MARINE MICROBIAL ENZYMES

M. Chandrasekaran and **S. Rajeev Kumar**
Microbial Technology Laboratory, Department of Biotechnology, Cochin University of Science and Technology, Cochin-682022, Kerala, India

Keywords: microbial enzymes, marine environment, mineralization, bacterial foraging, polysaccharases, amylase, chitinase, protease, lipase, extremozymes, thermostable, cold adapted, barophilic, halophilic, alkalophilic, nucleases, restriction endonucleases, enzyme engineering, immobilization, cloning, future prospects.

Contents

Summary

Several industrial enzymes are derived from terrestrial sources. Whereas, marine environment, which encompass about 71 percent of the earth's surface and a vast resource for useful enzymes, remain unexplored. Marine microorganisms take active part in the mineralization of complex organic matter through degradative pathways of their metabolism in the marine environments and contribute to the secondary production in sea. Microorganisms as the fastest means to search for food may often use cell-free extracellular enzymes.

Bacteria and fungi from marine environments secrete different enzymes based on their habitat and their ecological functions. Marine microbial enzymes have become the focal

point of interest and several enzymes have drawn the attention of the microbial prospectors and a few enzymes were isolated from seawater and marine sediments, purified and characterized for their properties and applications.

Enzymes known so far from marine microorganisms include α-Amylase, α-glucosidase, pullulanase, Cyclomaltodextrin-glucanotransferase, Agarase, alginate-lyase, κ-carrageenanase, α-galactosidase, beta-galactoside-alpha-2, 6-sialyltransferase, Cellulases, endo-1, 4-β-xylanase, β-galactosidase, β-glucosidase, β-fucosidase, endo-1,3-β-D-glucanase (I), endo-1,6-β-D-glucanase, chitinase, chitobiose-deacetylase, N-acetylglucosamine-deacetylase, N-acetylglucosamine-6-phosphate-deacetylase, lignin-modifying enzymes (LMEs): manganese-dependent peroxidase (MnP), lignin peroxidase (LiP), and laccase, Catechol-oxidase, Cresolase (monophenol-monooxygenase), beta-1,4-mannanases, Poly(3-hydroxybutyrate)-depolymerase, Poly-beta-hydroxyalkanoate (PHA)-depolymerase, alkaline protease, Subtilisin (EC-3.4.21.62)-like serine protease, neutral protease, metal neutral proteinases, thiol protease, Collagenase, lipase, L-asparaginase**,** L-glutaminase, Tyrosinase, Hydrogenases, Superoxide-dismutase, Glucose-dehydrogenase etc.

Extremophiles are the primary source of enzymes that are active at extreme conditions of life. Harsh marine environments, such as deep-ocean hydrothermal vents, polar oceans, and extremely saline bodies of water, have yielded valuable extremophilic microorganisms. Of particular interest are the enzymes that help extremophiles to function in markedly hot, cold, acidic, basic, pressurized, saline or mineral rich environments.

Extremozymes from extremophiles isolated so far include thermostable DNA-polymerases- *Taq* polymerase, and *Pfu* polymerase, Glutamate-dehydrogenase, beta-glucosidase, thermostable esterase, thiol protease, Adenylate-kinase, hydrogenases, alpha-glucosidase, lipase, alpha-amylase, beta-lactamase, protease, endo-1,4-beta-D-xylanase, cellulase and xylanase, extremely thermostable protease, thermostable serine protease etc. Psychrostable metallo protease (almelysin), protease, chitinase, glucanase, esterase, lipase, phospholipase and DNA-degrading enzymes have been obtained from marine bacteria isolated from different sites in permanently cold arctic and antarctic habitats. Some of the cold adapted enzymes from bacteria include α- amylase, isocitrate dehydrogenase, lipase, β- lactamase, triose phosphate isomerase, subtilisin. Alkaline protease, alkaline cyclomaltodextrin-glucanotransferase, lipases, alkaline cellulases, alkaline phosphatase, endo-1, 3-β-D-glucanase, and alkaline metallo endopeptidase; and halotolerant amylases, proteases lipases, Nuclease H, cyclophilin-type peptidyl-prolyl-cis-trans-isomerase (PPIase) are also known from extremophiles.

Enzymes that are capable of carrying out very specific molecular tasks, usually related to the modification of DNA or RNA, for the creation of genetically modified organisms or for diagnostic procedures include restriction endonucleases, RNA and DNA polymerases, DNA ligases, alkaline phosphatases, kinases, reverse transcriptases, Several such enzymes have been isolated.

In general traditional microbial enzyme technology ventures are undergoing rapid transformation through the process of evolution of innovative technologies facilitated

through techniques like molecular gene cloning, protein and enzyme engineering, metabolic engineering and immobilization of enzymes.

Despite the fact that more and more novel enzymes are yet to be discovered from marine microorganisms from diverse marine environments around the world, the prospects of isolating the genes coding for the novel enzymes with potential applications have already received the attention of the genetic engineers and biotechnologists. The art of gene cloning have paved the way for easy isolation of the concerned genes, characterization and cloning and expression in hosts such as *E.coli* successfully.

Extremozymes can be produced through recombinant DNA technology without massive culturing of the source extremophiles. Some of the success stories of cloning and expression of few genes of novel enzymes of industrial importance include alkaline serine protease (*apr*II, subtilase), endo-1,4-beta-D-mannanase, salt-tolerant glutaminase-I, agarase (*pja*A), *Age*I methylase, DNA-polymerase, *lux* genes encoding luciferase, tyrosinase, α-Amyalse, pullulanase type II, β-galactosidase, β-glucosidase, acid protease (Thermopsin), S-Adenosyl-homocysteine hydrolase, Glyceraldehyde-3-P-dehydrogenase, and Glutamine synthetase. Nitrite reductase genes (*nir*K and *nir*S), structurally different but functionally equivalent single-copy genes coding for nitrite reductases, a key enzyme of the denitrification process, were used as a molecular marker for denitrifying bacteria in pacific northwest marine sediment communities.

While considering the enormous microbial diversity native to the vast marine environments of this planet earth the efforts channeled into discovery of novel enzymes from marine microbes are inadequate and warrant launching of intensive screening programs by the scientists at global level. Such a mammoth attempt alone can return large number of novel enzymes for varied purposes and services of humanity, for the simple reason that marine environments are rich treasure of novel enzymes which probably may even avoid the need for enzyme engineering or molecular cloning for designing novel enzymes for specific requirements.

