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Role of mycology and mycologists in an era of industrial biotechnology

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ABSTRACT

While reflecting on the life and scientific contributions of Dr. M.J.Thirumalachar, I am amazed at the way he grew up with the times and acquired the necessary skills to contribute meaningfully as a mycologist to the rapidly advancing discipline of Industrial Biotechnology. He was truly ahead of his times in his thinking and vision and if we take a leaf out of his book and evaluate its relevance in the context of today's requirements for innovative research and development ,we will definitely see the need for mycologists to gear up and get prepared for making meaningful contributions to Biotechnology in the twenty first century. I will attempt to review the role of mycology and mycologists in an era of industrial biotechnology and offer it as my humble tribute to Dr.Thirumalachar in the Kavaka publication commemorating his birth centenary.

Keywords: Mycology, mycologist, fungal diversity, conservation, biotechnology

INTRODUCTION

Fungi during the early years have been recognised more as destructive unwanted entities robbing mankind's bread basket by causing destructive infections on plants and causing crop losses. They were also responsible for several diseases of humans and animals collectively termed as mycoses. Plant pathology advances resulted in the understanding of the disease cycles of several pathogens and methods to eradicate the disease by developing control measures. Medical mycology made significant progress through systematic investigations on the fungal infections in man and animals which led to development of effective chemotherapeutic control agents.

Beneficial activities of fungi contributing to human welfare have also received attention. The oriental fermented foods like **Miso, Tempeh** and others based on fungal fermentations have been known for long and practiced for long in countries like Indonesia, Japan and Thailand. During the late 19th and early 20th centuries, processes for the manufacture of useful metabolites such as Taka Diastase from *Aspergillus oryzae* and citric acid from *Aspergillus niger* were developed and reached commercial levels. Even metabolites from phytopathogenic fungi were discovered with medicinal properties and commercial applications such as the ergot alkaloids from *Claviceps purpurea* and related species and gibberellic acid from *Gibberella fujikuroi* (*Fusarium moniiforme*).

With the advent of the antibiotic era with penicillin from *Penicillium chrysogenum*, the focus on screening saprobic microbial diversity for bioactive metabolites increased and encompassed diverse fungi as well as actinomycetes. With reliable assay systems to identify and purify bioactive metabolites often produced in low yields from fermentation broths ,the technology of applying diverse fungal systems to intensively explore for novel metabolites and industrially useful enzymes has gained strong foothold globally.

Mycologists study fungi from diverse habitats and publish research papers identifying and naming them as well as classifying them primarily on the basis of the morphology of their spore forms. This has resulted in a well developed data base of fungal taxonomy and classification. Recent advances in molecular biology applied to fungal systems have added new dimensions to understand fungal taxonomy, classification and relationships.

In the present scenario of multidisciplinary involvement and inputs required for development of novel technologies, how should the mycologists adapt themselves to become an integral part of such teams? What is their specialization which counts seriously in biotechnological process development? I will focus on the following aspects:

a. Biodiversity exploration and "*in-vitro*" conservation of germ plasm for biotechnology research and development.
b. Ensuring morphological and genetic stability of wild strains as well as high productivity mutants during prolonged conservation in pure cultures.
c. Morphogenesis of fungal strains in submerged culture in relation to the production of bioactive metabolites.

KNOWLEDGE BASED EXPLORATION OF FUNGAL DIVERSITY

Understanding the ecology and distribution of diverse fungi in various habitats is a prime requirement for successfully culturing the rare and less investigated taxa. Also of great importance is the application of selective isolation techniques which enable the slow growing and less distributed forms to overcome competition from the abundant heavy sporing aspergilli, penicillia and fusaria which overrun the isolation plates. By the judicious application of special techniques combined with selective media formulations, several rare and relatively slow growing fungi have been successfully isolated and grown in pure culture. The topic of selective isolation of fungi is a vast one and has been comprehensively reviewed elsewhere which may be consulted for more details (Srinivasan, 2004; 2008). As an example we may consider screening fungal diversity in the forest litter ecosystem. Depending on the population diversity of the tree species present, the litter will vary widely in composition and in the different layers of the litter undergoing slow decomposition , the fungal diversity encountered will be most fascinating. Apart from the lignocellulosic residues contributed by the forest trees, the litter also has animal and bird droppings, hair and feather which are keratinaceous substrates. Some of the innovative

approaches to explore fungal diversity in the forest litter include the particle plating technique described by Bills and Polishook (1994) in which finely pulverised air dried plant litter was washed with distilled water followed by passage through fine filters ranging in size from 100 to 210 µm. Plating out the fine particles individually trapped on the different filters yielded a greater variety of fungal colonies many of which originated from the interior of the fine litter particles freed from surface borne propagules.

