Research Paper :

Biodiversity of endophytic fungiisolated from selected graminaceous hosts of Mercara region in Karnataka

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SUMMARY

Endophytes generally advocate a good tool for protection of host by various pathways. In the present study, six important plants belonging to Graminae of Mercara was investigated for endophytic micro flora as a possible source of bioactive secondary metabolites. 720 leaf segments from six plants collected from different locations during 2008-2009, were processed for the presence of endophytic fungi. A total of 46 fungal species were observed. Among the endophytic flora, *Aspergillus* and *Fusarium* were more predominant. Highest endophytic fungal colonization was observed in *Saccharum officinarum* and very least endophytes were isolated from *Cymbopogan citratus*. The importance of endophytes on Graminae members and the interaction between plant and fungus have been discussed in the present communication.

Key words : Endophytes, Graminae, Interaction, Secondary metabolites

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Endophytes are the endosymbionts, often may be a fungus and rarely a bacterium that lives within a plant for at least part of its life without causing apparent disease. They usually occur in above ground plant tissues, but also occasionally in roots. They are distinguished from mycorrhiza by lacking external hyphae or mantel (Kumerasen and Suryanarayan, 2002). Endophytes may be the 'treasure trove' for new pharmaceutical agents and agrochemical compounds. There is a strong need for new drug especially antibiotic, anticancer agent, immunomodulatory compounds and low toxic drought resistant agrochemicals (Huang *et al*., 2008) it is not surprising therefore, that the bulk of the world's food supply comes from this family. They also include plants that are used for medicinal purposes.

Graminae (Poaceae), is one of the largest families in monocots which include grasses along with rice, wheat, jowar, maize, sugarcane, corn, bamboo etc (Redlin and Carris,1996).In the present investigations, studies were focused on inventerlization of endophytic diversity on some important members of Graminae.

MATERIALS AND METHODS

Collection of sample:

Leaves and stem samples were collected from fifteen apparently healthy Graminae plants from several sites in Mercara in Karnataka. Samples were collected and brought to the laboratory in sterile bags and processed within a few hours after sampling, to reduce the chances of contamination.

Experimental site:

Mercara is located at 12.42^0 N and 75.73^0 E. It has an elevation of 1525meters (5003ft) above sea level. Mercara lies in the Western Ghats region of Karnataka. The temperature ranges from 8.6^0C in January to 35^0C in May. The humidity ranges from 20%-97%,it has an average rainfall of 2840.2mm and wind speed ranges from 1m-60m/sec.

Isolation of endophytic fungi from plants:

Isolation of endophytic fungi was carried out following the method described by (Petrini, 1986). The samples were rinsed gently in running water to remove dust and debris. Then leaves were cut into 3-4mm×0.5-1cm pieces with and without mid rib under aseptic condition. Treating the sample with 75% ethanol for 30secs made surface sterilization. Later, the segments were rinsed three times with sterile distilled water. The plant pieces were plotted on sterile blotting paper. The efficiency of surface sterilization procedure was ascertained for every segment of tissue following imprint method of (Schulz *et al*., 1993). In each Petri

dish 4-5segments were placed on PDA and MEA supplemented with streptomycin 250mg/litre concentration. The dishes were sealed with parafilm and incubated at $25^0C \pm 2^0C$ for 3-5weeks.Fungi growing out of the plant segments were purified and identified. Endophytic fungal colonization frequency was calculated as described by Suryanarayan *et al*. (2003). Samples were incubated and growth was examined daily during 3-5weks and colonization frequency was calculated by the following formula:

$$\text{Colonization frequency (\%)} = \frac{\text{Number of segments colonized by an endophyte}}{\text{Total number of segments analysed}} \times 100$$

RESULTS AND DISCUSSION

Plants materials were collected from Mercara and sample specimens were deposited in the Department of Biochemistry, University of Mangalore. Two thousand six hundred fifty eight species were screened. The high colonization frequency was observed by *Cladosporium herbarum* (115.03 isolates) and *Fusarium moniliforme* (109.82 isolates) in different plants. Among the six plants, high endophytic colonization was observed in *Saccharum officinarum* (542.67 isolates).A total of 46 fungal species were isolated, among them dominant endophytes were *Aspergillus oryzae* (119.99*), Phaeoisariopsis bambusae* (106.66), *Acroconidiellina chlorides* (85.0) *Pedosporium nilgirense* (85.0) *Curvularia tritica* (74.43), *Aspergillus vesicolor* (72.21), *Cladosporium herbarum* (69.72), and *Acrosporium monilioides* (64.99).

