	+	-	-	-	-
SFDDE-12	+	+	+	+	-
LFDHO-3	+	+	+	-	-
LFDLA -9	+	+	+	+	-
LFDKA-20	+	+	-	+	-
Effective bacterial endophytes					
RBDNA-4	-	-	+	+	+
RBDLA-5	+	+	+	+	-
RBDDE-14	+	+	+	+	+
SBHKA-2	-	+	+	-	+
SBDOF-6	+	+	-	-	-
SBDVA-9	+	+	-	-	+
SBBSA-11	-	-	-	+	+
LBHRA-5	+	+	+	+	+

+ : Presence **- :** Absence

Summary and conclusions

Study on hydrolytic enzymes and plant growth promotion activity of efficacious endophytes revealed that, among nine fungal and eight bacterial isolates, TRBBA-23, SBHKA-2 and LBDRA-5 showed positive reaction for all the tests (amylase, protease, lipase, siderophore production and phosphate solubilisation). Isolates RFBBE-19, SFDDE-12 and LFDLA-9 showed positive reaction for all the tests except for phosphate solubilisation.

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