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REVIEW ARTICLE

Marine Biotechnology in India

Anil Kumar
Department of Biotechnology & Zoology
Govt.V.Y.T.P.G. Autonomous College, Durg (C.G.) India, 491001
Email – aimum_aishley@yahoo.co.in

INTRODUCTION

India has to its advantage a favorable climate, diverse aqua fauna and its 8000 kilometers long coastline to support its marine life. Indian coastline stretches about 5700 kms on the mainland and about 7500 kms including the two island territories and exhibits most of the known geomorphological features of coastal zones.The mission of different leading Indian Institutes and its people is to work in partnership with visionary sponsors and collaborators to generate the knowledge and innovations required for efficient utilization of our coastal wasteland, sea water, marine algae, solar power and silicates. The Institutes will also harness its capabilities in biosciences, chemical transformation, process engineering, environmental monitoring, separation science and analysis to address focused needs of industries and organizations in the region and beyond.

Prominent Indian institutes such as the Central Institute of Brackish Water Aquaculture, Central Institute of Fisheries Technology, Cochin, Central Marine Fisheries Research Institute, Central Salt and Marine Research Institute, National Bureau of Fish Genetic Resources, National Centre for Antarctic & Ocean Research, National Institute of Oceanography, National Research Centre on Coldwater Fisheries, and MS Swaminathan Research Foundation, Chennai have been working on projects related to the marine sector.

Rich corals, diverse fish and a plethora of economically important arthropods and mollusks represent the marine biodiversity of India. A private company named Shantha Biotech has managed to produce a marine biotech product called beta- carotene using marine algae. The Company has been working on microbial metabolites sourced from the oceans. It offers pharmaceutical and nutritional supplement products and manufactures some critical bulk ingredients like beta-carotene Lutein, alpha carotene, zeaxanthin and cryptoxanthin. It also produces beta-carotene fortified products including jams, squashes, ketchups and sauces, cooking oils, chocolates, etc. Shantha Marine has notched up a turnover of INR 6 crore in its first year of operations and is estimated to achieve around INR 15 crore during the current year.

Several R&D activities are underway at CSIR funded laboratories such as National Institute of Oceanography (NIO), Goa and Central Salt and Marine Chemicals Research Institute (CSMCRI), Bhavnagar, in the areas of marine biotechnology, such as structural and functional genomics, cultivation of seaweeds and bioprospecting for marine organisms (seaweeds and microbes), microbial extremophiles and bioremediation, sustainable saline waste land cultivation technology, etc.

DBT has been focusing on semi-intensive prawn culture, intensive carp culture, feed and seed production, disease diagnostics/vaccine development, bioactive compounds, transgenics, etc. The programmes on semi-intensive prawn culture and intensive carp culture have demonstrated high production levels and could lead to process and product development for commercialization.

The Government of Andhra Pradesh is in the process of establishing a Marine Biotech Park (MBP) in Visakhapatnam, spread across 218 acres of well-developed land. A Marine Biotechnology Park at Mamallapuram is also being set up by Government of Tamil Nadu with an investment of USD 11.11 million to offer unique incubating facilities for commercial exploitation in pharmaceuticals, food supplements and cosmetics. Department of

Fisheries\University of Agricultural Sciences Dharwad, Karnataka proposes to establish a marine biotech park at Karwar for promoting research in marine biotechnology.

The University of Science and Technology at Cochin, Kerala, is proposing to establish a Centre for Marine Biotechnology that will offer a platform for R&D programmes in the field of marine biotechnology. The broad areas identified for research include genotypic characterisation and sequencing genes of interest, aquaculture and fisheries biotechnology, marine novel enzymes and marine natural products and biomaterials. The Centre will also work on the development of a database on marine biotechnology.