A group of fungi widely distributed in plant litter but not encountered in plating experiments is the saprophytic *Entomophthorales* represented by the genera *Conidiobolus* and *Basidiobolus.* The conidia are forcibly discharged from unbranched phototrophic conidiophores and this property was taken advantage by Drechsler (1952) who isolated several new species of these fungi by canopying moist litter attached to the inner surface of the petriplate lid over agar plates. Pure cultures developing from individual conidia were readily isolated by this technique. This technique was successfully adapted by Srinivasan and Thirumalachar who in a series of publications described several species including new ones from litter samples collected from different regions in India recording their widespread distribution in the tropical litter samples of the Indian subcontinent.

Because of the high protein content of the organic matter undergoing decomposition in forest litter, ammonia is released and within the litter niches are established with high alkaline pH. This encourages "enrichment"of alkalotolerant and alkalophilic microbes including fungi . In my studies on alkalophilic fungi from plant litter an alkalotolerant *Cephalosporium* secreting cellulase-free xylanase stable to and active at high alkaline pH was isolated. The technique consisted of plating out fine particles of detritus on media containing xylan adjusted to alkaline pH by addition of sterile sodium carbonate to autoclaved media before plating. Antibacterial tetracycline antibiotic was added at 10-25 µg/ml to suppress bacterial and actinomycete colonies while fungal development was unaffected, (Bansod *et al*,1993; Rele *et al*,1996).

Plant litter and compost heaps deserve intense investigations from mycologists for understanding the biodiversity of thermophilic species. Opportunities to identify hitherto unknown species or biotypes with thermo-alkalotolerant or thermo-halotolerant properties would be worthwhile investigating both from the mycological and biotechnological viewpoints.

"*IN-VITRO*" CONSERVATION OF FUNGAL DIVERSITY FOR TECHNOLOGICAL APPLICATIONS

In the era of industrial biotechnology which is making spectacular advances, mycologists must consider their fungal isolates in pure culture as valuable "mycological heritage" with potential for utilising it for discovering novel metabolites. They should spare no efforts to ensure their long term conservation under optimal conditions to ensure morphological and genetic stability. Presently morphological descriptions of new taxa are published with either no attempt to study them in pure culture or with a casual description of colony characters on routine mycological media like potato dextrose agar or malt agar. Little or no data is generated on the physiology and nutritional requirement for sustained morphological and genetic stability of pure cultures, which is most important for undertaking any biotechnological investigations on the novel isolates. Mycologists must seriously consider depositing their novel strains with the Germplasm collection centres in the country like the National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune; Microbial Type Culture Collection (MTCC); Institute of Microbial Technology, Chandigarh and National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. Deposition of the cultures with details of the optimal methods of conservation would ensure long term availability of the valuable indigenous germplasm for future biotechnological studies.

Prolonged subculture on sugar rich media like potato dextrose agar is preferably avoided since the tendency towards rapid vegetative growth consuming the sugar will adversely affect sporulation and it is often noticed that these cultures appear mycelial and pleomorphic. Also in cultures isolated for industrial enzyme technology, sugar rich media can cause catabolite repression from the high glucose content. In my studies on Cellulolytic and Xylanolytic enzymes, it was seen that the best method of conservation was on a weak potato carrot extract (Extract of 10 grams per litre of potato and carrot) solidified with 2% agar slants on which sterile filter paper and autoclaved grass leaf are placed after solidification. Cultures grown on these slants also colonise the filter paper and grass leaf and sporulate well on these substrates. Cultures of *Volutella, Abgliophragma* and *Beltrania* isolated from litter were successfully maintained with good sporulation on this medium while on sugar rich media sporulation declined and eventually totally lost.

