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Diversity analysis and characterization of antagonistic endophytic population from *Stevia rebaudiana*

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ABSTRACT

Antagonistic endophytic fungal community resident in medicinal plant *Stevia rebaudiana* Bertoni was studied at two sites within Bhopal, M.P. Among 339 recovered endophytic isolates from foliar tissues, 40 fungal isolates were found antagonistic against *Sclerotinia sclerotiorum*, casual agent of disease stem rot in stevia and soybean (Glycine max.). Antagonistic fungal population (40 isolates) consisted of 52.5% *Hyphomycetes* and 2.5% each of *Coleomycetes, Basidiomycetes, Ascomycetes* and *Sterile mycelia*. The percent colonization frequency of antagonistic endophytic community in foliar tissues ranged from 0.3%-5.3% whereas percent dominance was of the order, ranged from 2.31%-40.8%. Diversity analysis of the antagonistic endophytic population was determined in terms of Shanon index, Simpson index, Species eveness, Menhinick and Margalef richmess index .Antagonistic endophytic population was also evaluated for IAA production, siderophore and phosphorus solubilisation, considered as plant growth promotory attributes. Identification of the antagonistic endophytes was carried out by rDNAsequencing of the ITS region.

KEY WORDS: DIVERSITY INDEX, *S. SCLEROTIORUM*, ITS REGION, ANTAGONISTIC ENDOPHYTES, SEQUENCE PHYLOGENY

INTRODUCTION

Fungal endophytes possess huge diversity morphologically and biochemically (Strobel and Daisy, 2003). Endophytic fungi are known to reside in the tissues of plants above ground as well as below ground, parts of the plant (Zhang et al. 2006; Kusari et al. 2012). Endophytic fungi are an assemblage of microorganisms that chiefly belong to class *Ascomycetes* of kingdom fungi. A significant literature is available so far to show that these microorganisms, under laboratory culture conditions, produce numerous structurally diverse biologically active secondary metabolites that include antimicrobial substances. Different ecological factors such as seasonality, nearby vegetation and humidity influence the distribution of endophytic fungi in the host (Taylor

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et al; 1999; Toofanee and Dulymamode; 2002 Lumyong et al 2009, Dissanayake et al., 2016; Ratnaweera et al., 2017, Ratnaweera et al., 2018).

Stevia rebaudiana Bertoni is an herbaceous polyannual plant of the sunflower family (Fam. Asteraceae), generally known as candy leaf, sweet leaf, sweet leaf, or sugar leaf. The Stevia leaves also contain variety of glycosides compounds viz., flavonoid glycosides, Stevioside, Rebaudioside A, Rebaudioside C, coumarins, cinnamic acids, phenylpropanoids and some essential oils, (Midmore and Rank, 2002 Lavini et al., 2008).

Stevia rebaudiana Bert. Is a good source of sweeteners and is about 300 times sweeter than sucrose owing to presence of steviosides in its leaves. Previous studies have reported clearly the diversity of these fungal fungal endpphytes from *Stevia rebaudiana* which also indicates the presence of *Alternaria,, Aspergillus*, *Monodictys*, and *Curvularia* fungal genus from leaf of *Stevia rebaudiana* (Bert.). (Prakash et al., 2008; Madhumita and Chandra, 2013) Furthermore, these fungal isolates have been reported from almost all climatic regions of the globe viz., tropical, temperate and alpine (Arnold, 2007; Halmschlager et al 1993; Higgins et al 2007). The application of biocontrol agents has become one of the most promising tools for reducing the use of chemical pesticides in agriculture. The antagonism of biocontrol agent is based on different mechanisms i.e. nutrients, mycoparasitism, plant growth promotion and induction of the defense responses in plants (Howell, 2003, Sen et al, 2012; Hamzah et al, 2018).

In the present investigation the endophytes recovered from *Stevia rebaudiana* leaves have been tested for antagonistic abilities against *Sclerotinia sclerotiorum*, which is the major phytopathogen affecting varieties of crop plants in Central India (Prakash et al., 2008, Verma et al 2004).

Therefore, present investigation was carried out to understand the generic diversity of endophytic fungi in leaves of *Stevia rebaudiana* Bertoni and to compare the antagonistic endophytic assemblages in samples collected from two different sites in the same region. Thus a specific rationale for the selection of stevia plant for endophyte isolation and natural product discovery is used.

MATERIALS AND METHODS

Stevia rebaudiana **Bertoni:** *Stevia rebaudiana* Bertoni was selected as the target plant for isolation of fungal endophytes. Sampling was carried out from two sites. The first site was Misrod (23°16'N; 76°36'E), a village situated nearly 22 km away from the capital city Bhopal in the state of Madhya Pradesh. The second site of sampling was green house grown plants in the campus of (23°20'N; 77°45'E) Barkatullah University, Bhopal.

