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BIOPROSPECTING POTENTIAL OF MANGROVE RESOURCES

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INTRODUCTION

Bioprospecting is the search of useful products derived from bioresources. The useful products may be chemical compounds, genes, organisms and other valuable products that are used in medical, industrial, agricultural and food sectors. The bioprospecting helps to conserve and sustainably utilize the bio-resources. Once, mangrove wetland was considered as wasteland. But, mangrove wetland ecosystem has rich bioprospecting potential owing to presence of the valuable genes to tolerate salinity and flood, the novel chemicals to overcome adverse coastal conditions, and the biodiversity richness [1]. Mangrove extracts are used in indigenous medicine [2]. Scientific validation of these uses will help to develop drugs to cure human diseases. In this regard, my research team has done a pioneering work on mangrove bioprospecting. Further studies on this aspect will prove greater values of the mangroves in clinical medicines and for other useful products. This will also lead to development of patents, which can be a source of revenue and employment opportunities. Thus, there is a greater potential for raising mangroves as "cash crops" in the years to come.

Herbal tea from mangroves

Polyphenols such as tannins are commercially important phytochemicals. The level of tannin ranges from 2.41 to 21.42 mg g^{-1} in 14 mangrove species [3]. The mangroves contain gallotannins, which can be used in leather, medical, pharmaceutical, food, and beverage industries. The gallotannins range from 0.013 to 3.555 mg g^{-1}[4]. As the mangroves are rich in polyphenols, a protocol has been developed for making a beverage having similar quality of the commercial tea. The mangrove tea is proved to have no side effects or toxicity in rat animal model. The quality of mangrove tea could also be successfully improved by UV-irradiation treatments[5].

Nutraceuticals from mangrove-derived fungus

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid (PUFA), essential to humans owing to its functions in the brain and retina as well as in animal nutrition. The current commercial source of DHA is fish and fish oils. Because of low level of DHA in fish oil, the large scale productions of DHA are difficult. In this regard, thraustochytrids is a promising microbial source for commercial production of DHA that is important in human health, aquaculture and nutraceutical sectors. We have proposed the maximum DHA production by the thraustochytrids (*Aplanochytrium* sp.) in culture medium containing maida powder (6.32 $mg.l^{-1}$), yeasts powder (8.35 $mg.l^{-1}$), vitamin B 12.08 $mg.l^{-1}$ and vitamin C 10.08 $mg.l^{-1}$ [6]. The thraustochytrids are being attempted for mass scale cultivation, as a commercial source of polyunsaturated fatty acids.

Antioxidant activities of mangrove resources

Mangroves and other associates have a strong potential for antioxidant activity (Table 1). The activity is greater in mangrove leaves especially *Acanthus ilicifolius* followed by endophytic fungi such as *Trichoderma* sp. In general all the mangrove resources are capable of removing free radical oxygen thereby having the potential to prevent damages to biomolecules in cellular structure and function. This higher antioxidant property can be attributed to high levels of phenolics present in the mangroves (Table 1) [7].

Mangrove extracts to inhibit viral activities

We report the mangrove extracts to inhibit *in vitro* activity of human and animal pathogenic viruses such as human immunodeficiency virus (HIV), New castle disease virus, Vaccinia virus, Semiliki forest virus, encephalomyocarditis virus, and hepatitis-B-virus [8]. The extracts of Rhizophoraceae members are most effective against all the viruses. Purified active fractions contain acid polysaccharides (galactose, galactosamine, glucose and arabinose) as well as lignins, and these are responsible for potent anti-HIV activity [9]. Besides these animal viruses, the mangrove extracts also inhibit the activity of a plant virus (Tobacco Mosaic Virus-TMV). *Bruguiera*

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Table Antioxidant property of mangroves and microbial associates

Group	Material tested	% of DPPH radical scavenging
Mangroves	*Acanthus ilicifolius*	99.25
	Callophyllum inophyllum	99.25
	Excoecaria agallocha	98.59
	Avicennia marina (leaf)	75.82
	Avicennia marina (flower)	57.97
	Rhizophora annamalayana (leaf)	16.57
	Rhizophora mucronata (leaf)	74.8
	Rhizophora mucronata (flower)	45.97
	Sesuvium portulacastrum (callus)	47.30
Thraustochytrids (lower fungi)	*Schizochytrium* sp.	81
	Thraustochytrium sp.	64
	Aplanochytrium sp.	67
Sulphur Reducing Bacteria	*Desulfovibrio* sp.	86.35
Cyanobacteria	*Gloeocapsa* sp.	77
	Synechococcus sp.	75
Seaweeds	*Padina tetrastromatica*	78.6
	Turbinaria ornata	71.43
Fungi	*Trichoderma harzianum*	98
	Trichoderma estorium	84
	Trichoderma viride	87

cylindrica and *Excoecaria agallocha* show significant anti-TMV activity [10].