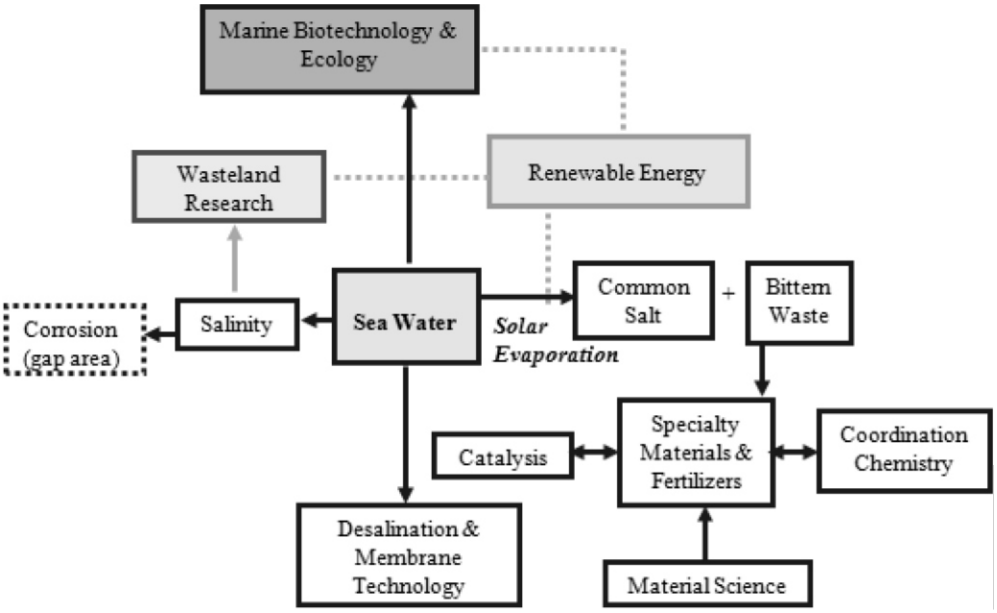

Fig. I. Schematic description of the Activities of Central Salt & Marine Chemicals Research Institute with Sea water as a Central Theme

MOLECULAR BIOLOGY AND BIOTECHNOLOGY

This group is actively pursuing research in the field of molecular biology and biotechnology to study and enhance the potentials of marine and halophytic resources. The studies focus mainly on understanding the molecular mechanism of stress tolerance, functional genomics, molecular taxonomy, biodiversity and bioremediation. The current research activities are centered on

1. Proteomics and genomics of halophytes and marine algae.

2. Selection and molecular characterization of improved clones developed through tissue culture of economically important seaweeds,

3. Exploitation of marine microbes for value added products and bioremediation of aquatic environment.

4. Biopigments from cyanobacteria/microalgae as a source of pharmaceutical, and nutraceutical substances.Yet another important activity is human resource development in the area of molecular biology and biotechnology of halophytes, macro algae.

PHYTOCHEMISTRY

The major thrust activity of this group is value addition of seaweeds and seaweed polysaccharides. Several environmental-friendly technologies have been developed and patented (US and PCT) for preparation of agarose, bacteriological agar, semi refined- and refined--carrageenans from different red seaweeds. Recently, US patent has been granted (USP 6,893,479) for an integrated process to simultaneously obtain liquid fertilizer and -carrageenan from fresh *Eucheuma* seaweed. Yet another area of focus with vast potential is development of biodegradable films and non-animal gelatin based on seaweed polysaccharides. Development of methods for physical and chemical modifications of agar and carrageenan is another emerging area. As a part of ongoing DOD-funded National

Programme on "Drugs from the sea", seaweeds as a source of bioactives are also being investigated.

BIOACTIVE MOLECULES FROM MARINE ENVIRONMENT

A program on “Development of Potential Drugs from the seas around India” is an ongoing project at the National Institute of Oceanography (NIO) in collaboration with Central Drug Research Institute (CDRI), Lucknow, Indian Institute of Chemical Technology (IICT), Hyderabad, Advanced Centre for treatment, research and education in Cancer (ACTREC) and ten other laboratories. The Ministry of Earth Sciences (MoES), New Delhi, supports this program. Under this program, therapeutic potential of several isolated and identified compounds have been explored and there are hopes of few of the lead compounds identified reaching the drug stage.

Bioactive molecules are isolated and purified using the latest state-of-art technology in the field of natural product research. Structural elucidation is affected using the most modern sophisticated instrumentation techniques which include Fourier Transform Infrared spectroscopy (FTIR), Nuclear Magnetic Resonance spectroscopy (NMR), Mass Spectroscopy (MS), etc.