Samples Collection and Surface sterilization: Healthy and mature plants were carefully chosen for sampling and leaves were collected randomly. Plant material was brought to the laboratory in sterile bags. Surface sterilization of foliar tissues were done using reagents like 70% ethanol, 4% sodium hypochlorite and sterile distilled water for different time period for effective surface sterilization process. Leaves were thoroughly washed several times in sterile distilled water (SDW) for 5-10 sec then sterilized by exposing them to 70% ethanol for 2 min followed by treatment with 4% sodium hypochlorite for 2 min. The leaves were now immersed in sterile water (SDW) for 2-5 sec and allowed to dry on blotting paper.

Isolation of endophytic fungi from foliar tissues of plants: The surface sterilized leaf segments of 5mm size were placed on Potato dextrose Agar (Howksworth et al 1995), supplemented with chloramphenicol (0.2gl-1) to avoid bacterial contamination. Plates were incubated at 28±2 °C in for 3-5 days and were observed regularly for fungal growth.

Analysis of data: The colonization frequency (% CF) of endophytic fungi was calculated according to Hata and Futai (1995) and dominance as per Kumaresan and Suryanarayanan (2002). Utilizing the data of percentage colonization of fungal endophytes in leaves, for two sites. Simpson's Diversity indices and Shanon-Wiener indices were calculated. Species evenness and species richness was calculated according to Simpson (1949), Shanon and Weaver (1949), Ludwig & Reynolds (1988) and Margelef and Menhinick (1964)

In vitro antagonistic activity of fungal endophyte

Fungal isolates were screened for antagonism against *Sclerotinia sclerotiorum* by a dual culture technique on Potato Dextrose Medium (Szekeres et al, 2005).

Characterization of endophytes: The endophytic fungi were identified by their macroscopic & microscopic characteristics such as the morphology of the fruiting bodies and spore morphology. Morphological characterization was done on the basis of color, margin, reverse pigmentation & texture. (Rifai 1969).Antagonistic endophytic fungi are characterized functionally employing plate assays for amylase cellulase, Protease, pectinase, lipase and xylanase (Paterson & Bridge. 1994; Teather & Wood. 1982; Shakeri et al. 2007; Pointing. 1999; Sierra. 1957, Mishra et al, 2013, Aneja, 2003).

Plant growth promoting attributes: Plant growth promoting attributes of antagonistic endophyte were also studied. This included IAA (Brick et al. 1991) and siderophore production (Schwyn & Neilands. 1987) and phosphate solubilisation efficiency (Pikovskaya. 1948). Both qualitative and quantitative estimation were made.

Molecular identification of antagonistic endophyte: - Morphological identification of the organism was car-

ried out at National Fungal Culture Collection of India (NFCCI), Agharkhar Research Institute, Pune. For molecular identification, total genomic DNA of the endophytic fungus was isolated directely from actively growing mycelium growing in Potato dextrose broth (PDB), using CTAB method (Sambrook and Russel, 2001). DNA amplification was performed by PCR using primer pair ITS1: TCCGTAGGTGAACCTGCGG and ITS4: TCCTCCGCTTGATATGC (White et al. 1990).PCR was carried out according to the following protocol: initial denaturation at 95 °C for 5 min; denaturation at 95 °C for 1 min; annealing at 55°C for 45 sec; extension at 72 °C for 10 min; steps 2-4 were repeated 35 times. Sequencing of PCR product was carried at Xcelris Labs Ltd, Ahmedabad. The sequenced data was subjected to BLAST algorithm and submitted to Genebank for accession number. The potential antagonistic endophytes were submitted at National Agriculturally Important Culture Collection (NAIMCC), culture collection facility at ICAR-NBAIM, Maunath Bhanjhan (U.P).

Phylogenetic analysis: To know the phylogenetic relationship among the isolates and also to confirm their taxonomical status, certain ITS rDNA sequences were chosen from GenBank databases via BLAST search analysis. The sequences were chosen from the top 20 database hits obtained in the blast search by querying the obtained sequences individually. These sequences were aligned using CLUSTAL W 1.83 (Thompson et al., 1994). Phylogenetic trees were generated by neighbourhood joining method with 100 bootstrapping replicates using MEGA version 5.