Mangrove-derived substances to control harmful microbes

Quorum sensing (QS) is a phenomenon through which bacteria communicate each other *via* secreted signal molecule or autoinducers for the expression of their phenotypic characters [11]. Gram positive bacteria utilize oligopeptides as autoinducer molecule, while Gram negative bacteria utilize N-acyl homoserine lactone (AHL). These autoinducer molecules stimulate the expression of bacterial characters especially virulence factors and biofilm formations, which are highly essential for the survival of pathogenic bacteria. Hence, interfering with bacterial QS by means of disturbing bacterial autoinducers will inhibit the virulence and biofilm formation of bacterial pathogens and this is a suitable alternative treatment for the eradication of pathogenic bacteria with emerging antibiotic resistance [12]. In this regard, our earlier studies have shown that the mangrove extracts inhibit bacterial growth. Lignins, extracted from *Ceriops decandra* have a proven antibacterial activity. The mangrove lignins are experimentally shown to protect the mice from lethal infection of *E. coli*. This antibacterial activity is due to the antioxidant property of the lignins [13]. Bearing this in mind, the mangroves and associated bacterial flora are being attempted for their anti-QS activity to prevent autoinducer activity of bacteria that cause diseases in human and aquatic organisms. This attempt is aimed towards the development of antipathogenic drugs as an alternative for antibiotic usage to control the diseases.

Biofoulers are of great environmental and economic concern, since they often cause problems by settling on any substrates, submerged in seawater. Antifouling paints such as tributyltin and tributyltin oxide are very effective in controlling the fouling organisms; however, they are toxic to many non-target marine organisms [14]. In this regard, mangrove extracts have been tested for antibacterial activity against the marine bacteria that are involved in fouling process. In such a study conducted to screen 16 mangrove plants against 15 strains of fouling bacteria, methanolic extract of *Sonneratia caseolaris* is active against all the bacterial strains tested [15]. In this line, our attempt is continuing to discover potential non-toxic antifouling compounds to replace toxic antifouling agents that are currently in use.

We report that marine actinomycetes, isolated from mangrove sediments are antagonistic with four phytopathogenic fungi namely *Rhizoctonia solani, Pyricularia oryzae* and *Helminthosporium oryzae* that cause sheath blight disease, blast disease and leaf spot disease of paddy crop respectively, and *Colletotrichum falcatum* that causes red rot disease of sugarcane crop. Of 160 strains, 10 exhibit potent antifungal activity against economically important pathogens of paddy and sugarcane. The active compounds from the strains may find place towards the development of agrochemicals [16].

Mosquito repellents and larvicides from mangroves

We report the mangrove extracts to kill the mosquito larvae of *Aedes aegypti*, *Culex tritaeniorhynchus*, and *Anopheles stephensi* respectively the vectors of Dengue fever, filariasis and malaria [17]. The extract of aerial root from *Rhizophora apiculata* exhibits high mosquito larvicidal activity at very low concentrations. The bioactive compound present in the mangrove extract is pyrethrum derivatives [18]. When mangrove extract is applied as an ointment on human skin, it also shows repellent activity against the adult mosquito of *Aedes aegypti*. Some extracts show potent smoke repellency and produce lethal effect on adult mosquito of *Culex quinquefasciatus* and *A. aegypti* [19].

Anti-diabetic activity of mangroves

India is becoming world capital of diabetics. This is a disorder characterized by increased fasting, postprandial glucose concentration, insulin deficiency and decreased insulin action. Our studies have given evidence that the mangrove plant *Ceriops decandra* is promising to have anti-diabetic activity at a dose of 120 mg/kg comparable to the commercial drug, glibenclamide [20]. This can be considered for further evaluation in clinical studies and drug development.