BIOPROSPECTING AND BIOTECHNOLOGY OF MARINE MICROORGANISMS

Following achievements have been recorded in the field of Bioprospecting and Biotechnology of marine microorganisms –

- Characterization of protease enzyme from a deep-sea fungus and increased production of the same using solif-substrate fermentation
- Decolorization of textile and paper mill effluents using laccase enzyme of a marine fungus NIOCC 2a, effect of different carbon and nitrogen sources in the culture medium for these processes
- Molecular sequencing of the ITS gene of rRNA of the marine fungus NIOCC 2a used for decolorization of colored effluents. The sequence has been deposited in the GenBank.
- Taxonomy of deep-sea yeasts using molecular sequencing of ITS region.
- Heavy metal tolerance and enzyme studies of thraustochytrids and bacteria isolated from hydrothermal vent
- Temporal changes in the diversity of fungi from Mandovi estuari in the oxic and anoxic zones.
- A species of thraustochytrid, new to science, was described using sequences of 18S rRNA gene
- Deciphering the phylogenetic relationship of several fungi and yeasts from deep-sea sediments, water column, anoxic sediments and mangroves.
- Physiological studies on fungi from coastal anoxic, and deep-sea sediments
- Application of lignin-degrading fungal enzymes for reducing color, phenolics and COD from factory effluents.
- Demonstration of mercury resistant bacterial potential in breaking down TBT
- Elucidation of physiological pathways of a bacterium of high de-inking ability
- Recognition of spatial difference in heterotrophic bacterial abundance and production in the Bay of Bengal.
- Isolation of marine derived, endophytic and wood litter fungal strains for harnessing their enzymes and pigments.

Recent Developments in the Field of Biotechnology in India with reference to Special features of marine organisms in the waters around India and their applications.

a) Relevance of biofilm bacteria in modulating the larval metamorphosis of Balanus amphitrite

Natural microbial communities found on different substrata- exposed marine environment, including barnacle shell surfaces, causing varied influences on the settlement and metamorphosis of competent larvae. Experiments were carried out to compare the influence of settlement-inducing compounds from the bacteria isolated from the shell surface of *Balanus amphitrite* on its larval metamorphosis. The effect of multispecies bacteria was also assessed. The production of different molecules by the bacteria was influenced by the nutrient media under which they were grown. It was observed that the promontory multispecies bacterial film turned to inhibition mode in the presence of the adult extract of the barnacle, indicating that bacteria–adult interactions alter the synthesis of different compounds produced by bacteria. Studies have also shown that the waterborne and the surface-associated cues from bacteria function differentially in mediating larval metamorphosis. Understanding the complexities involved in such interactions and identification of the condition governing them would be a step forward [1].

b) Aerobic degradation of highly chlorinated polychlorobiphenyls by a marine bacterium, PseudomonasCH07[2]

Hitherto, aerobic degradation of polychlorinated biphenyls (PCBs) has been reported to be limited to the less chlorinated biphenyls. A marine mercuryresistant bacterium, *Pseudomonas* CH07 (NRRL B- 30604) which was capable of degrading a variety of highly chlorinated congeners of PCBs from the technical mixture Clophen A-50 is reported. Of the two most toxic coplanar PCBs present in Clophen A-50, one coplanar pentachloro congener CB-126 and one toxic sterically hindered heptachloro congener CB-181 were found to be degraded completely and the other coplanar tetrachloro congener CB-77 was degraded by more than 40% within 40 h by this microorganism. The apparent absence of *bph*C in this bacterium leads to the proposal of a different mechanism for degradation of PCBs.

c) Molecular evidence of fungal signatures in the marine protest *Corallochytrium limacisporum* and its implications in the evolution of animals and fungi[3]

Fungi, animals, and single-celled organisms belonging to the choanozoans together constitute the supergroup Opisthokonta. The latter are considered crucial in understanding the evolutionary origin of animals and fungi. The choanozoan *Corallochytrium limacisporum* is an enigmatic marine protist of considerable interest in opisthokontan evolution. Several isolates of the organism were obtained from a coral reef lagoon in the Lakshadweep group of islands of the Arabian Sea. The ability of these cultures to grow on media containing inorganic nitrogen sources prompted us to examine the possible presence of fungal signatures, namely the enzyme aminoadipate reductase (AAR) involved in the aminoadipate (AAA) pathway for synthesizing lysine and ergosterol, in one of the isolates. These features, as well as the sterol C-14 reductase gene involved in the sterol pathway of animals and fungi, were detected in the organism. Phylogenetic trees based on the AAR gene suggested that *Corallochytrium limacisporum* is a sister clade to fungi, while those based on the C-14 reductase gene did not adequately resolve whether the organism was more closely related to fungi or animals.