RESULTS

Antagonistic Action and Diversity Analysis: A total of 339 recovered endophytic fungal isolates were screened for antagonistic ability against Sclerotinia sclerotiorum (culture obtained from Directorate of Soybean Research, Indore, M.P.) by using dual culture technique. The inhibition zone in dual plate assay averaged between 5 to 17 mm. Misrod field site harboured greater number of antagonists compared to the endophytes recovered from green house raised plants. Percent growth reduction of the pathogenic culture was recorded after 24 hrs. Isolate Aspergillus flavipes (NAIMCC-F-03153) strain 63 showed highest value of growth reduction i.e. 19% after 24 hrs followed by Alternaria alternata strain 99 and Aspergillus niger strain 89 (NAIMCC-F-03157) showed 17% and 18% growth reduction respectively (Fig 1); Least reduction of pathogen was recorded for isolate Alternaria brassicae strain 17 i.e. 6.9%. The percent growth reduction values ranged from 6.9% to 19%.

A total of 40 endophytic fungal isolates which showed antagonistic activity against *Sclerotinia sclerotiorum* consisted of 52.5% *Hyphomycetes* followed by 2.5% *Coleomycetes, Basidiomycetes, Ascomycetes* and Sterile mycelia each. The percent colonization in tissues samples ranged from 1% - 36.6% (site 1) whereas 1%-10.6% (site 2) (Fig 2) and percentage dominance of antagonistic endophytes ranged from 1.1%-40.6% (site 1) and 4.3%-45.3% at site2 (Fig 3). Diversity analysis of the antagonistic population was carried out which showed significant diversity index values at site 1 as compared to site 2, whereas Margalef & Menhinick's richness index value was maximum at site 2 as compared to site 1 (Table 1)

Characterization of antagonistic fungal endophytes

Morphological characterization of antagonistic fungal endophytes: Based on the morphology different antagonistic endophytic fungal isolates were recovered on PDA plate. Among 40 antagonistic endophytic isolates 62.5% showed reverse pigmentation, 15% showed velvety appearance on PDA plate while others appeared spory and cottony texture. Isolates *Aspergillus niger* strain 89

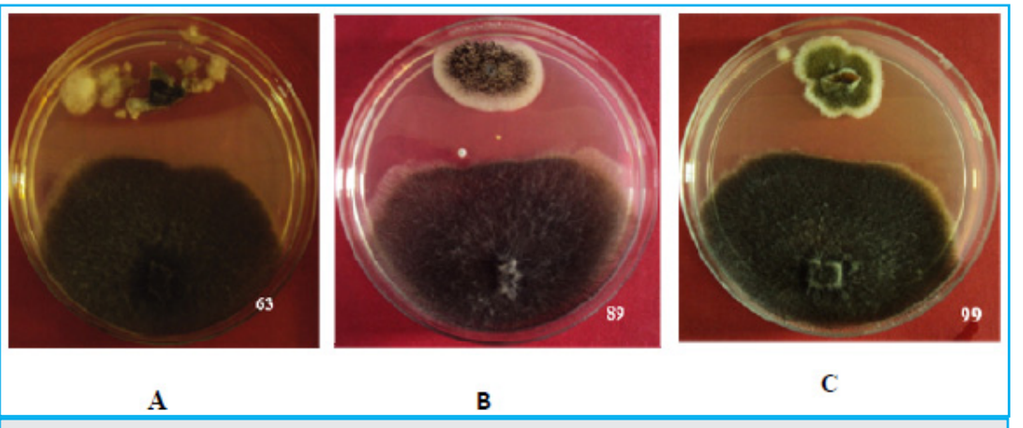

FIGURE 1. Dual culture assay shown by isolate A) Strain 63 B) Strain 89 C) Strain 99