Presently there is considerable interest in the study of endophytic fungi especially from medicinal plants with a view to develop fermentation processes for the valuable plant metabolite employing the fungal endophytes. This interest has originated from the study of the anticancer drug Taxol being shown to be produced by an endophytic fungus from *Taxus brevifolia* designated *Taxomyces andreanae* (Stierle *et al*, 1993). I believe serious consideration needs to be given to the conservation protocols of the endophytic fungal isolates. We need to appreciate that the genes for plant based metabolite "imbibed" by the endophyte through constant environmental association within the host plant, could very well be lost in pure cultures grown on routine sugar-rich mycological media. It may be worthwhile to culture and conserve them on media with low sugar content supplemented with key intermediates or biochemical building blocks essential for the biosynthesis of the plant based metabolites. By this we may hope to ensure that the growth environment of the endophyte *in-vitro* is an approximation of the nutrient composition within the host tissue. This aspect of endophytic fungi in relation to cultivation and conservation strategies deserves serious inputs from mycologists if successful technologies for the manufacture of plant metabolites from endophytic fungi are to be achieved.

Mycologists involved in long term conservation of fungi in

culture collections must possess expert knowledge on the physiology and nutritional requirements as well as conservation strategies in regard to the strains which are fastidious. Sensitivity of specific groups to the well practiced conservation protocols require to be given careful consideration prior to deciding upon the most suitable methods of conservation. For instance the strains of *Conidiobolus, Basidiobolus* and some members of *Mucorales* do not withstand lyophilisation and also revive poorly after being stored in the refrigerator at low temperatures. They are optimally conserved at 15 °C on agar slants and maintained through subcultures at 3-4 month intervals. Some success has been reported with liquid nitrogen storage but this needs to be studied more with a larger number of cultures before it can be recommended and accepted. Mitosporic fungi which form abundant conidia are best conserved by the grass leaf technique described by Srinivasan *et al.* (1971) for seed borne fungi. Young growing mycelia discs are inoculated on autoclaved grass leaf (*Pennisetum glaucum*) bits placed on water agar plates and incubated . Ready colonisation followed by rapid sporulation on the grass leaf surface is observed within 4-5 days in most cases. The sporulating material along with subtending agar blocks may be transferred to sterile water to remain afloat and conserved at 15 °C. By this technique the culture retains viability and ability to sporulate upon subculture over prolonged periods without undergoing pleomorphic degeneration often observed in serial subcultured agar cultures.

Strain selection through mutation for enhanced production of metabolites is a vital activity in which mycologists have a pivotal role to play along with fungal geneticists. Cloning and heterologous gene expression in yeast and fungal systems is now a practical technology for the production of mammalian proteins such as insulin and chymosin (enzyme used in cheese manufacture). Conservation of recombinant strains is a challenging one to ensure that the cloned heterologous genes are not lost during prolonged conservation, and requires specialist knowledge in molecular genetics.

MORPHOGENESIS IN SUBMERGED CULTURE

The majority of secondary metabolites and industrial enzymes produced by fungi are through submerged culture in which the fungal strains are cultivated under aerated agitated conditions in large volume fermenter tanks. In the development of technology and scale-up for large scale manufacture mycologists have several important assignments and these include devising mass sporulation techniques, standardisation of the protocols for inoculum development and microscopic follow-up of the mold morphogenesis and growth pattern during the fermentation.

Shake flask results need to be reproduced in the aerated agitated fermentation conditions and while in the shake flasks the mold growth is free mycelial or pellety and governed by strain, spore density, age of the spore inoculum, etc. In the fermenter the mold morphogenesis is influenced by the chopping effect of the agitator blades, shear forces and the availability of oxygen for the mold to utilise and grow. Mycological observations on mold morphogenesis in relation to metabolite production is a very important aspect contributing to the standardization of the process parameters and in collaboration with process bioengineers mycologists can contribute significantly to process optimization.

CONCLUSION

From the foregoing discussion it would be obvious that mycologists can have a leading role to play in the area of industrial biotechnology. While striving for excellence in their chosen field of specialization, mycologists need to expand the horizons of their interest and knowledge to include biochemistry, molecular biology and natural product isolation and purification. The era of multidisciplinary approach to scientific problems is in full swing and industrial biotechnology truly exemplifies it. The situation has been very well summed up by Kreeger (2003) in the following words:

"*We all realise that most scientists in the future will be part of multidisciplinary research teams...the shift is causing a change in the way scientists need to train... although they must still be an expert in their speciality they must also become conversant in techniques that once seemed beyond their domain... they also need to recognize where their knowledge ends and where they should seek the help from others who have expertise in a particular field*."

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