In the long run, probably, marine microbial enzyme based processes would substitute several of the current chemical processes under practice.

1. Introduction

Microbial enzymes have several advantage over the enzymes derived from plant or animal sources by virtue of their great variety of catalytic activities, cheaper in cost, regular abundant supplies at even quantity and relatively more stability [see also - *Enzyme production*]. Major targets of modern enzyme technology continue to be preservation of foods and food components, efficient use of raw materials, improvement of food quality such as texture and taste, manufacture of dietetic foods, eliminating antinutritive substances from certain nutritional raw materials, utilization of raw materials for preparation of animal feed, and optimization of process to reduce process costs. Enzymes are used as cost -effective and environmentally sensitive substitutes for chemical processing in several industries including pharmaceuticals, food, starch, laundry, detergents, for processing textiles, leather, wood pulp and paper, and for the

production of fine and specialty chemicals, and industrial catalysis, organic synthesis and transformation of compounds and bioremediation [see also - Enzyme production]. Further, enzymes are finding applications as research tools in biotechnology and molecular biology. As such the worldwide enzyme market is estimated at $2 billion and is expected to expand rapidly in the new millennium.

Further, several industrial applications demand that industrial enzymes must be stable at extremes of temperature, pH, and salt concentration. For this reason, the isolation and characterization of enzymes from microorganisms known as "extremophiles" [see also - Biotechnological potential of the Archae] may yield useful new enzymes for this purposes. For instance, the deep seabed provides many of these extremophiles, and consequently may be of interest to companies involved in developing enzymes for this sector.

Despite the fact that several industrial enzymes are derived from terrestrial sources, marine microorganisms are yet to be exploited to its full potential, and consequently warrant immediate attention for industrial exploitation. Nevertheless, marine microorganisms have drawn the attention of investigators in the last decade and there is lot of interest on microbial enzymes now.

Marine environment, which encompass about 71 percent of the earth's surface, is not only rich with biodiversity but also a vast resource for potential microorganisms of useful applications. Microbes inhabit various habitats of marine environment that include neuston, plankton, nekton, seston, and epibiotic, endobiotic, pelagic and benthic environments. These habitats harbor a diverse range of microbes including archaebacteria, cyanobacteria, eubacteria, actinomycetes, yeasts, filamentous fungi, microalgae, algae, and protozoa. Almost all of these groups are potential source of useful enzymes that remain unexplored.

Enzymes catalyze not only biochemical reactions in living cells but also in the mineralization processes and cycling of elements in various environments. Hence, every marine microorganism should act as a dependable source of useful enzymes such as protease, amylase, lipases, chitinase, cellulase, ligninase, pectinase, xylanase, nucleases (DNAses, RNAses, restriction enzymes), etc. Production of copious quantities of amylase, alginate lyases, chitinases, glutaminase, asparaginase, arylsulphatase, phosphatase and beta lactamase by marine bacteria are known. With the advent of biotechnology, enzyme engineering and introduction of other innovative technologies there is plenty of scope for efficient management of our rich marine microbial biodiversity towards deriving novel enzymes could also be recovered from marine microorganisms and efficiently exploited not only as a cost effective biocatalyst but also as an ecofriendly reagent in the coming years.

2. Role of Microbial Enzymes in Marine Environment

Marine microorganisms take active part in the mineralization of complex organic matter through degradative pathways of their metabolism in the marine environments and contribute to the secondary production in sea. Complex polysaccharides such as cellulose, lignin, pectin, xylan, starch, proteins, fats, sugar, urea, aromatic and aliphatic hydrocarbons, and several other organic compounds which reach marine environments,

besides the dead plants and animal residues, are degraded by marine microorganisms. Their participation in the degradation of organic compounds and retting of ropes and fibers testify their potential as rich source of hydrolytic enzymes of industrial importance.

Microorganisms as the fastest means to search for food may often use cell-free enzymes. Heterotrophic bacteria living in particle aggregates and sediments therefore must depend largely on extracellular enzymolysis and (or) desorption of particulate and particle-sorbed organic material (OM) to generate the dissolved, low-molecular-weight compounds required for uptake and metabolism. Transport of OM into bacterial cells is limited by cell permeability, probably to molecules smaller than about 600 Daltons.

Both substrate uptake and bacterial growth may be coupled with extracellular hydrolysis. Bacterial extracellular enzymes (EE) can effect partitioning of OM between particulate and dissolved pools in the pelagic ocean and probably contribute to significant dissolved OM fluxes out of the sediment.

Studies with size-fractionated samples and with radio labeled substrates suggest that cell-attached EE dominate extracellular hydrolysis by free-living marine bacteria, perhaps due to dilution of cell-free EE below detection limits. However, significant dissolved EE activity is sometimes observed in environmental samples, and for some aquatic and sedimentary environments, cell-free EE are produced by most of the cultivable bacteria.

Cell-free EE foraging should be a powerful bacterial feeding mechanism in high-surface-area, organic-rich, liquid-bathed environments, with the potential to support maximal growth rates. Where net energetic gain is the purpose of producing cell-free EE, bacteria can usually be expected to release enzyme at high rates. Many organisms may use cell-free EE to search for food, even in environments in which actual feeding is optimized by some other mechanism.

Polysaccharases release microorganisms from their natural seat, marine sediments for example. The enzymatic activity works both on the microbial adherence polysaccharides and on the support surfaces (cellulose, pectin, etc.). Dosages of glucose confirm polysaccharase activity. An association of bacitracine, thiophenicol and a few enzymes: cellulase, pectinase, amyloglucosidase, alpha amylase, hyaluronidase, release a considerable number of bacteria. High activities of exoenzyme aminopeptidase and alkaline phosphatase were detected even in a 124,000-year-old sapropel layer**.**

3. Enzymes from Marine Microorganisms

Bacteria and fungi from marine environments secrete different enzymes based on their habitat and their ecological functions. Marine microbial enzymes have become the focal point of interest and several enzymes have drawn the attention of the microbial prospectors and a few enzymes were isolated from seawater and marine sediments, purified and characterized for their properties and applications. Interestingly the enzymes reported from marine environments belonged to one or more of the major

classes of enzymes viz: Oxidoreductase, Transferases, Hydrolases, Lyases, Isomerases and Ligases.

