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Mission Mode Collections of Fungi with Special Reference to Entomopathogens and Mycopathogens

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ABSTRACT

Adequate development of entomopathogenic and mycoparasitic fungi as biocontrol agents requires a selection schedule of species and strains adapted to specific pests, environmental conditions and crops. For practical use of these "pathogens", a sound knowledge of their behaviour with respect to environmental factors is then of great significance. The biochemical and molecular aspects of fungus-host interaction, importance of minimum sub-culturing and regular passage through host, molecular markers for identification, horizontal transfer, short and long term storage, deposition in authentic culture collection center and maintenance in the natural habitat, etc are the issues with respect to the development of a commercially viable biocontrol agents. The mission-based culture collection can provide a range of strains which have different host specificities, virulent nature and different mechanisms for host interaction.

Keywords: Fungi, collections, entomopathogens, mycopathogens

MICROBIAL COLLECTIONS

Culture collections of microorganisms are the backbone of microbial research. Availability of authentic culture is vital to sustain progression of research. In addition to the supply of strains by Type Culture Collections, present generation researchers have access to gene libraries, cloned genes, cloning vectors, Expressed Sequence Tags (EST) and a variety of clones from joint ventures of sequencing projects. Several culture collections have originated as working collections of individual researchers. Most recent data released by the World Data Centre for Micro-organisms (WDCM) reveals existence of H"470 culture collections in sixty two countries. The number would increase if the collections in independent researcher's laboratories are included (Mc Cluskey, 2003). Genetic Culture Collection and Patent Depository are two types of collections with a specific mandate. The Fungal Genetics Stock Center (FGSC) at the Department of Microbiology in the University of Kansas Medical Center is one of the few genetic collections.

India has a long history of studying fungi and research with fungi which is being carried out in different institutes. General culture collections in India include: National Collection of Industrial Microorganisms (NCIM), Pune; Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi; National Fungal Culture Collection of India (NFCCI), Pune; Microbial Culture Collection (MCC), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. Added to these is the National Agriculturally Important Microbial Culture Collection (NAIMCC) - a recent establishment at the National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau. Apart from these general culture collections are the mission-based collections hosting a variety of strains whose biological characteristics have been catalogued without carrying out traditional genetics on the strains. In other words, the collection highlights specific characteristic or use of the organisms.

MISSION MODE COLLECTIONS OF ENTOMOPATHOGENIC, MYCOPARASITIC AND DUAL SPECIFIC FUNGI

One of the mission mode collections is the ARS entomopathogenic fungal (ARSEF) culture collection of the United States Department of Agriculture- Agriculture Research Service (USDA-ARS), USA. It is the world's largest and most diverse collection of cultures for fungal pathogens of insects, mites, spiders, nematodes, and other invertebrates. The collection currently comprises around 6000 isolates from more than 400 fungal taxa and approximately 930 diverse hosts, with isolates coming