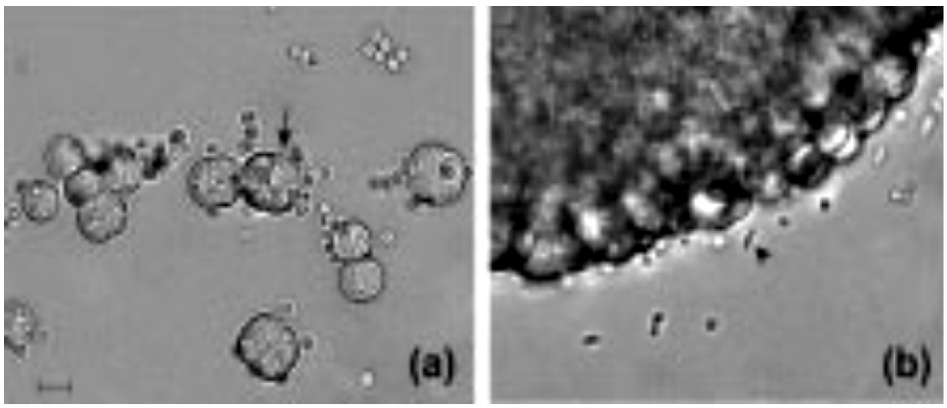

Fig. II. Photomicrograph of C*orallochytrium limacisporum* cells. (a) The organism ischaracterized by single cells, diads and tetrads (arrow). (b) Reproduction takes place by the production of limax-shaped amoeboid spores (arrowhead)

d)Glycolipids from the red alga Chondria armata (Kutz.) Okamura[4]

Three distinct fractions containing polar glycolipids (PF1–3) were isolated from the chloroform soluble fraction of crude methanolic extract of red alga *Chondria armata* (Kutz.) Okamura on gel chromatography over Sephadex LH20. Their structure was elucidated and indicated an exclusive configuration of the sugar molecules in the glycerolipids. Major glycolipids were identified as (2R)-2-*O*-(5,8,11,14-eicosatetranoyl)-3-*O*-□-D-galactopyranosyl-snglycerol (GL2), its pentacetate (GL1), and (2R)-1-*O*-(palmitoyl) -2-*O*-(5,8,11,14,17-icosapentanoyl)-3-*O*-□-D-galactopyranosyl-sn- glycerol (GL3). Each was methanolysed to give the same galactosylglycerol which on ESI-MS provided a pseudomolecular ion at m/z 309 representing deacylated glycolipid with the sodiated sugar moiety. Additionally, six minor glycolipids were also identified on the basis of ESIMS. These include 1,2-di-O-acyl-3-*O*-(acyl-6′-galactosyl)-glycerol (GL1a), sulfonoglycolipids 2-*O*-palmitoyl-3-O-(6′-sulfoquinovopyranosyl)-glycerol (GL2a) and its ethyl ether derivative (GL2b), 1-oleoyl-2-palmitoyl-3-*O*galactosyl glycerol (GL3a), and 1,2-diacyl phosphatidyl glycerol (GL3b). GL1, GL1a, and GL2b are new to the literature. The novelty of the remaining identified compounds lies in the diversity of their fatty acid composition. Antimicrobial properties of these glycolipids against pathogens were evaluated. The yeast *Candida*

albicans and the bacteria *Klebsiella sp.* were as sensitive as the standard Nystatin and antibiotic Streptomycin against PF3. Considerable activity was expressed by the same metabolite against the fungus *Cryptococcus neoformans* as compared to the control. Weak activity against the bacteria *Shigella flexineri* and *Vibrio cholerae* and the fungus *Aspergillus fumigatus* was also observed. Fraction PF2 was weakly active against some strains whereas all of them were resistant to its acetyl derivative PF1. Antimicrobial activity of glycolipids was reported here for the first time.