Among all these isolates majority of the endophytic fungi were saprophytic and many of them were *Aspergillus* sp. and *Curvularia* sp. Although they were saprophytic,they showed the endophytic nature in all the examined specimens of leaf and stems of Graminae. Endophytic pathogenic and saprophytic behaviour of fungi might be host/environment factor dependent.

Present investigation revealed the variation in distribution of fungal endophytes (Fig.1) in the members of Graminae which clearly shows that endophytes were not restricted to single species, genera or family. The same endophytic species were isolated from different hosts. No species specificity was observed among them (Table 1).

Incubated plant leaves showed a total isolates of 1316.71 in PDA and a total isolates of 1114.5 in MEA media. Plate 1 shows some important isolates of endophytes from PDA and MEA media. Several endophytic fungi were found in both PDA and MEA media. But more number of isolates were isolated from PDA than from MEA. Hence, PDA favours the growth of fungi and used as common medium for isolating and culturing of fungi.

In the present work, survey was conducted on the endophytic fungal diversity in the leaves of Graminae members. The used technique was to identify conidial, morphology and confirmed with culture techniques. The biodiversity of fungi is very vast. Ecological roles of endophytes are diverse and varied. *Cladosporium, Fusarium Aspergillus, Curvularia* are world wide plant pathogens that infect many plant species, apart from supporting the idea that pathogens may spend part of their life in an endophytic stage. This finding are consistent with early workers (Brown *et al*.,1998;Azor *et al*., 2007). The per cent of colonization, frequency and distribution of endophytes from the leaves of Graminae members suggest that the extent of host preferences in tropical leaf endophtyes is small.

The interaction may be strictly defined as those in the tropical ecosystem, which may be possibly related to more complex pattern of diversity of encountered grass species as only examined in the present study. It may be debatable whether fungal diversity estimates can be only on grasses and tropical fungal diversity may not be extensive as suggested by (Manoharachary *et al*., 2005). Thus, the present work is strongly supporting early workers contribution of diversity of endophytes on the leaves of Graminae which may be attributed to the differential leaf expansion, leaf chemistry and differential maceration of leaf whether infection of endophytes established before leaf expansion is to be studied (Rajgopal and Suryanarayan, 2000).This estimate can be compared to the number of fungal endophytes proposed for tropical tree leaves for *Manilkara Bidentata* (Lodge *et al*.,1996) and for *Guarea guidonia* (Gamboa and Bayman,2001) comparing these estimates with the result of present study which suggests that about half the leaf endophyte diversity in a population may be present in a 2×2cm piece of a single leaf. Similar observation had been made on other fungi in other nichens and substrates. Fungal populations may be highly variable on a very limited spatial scale (Bayman and Cotty, 1991). Thus, it appears that the occurrence of fungal endophytes are influenced by the type of host tissues and the chemicals present in the Graminae plants. The endophytic genera such as *Aspergillus,Alternaria, Cladosporium* and *Fusarium* that are ubiquitous were commonly isolated from the leaves of other hosts including many tropical trees and medicinal plants. Graminae being an important member

Table 1 : colonization frequency (%) in PDA and MEA media with total endophytic isolates

