



ENVIS CENTRE

MICROORGANISMS AND ENVIRONMENT MANAGEMENT



NEWSLETTER

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SPECIAL ISSUE ON SDNP-ENVIS PROGRAMME

Dear Readers,

The user community is aware that the ENVIS Centre on 'Microorganisms and Environment Management' functioning in the Department of Zoology, University of Madras is collecting, collating and disseminating environmental information since June 2002. In addition to the regular ENVIS activities, ENVIS Centre has been assigned a further responsibility of strengthening of its networking for Sustainable Development on the assigned thematic area under the **India-Canada Environment Fund (ICEF) Small Grant Programme**.

Microorganisms, are vital components in our daily lives and best tools for recycling and waste management. They can break down a wide range of pollutants and clean-up the polluted sites. Besides, the technology of **Effective Microorganisms (EM)**, manage the polluted environment in a beneficial and sustainable manner. Thus, the development of EM technology and research on genetically altered microorganisms will definitely make our environment cleaner and sustainable for us and the future generations. In view of microorganism's application on the sustainable development, separate web-page has been developed on the **Sustainable Development Networking Programme (SDNP)-ENVIS** thematic area and linkages are given in the ENVIS Centre's website. Besides, this is a **special issue under the SDNP-ENVIS Programme**. This issue includes research article, published reports, abstracts, references, e-resources, forthcoming conferences on microorganisms and sustainable development areas.

Your suggestions and comments are welcome to improve our ENVIS Centre activities and update services to user community.

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Are You Aware of?

UNESCO CHAIR IN FOOD AND ENVIRONMENT BIOTECHNOLOGY FOR SUSTAINABLE DEVELOPMENT (BIODEV) (578),

Established in 2002 at the University of Provence (Aix-Marseille 1), (France)

Objectives

- ⊗ To promote an integrated system of research, distance education, training and documentation activities in the field of food and environment biotechnology for sustainable development.
- ⊗ To facilitate collaboration between the high level researchers and the internationally recognized professors of the University, the INRA (Institute National de Recherche Agronomique) and the IRD (Institute de Recherche pour le Developpement) and the other participating teaching and research institutions.
- ⊗ The research activities will be related to the protection and development of the microbial genetic heritage from targeted biotopes (in particular tropical and extreme biotopes) of the sectors that constitute the intercontinental network. These research activities will lead to the creation of microorganism's genetic resources banks. Specific research activities will be undertaken in each geographical area concerned (Asia, North Africa and South America).
- ⊗ A system of distance education structured in complementary modules will be set up.
- ⊗ These studies will be sanctioned by an international certificate of biotechnology issued by the University.

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Biodiversity of *Basidiomycetes* and their role in Sustainable environment management

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Fungi have a vast range of biodiversity than in the plant kingdom, and possibly more than in the animal kingdom. Most fungi are closely associated with dead or living plants, and sometimes with animals. They degrade all kinds of biological wastes either transforming them as biofertilizer or mineralize into carbon-di-oxide and water ensuring a sustainable protection of environment by safeguarding soil fertility and water. They show a wide range of interactions with other organisms from mutualisms to pathogenicity. Fungi is also the second largest group of organisms in the world, after insects, only 5% of the world's fungi, 70,000 out of an estimated number of 1.5 million, are

currently known (Hawksworth, 1991; Hawksworth and Rossnab, 1997). Tropics are considered to be the largest repository of these unknown fungi as they encompass the largest range of climates and habitats on earth. Many countries are preparing red-data lists of endangered fungi (Arnold, 1997; Ing, 1992; Countecuisse, 1997), but most parts of India, a major tropical country remain mycologically unexplored. In maintenance of biodiversity, the conservation of fungi has received scanty attention in our country is of urgent necessity. Inventory and monitoring are important for proper management and conservation. There is growing awareness on the protection of biodiversity of all life forms including fungi throughout the world and various countries have initiated projects towards this end. International agencies like UNESCO and WWF are also involved in this endeavor. Many basidiomycetes are primary degraders and mineralize the lignocellulosic wastes. Lignin, a complex phenolic polymer resist microbial degradation and affect feed value by 35%, is successfully degraded by basidiomycetes, which

produces non-specific peroxidases. Employing these fungi for removal of lignin from feed improve milk production by improving the feed value. Further the substrate non specificity of these enzymes are exploited in bioremediation of many toxic pollutants like azodyes, herbicides, etc. from the environment. In the present study, the application of these organisms in sustainable management of environment and their biodiversity in South India are discussed.

National Status

A bird's eye view of the literature on mycological research in India reveals that agarics (Agaricales, Basidiomycota) are one of the least studied groups of fungi. In the updated list of agaricoid and boletoid basidiomycetes reported from India and Nepal. Manjula (1983) listed only 538 species of agarics. Recently Varma and Upadhyay (1998) enumerated a total of 912 species, belonging to 121 genera from 16 families of Agaricales, Tremellales and Auriculariales. According to Watling and Gregory (1980), the British Isles with a landmass almost hundred times lesser than that of India, supported two thousand five hundred species of mushrooms. The abysmally low figure of agaric species recorded from India, compared to that of the British Isles, very well depicts the present magnitude of ignorance on the diversity and distribution of this group of fungi in India.

Natarajan from Chennai, Sathe from Pune and Leelavathy from Calicut and their students have started systematic studies on the agarics of South India more than two decades, yet the task of preparing a preliminary inventory of agarics of this region is far from complete. Till date, around 1000 agarics are described in India, of which only few hundreds were brought into culture. Hence, it is utmost necessary to conserve the mushroom germplasm. This proposed herbarium and culture collection (genome bank) can serve the taxonomists as a source of reference and the biotechnologists who is looking for microorganism with variable potentials.