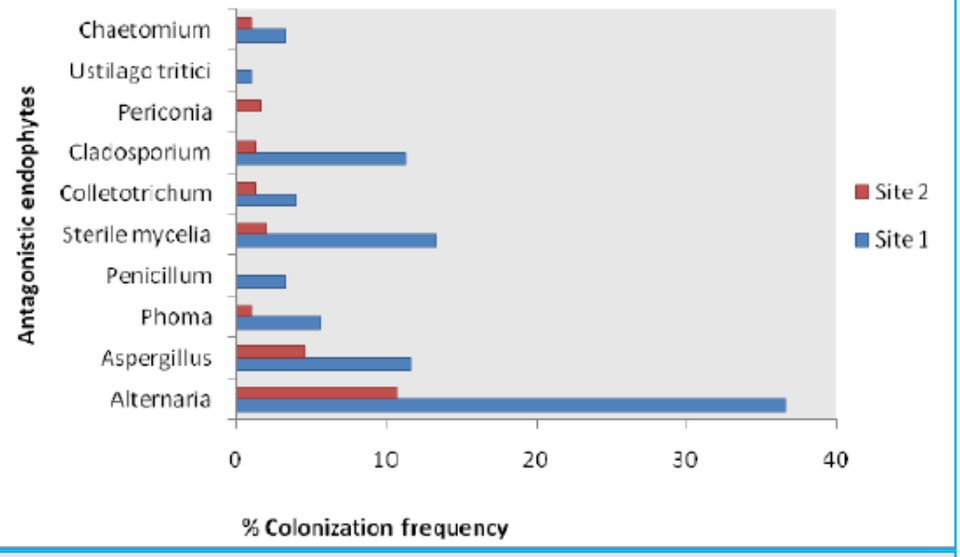

FIGURE 2. % Colonization frequency of the antagonistic endophytic population from both sites.

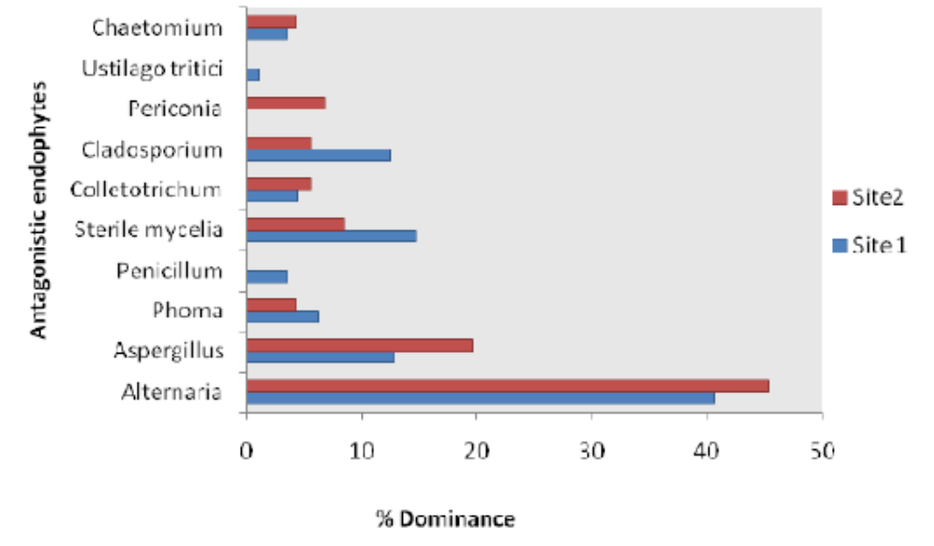

FIGURE 3. % Dominance of the antagonistic endophytic population from both sites.

(NAIMCC-F-03157), *Aspergillus niger* strain 88, *Aspergillus sp.* strain 37 (NAIMCC-F-03147), *Aspergillus flavipes* strain 63 (NAIMCC-F-03153) and *Aspergillus niger* strain 50 (NAIMCC-F-03151), showed dispersed growth on PDA medium.

Functional characterization of antagonistic fungal endophytes: The antagonistic fungal endophytes recovered from Stevia, were checked for their hydrolytic potential. They were screened for multiple enzyme activity on starch, pectin, lipid, carboxy methyl cellu-

Table 1. Diversity indices of two sites (Site 1 & Site 2)

Diversity indices	Site I	Site II
Simpson index (1-D)	0.821	0.7982
Shanon index (H')	1.856	1.781
Eveness (EH/s)	0.8001	0.8478
Menhinick S/√N	0.6305	1.121
Margalef S-1/ln(n)	1.378	1.638

lose, xylan and skim milk. Endophytes exhibited good amylolytic, cellulolytic, proteolytic, pectinolytic activities while xylanolytic and lipolytic activities were possesed by only few isolates. The confirmation of enzymatic activity was recorded by the presence of zone of clearance around the culture. *Alternaria alternata* strain 76 showed maximum zone of about 15 mm on starch agar plate while 11 mm zone was recorded for