3.1. Polysaccharases

3.1.1. Starch Hydrolyzing Enzymes

The enzymes involved in the conversion of starch to low molecular-weight compounds such as glucose, maltose and oligosaccharides are α-amylase, β-amylase, glucoamylase, debranching enzymes (pullulanase) and α-glucosidase.

α-Amylase, pullulanase and α-glucosidase from archaea are all active in the same pH and high temperature range and hence they could be used in a one-step process for the industrial bioconversion of starch [see also - Biotechnology of Archaea]. Improvement of the starch-conversion process using new efficient and thermoactive enzymes would significantly lower the cost of sugar syrup production.

3.1.1.1. α-Amylase (EC-3.2.1.1)

α-Amylase has a wide range of industrial applications particularly in the manufacture of alcoholic beverages like beer, alcohol, preparation of animal feed, laundry, detergent, in starch industry, confectioneries, as desizing agent in textile industry and in the production of sugar syrup. This is one among the few major industrial enzymes, which is in great demand and mainly produced using microbial sources [see also - Industrial Biotechnology].

α-Amylase has been obtained from thermophilic archaea Pyrococcus woesei, Pyrococcus furiosus, Thermococcus celer, Fervidobacterium pennavorans, Desulfurococcus mucosus and Thermotoga maritime; psychrotrophic Vibrio isolated from a deep-sea mud, Vibrio gazogenes, Alteromonas rubra, and Mucor sp. Pseudomonas-like strain MS300, from Deep-sea, produce two major and two miner maltotetraohydrolases (G4-amylase). Under high hydrostatic pressure, the strain MS300 produced more amylase than under atmospheric pressure.

3.1.1.2. α-Glucosidase (EC-3.2.1.2)

Alpha -Glucosidase (EC-3.2.1.2) is mainly used in the starch industry along with α-amylase**.** An extremely thermostable α-glucosidase (EC-3.2.1.2) was isolated from the hyperthermophilic marine archaebacterium, *Pyrococcus furiosus* and *Pyrococcus wesei*. The enzymes from these microorganisms appear to be the most thermostable α-glucosidases described.

3.1.1.3. Pullulanases (EC-3.2.1.41)-Debranching Enzymes

Similar to α-amylases and α-glucosidase, pullulanase are mainly used in starch industry. Pullulanase type I is a typical bacterial enzyme that is specific for α-1, 6 linkages in branched oligosaccharides. It is unable to attack α-1,4-linkages in a-glucan.

The production of pullulanases type I seem to be very rare amongst thermophilic microorganisms that produce a thermoactive pullulanase with a temperature optimum of 90°C. This enzyme has been reported only *in Fervidobacterium pullulanolyticum.* Pullulanase type II (amylo pullulanase), which is capable of hydrolysing the α-1,4-linkages and branching points (α-1,6-linkages) in polysaccharides and limit dextrins is mainly found in anaerobic bacteria and is widely distributed among thermophilic bacteria and archaea. Marine thermophilic *Thermococcus litoralis* (Tl) DSM 5473 (optimal growth temp. 90°C) and *Pyrococcus furiosus* (Pf) DSM 3638 (98°C) produce pullulanase type II whose thermoactivity and thermostability were enhanced in the presence of 5 mM Ca^{2+}, and under these conditions, enzyme activity could be measured at temperature of up to 130-140°C. Each of these enzymes was able to hydrolyze, in addition to the alpha-1, 6 linkages in pullulan, alpha-1, 4 linkages in amylose and soluble starch. The enzymes appear to represent highly thermostable amylopullulanase versions of those which have been isolated from less thermophilic organisms. *Pyrococcus woesei, Thermococcus celer, Fervidobacterium pennavorans,* and *Desulfurococcus mucosus* have also been reported to produce pullulanase type II.

3.1.1.4. Cyclomaltodextrin-glucanotransferase (CGase EC-2.4.1.19)

Cyclomaltodextrin-glucanotransferase (CGase EC-2.4.1.19), which has potential application in cyclodextrin production, was isolated from alkalophilic *Bacillus subtilis* sp. from a deep-sea mud.

3.1.2. Agarase (EC-3.2.1.81*)*

Agarase has been the subject of investigations for quite some time owing to its immediate applications in gene technology for the elution and isolation of DNA fragments from agarose gels after electrophoresis; in the preparation of algal protoplasts such as from red alga *Gelidium robustum;* seaweed polysaccharide characterization; in the production of simple sugars, including neoagarobiose, neoagarotetraose and neoagarohexaose; degradation of agarose to oligosaccharides, facilitating the liquefaction of agar and agarose gels; and in the defouling of fermentors and bioreactors. Moreover, the purified enzyme could effectively control red algae bloom contaminations, prevent the biofouling of submerged marine surfaces or pipes by contaminating complex polysaccharide layers, or treat such biofouled surfaces after contamination. Marine Bacterial agarase has a high level of activity for the depolymerization of complex polysaccharides, including agar and agarose. Agarase is obtained from few bacteria. Agarase, named agarase-0107, an endo-type β-agarase that hydrolyzed the β-1, 4-linkage of agarose to yield neoagarotetraose and neoagarobiose at a pH of around 8, have been isolated from *Vibrio* sp. JT0107, that requires seawater salts for growth. *Pseudomonas stutzeri*, *Aeromonas* sp and *Vibrio* sp, isolated from sea produce agarase, which have been characterized.

3.1.3. Alginate-lyase (EC-4.2.2.3)

The alginate-lyase may be useful for the conversion of brown algal biomass to methane. This observation has led to the isolation and characterization of an intracellular inducible alginate-lyase from marine *Bacillus* sp., *Alteromonas and Photobacterium*.

3.1.4. κ-carrageenanase (EC-3.2.1.83)

A Cytophaga-like, carrageenan-degrading bacterium, referred to as strain Dsij, isolated from the marine red alga *Delesseria sanguinea*, simultaneously produce extracellular κ-carrageenanase (EC-3.2.1.83) and iota-carrageenanase in the presence of crude λ-carrageenan.

3.1.5. α-Galactosidase (EC-3.2.1.22)

α-galactosidase is used in the sugar industry for enhancing the yield of sucrose by the hydrolysis of raffinose, an α-galactoside. α-galactosidase was obtained from marine *Alteromonas spp***.,** isolated from sponges and alga *Polysiphonia* sp., and from bacteria associated with mussel (*Crenomytilus grayanus)* and scallop (*Patinopecten jessoensis)***.**

A novel beta-galactoside-alpha-2, 6-sialyltransferase (EC-2.4.99.1) is produced by *Photobacterium damsela* JT0160 (FERM BP-4900), ATCC 33539 or ATCC 35083. The enzyme should be useful in modification of glycoproteins and industrial-scale production of sialosides.