Over the last decade, efforts have been made to

use the following lignolytic white-rot basidiomycetes in bioremediation technologies. They are *Bjerkandera adusta*, *Nematoloma frowardii*, *Phanaerochaeta chrysosporium*, *Phanaerochaeta sordida*, *Phlebia radiata*, *Stropharia rugosoannulata*, *Pleurotus ostreatus*, *Pleurotus florida*, *Tricholoma lobayense* etc. In spite of their excellent enzymatic capability a major disadvantage of these fungi is their less competitive potential in soil. However, many reports suggest that the litter decaying basidiomycetes, which preferentially colonize in upper soil layers of woodlands and pastures as well as on lignocellulosic materials (wood, straw). They form a huge mycelial colonies and it has been estimated that they can extend over an area of several hectares, while reaching an age of 1500 years and mycelial biomass of 100 tons, (Dix and Webster 1995). Hence, there is an urgent need to explore and characterize the litter decomposing and white-rot fungi, in order to innovate an eco-friendly bioremediation process.

In the present study, mushrooms were collected from South India, especially at Mudumalai forest Kodaikanal forest, Tirumala Hills and in Chennai city and preserved in the laboratory. Degradative enzyme profiles of these organisms were screened as a part of biodocumentation process. Further these enzymes are utilized in many industrial processes like biopulping, depectinisation of fruit juices and in bioremediation of industrial pollutants.

Materials and Methods

The shape size and the colour of the mushrooms collected were recorded in the field itself. The colour chart of Metheun was followed. Tissue from context region has been isolated in the medium and purified by subsequent sub culturing. The dried specimens were preserved with naphthalene in air tight polypropylene bags. For microscopic studies, the sections were treated with 5% KOH and stained in phloxine and cresol blue. Melzer's reagent was used to determine the amyloid nature of the spores. Industrially

important degradative enzymes such as Laccase (Daniel *et al.*, 2004), Manganese peroxidases (Boominathan *et al.*, 1998), Protease (Rajamani and Hilda, 1987) and lipase (Sierra., 1957) activities were screened for the isolated fungal cultures.

Results and Discussion

The culture of every people is closely allied to its landscape and their living inhabitants, and cannot be dissociated from them. Medicines and solutions to the pollution come both from flowering plants and microbes. Microbes are showing enormous promise in cleaning up pollution and providing novel enzymes to speed up chemical reactions in many industrial processes. Many macro fungi not only serve as medicines but also possess many bioactive agents, now considered as dietary supplement or mushroom nutraceuticals. Recent years have seen a surge of commercial interest in medicinal mushroom and dietary supplements. The common market value of these medicinal mushrooms is approximately \$ 9 million US dollars (Wasser *et al.*, 2004). Further, it is clearly demonstrated that plant communities are dependent on the mushroom / ectomycorrhizal community. Johnson *et al.*, (2005) demonstrated that nutrients are transported from the trees exposed to the sunlight to the trees growing under shade and under nourished ones and claimed this as wood wide web (www). Thus, mushrooms play a very important role in forest ecology and valuable source of bioactive compounds and efficient agent for bioremediation. Hence, in the present study an attempt has been made to collect mushrooms from various parts of Tamilnadu forest areas and from Tirumala Hills of Andhra Pradesh. The systematic position and their degradative enzyme profiles are determined. Their degradative enzyme profile were screened and employed in degradation of azo dyes and glyphosate.

Over three hundred specimens of macrofungi were collected from Tirumala hills, Kodaikanal forests and Mudumalai sanctuary for the past two years. Of

which, forty eight fungi were brought in cultures. Out of forty eight cultures, 20 are white rot in nature while others are litter decomposing. White rot fungi produce unique extracellular oxidative enzymes that initiate the attack on nature's complex aromatic polymer, lignin. Important examples are the haeme containing peroxidases copper containing laccases and manganese dependent Manganese peroxidase. Search for fibrin degrading proteases from non-toxic mushrooms is one of the thrust areas of medical research. Already few reports confirmed presence of fibrinolytic protease from edible mushroom *Pleurotus ostreatus* and a medicinal mushroom *Ganoderma lucidum*. (Choi and Shin, 1998; Choi and Sa, 2000) Some of the representative mushrooms collected are described and illustrated with photographs (Table 1).

Table 1. Agarics and their taxonomy

Family	Genera	No. of species
Polyporaceae	<i>Pleurotus</i>	3
	<i>Lentinus</i>	3
Tricholomataceae	<i>Clitocybe</i>	4
	<i>Lepista</i>	3
	<i>Ripartitella</i>	2
	<i>Marasmius</i>	16
	<i>Marasmiellus</i>	3
	<i>Mycena</i>	4
	<i>Laccaria</i>	1
	<i>Gerronema</i>	2
Entolomataceae	<i>Tricholoma</i>	2
	<i>Entoloma</i>	2
Amanitaceae	<i>Amanita</i>	3
	<i>Limacella</i>	1
Pluteaceae	<i>Pluteus</i>	3
	<i>Termitomyces</i>	4
Agaricaceae	<i>Chlorophyllum</i>	1
	<i>Macrolepiota</i>	1
	<i>Leucocoprinus</i>	6
	<i>Leucoagaricus</i>	5
	<i>Agaricus</i>	6
	<i>Lepiota</i>	12
Coprinceae	<i>Psathyrella</i>	6
	<i>Coprinus</i>	3
	<i>Paneolus</i>	1
Cortinariaceae	<i>Astrosporina</i>	5
	<i>Gymnopileus</i>	5
	<i>Rhodocybe</i>	2
	<i>Hebeloma</i>	4
	<i>Alinicola</i>	1
Russulaceae	<i>Russula</i>	6

Total no. of species 120

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Basidiocarps of various taxa of Basidiomycetes in their natural habitat

These basidiomycetous fungi preferentially colonize the litter and upper soil layers in woodlands and pastures as well as lignocellulosic materials (wood, straw). From ecophysiological point of view, they can be broadly classified into litter-decomposing, wood-decaying and mycorrhizal fungi.