Table 2. Plant growth promoting attributes of antagonistic endophytes

S.no	Isolates	Identity	IAA (µg/ml)	Siderophore (mg/ml)	% solubilising efficiency of Phosphate
1	SR/II/2	*Alternaria porri*	-	8.42±0.20	75
2	SR/II/4	*Alternaria alternata*	6.2±0.03	1.6±0.20	52
3	SR/II/5	*Alternaria brassicae*	-	3.79±0.05	71.4
4	SR/II/6	*Alternaria porri*	3±0.04	6.2±0.06	60
5	SR/II/42	*Alternaria sp.*	8±0.03	3.72±0.08	-
6	SR/II/45	*Penicillium mallochii*	6.3±0.05	4.2±0.04	32
7	SR/II/50	*Aspergillus niger*	4.8±0.01	-	6.25
8	SR/II/52	*Phoma sp.*	-	6.6±0.03	10
9	SR/II/54	*Phoma sp.*	4.6±0.02	8.7±0.03	20
10	SR/I/76	*Alternaria alternata*	-	3.2±0.02	5.4
11	SR/I/77	*Alternaria sp.*	5.5±0.03	6.9±0.02	22
12	SR/I/78	*Alternaria tenuissima*	6.5±0.02	8.02±0.03	11
13	SR/I/94	*Alternaria alternata*	3.8±0.01	2.8±0.03	8
14	SR/I/99	*Alternaria alternata*	5.3±0.3	-	36
15	SR/II/63	*Aspergillus flavipes*	4.6±0.04	11.12±0.16	22.2
16	SR/II/1	*Alternaria alternata*	-	17.09±0.1	-
17	SR/II/9	*Alternaria alternata*	9.8±0.6	7.45±0.12	17.3
18	SR/II/20	*Alternaria sp.*	6.3±0.06	8.03±0.11	70
19	SR/II/21	*Alternaria alternata*	-	8.03±0.11	50
20	SR/II/23	*Chaetomiumglobosum*	6.7±0.05	15.51±0.12	14
21	SR/II/24	*Alternaria alternata*	-	14.63±0.14	12
22	SR/II/26	*Alternaria alternata*	1.9±0.04	3.15±.10	10
23	SR/II/33	*Phoma sp.*	4.5±0.01	1.66±0.031	-
24	SR/II/36	*Alternaria brassicae*	8.2±0.7	27±20.22	65
25	SR/II/55	*Alternaria alternata*	-	4.42±0.14	14
26	SR/II/60	*Ustilago tritici*	5.5±0.04	9.51±0.17	-
27	SR/I/72	*Alternaria brassicae*	-	23±0.08	34
28	SR/II/81	*Alternaria alternata*	-	22±0.06	-
29	SR/I/85	*Alternaria alternata*	5.7±0.05	6.07±0.03	37
30	SR/I/95	*Alternaria alternata*	-	4.5±0.04	-
31	SR/II/100	*Alternaria alternata*	5.8±0.07	5.8±0.07	19
32	SR/I/83	*Alternaria alternata*	4.5±0.05	6.18±0.19	-
33	SR/II/18	*Phoma sp.*	-	1.3±0.02	85.7
34	SR/II/37	*Aspergillus sp.*	11±0.01	13.3±1.12	-
35	SR/II/46	*Aspergillus sp.*	6.8±0.06	8.06±0.07	-
36	SR/I/88	Aspergillus niger	8.1±0.2	-	-
37	SR/I/89	Aspergillus niger	11.2±0.4	6.72±1.4	-
38	SR/I/90	Alternaria alternata	-	-	35
39	SR/II/3	Sterile mycelia	-	8.42±0.20	85.7
40	SR/II/17	Alternaria brassicae	6.25±0.05	24.33±0.12	-

cellulolytic activity by isolate *Alternaria alternata* strain 26.Maximum pectinolytic activity was recorded by isolate *Alternaria alternata* strain 99, whereas isolate *Alternaria alternata* strain 4 (NAIMCC-F-03138), showed maximum zone (11 mm) of clearance on CMC plate. The isolates *Aspergillus sp*. strain 46, *Phoma sp*. strain18, *Phoma sp*. strain 33 and Alternaria alternata strain 94, *Aspergillus niger* strain 50, *Alternaria porri* strain 6 (NAIMCC-F-03139), and *Alternaria brassicae* strain 5 (NAIMCC-F-03140), showed minimum inhibition (2mm) on same substrate.

Plant growth promotiong attributes of antagonistic endophytes: The antagonistic endophytes were studied for plant growth promoting traits such as siderophore production, phosphate solubilisation, Indole acetic acid production. A comprehensive overview of the PGP traits of antagonistic endophytes is given in the Table 2. About 40% of the antagonistic endophytes were positive for siderophore production and the amount of siderophore produced ranged between 1.3-27 $mgml^{-1}$. On Pikovyaskya's agar, 31% of the antagonistic endophytes showed phosphate solubilisation; efficiency of P solubilisation ranged between 5.4%-85.7percent. Based on the results isolates *Phoma sp*. strain 18 and *Sterile mycelia* strain 3 were found to be most efficient P solubilizer. A total of 29% of the antagonistic endophytes were positive for IAA production which ranged between 1.9-11μgml^{-1} (Table 2).