e) Biotransformation of citrinin to decarboxycitrinin using an organic solvent-tolerant marine bacterium, Moraxella sp. MB1 [5]

Organic solvent tolerant microorganisms (OSTMs) are a novel group of extremophilic microorganisms that have developed resistance to withstand solvent toxicity. These organisms play an important role in biotransformation of organic compounds. An organic solvent-tolerant marine bacterium, *Moraxella sp.* MB1 is used. 16S rRNA sequencing revealed that the bacterium shows 98% similarity with an uncultured marine bacterium with GenBank accession no. AY936933. This bacterium was used for the transformation of a toxin, citrinin, into decarboxycitrinin in a biphasic system. This transformation was affected by decarboxylase enzyme produced by MB1. Transformation of citrinin to decarboxycitrinin was monitored spectrophotometrically and by thinlayer chromatography (TLC). Citrinin decarboxylase activity responsible for transformation was studied in cell-free growth medium and cell lysate of *Moraxella sp.* MB1. Citrinin decarboxylase was found to be intracellular in nature. The biotransformed product was purified and identified as decarboxycitrinin using electrospray ionization mass spectrometry (ESI-MS/MS) and nuclear magnetic resonance (NMR) spectrometry. The antibiotic activity of both citrinin and decarboxycitrinin is reported.

f) Antiviral property of marineactinomycetes against white spotsyndrome virus in penaeid shrimps [6]

Aquaculture farms, particularly in Southeast Asia are facing severe crisis due to increasing incidences of White Spot Syndrome Virus (WSSV). Actinomycetes have provided many important bioactive compounds of high prophylactic and therapeutic value and are continually being screened for new compounds. In this communication, the results of a study made to determine the effectiveness of marine actinomycetes against the white spot disease in penaeid shrimps are presented. Twenty-five isolates of actinomycetes were tested for their ability to reduce infection due to WSSV among cultured shrimps. When these actinomycetes were made available as feed additives to the post-larvae of the black tiger shrimp *Penaeus monodon* for two weeks and challenged with WSSV, the post challenge survival showed variations from 11 to 83%. However, six isolates have shown to be the most potential candidates for further study.

g) Isolation, purification and characterization of xylanase produced by Arthrobacter sp. MTCC 5214 when grown in solid-state fermentation [7]

Thermoalkalophilic *Arthrobacter* sp. Produced extracellular xylanase when wheat bran, rice husk,rice bran and bagassae were used as carbon source under solid-state fermentation (SSF). The xylanase enzyme was isolated by ammonium sulfate (80%) fractionation, and purified to homogeneity using size exclusion and ion exchange chromatography. The molecular mass of xylanase was ~20 kDa. Enzyme retained 100% activity at pH 7 and 8 for 24 h. It was interesting to note that at higher pH such as 9, 10 and 11 the enzyme activity increased over the period of incubation. The optimum temperature for the enzyme activity was 100°C at pH 9.0. At 80°C and pH 9, half-life of enzyme was 30 min. Half-life of enzyme at 70 and 60°C was 18 and 24 h, respectively. While at 50°C the enzyme retained 79% of activity even after 48 h. For xylan, the enzyme gave a Km value of 0.9 mg/ml, and V_{max} value of 3571 mol/min/mg when the reaction was carried out at 100°C and pH 9. In the presence of metal ions such as Co^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Mg^{2+} and Ca^{2+} and metal chelator EDTA the activity of the enzyme increased. Whereas strong inhibition of the enzyme activity was observed in the presence of Hg^{2+}. These are some novel characteristics that make this enzyme potentially very effective for industrial applications.

h)Alpha-L-Arabinofuranosidases: The potential applications in biotechnology [8]

Recently, alpha -L-arabinofuranosidases (EC3.2.1.55) have received increased attention primarily due to their role in the degradation of lignocelluloses as well as their positive effect on the activity of other enzymes acting on

lignocelluloses. As a result, these enzymes are used in many biotechnological applications including wine industry, clarification of fruit juices, digestion enhancement of animal feedstuffs and as a natural improver for bread. Moreover, these enzymes could be used to improve existing technologies and to develop new technologies. The production, mechanisms of action, classification, synergistic role, biochemical properties, substrate specificities, molecular biology and biotechnological applications of these enzymes have been reviewed.