Anti cancer activity of mangroves

Cancer is dreadful human disease and a leading cause of death worldwide, accounted for about 13% of all deaths. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths per year expected by 2030. Lung, oral, stomach, liver, colon and breast cancer cause the most cancer deaths each year. Lung cancer accounts for 27% of all cancer deaths. Breast cancer is the most common cancer in women worldwide.

In normal cells, the genes of BRCA1 and BRCA2 (breast cancer susceptible gene 1 and breast cancer susceptible gene 2) are stable thereby preventing cancer. Mutation of these genes is linked to the development of hereditary breast and ovarian cancers [21]. Cervical cancer is also leading cause of cancer death in women. Every year, approximately 4,70,000 new cases of cervical cancer are diagnosed and approximately 230,000 women worldwide die of the disease, with the majority (80%) of these cases and deaths occurring in developing countries [22]. Oral cancer is a malignant growth that affects any part of the oral cavity including the lips, upper and lower jaws, tongue gums, cheeks and throat. It is one of the sixth most common cancers in the world, and it is the third leading cancer in India [23]. The drugs used to treat the cancers are largely ineffective and also causing side effects and hence, new drugs of natural origin are being attempted. In this regard, we attempted the mangroves as a source of anticancer drugs.

The mangroves have long been traditionally used to treat the cancer and tumours. The earlier reports revealed that the *Avicennia africana, A. nitida, Bruguiera exeristata, B. parviflora* and some other mangrove associates show potent anticancer activities [2]. Initially, we have prepared a black tea from *Ceriops decandra* and shown to prevent the incidence of chemically induced oral cancer in the animal model (hamsters). This is scientifically proved by morphological, histopathogical and biochemical evidences. The histopathological study reveals that the carcinogenic effect of DMBA treated animals show well developed squamous cell carcinoma, along with well-defined epithelial and keratin pearls in the connective tissue with cellular pleomorphism. However, the animals treated with both carcinogenic DMBA and mangrove tea, exhibit only hyperplasia. In addition, the hair loss caused in the oral cancer bearing animals is prevented in the mangrove tea treated animals (Fig. 1a,b) [24]. The oral cancer is a serious issue with growing threat of global warming. We have experimentally proved that the oral cancer increases with increasing temperature[25].

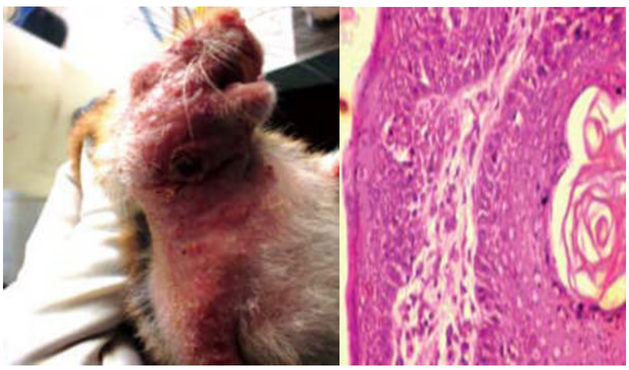


Fig. 1a. Animal treated with carcinogen (DMBA) alone showing tumour incidences and rapid hair loss as evident by hair sheath without follicle

We have observed that the tissue culture derived callus extract of *Acanthus ilicifolius* prevents the chemical benzopyrine (chemical carcinogen) induced lung cancer in albino mice.

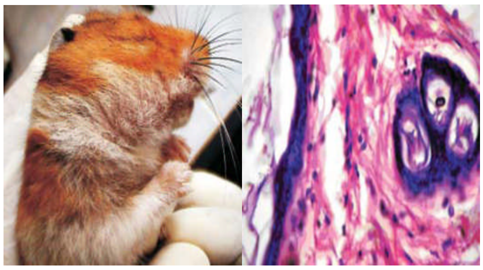


Fig. 1b. Animal treated with carcinogen (DMBA) + mangrove tea extract showing profound growth of hair as evident by hair sheath with follicle

Bearing the importance of tissue culture material as a source of anticancer compounds, further studies are progressing on stimulating secondary metabolites through hairy root culture using gene transfer techniques.

The mangrove plant *Ceriops decandra* is used traditionally and it is scientifically validated for its biological activities such as antiviral, antibacterial, antioxidant and chemo preventative potential [26]. In addition the DMBA induced oral cancer; this laboratory is also working on benzopyrine-induced lung cancer.