Sequence analysis of ITS region of rDNA gene fragments

All the selected isolates produced a single PCR product with approximately 600 bp. Purification of the PCR product was performed using Banglore Genei purification kit and sequencing was performed by Xcelris Genomics, Ahemdabad using the same set of primers as mentioned earlier. The full length sequences of the isolates were compared with the related fungal sequences in the GenBank databases and sequence similarities were determined using BLAST sequence similarity search tool (Altschul et al., 1990). The sequences of ITS region of rDNA gene of the fungal endophytes were deposited in the GenBank and given accession numbers (Table 3).

Phylogenetic analysis

To know the phylogenetic relationship among the isolates and also to confirm their taxonomical status, certain ITS rDNA sequences were chosen from GenBank databases via BLAST search analysis. The sequences were chosen from the top 20 database hits obtained in the blast search by querying the obtained sequences individually. These sequences were aligned using CLUSTAL W 1.83 (Thompson et al., 1994). Phylogenetic trees were generated by neighbourhood joining method with 100 bootstrapping replicates using MEGA version 5.(Fig:4)

DISCUSSIONS

Characterization of fungal endophytes from *Stevia rebaudiana* Bertoni was considered important because only few attempts have been made earlier to characterize fungal endophytes from this useful plant, (Begum et al 2008; Kumari and Chandra 2013). Various fungal diseases have been reported to pose serious problems to *S.rebaudiana* Bertoni commonly known as Stevia, a popular non calorific sweetener. These include *Verticillium dahlia* on leaves (Farrar et al., 2000), *S. sclerotiorum* reported in Canada (Chang et al., 1997), *S. rolfsii* in India (Kamalakannan et al., 2007) and *Botryis cinerea* in Itlay (Garibaldi et al., 2009). Sclerotinia stem rot (white mold) of soybean was first reported in Hungary in 1924 and since has been reported in Argentina, Brazil, Canada, India, Nepal, South Africa and United States. A great economic loss to crop plants by different phytopathogens results in low yields. The species composition of the endophytic assemblage and frequency of infection varies according to host species and site characteristic such as elevation, exposure, associated vegetation, tissue type (Fisher et al., 1994) and tissue age (Fisher et al., 1986; Rodrigues, 1994).

In the present study, foliar endophytic microorganisms were studied using various diversity indices viz Simpson index (Simpson, 1949), Shannon index (Shannon and Weaver, 1949), evenness index (Ludwig and Reynolds, 1988) and richness index (Margalef, 1958; Menhinick, 1964). Higher value of Shannon index and evenness index with lower values of Simpson index indicated greater diversity. Bills et al. (2002) described a significant difference between tropical and temperate endophytes, in terms of their ability to produce number of bioactive natural compounds isolated from endophytes. This observation suggests the importance of the host plant in influencing the general metabolism of endophytic microbes.

Among 339 recovered endophytic fungal isolates, 40 isolates were screened out as potential antagonistic endophytes against broad spectrum plant pathogen *S. sclerotiorum* following using dual culture technique. It was found that maximum numbers of antagonistic endophytes were recovered from site1 as compared to site 2. This may be explained as site 1 is open agricultural field which was exposed to wide variety of phytopathogens so in order to overcome these pathogens several bioactive compounds are produced by them whereas site 2 is closed area which was exposed to limited number of phytopathogens. Sadrati et al. (2013) screened 20 endophytic fungi from wheat which showed antimicrobial activities against 12 pathogenic bacteria, yeast and two phytopathogenic fungi. Percentage growth inhibition ranged between 6.9%-19% after 24 hr of incubation.

Table 3. Genetic relatedness of GenBank to antagonistic fungal endophytes recovered from Stevia rebaudiana Bertoni using ITS rDNA gene sequence analysis