i) Deep-sea fungi as a source of alkaline and cold-tolerant proteases [9]

Fungi from coastal environments have been widely studied with respect to the production of secondary metabolites and biotechnologically useful lignocellulolytic enzymes. A few studies on mycology of deep-sea sediments, however, have been carried out. This paper reports a study on alkaline, coldtolerant proteases from deep-sea fungi. A total of 221 deep-sea isolates of fungi from 5000 m in the Central Indian Basin were screened for the enzyme. Many of these grew and produced alkaline protease at 5 and 30°C and 1 bar pressure. *Aspergillus ustus* (NIOCC 20) producing the highest amounts of the enzyme was selected for further studies. The growth yield was substantial at 30 and 5°C at 1 bar and elevated hydrostatic pressures. The fungus produced alkaline, cold-tolerant protease when grown at 30°C and 1 bar pressure. The enzyme was active at combinations of 30, 5°C and 50 and 300 bar pressure. However, protease production was negligible when the fungus was grown at 5°C, under 1 bar or elevated hydrostatic pressures. The enzyme produced at 30°C and 1 bar pressure was further characterized. The fungus produced a maximum of 1639 ACUmL-1 of protease by day 7. The enzyme, with molecular mass of 32 kDa and pI values of 6.6 and 6.9 showed several interesting properties. It had a broad pH range of 6–10, with an optimum at pH 9. The optimum temperature for protease activity was 45°C and approximately 10% of the activity was retained at 2°C. The enzyme was totally inhibited in the presence of 2mM PMSF suggesting it to be a serine protease. It was active in the presence of several commercial detergents at 2 g L-1 concentration and in the presence of 0.5M NaCl, equivalent to 29 parts per thousand salinity. In the presence of stabilizing agents such as glycerol, CaCl2 its thermostability at 60°C was enhanced. Heavy metal ions Cu^{2+}, Hg^{2+}, Fe^{2+}, Ni^{2+} and Zn^{2+} did not inhibit the enzyme activity considerably. This study indicates that fungi from deep-sea sediments could be a useful source of proteases.

j) Purification and characterization of thermoalkalophilic xylanase isolated from the Enterobacter sp. MTCC 5112 [10]

Thermoalkalophilic *Enterobacter sp.* MTCC 5112 was isolated from a sediment sample collected from the Mandovi estuary on the west coast of India. This culture produced extracellular xylanase. The xylanase enzyme was isolated by ammonium sulfate (80%) fractionation and purified to homogeneity using size exclusion and ion exchange chromatography. The molecular mass of the xylanase was ~43 kDa. The optimal pH of the xylanase activity was 9, and at room temperature it showed 100% stability at pH 7, 8 and 9 for 3 h. The optimal temperature for the enzyme activity was 100°C at pH 9.0. At 80°C and pH 9, 90% of the enzyme activity was retained after 40 min. At 70 and 60°C, the enzyme retained 64% and 85% of its activity after 18 h, respectively, while at 50°C and pH 9 the enzyme remained stable for days. For xylan, the enzyme gave a *Km* value of 3.3 mg ml-1 and a *V*max value of 5000 µmol min^{-1} mg^{-1} when the reaction was carried out at 100°C and pH 9. In the presence of metal ions such as Co^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Mg^{2+} and Ca^{2+} the activity of the enzyme increased, whereas strong inhibition of enzyme activity was observed in the presence of Hg^{2+} and EDTA. To the best of our knowledge, this is the first report on the production of xylanase by this bacterium.

k) Genetic variation in skipjack tuna *Katsuwonus pelamis* (L.) using PCR RFLP analysis of the mitochondrial DNA D-loop region. *I* [11]

A high level of genetic diversity was observed in *Katsuwonus pelamis* populations from India (h =0.952, ne = 14.3) and Japan (h = 0.897, ne = 8.9). The log-likelihood (G)-based exact test revealed significant heterogeneity in the distribution of haplotypes between the two populations ($P < 0.01$,S.E. = 0.001). This result suggests that the two populations should now be treated as demographically independent and managed separately.