Computer aided drug discovery from mangrove-derived chemicals

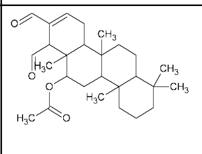
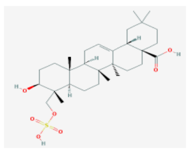
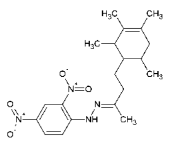
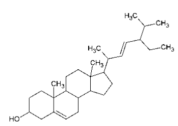
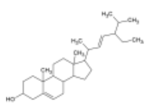
There are no reliable and potential drugs to cure the life threatening diseases such as cancers. As per the estimates, one successful drug takes about 12-15 years and requires about US$ 900 million. There are over US$ 45 billion every year invested by the pharmaceutical industry for 20-25 new potential drugs. Hence, we are attempting the computer based drug discovery to identify the mangrove-derived potential compounds, which can block the mutated gene/proteins responsible for the dreadful diseases.

We recorded that bark extract of the mangrove plant, *Xylocarpus mekongensis* exhibits potent antioxidant activities. This extract contains the predominance of three compounds namely 5-thio-D-glucose; 4-(cis-2,3,4, trans-6-Tetramethyl-3-cyclohexenyl)butan-2-one,2,4 dinitrophenylhydra-zone; and Dodecanoic acid, 2, 3-bis (acetyloxy) propyl ester, as evident by GC-MS analysis. These phytochemicals have been analysed by *in silico* docking against three cell survival proteins such as BCL-2, BCL-XL and MCL-1. The phytochemicals destabilize the over-expression of the proteins which possibly prevent carcinogenesis. Among the three compounds, dinitrophenylhydrazone is the most effective one which deserves further studies for developing it as a lead compound for anticancer drugs.

The fungus derived from mangroves, namely *Trichoderma* is found to contain abundance of some compounds (heptadecanoic acid, 16 methyl, methyl ester; 9,12-octadecadienoic acid; cis-9-octadecenoic acid) as evident by GC-MS analysis. Among these compounds, heptadecanoic acid, 16 methyl, methyl ester is the most potent having the docking score of −11.4592 Kcal/mol against the skin cancer protein (Hsp90) as revealed by *in-silico* docking method. This value is better than the standard drug "dyclonine". Similarly Phthalic acid is found to be the most potent secondary metabolite, isolated from the fungus to have activity against breast cancer protein BRCA1 [27].

In addition to human cancers, malarial fever is a serious one. Approximately three billion people, one half of the world's population, live in at-risk regions for malaria infection leading to about 250 million malaria cases every year and nearly one million deaths [28]. Once the malarial parasites *Plasmodium vivax* and *P. falciparum* enter the red blood cells, their growth is inhibited by Proguanil, a prophylactic antimalarial drug through inhibiting the enzyme, dihydrofolate reductase. The necessity of new improved drugs is being felt due to the side effects of antimalarial drugs. In this regard, stigmasterol is found to be potent in inhibiting the enzyme against malarial parasites.

Table 2 The mangrove-derived compounds with potential for developing drugs based on *in silico* docking

Disease	Compound	Structure	Docking energy (Kcal/mol)
Cervical cancer	Scalaradial		-12.06
Breast cancer	Triterpenoid		-13.06
Oral cancer	Dinitrophenylhydrazone		-11.52
Malaria (Dihydrofolate reductase)	Stigmasterol		-14.22
WSSV disease in shrimps	Stigmasterol		-15.03
Skin cancer	Heptadecanoic acid		−11.45

In addition to these human diseases, there is a shrimp viral disease, White Spot Syndrome (WSS), most disastrous to global shrimp aquaculture industries. The viral disease causes up to 100% mortality within a few days in commercial shrimp farms, resulting in heavy economic losses. The serious impact of WSS Virus (WSSV) in shrimp culture industry worldwide and the broad host range call for an efficient control strategy against the virus. VP28 is an envelope protein of WSSV, reported to play a key role in the systemic infection in shrimps. In this regard, stigmasterol is found to be potent to inhibit the protein, responsible for the shrimp disease.