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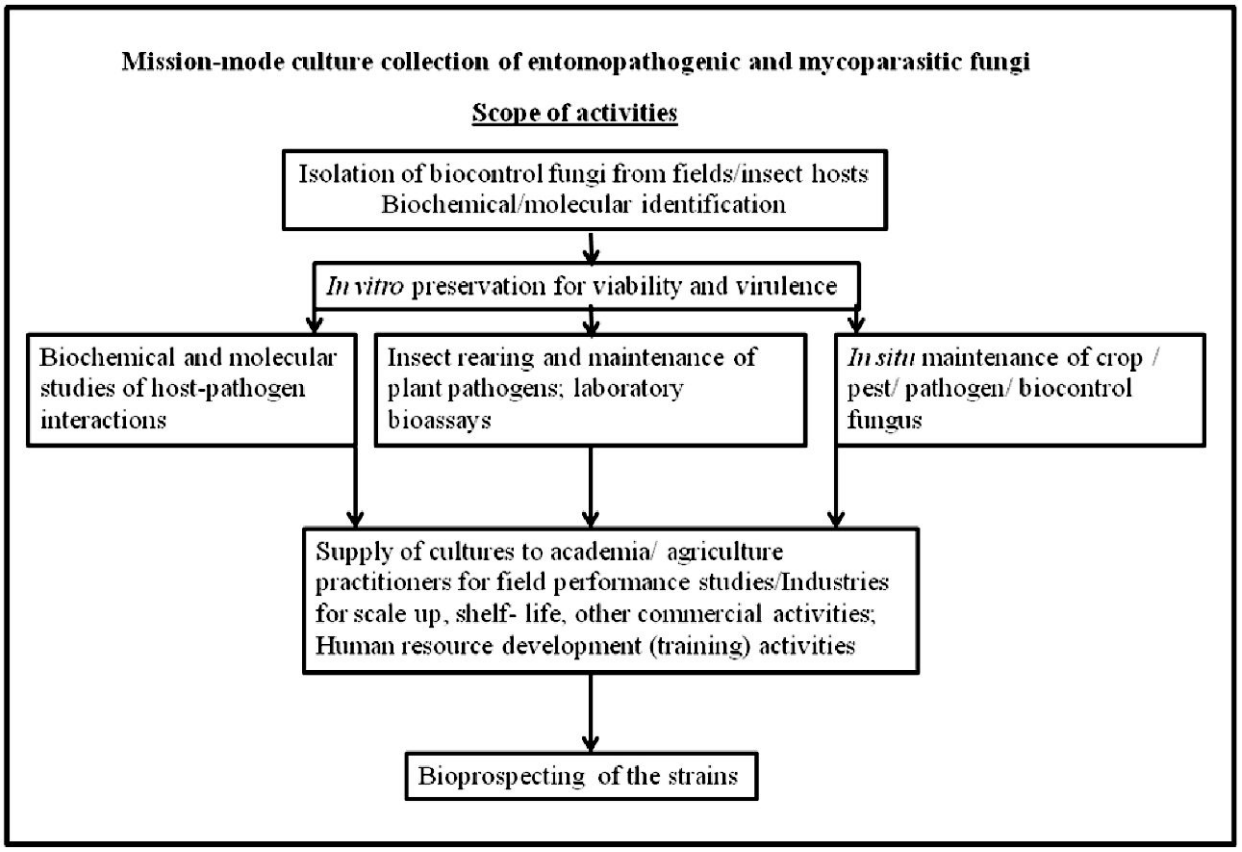
from every continent of the globe (http://www.ars.usda.gov/is/np/systematics/fungibact.htm). An additional feature of organism preservation in their natural environment, especially for a mycoparasitic and insect pathogenic fungi is to maintain their virulent nature for further exploitation. In India, so far there is no culture collection of fungi which are entomopathogenic, mycoparasitic and dual specific towards insects and fungi. The single or consortium of these fungi can be used in agriculture to control fungal pathogens and insect pests. The scope of activities is depicted in Fig. 1.

Fig. 1. Mission mode culture collection of entomopathogenic and mycoparasitic fungi.



ISOLATION OF ENTOMOPATHOGENIC FUNGI

As compared to bacteria and viruses, fungi infect a broader range of insects - lepidopterans (moths and butterflies), homopterans (aphids and scale insects), hymenopterans (bees and wasps), coleopterans (beetles), and dipterans (flies and mosquitoes). Commonly encountered insect pathogenic fungal genera with dry conidia and hydrophobic cell walls are *Metarhizium, Beauveria, Nomuraea* and *Paecilomyces* usually with a broad host range. Others though encountered frequently but less favoured are *Verticillium lecanii* and *Entomophaga grylli.* Sometimes the saprophytic genera such as *Aspergillus, Fusarium* and *Penicillium* are also mistaken as entomopathogens. Fungal genera such as *Metarhizium, Beauveria, Nomuraea, Verticillium,* and *Paecilomyces* have been studied extensively in the area of pest control (Butt et al. 2000; Kapoor and Deshpande 2013; Yadav and Deshpande 2010). The diversity of insect pathogenic fungi is listed in Table 1. Epizootics caused by *Nomuraea rileyi* and *Beauveria bassiana* were commonly reported on *Helicoverpa armigera* and *Spodoptera litura* in Indian fields (Uma Devi et al., 2003).