Host plant	Endophytic isolates	Colonization frequency (%)		Total isolates
		PDA	MEA	
Bambusa vulgaris L.	*Gliomastrix inflata*	49.01	15	64
	Pedosporium nilgirense	50	35	85
	Phaeoisariopsis bambusae	62.5	44.16	106.66
	Tubercularia coccicola	47.2		47.52
	Verticillium glaucum	27.5		27.5
	Xenosporium indicum		44.16	44.16
	Unidentified 1	22.5	14.16	36.66
	Unidentified 2	10.83	24.16	34.99
			Total	446.49
Cyanodon dactylon L.	*Acroconidiellina chloridis*	22.5	62.5	85
	Cercospora fusimaculans	27.5		27.5
	Cercospora sorghi	14.16	10.83	24.99
	Curvularia junata	32.5	24.16	56.66
	Curvularia senegalensis	12.22		12.22
	Fusarium graminearum	22.5	27.5	50
	Periconia jabalpurensis	33.33	16.66	49.99
	Ulocadium chartarum		10.83	10.83
	Unidentified 1	24.16	17.5	41.66
	Unidentified 2	12.22	10.83	23.05
	Unidentified 3	14.16	5.5	19.66
			Total	401.56
Cymbopogom citratus L.	*Aspergillus flavus*	5.5		5.5
	Aspergillus niger	25.5		25.5
	Cladosporium herbarum	47.5	22.2	69.72
	Cladosporium macrocarpum	25.2	16.6	41.8
	Gleocladium roseum		15.6	15.6
	Macrophoma sp.	16.66	14.43	31.09
	Pencillium notatum	22.22	11.11	33.31
	Trichoderma viride	16.66	15.6	32.26
	Unidentified 1	33.3	38.8	72.1
	Unidentified 2	22.2	5.5	27.7
			Total	354.58
Oryza sativa L.	*Alternaria humicola*	14.16	15.83	29.99
	Aspergillus oryzae	64.16	55.83	119.99
	Aspergillus flavus	14.16	11.11	25.27
	Bipolaris sorokiniana	12.2	22.2	34.4
	Cercospora longipes	14.16		14.16

Table 1 contd...

Contd… Table 1

	Curvularia geniculata	15.83	14.16	29.99
	Curvularia uncinata	22.3	16.6	38.8
	Fusarium moniliforme	16.6	17.5	34.1
	Pencillium purpurogenum	5.5	15.6	21.1
	Sclerotium oryzae	10.83	22.5	33.3
	Trichoderma viridae	14.16	12.22	26.38
	Trichosporum fuscum	27.5	22.2	50
	Unidentified 1	12.22		12.22
	Unidentified 2	22.5	11.11	33.61
	Unidentified 3		16.66	16.66
			Total	520
Saccharum officinarum L.	*Alternaria gomphrenae*	16.66	12.22	28.88
	Alternaria plurisepta	5.5	11.11	16.61
	Aspergillus niger	24.42	12.22	36.64
	Aspergillus vesicolor	55.5	16.66	72.21
	Cercospora longipes	26.2	18.2	44.4
	Cercospora vaginae	18.2		18.2
	Cladosporium chlorosephalum	24.42		24.42
	Cladosporium herbarum	34.2	11.11	45.31
	Cephalasporium sacchari	42.23	24.42	66.65
	Fusarium moniliforme	26.2	18.2	44.4
	Fusarium oxysporum	16.66	20.2	36.86
	Pencillium purpurogenum	11.11	32.2	43.31
	Stachybotrys pulchra	16.66		16.66
	Unidentified 1		18.2	18.2
	Unidentified 2	24.42	5.5	29.92
			Total	542.67
Triticum aestivum L.	*Acrosporium monilioides*	39.16	25.83	64.99
	Alternaria triticina	16.6	11.11	27.77
	Alternaria triticicola	27.5	5.5	33
	Aspergillus lutescens	18.5		18.5
	Bipolaris sorokiniana	24.42	10.83	35.25
	Curvularia tritica	42.23	34.2	74.43
	Embellisia chlamydospora		12.2	12.2
	Fusarium moniliforme	11.11	20.2	31.32
	Fusarium oxysporum	24.2	18.2	42.62
	Sclerotium rolfsii		10.83	10.83
	Unidentified 1	25.83		25.83
	Unidentified 2		16.66	16.66
			Total	393.4