Isolate No.	Site	Accession no.	% Similarity	Organism
SR/II/2	1	KJ592050	99%	*Alternaria porri*
SR/II/4	1	KJ592051	100%	*Alternaria alternata*
SR/II/5	1	KJ592052	99%	*Alternaria brassicae*
SR/II/6	1	KJ603463	98%	*Alternaria porri*
SR/II/42	1	KJ713969	100%	*Alternaria sp.*
SR/II/45	1	KJ713970	99%	*Penicillium mallochii*
SR/II/50	1	KJ648618	100%	*Aspergillus niger*
SR/II/52	1	KJ713971	99%	*Phoma sp.*
SR/II/54	1	KJ648619	99%	*Phoma sp.*
SR/I/76	1	KJ713972	99%	*Alternaria alternate*
SR/I/77	2	KJ713973	100%	*Alternaria sp.*
SR/I/78	1	KJ728832	100%	*Alternaria tenuissima*
SR/I/94	1	KJ728833	100%	*Alternaria alternate*
SR/I/99	1	KJ728834	99%	*Alternaria alternate*
SR/II/63	1	KF671231	94%	*Aspergillus flavipes*
SR/II/1	1	KJ728835	100%	*Alternaria alternate*
SR/II/9	1	KJ735925	99%	*Alternaria alternate*
SR/II/20	1	KJ728836	98%	*Alternaria sp.*
SR/II/21	1	KJ728837	99%	*Alternaria alternata*
SR/II/23	1	KJ728838	100%	*Chaetomiumglobosum*
SR/II/24	1	KJ728839	99%	*Alternaria alternate*
SR/II/26	1	KJ728840	100%	*Alternaria alternate*
SR/II/33	1	KJ728841	100%	*Phoma sp.*
SR/II/36	1	KJ728842	99%	*Alternaria brassicae*
SR/II/55	1	KJ728843	99%	*Alternaria alternata*
SR/II/60	1	KJ735919	100%	*Ustilago tritici*
SR/I/72	1	KJ735920	99%	*Alternaria brassicae*
SR/II/81	1	KJ735921	100%	*Alternaria alternata*
SR/I/85	1	KJ735922	100%	*Alternaria alternata*
SR/I/95	1	KJ735923	100%	*Alternaria alternata*
SR/II/100	1	KJ735924	100%	*Alternaria alternata*
SR/I/83	2	KJ748009	100%	*Alternaria alternata*
SR/II/18	1	KJ748010	100%	*Phoma sp.*
SR/II/37	1	KJ767528	100%	*Aspergillus sp.*
SR/II/46	1	KJ767529	100%	*Aspergillus sp.*
SR/I/88	1	KJ767530	100%	*Aspergillus niger*
SR/I/89	1	KJ767531	100%	*Aspergillus niger*
SR/I/90	1	KJ767532	100%	*Alternaria alternata*
SR/II/3	1	NS	NS	*Sterile mycelia*
SR/II/17	1	KJ767533	100%	*Alternaria brassicae*