For the isolation of entomopathogenic fungi, soil dilution method (Goettel and Inglis, 1996; Meyling, 2007) and *Galleria* bait method (Zimmermann, 1986) are commonly used. In the soil dilution method, the medium containing streptomycin, tetracycline, cycloheximide and dodine was reported to be useful (Kulkarni et al, 2008). Selective isolation of Entomopathogens is undertaken by allowing *Galleria* larvae to move through the soil in a small vial which is moved upside down every day. After 14 days, one can find dead or even mycosed *Galleria* larva from which fungus can be isolated. This method is more successful for the isolation of *Beauveria* isolates rather than other entomopathogenic fungi like *Metarhizium* and *Nomuraea* (Maranhao and Santiago-Alvarez, 2003). Insects showing abnormal behaviour with poor coordination collected from the fields were sometimes found to be infected with entomopathogenic fungi (Nahar et al., 2003).

Table 1. **Diversity of entomopathogens**

Known insect pathogens	**Opportunistic pathogens**
Beauveria bassiana	*Aspergillus flavus*
Metarhizium anisopliae var. anisopliae	*A. sydowii*
Lecanicillium lecanii	*Cladosporium cladosporioides*
Paecilomyces farinosus, P. fumosoroseus	*Clonostachys rosea f. catenulatum*
Tolypocladium inflatum	*C. rosea f. rosea*
Secondary colonizers	*Fusarium avenaceum*
Absidia glauca	*F. oxysporum*
Chaetomium globosum	*F. redolens*
Clonostachys sp.	*F. solani*
Fusarium equiseti	*Fusarium spp.*
F. sambucinum	*Geomyces pannorum*
F. tricinctum	*Gloeotinia temulenta*
Penicillium simplicissimum	*Lecythophora sp. 2.0*
P. italicum	*Mariannaea elegans*
Periconiella mucunae	*Mortierella spp.*
Pseudeurotium zonatum	*Mucor spp.*
Rhizopus oryzae	*Penicillium brasilianum*
Talaromyces flavus	*P. chrysogenum*
T. trachyspermus	*P. corprophlium*
Trichoderma aureoviride	*P. thomii*
T. koningii	*Pestalotiopsis theae*
T. parceramosum	
T. virens	
Williopsis satumus (yeast)	

Thakur and Sandhu (2010) collected insect cadavers infected with fungi from different regions of central India and reported diversity of insect hosts and their fungal pathogens. This can be one of the mandates to identify novel entomopathogenic fungi and to understand their co-evolution with insect hosts. Similarly, Sun and Liu (2008) studied the occurrence and species diversity of insect-associated fungi which include: known insect pathogenic fungi, opportunistic pathogens and secondary colonizers, in forest soils collected from different regions of China using the 'Galleria bait method'. A total of 377 fungi belonging to 46 species and 27 genera were isolated and identified.

Bidochka et al (2001) suggested that the habitat from where the soil is taken affected the nature of isolate. For instance, Vimala Devi et al (2003) reported that *N. rileyi* isolates from different geographical locations showed different levels of virulence against two insect hosts, *H. armigera* and *S. litura*. Rhizospheric soil samples usually showed the presence of more number of isolates with high infectivity against insect pests that could be correlated with lesser quantities of chemical pesticides in the vicinity of the rhizosphere. *Metarhizium* isolates from fields of tomato, okra and other vegetables heavily sprayed with chemicals were reported less virulent to *H. armigera* in comparison to isolates from custard apple fields rarely sprayed with chemicals (Nahar et al., 2003). Natural insect flora of host plant is known to affect the virulence of the entomopathogenic fungi isolated from that region.

ISOLATION OF MYCOPARASITIC FUNGI

The mycoparasitism was studied extensively by Richard Weindling from 1930s and 1940s (Yadav and Deshpande, 2010). For the first time Weindling noted the potential of *Trichoderma lignorum* in controlling plant-pathogenic fungi and also identified the killing component, gliotoxin from another mycoparasitic fungus, *Gliocladium*.

Interaction of various isolates of *Trichoderma* species such as *T. viride, T. harzianum, T. hamatum, T. longibrachiatum, T. koningii, T. pseudokoningii, Gliocladium virens* and *Laetisaria arvalis* with a plant pathogen *Botryodiplodia theobromae* was studied using scanning electron microscopy (SEM) (Gupta et al 1999). *Dimargaris cristalligena* is a mycoparasite of members from Mucorales like *Cokeromyces recurvatus*. Interestingly, *D. cristalligena* exhibited self-penetration, a relatively rare phenomenon which is similar to the conventional infection of a compatible host (Jeffries and Cuthbert, 1984).

