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Marine Enzymes Production Tools to the Pharmaceutical Industry

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Marine environment contains organisms that make it a profitable natural reservoir, having a tremendous potential to produce functional bio-catalysis, such as amylase, lipase, protease, curtains etc. The enzymes isolated from marine organisms, especially extremophiles, are distinguished by their habitat-related features through bioprospecting processes. These novel features include barophilicity, cold adaptively, salt tolerance and hyperthermo-stability which can alter industrial processes to facilitate mass transfer, energy savings, cost reduction, etc. . This review gives details about the marine enzymes and their, historical discovery followed by isolation processes, introducing seven special marine enzymes with emphasis on their potential applications in chemical, food and pharmaceutical industry.

[Keywords: Marine enzyme invention; *In vitro* studies; Amylase, Lipase; Protease; Chitinase; Pharmaceutical industry]

Introduction

Ocean contains a huge variety of marine organisms which produce enzymes and bioactive metabolites that are remarkably applicable in a variety of industrial products, such as pharmaceutical and food products and also in the renewable processes to obtain raw materials. Marine material is also useful in the production of food, fertilizers, animal feed additives, cosmetics and medicines, etc. This review focuses on the marine organisms and their components useful as tools for enzyme production and their application in terms of food, feed, and medical and industrial products. More than 30000 marine species provide new tools for advancement in biomaterials, health care diagnostics, aquaculture and seafood safety, bioremediation, biofilms, and bio-corrosion. . Marine seashore species have a high potential in progressive areas, such as healthcare, food, feed, aquaculture, and industrial products especially cosmetics and phycocolloids. However, there are several limitations in terms of conserving sustained biomass of organisms for research and operations as well as providing a proper condition for the culture of marine organisms in the laboratory. Due to the competition amongst organisms for space and nutrients in the marine and terrestrial environments, as a selective force, a complex network of reactions has led to evolving organisms and microorganisms to produce diverse enzyme systems. Marine organisms that have been studied include: Shrimps, oysters, abalone, sea cucumber, fish, algae or seaweeds, archaea, actinomycetes, and fungi. These have demonstrated several applications in aquaculture, pharmaceuticals, nutraceuticals, cosmeceuticals, and biomaterials[1] .

Extraction of marine enzymes, chitosan, carotenoids, polysaccharides, omega-3 fatty acids and phenols from oils of fish, shellfish waste and algae can be done through traditional methods (solvents, pressing), subcritical fluid extraction (pressurized hot water, pressurized ethanol), and supercritical fluid extraction (CO_2). In a biological system, the enzyme is completely soluble and most of the time, it is linked to the membrane. There are two kinds of enzymes, those existing inside the cell are intracellular and those present outside the cell are known as extracellular. Extracellular enzymes are usually powerful and endure changeable conditions compared to intracellular ones that have more stable situations. Extracellular enzymes may exist in immobilized form, but maintain their catalytic activity for thousands of years, for example, phosphatase enzyme in soil. Artificially immobilized enzymes, which are held in their place throughout the reactions, are utilized for various targets from detoxifying environmental

pollutants to industrial biocatalysis. For as much as all enzymes have optimal activity at a different value of salt concentration, pH, and temperature, they are effective to carry on appropriate reaction between the substrate and stereochemical asset of catalysis. The enzymes involved in catalytic chemical reactions in living things are responsible for stability or changing. For instance, animal cells may contain up to 4000 different enzymes responsible for enhancement of their compatibility or differentiation happening in the cell[2].

Microalgae and seaweeds are remarkable organisms used in enzyme production, containing several useful compounds, especially polysaccharides with high molecular weight . Seaweeds are classified as Chlorophyta (green seaweeds), Rhodophyta (red seaweeds) and Phaeophyta (brown seaweeds). The annual global value of seaweeds from the US is reported as US 5.5-6 billion . The cultivation of seaweeds has been successfully done in 35 countries, including China, Japan, Korea, Philippines, Indonesia, and Chile. A bioactive compound from microalgae is a nutraceutical; for example, spirulina (blue-green algae) by way of enzymatic hydrolysates activity helps to increase skin metabolism and interrupt or stop keratinization. This characterization is good for ulcers, burns, and eczema. Of course, the presence of chlorophyll, carotenoids, and vitamin B enhances the enzymatic activity. In fact, algae contains numerous bioactive compounds, such as enzymes, growth substances, antibiotics, and toxic compounds which could be helpful to improve the physiological, biochemical and molecular strategies to cope with stress. Therefore, algae are capable of synthesizing in a variety of bioactive conditions[3].