3.1.6. Cellulases and Related Enzymes

Cellulases are used in the manufacture of alcohol, flavors, maize gluten, silage, in laundry & detergents, and in wastewater treatment. A symbiotic bacterium found in the gland of *Deshayes* of the marine shipworm and *Aspergillus terreus* isolated from the seawater produce cellulase. Thermophilic *Thermotoga* sp produces thermostable exo-1, 4-β-cellobiohydrolase (105^{o}C).

-
-
-

Bibliography

Ahuja S.K., Ferreira G.M. and Moreira A.R. (1997) Production of cellulase by a shipworm bacterium' Abstr.Pap.Am.Chem.Soc.; 213 Meet., Pt.1, BIOT226 [A cellulase (EC-3.2.1.4) was isolated from a symbiotic bacterium found in the gland of Deshayes of the marine shipworm. The enzyme is thermostable, halostable and pH stable]

Ando S., Yoshida A. and Hatano M. (1991) Occurrence of marine bacterial lipase hydrolyzing fish oil. Agric.Biol.Chem., **55(**10) 2657-59. [Production of a lipase that hydrolyzed n-3 polyunsaturated fatty acid (PUFA)-containing fish oil, from Vibrio sp.]

Arnosti C. and Repeta D.J.(1994) Extracellular enzyme activity in anaerobic bacterial cultures: evidence of pullulanase activity among mesophilic marine bacteria *Appl.Environ.Microbiol*., **60**(3)840-46.*[This is the 1st report of pullulanase activity among mesophilic marine bacteria]*

Bakunina I.Y., Ivanova E.P., Nedashkovskaya O.I., Gorshkova N.M., Elyakova L.A. and Mikhailov V.V. (1996) Searches for alpha-galactosidase producers among marine bacteria of the genus *Alteromonas. Prikl.Biokhim.Mikrobiol*.; **32**(6) 624-28 *[Isolation and partial purification of Alpha-galactosidase and agarase from agarolytic bacteria associated with mussel]*.

Barbeyron T., Henrissat B. and Kloareg B.(1994) The gene encoding the kappa-carrageenase of *Alteromonas carrageenovora* is related to beta-1,3-1,4-glucanases *Gene*; **139**(1) 105-09 [*gene isolation and DNA sequence determination.It is proposed that residue Glu-163 in the kappa-carrageenase from A. carrageenovora is important for biocatalysis]*

Bok J.D., Goers S.K. and Eveleigh D.E. (1993) Thermostable cellulase and xylanase from the hyperthermophile *Thermotoga neopolitana Abstr.Pap.Am.Chem.Soc*., 205 Meet., Pt.2, BTEC20

Bok J.D., Goers S.K. and Eveleigh D.E.(1992) Cloning and characterization of an endoglucanase and a xylanase from the hyperthermophilic eubacterium *Thermotoga maritima* Abstr.Gen.Meet.Am.Soc.Microbiol., 92 Meet., 312

Brown B.J. and Preston III J.F. (1991) L-Guluronan-specific alginate-lyase from a marine bacterium associated with *Sargassum*. *Carbohydr.Res*. **211**(1) 91-102

Brown B.J., Ingram L.O. and Preston J.F. (1990) Cloning of alginate-lyase gene, alxM, and expression in *Escherichia coli* Abstr.Pap.Am.Chem.Soc.; 199 Meet., Pt.1, BIOL77

Cammack R. (1995) Role of Desulfovibrio gigas hydrogenase in energy e.g. hydrogen, methane generation. Nature, **373**(6515), 556-57. [The hydrogenase from the marine and freshwater sediment bacterium Desulfovibrio gigas catalyzes hydrogen production and consumption]

Chandrasekaran M. (1994)Economic utilization of marine microorganisms employing solid state fermentation. In A. Panday(ed) *Solid State Fermentation*, Wiley Eastern. New Delhi.pp168-172.ISBN81-224-0661-0.

Chandrasekaran M..(1996) Harnessing of Marine Microorganisms through solid state fermentation. J. Sci.Ind. Res. **155**(5&6) 468 - 471.[First paper to propose propspects of employing solid state fermentation for production of marine microbial enzymes]

Chandraskaran M..(1997). Industrial Enzymes from marine microorganisms- Indian scenario. *J. Mar. Biotechnol.* **5**, 86-89[*First Review on marine microbial enzymes*]

Chiura H., Noro Y., Kanayama S., Ueda Y., Simidu U. and Takagi J. (1988) Site specific deoxyribonuclease produced by a marine bacterium *Flavobacterium* I 16-04 *Agric.Biol.Chem*., **52**(8) 2107-09 *[isolation and characterization of FspI]*

Chiura H.X., Kamiyama T., Hirano H., Futagami M., Watahiki M. and Kobayashi K. (1992) Purification and characterization of *Asp*MDI, an isoschizomer of *Sau*3AI, from a marine bacterium, *Alcaligenes* sp. MD1. *Nucleic-Acids-Res*., **20**(8) 1996

Clark D.S., Sun M.M., Giarto L., Michels P.C., Matschiner A. and Robb F.T. (1996) Stabilization of thermophilic enzymes by pressure. *Prog.Biotechnol*. **13**, 195-202 *[Improved thermostability under pressure appears to be closely related to enzyme thermostability. Pressure-induced enzyme stabilization of glutamate-dehydrogenase from the deep sea hyperthermophile Pyrococcus endeavori, was studied]*

Costantino H.R., Brown S.H. and Kelly R.M.(1990) Study of an extremely thermostable alpha-glucosidase from the hyperthermophilic archaebacterium, *Pyrococcus furiosus*. Abstr.Pap.Am.Chem.Soc., 199 Meet., Pt.1, BIOT17

Dahlberg L., Holst O., and Kristjansson J.K. (1992) Thermostable xylanolytic enzymes from *Rhodothermus marinus*. *Thermophiles-Sci.Technol*., **61**

Danaher R. and Stein D.C. (1988) Characterization of a restriction enzyme isolated from a hydrothermal vent community bacterium, *Hyphomonas jannaschiana* Abstr.Annu.Meet.Am.Soc. Microbiol., 88 Meet., *213 [A restriction endonuclease, HjaI, was purified from Hyphomonas jannaschiana]*