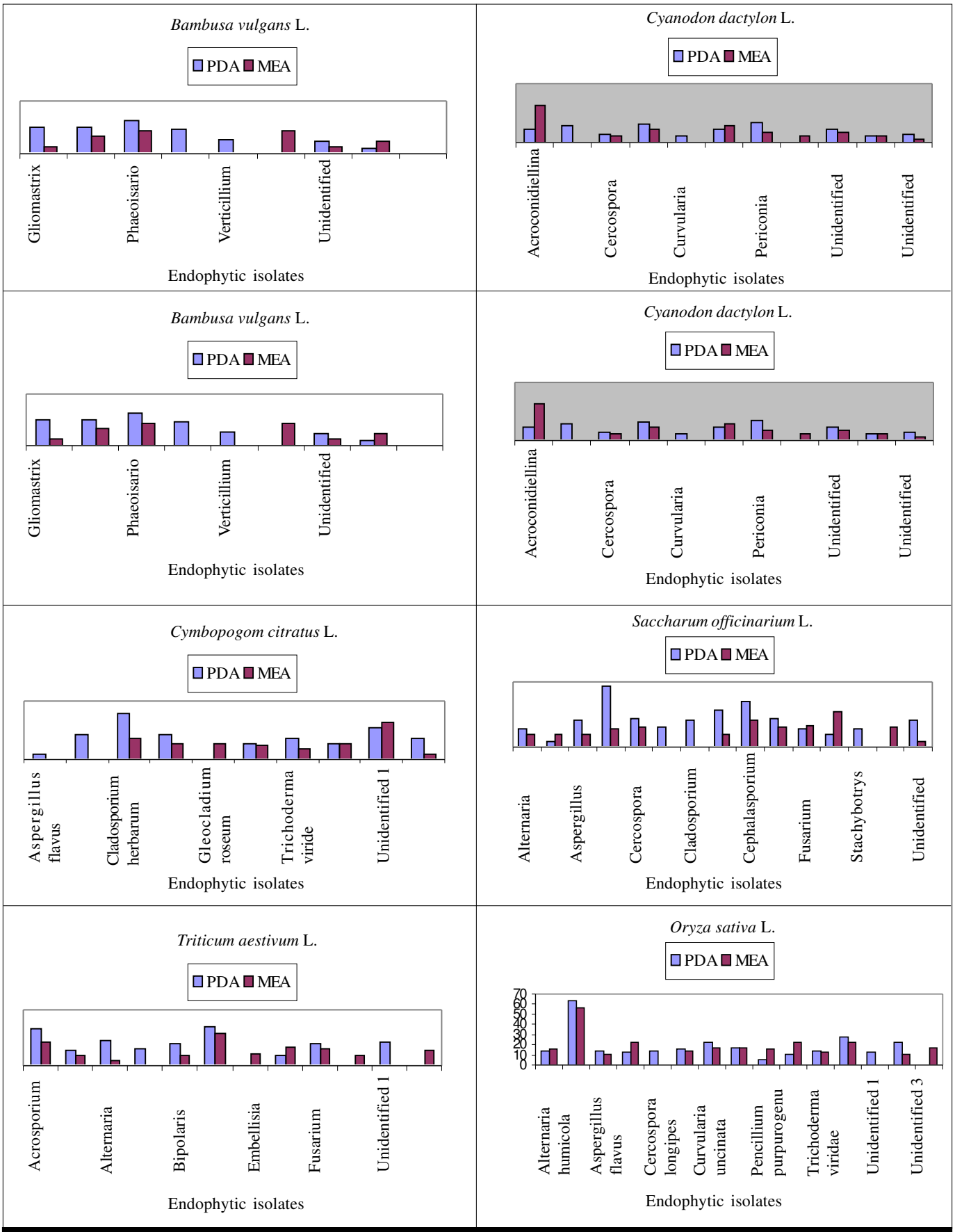

Fig. 1 : Colonization frequency (%) in PDA and MEA media with total endophytic isolates