However, all these fungi possess an efficient degradative enzyme system. Lignin peroxidase and Manganese dependent peroxidases (MnP) as well as the copper containing peroxidase, laccase are the major degradative enzymes produced by fungi. These enzymes biotransform the substrates like lignin, cellulose and hemi-celluloses for its absorptive nutrition and thus these microorganisms play an important role in maintaining the global carbon cycle (Griffin, 1994). These enzymes are non specific in their substrate range. It is the only enzyme system that converts aromatic compounds including lignin partly to carbon dioxide (CO₂). This means lignin and other aromatic compound can be mineralized at least in part outside the fungal hypha and has been described as enzymatic combustion (Hofrichter *et al.*, 1999).

The unique lignolytic enzyme system of basidiomycetous fungi would be ideal for the biodegradation of organopollutants in the environment (Fernando and Aust, 1990). Compared to other potential bioremediation systems, the extracellular, non-specific, non-stereoselective lignin-degrading system of basidiomycetous fungi has the advantage of being applicable to a variety of recalcitrant and toxic chemicals. Examples for such chemicals and selected fungi responsible for the biodegradation are given in Table 3. Among these substances, are hazardous xenobiotic compounds like polychlorinated dibenzodioxins and dibenzofurans, other chlorinated aromatics, nitroaromatic compounds (explosives) and cancerogenic organopollutants belonging to the polycyclic aromatic hydrocarbons. However the recent research has been focused on litter decaying basidiomycetes, since they have competitive potential in soil and naturally colonize the upper soil layer. *Stropharia rugosoannulata* is an example of such a

fungus which has already been successfully tested for TNT decontamination of soils (Herre *et al.*, 1997). Both Mn peroxidase and Laccase have been shown to be involved in the transformation of organopollutants (Bollag *et al.*, 1988 Barr and Aust, 1994; Hofrichter *et al.* 1988a).

Hence in the present study many basidiomycetes were screened for the Mn, Lip and Laccase and many of the organisms have showed positive results. From the screening, it is clearly evident that majority of the fungi have laccase which is responsible for degradation of phenolics and lignin besides Mn or Lip. Further it is necessary to identify indigenous organisms from polluted soils and to enhance their degradative potential by bioaugmentation. Hence our laboratory is investigating and screening novel litter decomposing fungi which can be used in biological remediation.

Table 2 : Selected Organopollutants that are Transformed or Mineralized by Ligninolytic Basidiomycetes and/or their Ligninolytic Enzymes (according to SACK *et al.*, 1997b ; HAAS *et al.*, 1998a)

Fungus	Organopollutants
<i>Bjerkandera adusta</i>	Benzo(a)pyrene, other PAHs, TNT, dyes
<i>Nematoloma frowardii</i>	Benzo(a)pyrene, other PAHs, TNT, DCP, PCP, AsO
<i>Phanerochaete chrysosporium</i>	Benzo(a)pyrene, other PAHs, BTX, DNT, TNT, DDT, DCP, PCP, PCBs DCA, dyes, polystyrenes, KCN
<i>Phanerochaete sordida</i> <i>Phlebla radiata</i> <i>Pleurotus ostreatus</i>	PAHs, polychlorinated DBDs and DBFs TNT, dyes Benzo(a)pyrene, other PAHs, dibenzothiophene, TNT
<i>Stropharia rugosoannulata</i>	DCP, PCP, TNT
<i>Trametes versicolor</i>	Benzo(a)pyrene, other PAHs, DCA, DCP, PCP, dyes.
<i>Tricholoma lobayense</i>	Azo dyes, herbicide (Glyphosate), (Kaviyaran <i>et al.</i> , 2003)
<i>Pleurotus cornucopiae</i> <i>var. citrinopileatus</i>	Paper mill effluents and sludges. (Kaviyaran and Natarajan 1996)

Azodyes a potent carcinogenic textile dyes constitute a major class of environmental pollution accounting for 60 -70 % of dyes used in industries. *Laccaria fraterna* decolourized the dye congo red.(Fig. 1). Reduction and disappearance of the peaks from the absorption spectrum of both fungi clearly indicated the degradation of the dye. The degradation may be due to the extracellular degradative enzymes such as laccase, lignin peroxidases and some dye decolouring azoreductases. Involvement of laccase was well demonstrated by Muralikrishna and Renganathan (1993.). Papermill effluents and sludges were decolourized by *Pleurotus cornucopiae* var. *citrinopileatus*. (Kaviyarasan and Natarajan 1996). Glyphosate , the herbicide used indiscriminately not only spoils flora but also affect human beings is easily degraded by *Pleurotus ostreatus* (Fig 2).

These experiments were very well demonstrated the potential ability of these organisms in degradation of xenobiotic compounds such as azo dyes and paper mill effluents the threatening pollutants. Amending the polluted soil with the compost of litter decomposing fungi not only reduced the pollutants in the soil but also reduced the cost of bioremediation by 10 times (Hofrichter *et.al.*,1999). *Pleurotus* sp mycelium reduced the hydrocarbon level from 10,000ppm to 200ppm in the contaminated soil and also eliminated coliforms from the effluents (Stammet, 2003). Municipal wastes composted with these fungi and suitable bacteria are utilized for landfilling of contaminated sites and forests, ensures the sustainability, beneficial effects on soil and environment quality(Magesan and Wang,2003). Using basidiomycetes to remove contamination from soil is beyond the proof of concept phase but remains in stillborn status compared to physico- chemical technologies.

Mycoremediation works because mycelia produce both enzymes and acids which breakdown carbon and hydrogen bonds.