Mycoparasitism initiates with location of the host mycelium or spores by the parasitic fungus in response to the chemical signals from the host fungus followed by attachment and lectin-mediated recognition between the parasite and its host (Inbar and Chet., 1997). Necrotrophic *Trichoderma* attaches to the host hyphae by coiling, hooks or appressorium-like bodies and penetrates the host by secreting cell wall lytic enzymes (Carsolio et al., 1999; Elad et al, 1999; Lorito et al., 1993) while *Gleocladium roseum* was reported to grow through *B. cinerea* cell walls by producing germ tubes without the formation of appresoria, produced chitinase and b-1,3-glucanse in the interaction with *Botrytis allii* (Li et al 2002). On the other hand, a biotrophic fungus like *Sporidesmium sclerotivorum* produces the haustoria in the host *Sclerotinia sclerotiorum* and the host itself triggers the production of cell wall lytic activities in the pathogen (Schroth and Hancock, 1981). As a result of different mechanisms of mycoparasitism there existed a strain dependent variation for the interaction between

Colletotrichum muscae, a pathogen of banana and mycoparasites (Krauss et al., 2001). Use of a mixture of different mycoparasitic strains could therefore be beneficial. Similarly Krauss and Soberanis (2001) reported the use of a mixture of mycoparasitic strains to control cocoa pod diseases caused by *Moniliophthora roreri, Crinipellis pernicosa* and *Phytophhora palmivora,* respectively. Five strains of *Clonostachys rosea* and three strains of *Trichoderma* sp. in different combinations were reported to be effective in controlling cocoa pod diseases.

The mycoparasitic fungi *G. roseum, G. virens*, *Chaetomium globosum* and *Coniothyrium minitans* were reported to parasitize hyphae as well as sclerotia of *Sclerotium cepivorum*, a causal agent of onion, garlic white rot (Stewart and Harrison, 1989). However, melanization protected the rind cells. Similarly, *T. virens* was found to parasitize sclerotia of *R. solani* (Liu et al., 2009). *Talaromyces flavus* was reported to parasitize microsclerotia of *Verticillium dahliae* (Fahima et al., 1992). However, Ulacio-Osorio et al (2006) reported inability of *Trichoderma* spp. to parasitize sclerotia of *S. cepivorum*.

Rey et al (2005) reported that sclerotial morphology of *Botrytis cinerea* and *Sclerotinia minor* was important in the mycoparasitism. In case of *B. cinerea* the mycoparasite, *Pythium oligandrum* entered through breaches at the junction of the rind wall corresponding to gaps in melanin deposits. However, the sclerotia of *S. minor* did not show the breaches and therefore penetration by *P. oligandrum* was up to the inner rind wall (Rey et al., 2005).

V. psalliotae was reported to grow on the rust sori masses of *Hemileia vastatrix* in coffee plantings in Malaysia (Mahfud et al., 2006). *R. solani* a plant pathogen with an extremely wide plant-host range is reported to be a parasite of fungi, especially mucoraceous ones, by coiling and penetrating aerial hyphae and sporophores of hosts (Butler, 1957). These hyphae also attacked sporangia of *Mucor recurvus* which were reported to be morphologically abnormal. Hoch (1978) reported short globose protruberances in *Stephanoma phaespora* during parasitism of *Fusarium* species. The mode of attack and the infection structures of *Pythium acanticum* in the mycoparasitism on taxonomically distinct hosts were studied both by light and electron microscopy (Hoch and Fuller, 1977).

All the above examples of fungus-fungus interactions and the knowledge of their different mechanisms can be used to isolate new strains for the culture collection.

One of the methods to isolate mycoparasitic strains is dual culture technique. Sometimes a potential mycoparasitic fungus was identified by its capability to produce extracellular metabolites/hydrolytic enzymes and/ or volatile compounds. The physical contact between two organisms was not required (Calistru et al., 1997). However, most of the researchers believed that the intimate contact is one of the pre-requisites to identify the mycoparasitic strain while some related the mycoparasitism to coiling around and penetration in to the host (Fenice and Gooday, 2006).