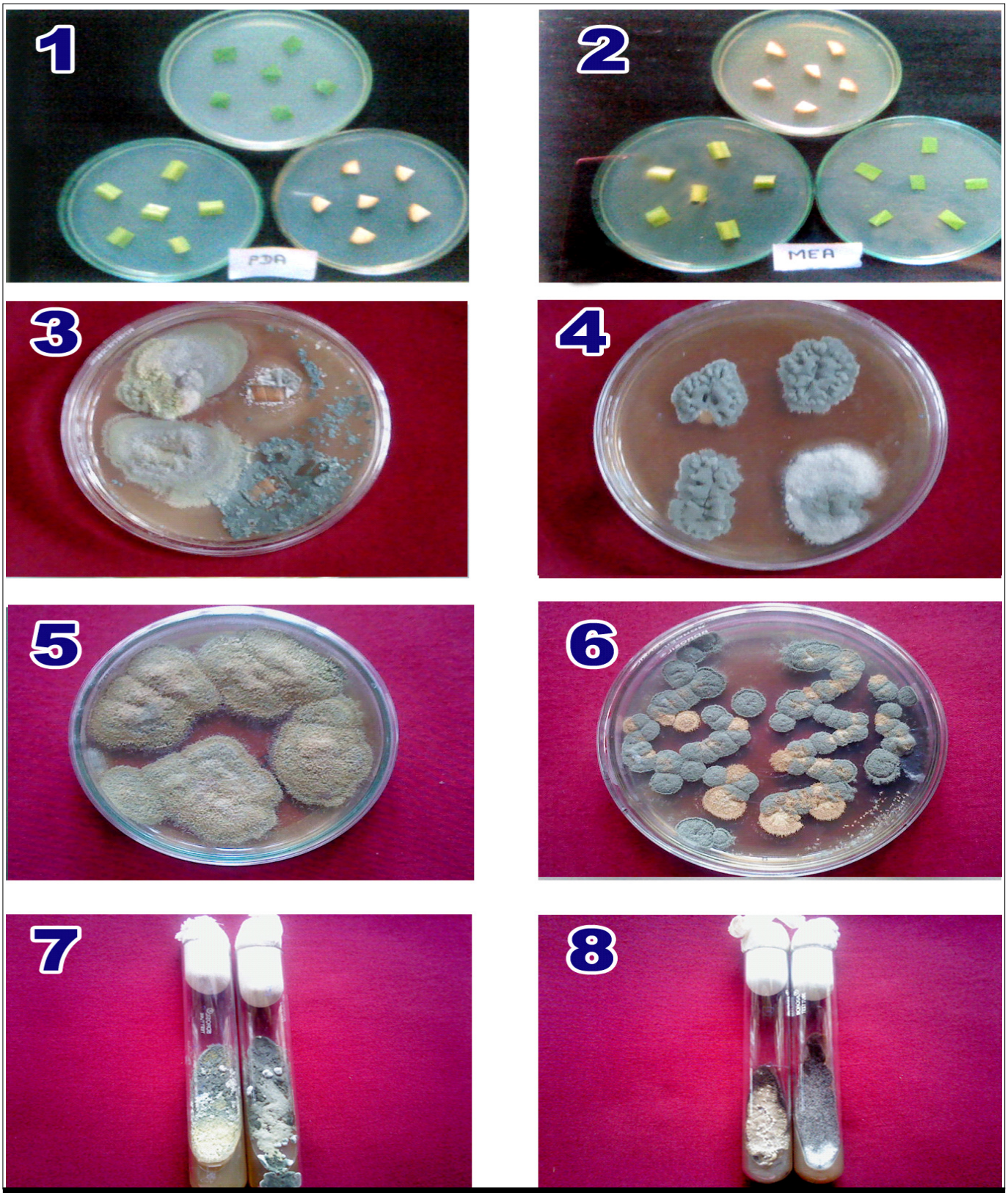

Plate 1 : Endophytes Isolated from different plant segments of graminae
1-2 Plant segments plated on different media
3. Potato dextrose agar (Emergence of endophytes on leaf and stem segments)
4. Malt extract agar ((Emergence of endophytes on leaf and stem segments)
5-6. Pure culture of *Cladosporium* sp. (5) and *Pencillium notatum* (6)
7-8. Slant tubes of *Trichoderma* sp. (7) and *Aspergillus oryzae* (8)

for the food source may be a good candidate for exploitation of its endophytic fungi in biological control. Further study needs to focus on the molecular level.

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