Demina N.S., and Lysenko S.V. (1996) Collagenolytic enzymes from microorganisms .*Mikrobiologiya*; **65**(3) 293-304 *[an excellent review on collagenolytic enzymes]*

Doubet R.S. and Quatrano R.S.(1984) Properties of alginate-lysases from marine bacteria. *Appl.Environ.Microbiol*., **47**(4) 699-703 *[Alginate-lyases from 2 marine bacteria were isolated and characterized]*

Feller G., Narinx E., Arpigny J.L., Zekhnini Z., Swings J. and Gerday C. (1994). Temperature dependence of growth, enzyme secretion and activity of psychrophilic Antarctic bacteria. *Appl.Microbiol.Biotechnol*., **41**(4)477-79 *[Production of lipase by Moraxella sp., alpha-amylase by Alteromonas haloplanctis, beta-lactamase by Psychrobacter immobilis and protease by Bacillus sp]*.

Fujishima S., Yamano N., Murayama A., and Higashihara T. (1996) Preparation of N-acetyl-D-glucosamine-deacetylase PN: EP-; EP 732400; 18.09.96 *[useful as a drug or in the production of D-glucosamine, which is a material useful in the pharmaceutical, agricultural and food industries. D-glucosamine is useful for production of glucosamino-oligosaccharides with antimicrobial and antitumor activities.]*

Fukuda K., Hasuda K., Oda T., Yoshimura H. and Muramatsu T. (1997) Novel extracellular alkaline metalloendopeptidases from *Vibrio* sp. NUF-BPP1: purification and characterization. *Biosci.Biotechnol.Biochem*. **61**(1) 96-101

Fukusawa S., Nakamura K., Miyahira M., and Kurata M. (1988) Some properties of two proteinases from a luminous bacterium, Vibrio harveyi strain FLN-108 *Agric.Biol.Chem*. **52**(12) 3009-14 *[thermostable alkaline protease purification and characterization]*

Gavrilovic L., O'-Brien R.W. and Sanders R.L. (1982) Secretion of amylase by the marine bacterium, *Alteromonas rubra*. *Rev.Drug-Metab.Drug-Interact*. **35**, 111-24.

Georganta G., Kaneko T., Nakamura N., Kudo T., and Horikoshi K.(1993) Isolation and partial properties of cyclomaltodextrin-glucanotransferase-producing alkaliphilic Bacillus spp. from a deep-sea mud sample. *Starch* **45**(3) 95-99

Gildberg A. (1993) Enzymic processing of marine raw materials. Process-Biochem. **28**(1) 1-15 [Enzyme treatment of fish, shellfish, alga, etc.; a review]

Greene R.V., Griffin H.L., and Cotta M.A. (1996). Utility of alkaline protease from marine shipworm bacterium in industrial cleansing applications. *Biotechnol. Lett*.**18** (7) 759-64 *[Psiloteredo healdi-derived alkaline protease was evaluated as a cleansing agent in standard and non-phosphate surfactants and for cleaning contact lenses. The cleaning power of a non-phosphate surfactant was improved by added protease, independent of the pH range 10 to 12.]*

Ha J.C., Kim G.T., Kim S.K., Oh T.K., Yu J.H., and Kong I.S. (1997) Beta-agarase from *Pseudomonas* sp. W7: purification of the recombinant enzyme from *Escherichia coli* and the effects of salt on its activity. *Biotechnol.Appl.Biochem*. **26**, Pt.1, 1-6 *[pjaA gene was cloned and characterized.The enzyme was halophilic, with maximum activity at 0.9 M sodium chloride, and may be useful in formation of protoplasts from marine algae]*

Hamamoto T., and Horikoshi K. (1991) Characterization of an amylase from a psychrotrophic Vibrio isolated from a deep-sea mud sample *FEMS-Microbiol.Lett*. **84**(1) 79-84

Hanzawa S., Hoaki T., Jannasch H.W., and Maruyama T. (1992) Extremely thermostable protease of a hyperthermophilic archaeum isolated from hydrothermal vents *Thermophiles-Sci.Technol*. **25** *[purification and characterization of serine protease from Desulfurococcus sp]*

Hanzawa S., Hoaki T., Jannasch H.W., and Maruyama T. (1996) An extremely thermostable serine protease from a hyperthermophilic archaeum, *Desulfurococcus* strain SY, isolated from a deep-sea hydrothermal vent. *J.Mar.Biotechnol*. **4**(2) 121-26*[A thermostable serine protease was produced and purified (283-fold with 1.6 % yield) from deep-sea hyperthermophilic Desulfurococcus sp. strain SY, which requires 11 amino acids for growth]*

Hei D., Nelson C., and Clark D.S. (1991) Pressure-temperature effects on the structure and function of enzymes from deep-sea thermophiles Abstr.Pap.Am.Chem.Soc., 201 Meet., Pt.1, BIOT86.*[Pressure effects on the catalytic and structural properties of hydrogenase enzymes obtained from thermophiles, e.g. Methanococcus jannaschii, an extremely thermophilic methanogen isolated from a deep-sea hydrothermal vent are presented]*

Hei D.J., and Clark D.S. (1994) Pressure stabilization of proteins from extreme thermophiles Appl.Environ.Microbiol. **60**(3) 932-39 [The stabilization of enzymes by pressure is described, using a hydrogenase from Methanococcus jannaschii, an extremely thermophilic deep-sea methanogen.. Pressure stabilization of an enzyme may be related to its thermophilicity]

Helmut Uhlig (1998) Industrial enzymes and their applications. John Wiley &Sons Inc. New York. ISBN 0-471-19660-6 [An excellent book on industrial enzymes]

Hiraga K., Shou L., Kitazawa M., Takahashi S., Shimada M., Sato R., and Oda K. (1997) Isolation and characterization of chitinase from a flake-chitin degrading marine bacterium, *Aeromonas hydrophila* H-2330. *Biosci. Biotechnol. Biochem.* **61**(1) 174-76 *[Reports the A. hydrophila chitinase as an inducible enzyme.]*

Hirayama S., Ueda R., Sugata K., and Kamiyoshi H. (1993) Production of bacteriolytic enzyme by bacteriophage from seawater *Biosci.Biotechnol.Biochem.* **57**(12) 2166-67 *[A system of phage pA1 and the host bacterium Vibrio sp. A1 was isolated from sea-water. Overnight cultivated Vibrio sp. A1, Bacillus subtilis, Bacillus megaterium, Micrococcus luteus, Arthrobacter simplex, and Escherichia coli were susceptible to the lysate, but Lactobacillus casei and Streptococcus faecalis were resistant.]*