Some fungal strains have the capability to show dual pathogenesis. For instance, Jassim et al. (1990) reported that mycoparasitic *T. harzianum* parasitized the elm bark beetle, *Scolytus* while *V. lecanii*, pathogen of aphids (Alavo and Accodji, 2004), can parasitize rust, mildew and green mold pathogens (Benhamou, 2004). The dual specificity was attributed to the production of specific hydrolytic enzymes. It was suggested that entomopathogenic, mycoparasitic and plant pathogenic strains within *Verticillium* species can be separated on the basis of morphological and biochemical characteristics (Jun et al., 1991). Conidial size, shape, melanization of mycelium, colony characteristics, presence of different hydrolytic enzymes etc. were used to identify the strains from 3 groups. For instance, majority of *Verticillium* isolates from insects exhibited chitobiosidase (chitobiohydrolase, enzyme releasing chitobiose from the non-reducing end of the chitin chain) activity in contrast to mycoparasitic isolates (Chavan and Deshpande, 2013; Jun et al., 1991). However, recently Sharma and Shanmugam (2012) reported chitobiosidase activity from *T. saturnisporum*, antagonistic to *Fusarium oxysporum* f. sp. *dianthi.*

The fungal endosymbionts have significant contribution in insect nutrition, broadening the range of available resources by supplying enzymes for degradation or detoxification of plant material (Suh et al, 2001). Leufven and Nehls (1986) studied the yeasts associated with spruce bark beetles and reported *Hansenula holstii*, *H. capsulate, Candida diddensii, C. nitratophila, Pichia pinus,* and *Cryptococcus* species to affect the beetle behavior. On the other hand mushrooms have become a source for ascomycete and basidiomycete yeast species. Yurkov et al (2012) collected *Paxillus* and *Xerocomus* fruiting bodies for yeast isolations. The yeast genera reported were *Cryptococcus, Cystofilobasidium, Holtermanniella, Kluyveromyces, Leucosporidiella, Mastigobasidium, Rhodotorula, Rhodosporidium* and *Trichosporon.* The influence of yeast strains on the mycoparasite, *Sepedonium chrysospermum* and host *Paxillus involutus* was growth inhibitory and in some case stimulatory too (Yurkov et al 2012).

Strain selection criteria

Performance of entomopathogenic fungi can be attributed to their ability to cause mortality of target insects necessitating evaluation of parameters such as kill time (LT_{50} value), comparison of the performance of the isolates under laboratory and field conditions as well as effect of

laboratory maintenance (serial transfer) on the virulence of the fungus. Attenuation of insect pathogenic fungi especially hyphomycetes after serial transfer *in vitro* has been reported. However, passage through a host may improve virulence and expand host range (Nahar et al., 2008; Vandenberg and Cantone, 2004). Furthermore, the ability of the strains to produce differential levels of various cuticle degrading enzymes was reported to be important in the strain selection (Krieger de Moraes et al., 2003; Kulkarni et al., 2008; Nahar et al., 2003; Nahar et al., 2004; St Leger et al., 1986). The conidial size, viability, production, germination rate, hyphal development and the effect of environmental perturbations on differentiation were reported to significantly contribute in strain selection (Liu et al., 2003). For instance, conidial size in *P. fumosoroseus* showed correlation to virulence towards diamond back moth, *Plutella xylostella* (Altre et al., 1999). Genetic properties were also significant along with conidial morphology for the virulence of *V. lecanii* towards different insect hosts (Sugimoto et al., 2003). Thus, in mission mode collection of entomopathogens isolation and selection of fungal strains based on above mentioned parameters is important in the supply of cultures to the agriculture practitioners.