Our computer based drug discovery has identified mangrove-derived compounds as lead molecules to develop drugs. The compounds are the most potent against the target proteins based on their docking scores and high binding affinity and they are Scalaradial (-12.06 Kcal/mol), Triterpenoid (-13.06 Kcal/mol)[29], Dinitrophenylhydrazone (-11.52 Kcal/mol) Stigmasterol (-14.22 kcal/mol)[30], Stigmasterol (-15.03 Kcal/mol) and Heptadecanoic acid (-11.45 Kcal/mol) against cervical cancer, breast cancer, oral cancer, antimalarial (Dihydrofolate reductase enzyme) ,WSSV virus and skin cancer respectively (Table 2). These compounds are being tested under *in vitro* and *in vivo* conditions to demonstrate the effectiveness of these potential compounds towards the development of drugs.

Mangrove-based nanoparticle synthesis

Nanotechnology is an exciting and fast growing area of research. The biologically diverse marine environment has a great promise for nanotechnology. Most of the studies on biosynthesis of nanoparticles, however, are restricted to terrestrial organisms. For the first time, we report that mangroves are capable of synthesizing nanoparticles. Among the mangrove species tested, *Xylocarpus mekongensis* displays the maximum production of silver nanoparticles [31]. The compound responsible for reducing the substrate chemical to nanoparticle is mainly gallic acid, and glucose is responsible for stabilization of the nanoparticles. It is also proved that the callus derived from tissue cultured *Sesuvium portulacastrum* produces silver nanoparticles better than intact plant tissue [32].

In addition to mangrove extracts, marine microorganisms derived from mangrove habitat are also capable of synthesizing the nanoparticles. The marine fungus *Penicillium fellutanum* isolated from mangrove sediment extracellularly produces silver nanoparticles, when it is exposed to $AgNO_3$. The strains of *Escherichia coli* AUCAS 112 and *Aspergillus niger* AUCAS 237 isolated from mangrove sediments, are capable of reducing the silver ions at a faster rate. The antimicrobial activity of nanoparticles produced by *E. coli* is more pronounced than that of *A. niger* and is also enhanced with the addition of polyvinyl alcohol as a stabilizing agent. The synthesized silver nanoparticles are monodispersed and spherical in nature [33]. The culture filtrate of mangrove-derived yeast *Pichia capsulata* exhibits the most efficient production of silver nanoparticles, within minutes. The protein present in the culture filtrate of the yeast species is responsible for the synthesis of silver nanoparticles. We have also recorded the silver and gold nanoparticle synthesis by the fungi, the cyanobacteria and thraustochytrids [34]. We are now focusing on the synthesize of different nanoparticles such as gold, silver, calcium, copper, magnesium, zinc and lead, with the use of mangroves and associated microorganisms.

The mangrove-based nanoparticles possess different biological activities. The silver nanoparticles, synthesized by the mangrove leaf extract of *Rhizophora mucronata* exhibit mosquito larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* [35]. We have found that silver nanoparticles, synthesized by coastal stand of *Prosopis chilensis* have strong antibacterial activity in controlling the vibriosis, a common shrimp disease and inhibiting the human pathogenic bacteria. We have proved the use of nanoparticles in stabilizing the cotton fabrics and making them odour resistant, preserving the apple fruits, purifying the drinking water from microbial contaminants, detoxifying the carcinogenic ethidium bromide as well as in controlling the cancer cells that cause oral and skin cancers. Toxicity of the nanoparticles on non-target organisms is being assessed for their application in the field condition.

Removal of toxic metals by mangrove-derived microbes

Mangrove habitats act as sink for toxic heavy metals. The mangrove soil holds higher levels of metals than plant parts. Bark and root accumulated higher levels of trace elements in a magnitude of 10-80 folds than other plant parts. Essential elements (B, Cu, Fe, Mg, Mn, Zn) accumulate high in the unpolluted mangroves forest while non-essential elements (Al, Cd, Co, Cr, Ni, Pb) in the industrially polluted mangrove habitats. The ratio between essential and non-essential elements is found higher in young mangrove forest than that in mature mangrove forest and polluted mangrove areas. Thus, we have suggested that the ratio of metal accumulation can be used as an index of the growth and pollution status of mangroves [36].