l) Enhanced production of laccase by a marine fungus during treatment of colored effluents and synthetic dyes [12]

Paper and pulp mills, textile and dye-making industries and alcohol distilleries release highly colored effluents that are relatively difficult to decolorize by chemical and physical treatments. White-rot basidiomycetous fungi that produce lignin-degrading enzymes are reported to be most efficient in decolorizing such effluents. Decolorization

of all the three effluents by a marine fungal isolate, NIOCC#2a cultured from decaying mangrove wood is reported. The fungus also decolorized several synthetic dyes. Laccase was the most dominant lignin-degrading enzyme produced by this fungus with very low activities of manganesedependent peroxidase and no lignin peroxidase activity. The growth and production of laccase was best in a medium prepared with seawater having salinity in the range of 25–30 ppt. The pH optimum for the laccase activity was 3.0 and 6.0 and the temperature optimum was 60°C. Laccase production was increased in the presence of phenolic and nonphenolic inducers. A several fold enhancement in laccase production was found during treatment of colored effluents from textile, paper and pulp mill and distillery waste. Industrial effluents and synthetic dyes added to the growing culture of this fungus were decolorized to a great extent. The culture supernatant without the fungal biomass was also effective in decolorization of these effluents to various degrees within h of incubation. Extracellular polymeric substances (EPS) produced by this fungus were also useful in decolorization of these effluents. Thus, efficiency of this fungus in decolorization of various effluents with laccase that is active at pH 3.0 and 6.0 and 60°C in the presence seawater has great potential in bioremediation of industrial effluents. Enhanced laccase production in the presence of industrial effluents by this fungus is an added advantage during bioremediation of effluents

m) Pink-line syndrome, a physiological crisis in the scleractinian coral *Porites lutea*[13]

Coral diseases are one of the major factors that alter coral cover and their diversity. The 'Pink-line syndrome' (PLS) in the scleractinian coral *Porites lutea* wherein a colored band appears between the dead and healthy tissue of a colony is reported earlier. About 20% of the *P. lutea* colonies were affected in Kavaratti of the Lakshadweep Islands in the Arabian Sea during April 1996 and the incidence increased fourfold within the next 4 years. Fungi were associated in both PLS-affected and healthy specimens, whereas the cyanobacterium *Phormidium valderianum* occurred exclusively in the PLS-affected specimens. There was an increased expression of a 29 kDa protein without any significant increase in total protein content in the PLS affected colonies. A reduced number of zooxanthellae and an increase in zooxanthellae size, mitotic index, and chl *a* concentrations were some of the characteristics of the PLS-affected colonies. PLS induction experiments conducted using selected fungi and the cyanobacterium *P. valderianum* isolated from the affected colonies and abiotic factors, such as CO2 enrichment and the effect of cyanobacterial photosynthesis inhibition, indicated that the CO2 build-up around the host tissue caused the pink coloration. It is hypothesized that these physiological changes disturb the mutualism between the zooxanthellae and the host. When the symbiosis is disturbed by the external CO2, the host loses control over the zooxanthellae, causing their uncontrolled division. This process may lead to a break in photosynthate transfer to the host, thereby resulting in starvation and finally leading to partial mortality. It is hypothesized that these degenerative processes are triggered by the CO2 produced by *P.valderianum* through its carbon concentration mechanism. In this context, any opportunistic cyanobacteria or other agents having potential to interfere with the physiology of the host or the symbiont can cause such a physiological disorder. The mechanism of PLS formation is an early warning to protect corals as the increasing atmospheric CO2 could induce PLS-like physiological disorder in corals.