STRAIN-SPECIFIC MARKERS FOR IDENTIFICATION, PERSISTENCE, VIRULENCE AND LATERAL TRANSFER

Several methods have been used to describe the variation within a species of insect-pathogenic and mycoparasitic fungi. The morphological characteristics of spores and colonial characteristics, extracellular protein profiles, host specificity and pathogenicity, nutrient requirements, immuno-taxonomy, chemo-taxonomy and ultimately molecular methods are being used (Bielikova et al, 2002). Study of diverse areas of biology covering questions of phylogeny, evolution, ecology and population dynamics is facilitated by molecular markers (Chart 2) (Meyling 2008; Michelmore and Hulbert, 1987). Various techniques have been used to characterize entomopathogenic fungi and their population structure, habitat association and their interactions with hosts (Enkerli et al 2005). For instance, St. Leger et al (1992) reported use of allozyme variation, while randomly amplified polymorphic DNA (RAPD) was suggested by Leal et al (1997) and restriction fragment length polymorphism (RFLP) of protease *Pr1A* was used by Bidochka (2001) and microsatellite markers from *B. brongniartii* (Enkerli et al 2001), *M. anisopliae* (Enkerli et al 2005) and *P. fumosoroseus* (Gauthier et al 2007) were reported earlier. Bieliková et al (2002) reported strain-specific RAPD fingerprints for different entomopathogenic and mycoparastic fungi such as *P. fumosoroseus*, *G. virens* and *V. lecanii*. Ghormade et al (2007) evaluated *M. anisopliae* isolates for biocontrol of wire worm, *Agriotes* sp. using virulence markers such as protease (*Pr1A* and *Pr1B*) and chitinase (*ChiIII, Chi Va* and *Vb*) and microsatellite markers. Liu and Yang (2005) extensively used EST analysis to discover novel genes for exploring gene expression patterns and for identifying differentially regulated genes.

Markers for diversity studies

- Host recognition
- Cell surface lectins and carbohydrates
- Enzymes/ toxins involved in killing process
- Isoenzymes
- DNA-based
- RFLP (Restriction Fragment Length Polymorphism)
- RAPD (Random Amplified Polymorphic DNA)
- AFLP (Amplified Fragment Length Polymorphism)
- Microsatellite (Variable Nucleotide Tandem Repeat)
- SSCP (Single Strand Conformation Polymorphism)
- SNP (Single Nucleotide Polymorphism)

Fig. 2. Markers for diversity studies in entomogenous fungi.

The cuticle degrading enzyme (CDE) activities (protease, chitinase, chitin deacetylase, chitosanase and lipase) and mortality of *H. armigera* of sixty plus *Metarhizium* isolates showed eight major groups on the basis of variability in mortality (Kulkarni et al 2008). To understand a relative significance of individual activities further analysis was also carried out which suggested that all enzymes excepting chitosanase could be used as virulence markers (Unpublished data). The polymorphism in protease *Pr1A* gene of sixty eight *Metarhizium* isolates was assessed by PCR-RFLP and compared with *in vitro* enzyme activity and mortality against *H. armigera*. The correlation between the profile of restriction fragments and virulence of *Metarhizium* isolates as well as the geographic origin was observed. The *Pr1A* could be used as a virulence marker for *Metarhizium* isolates (Unpublished data).

The fungus-insect interaction is mainly affected by different environmental factors, such as heat shock and UV-B radiation, and also by responses of the host insect, such as oxidative stress, osmotic stress and fever. Earlier it was reported that adenylate cyclase regulates a variety of physiological processes in phytopathogenic fungi, which include: conidiation, conidial germination, vegetative growth, appressoria formation and virulence. Liu et al (2012) using RNA silencing approach reported that *M. acridium* adenylate cylcase gene (*MaAC*) significantly contributes in virulence and tolerance of the fungus to adverse environmental and host factors. *MaAC* was found to affect virulence by way of controlling the rate of vegetative growth inside the insect host coupled with tolerance of the fungus to oxidative and osmotic stresses and immune response of the host. These markers are important to identify virulent strains, their lateral

transfer and persistence in the field. This could prove to be an important mandate for the mission-based culture collection.

Fungal entomopathogens have been reported to occur as endophytes, both naturally and in response to various inoculation methods (Parsa et al., 2013). *B. bassiana* is the best-studied endophytic fungal entomopathogen. Parsa et al (2013) reported different inoculation methods used to establish *B. bassiana* as an endophyte such as soil drenches, seed coatings and immersions, radicle dressings, root and rhizome immersions, stem injection, foliar sprays and flower sprays. Using these methods, researchers have introduced *B. bassiana* into different fruit and vegetables, pulses and cereals. It has been observed that endophytic *B. bassiana* protected plants not only from arthropod pests, but also from some plant pathogens.