The ocean covers 71 % of the earth's surface 61 % of the northern hemisphere and 80 % of the southern hemisphere is covered with the ocean. This vast area contains many strange and wonderful creatures, including microbes and nearly 300,000 described species that have been detected by high throughput DNA sequencing and through computational genomics processes[4]. In the marine environment, extracellular enzymes recycle organic carbon and nitrogen compounds. High molecular weight organic compounds (chitin) cannot be transported directly into bacteria or other host organisms which live in symbiosis with bacteria. The marine extracellular enzyme helps the bacteria to hydrolyze and convert the organic polymer to smaller molecules to facilitate their metabolism. By this feature, these find their application in agriculture and pharmaceutical industries[2,5]. Chitin is abundant in the sea, degraded by the actinomycetes which decompose the recalcitrant organic materials. Actinomycetes account for approximately 7000 compounds reported in the Dictionary of Natural Products Production, which is about half of the discovered bioactive metabolites[6], including antibiotics, antitumor agents, immunosuppressive agents, and enzymes[7], that inhibit cancer cell growth[8]. *Pseudoalteromonas* sp. Is a marine bacteria and the best producer of intracellular and cold-adapted β-galactosidase. It can be applied for the production of lactose-free milk-derived foods for individuals with lactose intolerance (approximately 30 % of the world population)[9].

Marine fungi grows in wood, sediments, mud, soil, algae, corals, calcareous tubes of mollusks, decaying leaves of mangroves, intertidal grasses and living animals, and guts of crustaceans. It has main role to decompose the woody, herbaceous substate, dead animal and digest lignocellose. For example, *Aspergillus niger* contains xylanases (a thermostable enzyme active at alkaline pH) which could be used in bio-bleaching to degrade residual lignin from wood pulp in the making of paper[10]. Marine sponges are one of the enzyme production sources as well as bioactive compounds like antiviral, anti-inflammatory, antitumor antimalarial, neuro-suppressive and a muscle relaxant[11]. Many enzymes are extracted from sponges: Gastric proteases from aspartic proteases (pepsin, pepsinogen, chymosin and gastricsin), intestinal proteinases from serine proteases (trypsin, chymotrypsin, collagenases, and elastases) and chitinolytic enzyme (chitinases and lysozyme), transglutaminases and lipases. This review describes the recent advances in cultivation and isolation of marine enzymes and their applications through marine biotechnology, with a focus on saccharolytic enzymes (amylases, cellulases, and chitinases), proteolytic enzymes (proteases), and some other enzymes (lipases, peroxidase, and pectinase). These enzymes have a different production applications, including food, feed, medicine preparation and industrial goods such as detergent, paper, and textile industries[12].

Marine Enzyme Invention

To enhance the stability of enzymes extracted from a marine organism and apply them into industrial development processes, the knowledge of about optimum pH and temperature is very crucial. The thermopile marine micro-organisms contain:

Phototrophic bacteria domains (cyanobacteria, purple and green bacteria); bacteria domains (gram positive bacteria which lives in both terrestrial and aquatic domains ; aerobes and anaerobes gram-positive spore-forming bacilli (*Bacillus* sp., *Clostridium* sp.); sulfate reducing bacteria (*Desulfotomaculum* sp., *Thermos* sp.); gram negative and beta-proteobacteria (*Thiobacillus* sp.),; fermenting bacteria (spirochetes and numerous other related genres); and the archaea domains, including *Pyrococcus* sp. which its optimal growth temperature is 100 °C, *Sulfolobus* sp. which its optimal growth is happening in pH 2-3 and belongs to the volcanic springs area with temperatures of 75-80 °C[13], *Thermococcus* sp. (thermophilic and hyperthermophilic archaea), *Thermoplasma* sp. (standing acidic and high-temperature environments) and methanogenic archaea[14]. Polychaeta belongs to the segmented marine worms and is a food source to other organisms. The specified marine enzyme sources, their preparation method and application in various fields are explained in Figure 1. In many studies, the marine enzymes are used as catalyst in industrial biotransformation and have potential application in the marine pharmaceutical product development. During the transfer of organisms from their native habitat to laboratory environment, several difficulties are faced with regard to the revival of enzyme activities and cultivation system in contrast to a natural system which should be considered.