It matters not to the mycelia that these bonds are found in cellulose or petroleum products, pesticides, or other pollutants. The ability of mycelia to break these bonds make them predictable, effective and sustainable forms of hazardous waste remediation. Research continues on the effectiveness of mycelialstrains in breaking down a variety of other toxic and hazardous compounds.

It has been clearly demonstrated that the peroxidases oxidizes pollutants in the presence of Mn and Fe. Our studies also proved that these metal

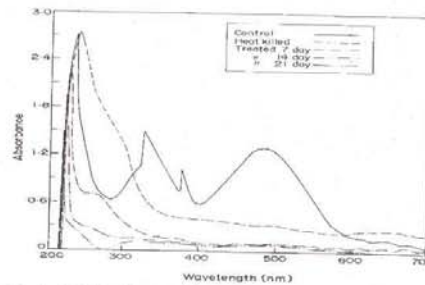


Fig 1: Azodye (Congo red) degradation by *Laccaria fraterna* under submerged condition.

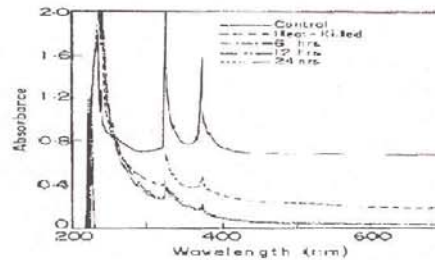


Fig2: Degradation of Herbicide - glyphosate by *Pleurotus ostreatus* under submerged condition.

complexes enhanced the enzyme activity to hundred folds. In addition, these degradative enzymes are increasingly used in a wide range agrobiotechnological processes, like enzyme assisted ensiling, bioprocessing of crop residues, fiber processing, biopulping for paper industries and feed supplements. Hence, the preservation of these species in our biosphere not only help in bioremediation but also helpful in many biotechnological purposes

In this study we have successfully isolated many kinds of macro fungi belongs to both Ascomycetes and Basidiomycetes; Of which, many edible fungi such as *Volvariella volvaceae*, *Morchella esculenta* were isolated which belongs to Basidiomycetes and Ascomycetes respectively. One unidentified polyporaceae member was consumed by the local tribals of Kodaikanal was also collected and brought into culture which may be new to science. The trials are on for cultivating these mushrooms. Further these specimens are also available for screening of bioactive compounds for the pharmaceutical science and industry. Thus the biodiversity of these higher basidiomycetes yield potential organisms to degrade and mineralize the pollutants at a cost effective rate in an eco-friendly way. As for India is concern it is a humble beginning but miles to go before we list the red data.

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(*Cited references in the text can be obtained from the author by request. E-mail address: manikavi53@yahoo.com)

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R.Siva and **N. Kumar** are research scholars pursuing their Ph.D programme).

Reports on Microorganisms and Sustainable Development

Bioprospecting for Novel Microorganisms

Studies concentrated on consolidating the leads established and taking them to the next stage of product development, especially with reference to the potential biofertilizer strains, initiating the field evaluation of the

potential bio-control strains and conducting awareness programmes for the farmers on the use of biological software

Basic research continued in the area of microbial diversity, to understand the mechanism of salt adaptation in the beneficial bacterial strains. Molecular tools like PCR-RFLP, PCR-SSCP and RFLP-SSCP are being employed to study the diversity among soil bacteria in the mangrove rhizosphere ecosystem. The osmolyte and salt shock protein profile in *Pseudomonas fluorescens* is also studied using advanced biochemical tools.

Field Testing and Product Development of Plant Growth Promoting Bacteria in the Coastal Agri-ecosystem

Potential bio-fertilizer strains (*Azospirillum*, *Phosphobacteria*, *Swaminathania salitolerans*) were tested under multi-locational trials in nearly 100 acres of land and their efficacy evaluated. Up to 30 % increase in the yield in biofertilizer treated plants over control was observed. There was 14 to 70 % increase in the number of tillers in the bacterized plants compared to the non-bacterized control. Farmers were impressed with the outcome of the field trials.

An MoU is being signed with a private entrepreneur for the formulation of these tested strains. The 14 formulated strains *Azospirillum* spp. such as (strains, MSA-148, MSA-160, MSA-274, MSA-289), *Rhizobium* spp. (strains GR55, GR57, GR59), *Swaminathania salitolerans* (strains PA-6, PA-12, PA-51) and *Phosphobacteria* (strains PS-4, PS-5, PS-9, PS-10) are now ready for large-scale usage.

Biocontrol trial at Aduthurai Rice Research Station

The trial was carried out with 4 strains of *Pseudomonas*, which had been tested and proved efficient in the greenhouse. The experiment was carried out for two major diseases of rice, Bacterial blight (*X. oryzae*) and Bacterial leaf streak (*X. oryzaicola*). The seed treatment was carried out by soaking the seeds in the bacterial suspension overnight. The germinated seeds were sown in the nursery and after 25 days they

were transplanted to the field after root treatment with the respective bacterial cells. After 20 days in the field, the plants were given the second dose of biocontrol application as foliar spray. Two days after the application of *Pseudomonas* strains, the plants were challenge inoculated with the virulent pathogens, *X. oryzae* and *X. oryzaicola* in the respective fields by the clip inoculation method. The plants were again sprayed with the biocontrol agents and the disease was scored after two weeks. The *Pseudomonas* strains imparted up to 60 % disease suppression against bacterial blight. The trial needs to be repeated for consistent results, before release to the farmers.

Mechanism of salt tolerance in *Pseudomonas* using proteomics and genomics tools

Basic studies were carried out in order to understand the mechanism of salt tolerance in the biocontrol strains of *Pseudomonas*. Greenhouse studies revealed that the root colonization potential of the strain was not hampered with higher salinity in soil. As a means of salt tolerance, the strain de novo synthesized the osmolytes, Ala, Gly, Glu, Ser, Thr, and Asp in the cytosol, as revealed by HPLC-Amino acid analyzer profile. Accumulation of Aspartic acid and Glutamic acid was found to be nearly 5 fold in cells with increasing concentration of salt, while concentration of Serine increased more than 7 fold. Among the compatible amino acids synthesized by the strain, concentration of Aspartic acid was the least (up to 500 nM), while that of Glutamic acid was nearly up to 25,000 nM.