Horikoshi K. (1996). Alkaliphiles - from an industrial point of view a review. *FEMS-Microbiol.Rev.* **18**(2-3) 259-70

Inagaki K., Terada I. and Arita M. (1995) Purification and characterization of a Ca2+-dependent neutral proteinase from *Bacillus* sp. strain JT0127 *J.Mar.Biotechnol.*. **2**(4)187-92 *[describes a protease significantly different from other Bacillus metallo proteases]*

Isolated gene encoding marine tyrosinase from *Alteromonas colwelliana* (1991) PN: WO-; WO9114698; 03.10.91

Kaerst U., Woehl M., Czempinski K., and Schmid R.D.(1994) Characterization of extracellular hydrolases from marine psychrophilic bacteria *Prog. Biotechnol.* **9**, Pt.1, 263-66 *[extracellular hydrolase e.g. protease, chitinase, glucanase, esterase, lipase, phospholipase and DNA-degrading enzyme were isolated from marine bacterium cold arctic and antarctic habitats]*

Keerthi T.R., Suresh P.V., Sabu A., Rajeev Kumar S., and Chandrasekaran M.. (1999) Extracellular production of L-glutaminase by marine *Beauveria bassiana* BTMF S10 isolated from marine sediment. *World J of Microbiol. and Biotechnol.* **15**, 751-752.*[First report on production of extracellular L-glutaminase by a marine fungi]*

Klump H., Di-Ruggiero J., Kessel M., Park J.B., Adams M.W.W. and Robb F.T.(1992) Glutamate-dehydrogenase from the hyperthermophile *Pyrococcus furiosus*: thermal denaturation and activation. *J.Biol.Chem.* **267**(31) 22681-85

Kondratieva L.M., and Vakhrusheva E.V. (1991) Biosynthesis of beta-glucanase by marine bacteria Cytophaga. Mikrobiol.Zh. **53**(1) 53-58 [effect of culture medium C-source (laminarin or pustulan), peptone, NaCl and metal ion concentration, temperature, and pH on endo-1,6-beta-D-glucanase production]

Konisky J., Michels P.C., and Clark D.S. (1995) Pressure stabilization is not a general property of thermophilic enzymes *Appl.Environ.Microbiol.* **61**(7) 2762-64. *[Adenylate-kinase, which is highly flexible and known to undergo substantial conformational changes during its catalytic cycle, is cited as an example of enzyme whose catalytic property itself impose structural constraints that are not compatible with pressure stabilization]*

Leuschner C. and Antranikian G. (1995) Heat stable enzymes from extremely thermophilic and hyperthermophilic microorganisms. *World J. of Microbiol. Biotechnol.* **11**(1) 95-114. *[An excellent review covering extracellular and intracellular enzymes of thermophilic and hyperthermophilic microbes, their properties etc]*

Manachini P.L., and Fortina M.G. (1994) Proteinase production by halophilic isolates from marine sediments. *Folia-Microbiol.* **39**(5) 378-80 *[3 Strains produced high levels of proteolytic activity only when cultured in the presence of sea-water]*

Mathur E., Scott B., Nielson K., Schoetiin W., and Cline J. (1992) Cloning, expression and characterization of a high fidelity DNA-polymerase from *Pyrococcus furiosus*. Thermophiles-Sci.Technol.; 99

Mathur E.J., Lundberg K.S., Shoemaker D.D., Nielson K.B., Mathur S.A., and Scott B.R. (1991) Characterization of a DNA-polymerase isolated from the extremely thermophilic archaebacterium, *Pyrococcus furiosus J.Cell-Biol*. **115**(3) Pt.2, 80a

Mizuno H., Suzuki T., Yamada Y., Akagawa M., and Yamasato K. (1990) Purification and properties of restriction endonuclease from *Deleya marina* IAM 14114, a marine bacterium (DmaI) *Agric.Biol.Chem.* **54**(11) 2863-67 [Isoschizomer of PvuII]

Mohapatra B.R., Sani R.K., and Banerjee U.C. (1995) Characterization of L-asparaginase from *Bacillus* sp. isolated from an intertidal marine alga (Sargassum sp.) *Lett.Appl.Microbiol*. **21**(6) 380-83

Moriguchi M., Sakai K., Tateyama R., Furuta Y., and Wakayama M. (1994) Isolation and characterization of salt-tolerant glutaminases from marine *Micrococcus luteus* K-3. *J.Ferment.Bioeng*. **77**(6) 621-25 *[Conserved sequences LA--V and V--GGT-A were observed at the glutaminase-I N-terminus, similar to that for other glutaminases.]*

Morikawa M., Izawa Y., Rashid N., Hoaki T., and Imanaka T.(1994) Purification and characterization of a thermostable thiol protease from a newly isolated hyperthermophilic *Pyrococcus* sp. *Appl.Environ.Microbiol*. **60**(12) 4559-66

N-acetylglucosamine-6-phosphate-deacetylase(1997) PN: JP-; JP09234064; 09.09.97 [N-acetylglucosamine-6-phosphate-deacetylase (EC-3.5.1.25) is produced by a marine psychrophilic Vibrio sp, isolated from sea-water at a depth of 6,000 m, and may be used for low-cost enzyme production]

Nagendra Prabhu G. and Chandrasekaran M. (1998) Use of inert supports in Solid State Fermentation- A polystyrene experiment. *In* Advances in biotechnology Ed. Ashok Pandey. Educational Publishers & Distributors, New Delhi. p 51-58

Nagendra Prabhu G. and Chandrasekaran M. (1997) Impact of process parameters on L-glutaminase production by marine *Vibrio costicola* under solid state fermentation using polystyrene as inert support. *Process.Biochem*. **32** (4) 285-289.*[First report on bioprocess optimization of solid state fermetnation production of extracellular L-glutaminase by marine bacteria using polystyrene as inert support]*

Nagendra Prabhu G. and Chandrasekaran M. (1996) L-Glutaminase production by marine *Vibrio costicola* under solid state fermentation using different substrates. *J.Mar. Biotechnol*. **4**, 176 -179*[First report on use of solid state ferementation for extracellular L-glutaminase production by a marine bacteria]*