1= Misrod Agriculture Field 2= Green House NS= Not Sequenced

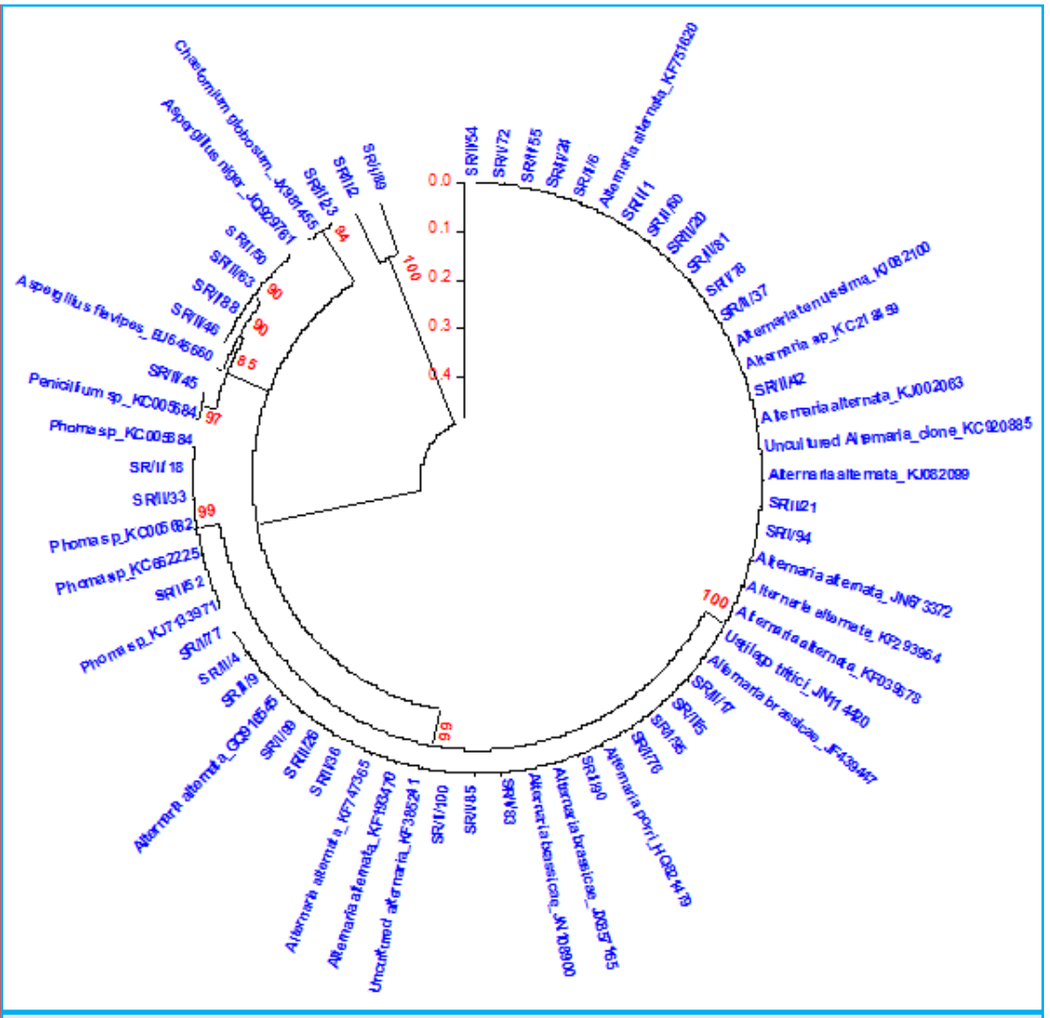

FIGURE 4. Phylogenetic tree generated on the basis of ITS rDNAsequences of antagonistic endophytic fungal isolates aligned with sequences in GenBank databases

Diversity analysis of the antagonistic endophytic population showed significant diversity index values. Maximum antifungal activity against *S. sclerotiorum* was recorded by *Aspergillus flavipes* strain 63 followed by strain *Aspergillus niger strain* 89 and *Alternaria alternata* strain 99 after 24 hrs of incubation.

For instance, site1 is open agricultural field with surrounding vegetation like wheat and soybean, which favours the establishment of endophytic colonization whereas site 2 is devoid of natural open conditions of environment which seems to be the major factor for tissue specific fluctuations in the recovery of endophytes The increased species richness in foliar tissues may be a result of super infection of the leaves overtime by airborne inocula (Carroll et al., 1977; Suryanarayanan and Vijaykrishna, 2001).

The 40 antagonistic endophytic fungal isolates were studied for morphological, functional and genotypic characterization. In the present study, significant functional diversity was observed among the antagonistic endophytes with respect to their hydrolytic potential viz. amylolytic, cellulolytic, lipolytic, proteolytic, pectinolytic and xylanolytic activities. 19% of the antagonistic endophytic fungal population showed amylase, cellulase, pectinase and protease production whereas 17% were xylanase producing and rest 7% were lipase producers. Fifty fungal strains isolated from medicinal plants (*Alpinia calcarata*, *Bixa orellana*, *Calophyllum inophyllum* and *Catharanthus roseus*) showed 64% were lipase producers, 62% were amylase and pectinase whereas 32% were cellulase and 30% were laccase producers (Sunitha et al 2013). Begum et al. (2008) reported

that majority of the endophytes from Stevia leaves were cellulolytic in nature. Suganthi et al. (2011) isolated and characterized *Aspergillus niger* (BAN 3E) out of five fungal isolates as the most potent α-amylase producer. Sidkey (2011) found endophytic strain F2Mbb to produce extracellular amylase.

These potential antagonistic endophytes were screened for plant growth promoting attributes like siderophore production, Indole acetic acid production and phosphate solubilisation. In present study, about 40% of the antagonistic endophytes were positive for siderophore production and amount of siderophore produced ranged between 1.3-27 mg ml^{-1}, 31% of the antagonistic endophytes showed positive results for phosphate solubilisation. Phosphate solubilisation efficiency ranged between 5.4-85.7% whereas 29% of the antagonistic endophytes were recorded positive for IAA production between the range 1.9-11 µg ml^{-1}.Certain endophytes were observed to improve the ecological adaptability of host enhancing their tolerance to environmental stress and resistance to phytopathogens (Kimmon et al., 1990; Struz et al., 1999). In a study performed on *Absidia corymbifera*, fungi isolated from rhizospheric soil, were found to produce siderophore in the range of 4-4.55 µg ml^{-1} (Holzberg and Artis, 1983). Maliha (2004) found *Aspergillus flavus, Aspergillus niger* and *P.canescens* as the most potent phosphate solubilizers, (Bilal et al, 2018).

Sequence analysis revealed that majority of fungal endophytes belonged to *Alternaria alternata* followed by *Aspergillus niger, Phoma, Chaetomium globosum,* and *Ustilago tritici*. Mandyam et al. (2010) employed sequencing of ITS region for studying Dark septate endophytes (DSE) in annually burned tallgrass prairie. In a nut shell, present investigation has shown that *Stevia rebaudiana* Bertoni harbours a good deal of antagonistic fungal endophytic community. These endophytes have exhibited various characteristics features which may pose better fitness to Stevia plant and reveal ecological significance of endophyte- host relationship.

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