The protein profile (SDS-PAGE) of the normal and induced bacterial cells showed that there is one newly induced protein, five over repressed and six repressed proteins. One newly induced and repressed proteins were observed in the SDS-PAGE profile carried out for the membrane proteins. In order to understand this phenomenon better, proteome analysis of the bacteria was carried out, employing 2D gel electrophoresis and MALDITOF. This technique was used to investigate the regulation of gene product

expression of *P. fluorescens* MSP-393 grown under high osmolarity and peptide mass fingerprinting and in silico investigation done to identify those proteins with altered expression. With peptide mass fingerprinting and using bioinformatic tools, function was assigned to 13 induced proteins and two repressed proteins. All proteins identified had molecular weights between 12 and 77 kDa and isoelectric points between 4 and 7 pI respectively. The majority of proteins identified were homologous to stress proteins in prokaryotes.

Differential Display (DD) gene expression profiling for salt stress carried out using random primers, revealed an array of up-regulated and down regulated-genes that are being characterized.

PGPRs and Mangroves

The functional population of rhizosphere microbes has been addressed with respect to nitrogen fixation, phosphate solubilization and biocontrol potential. It showed that the phosphate solubilisers were too low and that it could be because of the low content of phosphate in the mangrove soils, as reported by many researchers earlier. The diversity among the nitrogen-fixing organisms was studied. The nitrogenase activity of the strains (based on ARA) ranged from 10.3 to 32.34 nmole/ml/hr. Molecular detection of *nif* genes was also carried out. Strains that can effectively fix nitrogen (10.3 to 32.34 nmole/ ml/hr) were isolated. There were also certain antagonistic microbes, which showed good activity against chilly pathogen, *Colletotrichum capsici*. Detailed studies have to be undertaken in order to understand the functional role of these microbes in mangrove rhizosphere. A novel filamentous fluorescent endophytic Bacillus has been isolated from wild rice, *Proteresia coarctata* and 16S rDNA partial full length sequence, deposited in NCBI (Acc. No. AY 941162). The strain needs to be characterized further.

Diversity analysis with soil DNA for the non-culturables

Fifty different samples of soil DNA were extracted from five mangrove plants, *Avicennia marina*,

Rhizophora mucronata, *Excoecaria agallocha*, *Porteresia coarctata* and *Sonneratia apetala* rhizospheres. All the extracted DNA samples were subjected to PCR for amplification of 16S rDNA, *nifD* and *nifH* genes of soil microbes. After the PCR amplification, these genes were analyzed by PCR-RFLPSSCP to understand the genetic diversity among the DNA samples. The bands need to be cloned, sequenced and characterized to assess the microbial diversity.

The amplified products were digested with six four-base recognizing enzymes. The restriction enzymes used were Csp 61, Hae III, Msp I and Taq I (Amersham Pharmacia Biotech, UK). All the enzymes gave polymorphic banding patterns. With each enzyme, 4 to 7 restriction fragments were resolved. Msp I and Csp 61 produced 10 and 8 restriction patterns. The Hae III showed least polymorphism, with 4 restriction patterns. In the Cluster analysis (UPGMA), 50 % of major genetic similarity could be identified based on the number of restriction sites in the 16S rDNA. In the dendrogram, there were two major clusters at the similarity co-efficient value of 0.50 and each cluster consisted of two clusters and many sub clusters at different co-efficient values. Currently, SSCP-heteroduplex analysis is being performed to study the sequence similarity and dissimilarity in the 16S rDNA, *nifD* and *nifH* genes of 50 DNA samples.

(Courtesy: M.S.Swaminathan Research Foundation, Chennai)

Microbial Diversity and Sustainability

Introduction

Microorganisms play an integral and often unique role in the functioning of ecosystems and in maintaining a sustainable biosphere. Indeed, the role of microorganisms in maintaining the dynamic equilibrium and integrity of the biosphere is so critical that the continued existence of life is dependent upon the sustained, microbially-mediated transformation of

matter in both terrestrial and aquatic environments. That almost all biological processes in the environment, either directly or indirectly, involve microorganisms is often overlooked with the potential benefits of regulating, optimizing and exploiting microbial activity largely unexplored (Zedan, 1993). Thus, it is now generally accepted that the extent of microbial diversity has not been adequately characterised and that there is an immense mismatch between our knowledge of that diversity and its importance in both ecosystem processes and economic development (Zedan, 1993).

Quantifying Microbial Diversity

Unlike macroecologists, microbial ecologists working on natural communities are faced with quite unique challenges posed by, for instance: (i) the very large number of individuals per sample (*e.g.* more than 109 organisms per gram of soil (Torsvik *et al.*, 1990a;b); (ii) the problem of differentiating between different populations and the very high diversity at a relatively small scale (*e.g.* more than 104 species per gram of soil (Klug and Tiedje, 1994); and (iii) the difficulty of defining a microbial species (Goodfellow and O'Donnell, 1993; Claridge and Boddy, 1994) or some other unit that encompasses the appropriate level of diversity (O'Donnell *et al.*, 1994). The immediate challenge facing microbial ecologists is how best to quantify microbial diversity in natural environments.

Estimates of the microbial diversity must accommodate the spatial and temporal variability in microbial populations. Scale effects, both temporal and spatial, are not only of fundamental importance in the quantification of biodiversity, but pose basic questions for microbial ecology the resolution of which could lead to the development of fundamental theories and hypotheses as to how microbial communities are structured in space and time; how they respond to environmental pressures and how diversity is connected to function (Klug and Tiedje, 1994).