The mangrove-derived microbes are efficient in accumulation of toxic heavy metals. For example, the dried biomass of cyanobacterium, *Synechococcus elongatus* removes lead by 85.84% in sewage water under optimized condition of temperature (40ºC), adsorbent dosage (0.63 $g.l^{-1}$), pH 9 at 60 minutes of incubation [37]. Similarly, the marine cyanobacterium (*Gloeocapsa* sp.) is efficient to remove calcium and cadmium up to 92.9% from seawater. Dried fungal biomass of *Aplanochytrium* sp., removes the toxic chromium by 69.4% in waste water treatment. Further studies are at progress on the application of mangrove system to treat the wastewaters.

Dye degradation by mangrove-derived microbes

Mangrove-derived microbes are efficient in dye degradation. For example, the thraustochytrids *Aplanochytrium* sp., is found to remove the azo-dye (malchite green) significantly up to 86.32% within five and half days of incubation under optimized conditions of pH 7.8 and at temperature of 27.8°C. Another example is that cyanobacterium, *Synechococcus elongatus ARKK1* degrades methyl red dye as much as 82.59% under optimized conditions of *p*H of 7.9, temperature of 23.08°C, 10 days of incubation. Dye degradation in terms of changes in chemical groups is confirmed by Fourier Transform Infra Red (FT-IR) [34].

Polythene and plastic degrading microbes

Microbes have been isolated from mangrove sediment and assessed for their ability of degrading the polythene and plastics. A bacterial species (*Pseudomonas* sp.) is capable of degrading 20.54% of polythene and 8.16% of plastics. A fungal species (*Aspergillus glaucus*) has an efficiency of degrading 28.8% of polythene and 7.26% of plastics. Studies on the microbial strains in clearing plastic wastes are progressing for their application in the field

Mangrove-derived enzymes

Mangrove habitat is a rich source of microbial enzymes. We have recorded the fungal production of enzymes such as amylase, glucose isomerase, cellulase, glucose oxidase and alkaline protease [38] by *Penicillium fellutanum*, isolated from mangrove sediment. We have also reported that the microbes isolated from decomposing leaves of mangroves exhibit extracellular enzymes such as amylase, protease, cellulase, chitinase and lipase. Among the microbes, fungal *Trichoderma* species are more efficient in producing the enzymes than the bacterial isolates, revealing the significance of fungi in detritus-based mangrove systems.

The value of mangrove-derived microbial enzymes has been proved. For example, protease derived from *Trichoderma estonicum* is capable of removing blood stain by 59.7% instantly, which is greater than that of the commercial detergent. Therefore, *Trichoderma* derived enzyme is a promising detergent in removal of blood stain. Another example is the enzyme adenosine deaminase, produced by *Lysinibacillus* sp. (JQ710723) being the first report as a bacterial endophyte in the mangrove species, *Avicennia marina*. This enzyme finds its application in disease diagnosis [36].

Bioethanol production by mangrove-derived yeasts

Yeasts are well known for bioethanol production. However, marine yeasts are less known for the activity. In the present context of increasing demand for energy and biofuel, the microbial synthesis of ethanol using cellulosic waste materials has gained importance. We have proved that mangrove-derived marine strain *S. cervevisiae* is promising for bioethanol production over the terrestrial yeast. The ethanol production by the yeast *Pichia salcaria* is found maximum (26.2±8.9 g/l) in 72 hours of fermentation in the culture broth supplemented with 2% sawdust that has been preprocessed with dilute phosphoric acid. This condition of fermentation coincides with high activity of cellulase in the culture broth, which also reveals a single prominent protein band of 68 kDa molecular weight [39]. We have found among 10 species of mangrove-derived yeasts, *Candida albicans* exhibits the maximum production of ethanol (47.3±3.1 g/L) within 96 h, when glucose is used as carbon source. The ethanol production by this species is found increased when the yeast cells are immobilized in sodium alginate, as compared to non-immobilized cells [29]. Work on bioethanol production by using marine-derived yeasts is at progress for its application.

Biofertilizer

The fungus *Trichoderma* isolated from mangrove soil is capable of phosphate solubilization and phytase enzyme production. We have also proved that the fungal treatment improves the mangrove soil fertility as well as growth and biomass production of mangrove seedlings of *Avicennia marina* (Saravanakumar *et al.*, 2012c). A similar biofertilizer effect is proved with *Rhizophora mucronata.* Work is at progress to develop the fungus based biofertilizer for its application in mangrove plantations.

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