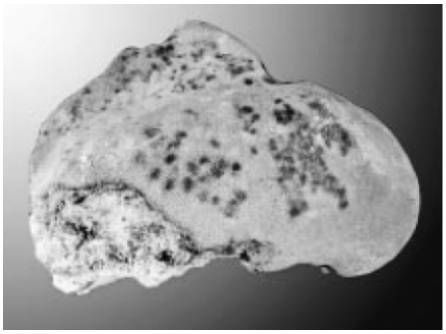

Fig. III. *Porites lutea* colony showing pink lesions where no associated biotic agent was found.

n) Reaction of elemol with acetic acid/perchloric acid: Characterization of a novel oxide and (+)-□-cyperone[14]

The minor unidentified compounds of the acetic acid/perchloric acid dehydration of elemol (1) were fully characterized. The structure and relative configuration of the less polar fragrant compound 2, named elemoxide,

was deduced by ID- and 2DNMR data including C,C-connectivity, NOE, and NOESY experiments. The absolute configuration was established as (3S, 3aft, 7aft)-l,3,3a,4,7,7a-hexahy-dro-6-isopropyl-l,l,3,3a-tetramethylisobeiizofuran(2) on the basis of its preparation from elemol (1). (+)-□-cyperone (3), a known sesquiterpene, was also identified as a minor product of the reaction. A plausible mechanistic explanation for the formation of elemoxide (2) and (+)-□-cyperonc (3) is presented.

o) Effect of nutrient nitrogen on laccase production, its isozyme pattern and effluent decolorization by the fungus NIOCC No. 2a, isolated from mangrove wood[15]

Carbon and nitrogen sources in the growth medium play an important role in the production of lignindegrading enzymes in the white-rot basidiomyceteous fungi. The role of nutrient nitrogen sources in growth media on production of lignin degrading enzymes namely laccase, lignin peroxidase and manganese peroxidase as well as on the decolorization of industrial effluents like black liquor, molasses spent wash and textile mill effluents was studied using the basidiomycetous fungus NIOCC No.2a isolated from mangrove wood. The results indicated that the type of nitrogen source used, not only influences the amount and type of lignin-degrading enzymes produced but also has an effect on the decolorization of these effluents. The amount of extracellular peroxidases increased by several fold in the presence of effluents whereas in their absence they were of negligible quantity. Some of the effluents had an inhibitory effect on laccase production. The effect of nitrogen sources in the absence as well as presence of the effluents, on the expression of laccase isoenzymes was studied by non-denaturing SDS-PAGE. It was noticed that a few new isozymes of laccase were induced in the presence of industrial effluents. Decolorization of these effluents by the concentrated culture filtrate obtained from media containing different nitrogen sources further proved the importance of the type of nitrogen source in decolorization of colored industrial effluents.

p) Removal of polycyclic aromatic hydrocarbons from aqueous media by the marine fungus NIOCC 312: Involvement of lignin-degrading enzymes and exopolysaccharides[16]

The removal of polycyclic aromatic hydrocarbons from aqueous culture medium by the lignindegrading marine fungus NIOCC 312, obtained from decaying seagrass from a coral reef lagoon is reported here. The percentage of phenanthrene removed from the culture supernatant and the fungal biomass after 6 days is estimated. About 60-70% of phenanthrene, at a concentration of 12 mgl-1 (12ppm) was removed from the culture medium containing live or heat-killed fungus, as estimated by fluorescence spectroscopy method. Nuclear magnetic resonance spectra of the phenanthrene extracted from the fungal biomass revealed that in the heat-killed fungal biomass, the phenanthrene remained undegraded till day 6. On the other hand in the live fungal biomass, no phenanthrene was detected on day 6 suggesting that it was metabolized or transformed into non-aromatic fragments. It is concluded that the disappearance of phenanthrene from the aqueous culture medium is due to its instant adsorption to the fungal biomass owing to the presence of the exopolymeric substance (EPS) around the fungal hyphae. The EPS produced by the fungus was partially characterized. It is hypothesized that phenanthrene thus adsorbed by the live fungal biomass was subsequently degraded by the lignin-degrading enzymes present in the cell wall and the EPS envelope. Thus, the heat-killed fungal biomass could be used only for adsorption of PAHs from contaminated sites whereas use of the live fungal biomass would result in degradation of PAHs.

q) New Caulerpenyne-derived metabolites of an *Elysia* Sacoglossan from the south Indian coast[17]

Chemical analysis of the secondary metabolite pattern of the sacoglossan mollusc *Elysia cf.expansa*, collected along South Indian coasts, showed the presence of the typical *Caulerpa*-derived sesquiterpene caulerpenyne (1) and two new minor cooccurring metabolites, the compounds dihydrocaulerpenyne (4) and expansinol (5). The chemical characterization of these molecules, structurally related to 1, is reported.

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