Spatial effects include an assessment of the relationship between community composition and scale. This is analogous to the area-species curve in

macroecology but would require that appropriate measures of microbial diversity are substituted for the classical eukaryotic species. The structure of such curves would be particularly important in predicting the location of undiscovered diversity and would provide insight into how microbial diversity changes relative to the environment. Such studies could be extended to include an evaluation of biodiversity in 'comparable' (e.g. the same soil type or similar vegetation cover) but geographically isolated habitats thereby providing information on microbial dispersal, evolution and selection (Klug and Tiedje, 1994).

Temporal shifts in microbial diversity are brought about by changes in the environment of the microorganisms and may be induced by the organisms or imposed on the community from outside. A prerequisite to the quantification of diversity in natural samples is an understanding of the magnitude and level at which such changes operate. There is a need to know which taxonomic rank is most susceptible to change, what are the implications for estimates of microbial diversity at a given site, and can these data be used as quantitative indices of sustainability. Advances in the analysis and quantification of microbial diversity will undoubtedly require extensive, collaborative, interdisciplinary studies.

In addition to the development of new procedures, the efficacy and importance of existing techniques will need to be re-evaluated, and protocols developed to enable extrapolative approaches to be used in sites where limited resources preclude intensive studies. Biodiversity estimates will need to be based on stable, readily analysed properties of the microbial community. Estimates based on phenotypic, and to a lesser extent chemical techniques, are likely to reflect the physico-chemical environment and as such may be influenced by community composition and function at a particular site, at a given time. Thus, sampling and collecting procedures need to be standardised temporally. Analysis of microbial communities using rRNA sequences is likely to be less sensitive to variability in the physico-chemical environment providing the methodology can be improved and the

bias in nucleic acid extraction and amplification procedures removed (Embley and Stackebrandt, 1994).

Although significant steps have been taken over the last few years, the required technology remains in its infancy and is as yet unable to provide the tools necessary to quantify microbial diversity in anything but the simplest of natural habitats. Nevertheless, with an awareness of the limitations of existing methods and human resources, a re-assessment of the 'species' as the basic unit of microbial diversity, and the introduction of new methodologies, significant progress in the quantification of microbial diversity can be expected.

Conclusions and Assessment

The management and exploitation of microbial diversity has an important role in sustainable development with the industrial and commercial application of microbial diversity worth millions of pounds. Given the heterogeneity of natural environments and the enormous potential of microorganisms to provide novel pharmaceuticals, fine chemicals and new technologies, the biotechnology industry has a vast, largely untapped resource for the discovery of new chemicals and novel processes. However, despite the obvious economic value of microbial diversity, microorganisms have been largely ignored in debates on the conservation and management of global diversity. There is, therefore, an urgent need to persuade policy-makers to be more concerned about the conservation, management and exploitation of microbial diversity. There are a number of reasons why the conservation of microbial diversity has not received the same attention as plants and animals. For example, microorganisms are invisible, less familiar and perceived primarily as agents of disease. With respect to the role of microorganisms in sustainable development, little is known about the potential contribution of microbial diversity to the national economy, to wealth creation and to improvements in the quality of life. An appreciation of these factors might be one way of changing government and public perception of microorganisms by showing that the sustainable use of microbial diversity has positive economic value. This

would help justify the costs involved in conserving microbial diversity, but equally provide a useful indicator of the costs of inaction. In terms of the scientific rationale needed to underpin policy, we are limited by our inability to quantify microbial diversity. This makes it difficult to indicate what needs to be conserved in order to support our biotechnology industries and to understand fully the interactions between organisms responsible for maintaining a functional ecosystem.

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- (Courtesy: Author : Tony O' Donnell - Published in 'The UK Strategy for Sustainable Agriculture: A Critical Analysis' Martin Whitby and Neil Ward (eds); Centre for Rural Economy Report , Department of Agricultural Economics and Food Marketing, University of Newcastle upon Tyne; 1994).

Beneficial and Effective Microorganisms for Sustainable Agriculture

Integration of Essential Components for Optimum Crop and Livestock Production. Agriculture, in a broad sense, is not an enterprise which leaves everything to nature without intervention. Rather it is a human activity in which the farmer attempts to integrate certain agroecological factors and production inputs for optimum crop and livestock production. Thus, it is reasonable to assume that farmers should be interested in ways and means of controlling beneficial soil microorganisms as an important component of the agricultural environment. Nevertheless, this idea has often been rejected by naturalists and proponents of nature farming and organic agriculture. They argue that beneficial soil microorganisms will increase naturally when organic amendments are applied to soils as carbon, energy and nutrient sources. This indeed may be true where an abundance of organic materials are readily available for recycling which often occurs in small-scale farming. However, in most cases, soil microorganisms, beneficial or harmful, have often been controlled advantageously when crops in various agroecological zones are grown and cultivated in proper sequence (*i.e.*, crop rotations) and without the use of pesticides. This explains why scientists have long been interested in the use of beneficial microorganisms as soil and plant inoculants to shift the microbiological equilibrium in ways that would enhance soil quality and the yield and quality of crops (Higa and Wididana, 1991b; Higa, 1994, 1995). Most would agree that a basic rule of agriculture is to ensure that specific crops are grown according to their agroclimatic and agroecological requirements. However, in many cases the agricultural economy is based on market forces that demand a stable supply of food, and thus, it becomes necessary to use farmland to its full productive potential throughout the year. The purpose of crop breeding is to improve crop production, crop protection, and crop quality. Improved crop cultivars along with improved cultural and

management practices have made it possible to grow a wide variety of agricultural and horticultural crops in areas where it once would not have been culturally or economically feasible. The cultivation of these crops in such diverse environments has contributed significantly to a stable food supply in many countries. However, it is somewhat ironic that new crop cultures are almost never selected with consideration of their nutritional quality or bioavailability after ingestion (Hornick, 1992). To enhance the concept of controlling and utilizing beneficial microorganisms for crop production and protection, one must harmoniously integrate the essential components for plant growth and yield including light (intensity, photoperiod, and quality), carbon dioxide, water, nutrients (organic-inorganic), soil type, and the soil microflora. Because of these vital interrelationships, it is possible to envision a new technology and a more energy-efficient system of biological production.