National Sea Grant College Program(USA) - [National Sea Grant Program encourages the wise stewardship of our marine resources through research, education, outreach and technology transfer. Sea Grant is a partnership between the nation's universities and National Oceanic and Atmospheric Administration () It produces and makes available a wealth of information on marine topics]

New alkaline protease from symbiotic bacterium of shipworm (1992)PN: US-; US7880912; 15.09.92 [Purification and characterization for use in surfactant composition, silver metal recovery from photography film, beer dehazing, etc]

Nishihara H., Miyashita Y., Aoyama K., Kodama T., Igarashi Y., and Takamura Y. (1997) Characterization of an extremely thermophilic and oxygen-stable membrane-bound hydrogenase from a marine hydrogen-oxidizing bacterium *Hydrogenovibrio marinus Biochem. Biophys. Res. Commun*. **232**(3)766-70 *[A membrane-bound hydrogenase was characterized as highly oxygen-tolerant, extremely thermophilic and thermostable in its membrane-bound form]*.

Ochoa J.L., Ramirez-Orozco M., Hernandez-Saavedra D.; and Sanchez-Paz A. (1995) Halotolerant yeast *Debaryomyces hansenii* as an alternative source of Cu/Zn superoxide-dismutase (SOD) *J.Mar.Biotechnol*. **3**(1-3) 224-27 *[D. hansenii may be an excellent alternative for producing superoxide-dismutase in an economically attractive manner]*

Ohishi K., Yamagishi M., Ohta T., Suzuki M., Izumida H., Sano H., Nishijima M., and Miwa T. (1996) Purification and properties of two chitinases from Vibrio alginolyticus H-8. *J.Ferment.Bioeng*. **82**(6) 598-600 *[Purification of Chitinase C1 and C3 from Vibrio alginolyticus H-8]*

Raghukumar C., Chandramohan D., Michel Jr F.C., and Reddy C.A. (1996) Degradation of lignin and decolorization of paper mill bleach plant effluent (BPE) by marine fungi. Biotechnol. Lett., 18(1) 105-06 *[Marine fungi possessing laccase alone are able to effectively decolorize BPE and mineralize 14C-ring labeled synthetic lignin to $^{14}CO2$]*

Raghukumar C., Raghukumar S., Chinnaraj A., Chandramohan D., D'-Souza T.M., and Reddy C.A. (1994) Laccase and other lignocellulose modifying enzymes of marine fungi isolated from the coast of India., *Bot. Mar*. **37**(6) 515-23 *[Results indicate the wide distribution of laccase and other lignocellulose-modifying enzymes in some strains, except for LiP and MnP, which are less common]*

Renu. S. and Chandrasekaran M**.** 1992. Extracellular L-Glutaminase production by marine bacteria. *Biotechnology Letters*. **14**(6) 471-474.

Robinson K.A., Bartley D., Robb F.T., and Schreier H.J. (1994) A gene from the hyperthermophilic archaeum *Pyrococcus furiosus* displaying homology to prolyl endopeptidase Abstr.Gen.Meet.Am.Soc.Microbiol., 94 Meet., 297 *[Pyrococcus furiosus produces at least 13 distinct proteases, including several whose biological activity is resistant to surfactants as well as treatment at temps. in excess of 100 deg. Protease characterization by gene cloning]*

Romanenko L.A., Plisova E.Yu., and Fedosov Yu.V. (1995) Strain of Alteromonas haloplanktis B-3906 PN: RU-; RU 2026347; 10.01.95 *[Polyuridyl-endo-RNA-ase production]*

Sabu A., Keerthi T.R., Rajeev Kumar S., and Chandrasekaran M. (2000) L-glutaminase production by marine *Beauveria sp* under solid state fermentation. *Process Biochem*. **35** (7) p 705-710*[First report on use of solid state fermentation for production of extracellular L-glutaminase by a marine fungi]*

Shashirekha S., Uma L., and Subramanian G. (1997) Phenol degradation by the marine cyanobacterium Phormidium valderianum BDU 30501 J.Ind.Microbiol.Biotechnol. **19**(2) 130-33 [pollutant degradation for waste-water treatment; catechol--oxidase and laccase activity]

Shibata M., Takahashi S., Sato R., and Oda K. (1997) A novel metalloproteinase, almelysin, from a marine bacterium, *Alteromonas* sp. No. 3696: Purification and characterization. *Biosci.Biotechnol.Biochem*. **61**(40) 710-15 *[psychrostable metallo protease production in 20 l of culture medium in a fermentor]*

Southworth M.W., Kong H., Kucera R.B., Ware J., Jannasch H.W., and Perler F.B. (1996) Cloning of thermostable DNA-polymerases from hyperthermophilic marine Archaea with emphasis on Thermococcus sp. 9N-7 and mutations affecting 3'-5' exonuclease activity. *Proc.Natl.Acad.Sci.U.S.A.* **93**(11) 5281-85 *[enzyme engineering by site-directed mutagenesis and vector plasmid expression in Escherichia coli]*

Sova V.V., Elyakova L.A., Ivanova E.P., Fedosov Y.V., and Mikhailov V.V.(1994) Induced beta-1,3-glucanase from the marine bacterium *Alteromonas* sp. *Biokhimiya* **59**(9) 1369-77 *[Laminarin-induced endo-1,3-beta-D-glucanase (I) (EC-3.2.1.39) was isolated from the marine bacterium Alteromonas sp.*

Sugano Y., Matsumoto T., Kodama H. and Noma M. (1993) Cloning and sequencing of *agaA*, a unique agarase 0107 gene from a marine bacterium, *Vibrio* sp. strain JT0107 *Appl.Environ.Microbiol*. **59**(11) 3750-56

Sugano Y., Nagae H., Inagaki K., Yamamoto T., Terada I., and Yamazaki Y. (1995) Production and characteristics of some new beta-agarases from a marine bacterium, *Vibrio* sp. strain JT0107. *J. Ferment. Bioeng*. **79**(6) 549-54 *[Culture conditions for the production of agarase (EC-3.2.1.81) by Vibrio sp. strain JT0107 were optimized.]*

Sugano Y., Terada I., Arita M., Noma M. and Matsumoto T.(1993) Purification and characterization of a new agarase from a marine bacterium, *Vibrio* sp. strain JT0107 *Appl.Environ.Microbiol*. **59**(5) 1549-54 *[The novel Agarase-0107, with an optimum pH of 8, allowed the recovery of a DNA fragment from a standard agarose gel at 8-fold higher yield that those of commercial agarases]*