Preservation of entomopathogenic fungi

In general, fungal spores are stored at -20°C or -80°C using skimmed milk, or water or mineral oil. However, in case of entomopathogenic and mycoparasitic strains in addition to the viability, preservation of virulence is important too. López Lastra et al (2002) studied nine species of entomopathogenic fungi for viability when stored in deionized water, mineral oil, or silica gel or frozen at -20°C or -80°C for 3, 6, 12, and 18 months. *P. fumosoroseus* survived through 18 months when stored with water / mineral oil and when frozen at -80°C. For the majority of the fungal species storage at -80°C was best. However, Lopez Lastra et al (2002) observed that for *Smittium culisetae* and *Leptolegnia chapmanii*, freezing at -80°C was not useful. In addition to viability, virulence against *Aedes aegypti* larvae was evaluated after 18 months of storage for *L. chapmanii* and *S. culisetae*. The species stored in water or mineral oil maintained their viability and infectivity maximum.

Toegel et al (2010) assessed the stability of aerial conidia of *BEAUVERIA BRONGNIARTII* and *M. ANISOPLIAE* after lyophilization. The spores without any protectant exhibited high cryosensitivity. However, the sugars such as fructose, glucose, and saccharose significantly enhanced viabilities for *B. BRONGNIARTII* and *M. ANISOPLIAE* spores following freeze drying. The combination of fructose and a bulking agent, dextran 4 was beneficial for viability as well as for the production of virulent secondary metabolites such as destruxin A, destruxin B, or oosporein.

Fungal isolates can be stored for long period as dry alginate pellets containing wheat bran at -23°C. Fungal conidia can also be stored in carriers such as mineral oils, wheat bran, talc powder, silica etc. Kim et al (2008) suggested that the conidia of *Beauveria* species adsorbed onto mineral adsorbents mixed with vegetable oils (soyabean, olive, cotton seed and corn) had increased thermostability. Oliveira et al (2009) isolated *B. bassiana* strains from naturally infected *Prays oleae* pupae which were stored either in 30% (v/v) glycerol at -20ºC or lyophilized. After one year of storage *B. bassiana* viability was strain as well as storage method specific. The 30% glycerol and storage at -20ºC was found to be useful for all the isolates while only two isolates showed good viability after freeze-drying. The capacity to produce conidia was significantly lower for strains preserved by lyophilisation than the cultures preserved in glycerol.

***IN SITU* MAINTENANCE**

Both biology and physiology of entomopathogenic fungi depend on environmental parameters such as temperature and relative humidity or nutrient availability. However, for use of these entomopathogens in fields, *per se*, the understanding of their interactive behavior with host with respect to environmental factors is of great significance. Therefore morphological homogeneity and virulence are essential steps to develop any mycoinsecticide for field performance. Many insect-pathogenic fungi are known to attenuate during successive sub-culturing, resulting not only in a loss of virulence but also variation in conidiation characteristics (Nahar et al., 2008). In most of the cases the virulence can be regained by host passages for few times. The pests which have some part of their life cycle in soil can be maintained regularly with their respective hosts along with entomopathogens. Thus *in situ* preservation can be one of the methods to supply active strains. Alternately, minimum sub-culturing on artificial media and regular passage through insect host can be employed to give active cultures.

BIOPROSPECTING OF ENTOMOPATHOGENS AND MYCOPARASITIC FUNGI

The pest and pathogen control in the field using fungi has gone beyond 'proof of concept'. However, in view of the performances of these organisms in the fields and moreover acceptability by the end users regarding cost-effectiveness, shelf life, intellectual property rights (IPR), the additional roles and possible applications are being explored (Vega et al., 2009). Dual pathogenicity of either entomopathogens or mycoparasitic strains has added advantage of wide spectrum of biocontrol activity. In addition to plant protection these fungi can promote plant growth too (Ondrackova et al., 2013). The phylloplane yeast, *Sporothrix flocculosa* was reported to colonize hyphae and conidia of a wheat powdery mildew pathogen, *Erysiphe graminis* var. *tritici* (Hajlaoui and Langer, 1993). Suzzi et al (1995) reported biocontrol potential of natural wine yeasts while a number of natural yeast isolates from grapes exhibited pesticide (azoxystrobin) degrading activity (unpublished data). Epiphytic *Pseudozyma* yeast group also showed biocontrol activity against a number of plant pathogenic fungi (Avis and Belanger, 2002). These

types of organisms have lot of bio-prospecting potential which could be one of the main activities of mission mode culture collection. However, safety to beneficial insects and fungi and biodiversity, in general, indeed is important before increasing the scope of these organisms for a variety of applications.

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