(Source: <http://www.livingsoil.co.uk/learning/higa.htm>)

ABSTRACTS OF RECENT PUBLICATIONS

001-M. J. Terpstra ,From the Department of Agro technology and Nutrition, Section of Consumer Technology and Product Use, Wageningen University. **The correlation between sustainable development and home hygiene.** *American Journal of Infection Control*, 29 (2001)4, 211-217.

Hygiene refers to the science of the establishment and maintenance of (human) health. In everyday life, hygiene is closely associated with good housekeeping. This article will focus on home hygiene in relation to cleaning, on **micro -organisms, and on sustainable** development of domestic technology. In domestic cleaning there are two beneficial effects: the aesthetic aspects of cleanness and the removal of **microorganisms**. In cleaning science substantial attention is paid to the interrelation between cleaning and removal of **microorganisms**. It appears that the parameters of the cleaning process and the detergent properties play a significant role in this interrelation. Changing technology to reduce the environmental

impact of household cleaning not only influences the household activities and the functional performance of the cleaning processes but also has an impact on the level of hygiene. Results are presented of research in which the hygiene has been evaluated in relation to such changes. One option recently studied to reduce the environmental impact of households is the reduction of water consumption by water reuse. In such concepts water is used in successive steps for various processes before it is drained. The potential impact of such systems and of the use of rain water on the level of home hygiene is discussed.

002 -Robert J. Kremer^a and Jianmei Li^b. United States Department of Agriculture, Agricultural Research Service, Cropping Systems and Water Quality Research Unit and Environmental Soil Science Program, School of Natural Resources, University of Missouri, 302 Anheuser-Busch Natural Resources, Columbia, MO 65211, USA. **Developing weed-suppressive soils through improved soil quality management.** *Soil and Tillage Research*, 72 (2003)2, 193-202.

Manipulating soil microbial communities using soil and crop management practices is a basic strategy in developing sustainable agricultural systems. Sustainable farming is based, in part, on the efficient management of soil microorganisms to improve soil quality. However, the identification of biological indicators of soil quality that can be used to predict weed suppression in soils has received little attention. We investigated differences in soil microbial activity among various crop and soil management systems to assess: (i) the microbiological characteristics of these soils; (ii) determine whether any relationships existed that might be used in the development of weed suppression. Soil enzyme activity, water-stable aggregates, and the proportions of weed-suppressive bacteria were compared among seven cropping systems and one native-prairie ecosystem in mid-Missouri, USA. Assays of soil enzymes (fluorescein diacetate hydrolase, dehydrogenase, phosphatase) revealed that organic and integrated cropping systems, and the native-prairie ecosystem had the highest levels of soil activity. Weed rhizospheres from these same ecosystems also had greater proportions of bacterial isolates characterized as "growth suppressive" to green foxtail (*Setaria viridis* [L.] Beauv.) and field bindweed (*Convolvulus arvensis* L.): 15 and 10%, respectively. The proportion of water-stable soil aggregates was the greatest in soils with the highest organic matter and was found to be related to

higher enzyme and weed-suppressive activity. Selected biological indicators of soil quality were associated with potential weed-suppressive activity in soil when that soil was managed for high organic matter content under reduced tillage systems. This research study provides further evidence that soil quality and sustainable agricultural practices may be linked to integrated weed management systems for the biological suppression of weeds.

Keywords: Conservation biological control; Organic matter; Soil enzyme activity; Sustainability; Weed-suppressive bacteria; Water-stable aggregates.

003-Jonas F. Johansson , Leslie R. Paul and Roger D. Finlay. Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, SE-750 07, Uppsala, Sweden. **Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture.** *FEMS Microbiology Ecology*, 48 (2004) 1, 1-13

In sustainable, low-input cropping systems the natural roles of microorganisms in maintaining soil fertility and biocontrol of plant pathogens may be more important than in conventional agriculture where their significance has been marginalised by high inputs of agrochemicals. Better understanding of the interactions between arbuscular mycorrhizal fungi and other microorganisms is necessary for the development of sustainable management of soil fertility and crop production. Many studies of the influence of mycorrhizal colonisation on associated bacterial communities have been conducted, however, the mechanisms of interaction are still poorly understood. Novel approaches including PCR-based methods, stable isotope profiling, and molecular markers have begun to shed light on the activity, identity and spatiotemporal location of bacteria in the mycorrhizosphere. This paper reviews current knowledge concerning the interactions between arbuscular mycorrhizal fungi and other microorganisms particularly bacteria, and discusses the implications these interactions may have in sustainable agriculture.

Keywords: Arbuscular mycorrhiza; Bacteria; Biocontrol; Mycorrh -izosphere; Soil fertility; Sustainable agriculture.

004-Gregory E. Welbaum, Antony V. Sturz, Zhongmin Dong and Jerzy Nowak. Department of Horticulture, Saunders Hall (0327), Virginia Polytechnic Institute and State University, Blacksburg, VA 24060, USA. **Managing soil microorganisms to improve productivity of agro-ecosystems.** *Critical Reviews in Plant Sciences*, 23 (2004), 2, 175-193.