Suresh P.V. and Chandrasekaran M. (1999). Impact of process parameters on chitinase production by an alkalophilic marine *Beauveria bassiana* under solid state fermentation. *Process Biochem*. **34**,257-267

Suresh P.V. and Chandrasekaran M. (1998)Utilization of prawn waste for chitinase production by marine *Beauveria bassiana* under solid state fermentation. *World J. Microbiol. Biotechnol*. **14**(5), 655-660

Suzuki T., Sugimoto E., Tahara Y., and Yamada Y. (1996) Cloning and nucleotide sequencing of the AgeI methylase gene from *Agrobacterium gelatinovorum* IAM 12617, a marine bacterium *Biosci.Biotechnol.Biochem*. **60**(3) 444-47

Svitil A.L., Chadhain S.M.N., Moore J.A., and Kirchman D.L. (1997) Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of *chitin Appl. Environ. Microbiol*. **63**(2) 408-13*[marine bacterium appeared to synthesize separate chitinases for the efficient utilization of different forms of chitin and chitin by-products]*

Tamaru Y., Araki T., Morishita T., Kimura T., Sakka K. and Ohmiya K. (1997) Cloning, DNA sequencing, and expression of the beta-1,4-mannanase gene from a marine bacterium, *Vibrio* sp. strain MA-138. *J.Ferment.Bioeng*. **83**(2) 201-05 *[recombinant enzyme purification, characterization and expression in Escherichia coli]*

Tsugawa W., Horiuchi S. Tanaka M., Wake H., and Sode K. (1996) Purification of a marine bacterial glucose-dehydrogenase from *Cytophaga marinoflava* and its application to measurement of 1,5-anhydro-D-glucitol *Appl.Biochem.Biotechnol*. **56**(3) 301-10

Tsujibo H., Fujimoto K., Kimura Y., Miyamoto K., Imada C., Okami Y., and Inamori Y. (1995) Purification and characterization of beta-N-acetylglucosaminidase from *Alteromonas* sp. *Biosci.Biotechnol.Biochem*. **59**(6) 1135-36

Tsujibo H., Yoshida Y., Miyamoto K., Imada C., Okami Y., and Inamori Y.(1992) Purification, properties, and partial amino acid sequence of chitinase from a marine *Alteromonas* sp. strain O-7 *Can. J. Microbiol*. **38**(9) 891-97

Ventosa A., and Nieto J.J. (1995) Biotechnological applications and potentials of halophilic microorganisms. World J. of Microbiol. Biotechnol. **11**(1) 85-94. [Review on several present or potential applications of halaophiles, including production of polymers, enzymes etc and use of these in industrial and biotechnological applications]

Vetter Y.A., Deming J.W., Jumars P.A., and Krieger-Brockett B.B. (1998) Microbial foraging by means of freely released extracellular enzymes: consequences of diffusive solute transport. *Microb. Ecol.* **36**(1), 75-92. *[A modelling approach to analyse the costs and benefits of individual immobile microbe by releasing extracellular enzymes into its environment]*

Vilter H. (1986) Alginate-lyase from *Alteromonas* sp. strain KL1a: a useful aid for isolation of enzymes and other biopolymers from brown algae. *Planta-Med.* **5**, 417

Wachi Y., Burgess J.G., Takahashi J., Nakamura N., and Matsunaga T. (1996) Production of superoxide-dismutase by marine cyanobacteria *J.Mar.Biotechnol*. 3(4) 258-61 *[enzyme preparation from Synechococcus sp. and culture conditions optimization]*.

Wakayama M., Nagano Y., Renu N., Kawamura T., Sakai K., and Moriguchi M.(1996) Molecular cloning and determination of the nucleotide sequence of a gene encoding salt-tolerant glutaminase from Micrococcus luteus K-3. *J.Ferment.Bioeng*. **82**, (6) 592-97 *[First report on cloning of the gene encoding salt-tolerant glutaminase-I from marine Micrococcus luteus K-3 in Escherichia coli JM109]*.

Weiner R.M., Colwell R.R., Bonar D.B., Coon S.L., and Walsh M. (1995) Tyrosinase production by culturing melanin-synthesizing marine bacterium PN: US-; US 5451515; 19.09.95

Yamada Y., Mizuno H., Sato H., Akagawa M.., and Yamasato K.(1989) A new restriction endonuclease from '*Agrobacterium gelatinovorum*', a marine agrobacterium (AgeI) *Agric.Biol.Chem.* **53**, (60) 1747-49 *[Purification and characterization of restriction endonuclease AgeI]*

Biographical Sketches

Dr. M. Chandrasekaran is professor of Biotechnology, in the Department of Biotechnology, Cochin University of Science and Technology. He graduated in marine biology from Annamalai University and took his PhD in marine microbiology from the Cochin University of Science and Technology in 1985. He is a career awardee in microbiology, of the University Grants Commission, Government of India. He was an overseas associate of the Department of Biotechnology, Govt. of India during 1997-98 at Hiroshima Universtity, Department of Fermentation Technology. He has 23 years of research experience in the field

of marine microbiology. He has been working on marine microbial enzymes since 1988 and has contributed significantly to the growth of marine microbiology, through several publications in peer reviewed international journals. He is one among the pioneers who have introduced bioprocess technologies particularly solid state fermentation for the production of marine microbial enzymes.

He has a total of 95 publications including presentations in symposia/seminars. He had supervised 15 doctoral theses, of which 10 theses were on microbial enzymes. He is also the founder head of the Department of Biotechnology of the Cochin University of Science and Technology, which is offering masters degree program in Biotechnology. He has served as subject expert in several academic /assessment and selection committees at various levels. He has also served as referee for reviewing of papers for several international journals. He is a consultant several industries for microbial bioprocesses. He founded the society for Biotechnologists of India in the year 1995. His current research interest includes marine microbial enzymes, extremozymes, strain improvement and enzyme engineering.

S Rajeev Kumar is a research associate working with Prof. M. Chandrasekaran. He took his masters degree in Biotechnology from Cochin University of Science and Technology, Cochin, India and is submitting his doctoral thesis on marine bacterial enzymes. He has been specializing in use of immobilized whole cell processes for production of marine bacterial enzyme. He has over five years of research experience in the field of marine microbial enzymes.