Historically, agricultural production has relied on practices designed to manage nutrients, water, weeds,

and crop diseases. Precision agriculture and integrated pest management programs have gone one step further by recognizing the need to target inputs where they are required in the field. The major objective of these programs has been to minimize adverse environmental impacts of intensive agriculture practices and reduce per unit production costs. This review surveys the literature, examining the manipulation of microbial (primarily bacterial) populations as linked to agricultural production, and discusses new approaches that involve the precision management of microorganisms in the agro-ecosystem. It is proposed that our understanding of plant-soil interactions can be greatly refined through the development of "smart" field technology, where real-time, computer-controlled electronic diagnostic devices can be used to monitor rhizosphere and plant health. We submit that "smart field" generated information could be used to develop a prescription for timely and low-level production interventions that will avoid the traditional inundative approaches to crop maintenance and soil husbandry. Consequently, a lesser impact on the agricultural soil environment is envisioned. The maximization of production efficiencies will also involve the development of crop cultivars that are bred specifically to capitalize on beneficial plant-microbial associations.

Keywords: soil microbial communities; diversity; management; plant-microbial interaction; feedin-microbes.

005-Maria Gavrilescu^a and Yusuf Chisti^b. Department of Environmental Engineering and Management, Faculty of Industrial Chemistry, Technical University Iasi, 71 Mangeron Blvd, 700050 Iasi, Romania. **Biotechnology a sustainable alternative for chemical industry.** *Biotechnology Advances*, 23 (2005) 7-8, 471-499.

This review outlines the current and emerging applications of biotechnology, particularly in the production and processing of chemicals, for sustainable development. Biotechnology is "the application of scientific and engineering principles to the processing of materials by biological agents". Some of the defining technologies of modern biotechnology include genetic engineering; culture of recombinant microorganisms, cells of animals and plants; metabolic engineering; hybridoma technology; bioelectronics; nanobio - technology; protein engineering; transgenic animals and plants; tissue and organ engineering; immuno - logical assays; genomics and proteomics; bioseparations and bioreactor technologies. Environmental and economic benefits that biotechnology can offer in manufacturing, monitoring and waste management are highlighted. These benefits include the following: greatly reduced dependence on nonrenewable fuels and

other resources; reduced potential for pollution of industrial processes and products; ability to safely destroy accumulated pollutants for remediation of the environment; improved economics of production; and sustainable production of existing and novel products.

Keywords: Industrial sustainability; Biotechnology; Chemicals; Biocatalysts; Environment.

006-Agron. Sustain. Dev. Mohammad Saghir Khan, Almas Zaidi and Parvaze A. Wani. Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh U.P., 202002, India E-mail: khamms17@rediffmail.com. **Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review.**(2006)

Compared with the other major nutrients, phosphorus is by far the least mobile and available to plants in most soil conditions. Although phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major or even the prime limiting factor for plant growth. The bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species, nutritional status of soil and ambient soil conditions. To circumvent phosphorus deficiency, phosphate-solubilizing microorganisms (PSM) could play an important role in supplying phosphate to plants in a more environmentally-friendly and sustainable manner. The solubilization of phosphatic compounds by naturally abundant PSM is very common under in vitro conditions; the performance of PSM in situ has been contradictory. The variability in the performance has thus greatly hampered the large-scale application of PSM in sustainable agriculture. Numerous reasons have been suggested for this, but none of them have been conclusively investigated. Despite the variations in their performance, PSM are widely applied in agronomic practices in order to increase the productivity of crops while maintaining the health of soils. This review presents the results of studies on the utilization of PSM for direct application in agriculture under a wide range of agro-ecological conditions with a view to fostering sustainable agricultural intensification in developing countries of the tropics and subtropics.

E-resources on Microorganisms and Sustainable Development

Sustainable Development Communications Network
<http://www.sdcn.org>

Sustainable Livelihoods
<http://sdgateway.net/livelihoods/>

Institute for Trade, Standards and Sustainable Development
<http://www.itssd.org/>

International Institute for Sustainable Development
www.iisd.org

Institute of Environmental Engineering
<http://www2.nsysu.edu.tw/IEE/eng/eri.htm>

The Asia Pacific Natural Agriculture Network
www.apnan.org

Sustainable Community Development
<http://www.scdworld.com>

Sustainable Agriculture Research and Education
<http://www.sare.org/highlights/1996/soil.htm>

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Conference / seminars / meetings 2006 & 2007

2nd ASM Conference on Salmonella: From Pathogenesis to Therapeutics: 9-13 September, 2006. **Venue:** Victoria, BC, Canada. **Website:** <http://www.asm.org>

Biofilms: From Basics to Emergent University of

Minho: 11-16 September, 2006. **Venue:** Department of Biological Engineering, Braga, Portugal. **Website:** <http://www.ceb.uminho.pt/biofilm/Workshop.htm> **Email:** biofilm@deb.uminho.pt

The 10th Conference of the International Environment Forum Science, Faith and Global Warming: Arising to the Challenge: 15-17 September, 2006. **Venue:** Balliol College, Oxford University, Oxford, England. **Website:** <http://www.bcca.org/ief/conf10.htm> **Email:** IEF Sep2006@yahoo.co.uk

Biofilm Systems VI: 24-27 September, 2006. **Venue:** Amsterdam RAI, The Netherlands. **Website:** <http://www.moorga.com/> **Email:** info@moorga.com

Genomes, Medicine and the Environment Conference: 16-18 October, 2006. **Venue:** Hilton Head Marriott Beach & Golf Resort, South Carolina. **Website:** <http://www.venterlinstitute.org/gmc/>

Forestry: A Sectoral Response to Climate Change: 21-23 November, 2006. **Website:** <http://www.wiltonpark.org.uk/themes/environment/conference.aspx?confref=WPS06/9> **Email:** Roger Williamson

Biotechnological Applications of Attached & Suspended Microbial Aggregates IWA Biofilm Conference Series: 12 March - 12 June 2007. **Venue:** Singapore. **Website:** <http://www.iwahq.org> **Email:** cvivanov@ntu.edu.sg

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