History of the enzyme dates back to the 17th century with the yeast-catalyzed transformation of juices into alcoholic beverages. After a long time, around 100 years later, *A. Lavoisier* in 1789 explained that sugar gets converted in to carbon dioxide and alcohol. Since then, several extended applications of enzymes have been found ranging from industrial production of alcohols and drugs to chemical warfare[5]. Earlier, marine biotechnology scientists

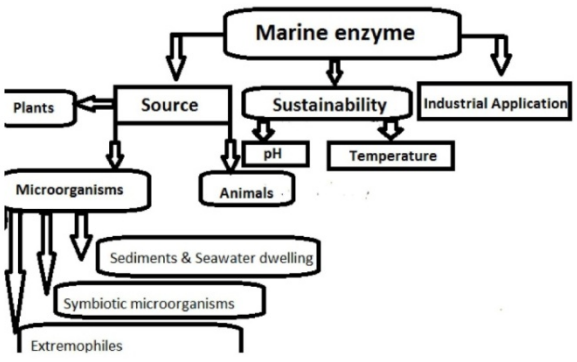

Fig. 1 — Marine enzyme source, Preparation and Application in different field

invented and identified bioactive compounds from marine-based micro-and macro-organisms. For the first time, the sponge *Cryptotethya crypta* was studied by Bergmann as a step toward of production of artificial nucleosides, such as ara-A (arabinofuranosyl adenine**)** or vidarabine, an active compound against herpes simplex and ara-C (arabinofuranosylcytidine) or cytarabinea, a chemotherapy agent effective in treatment of cancers related to leukocytes such as acute myeloid leukemia (AML) and non-Hodgkin lymphoma. peptide Ziconotide (pain killer especially severe chronic pain) is the first drug isolated , from a marine cone snail, *Conus magus*. This medicine has been used for spinal cord injury to control chronic pain, under the trade name *Prialt* [6]. A second drug, Trabectedin known as ET-743 was isolated from *Ecteinascidia turbinata* (a sea squirt), accepted by the European Union for the treatment of soft-tissue sarcoma[15].

Cultivation Methods and Enzyme Preparation

The first step in the supply of marine enzyme and enumeration of bacteria or sedentary organisms is the cultivation. The phototrophic microorganisms like cyanobacteria and microalgae under study are primarily found within the zones affected by sunlight. One important point in a study about cultivation of a marine organism is a situation of transfer from its natural habitat to laboratory and reproduction in new conditions. In addition, nutrient conditions for development of marine organisms are different and related to type of situations of their habitat; for example, bacteria or fungi originated from the water cylindrical column showed different nutrient demands to support growth and production. A nutrient media with significant carbon substrate is suitable for production of a marine strain. Through dilution technique, a putative number of cells from the sample is diluted in the medium culture or inoculated into decontaminated seawater for study during definite time. To produce a special product from marine bacteria, related nutrient is added to their culture media . The primary medium culture that was created for the growth of marine organisms is Marine Broth 2216. Its formulation was invented by *Zobell* in 1941[16]. It was used demonstrate that nutritional needs and adaptation of distinct sedentary microorganism require dilution. Iron salt (0:1 % iron-citrate) is one of the important ingredients. For this medium culture, organisms are finely washed and acclimatized uniformly in decontaminated 50 % [v/v] aged

seawater. Then a serial dilution of the sample is prepared and 0.05 ml part of the dilution is put over ZOBELL's marine (ZM) agar 2216E plates. For optimum results, the medium culture is required to be included at room temperature, (28-30 °C) for approximately 9-10 days. Thereafter, counting is done based on each recognizable morphological characteristic. The isolation and preservation of the samples is done through three-time culturing of each distinct bacterial colony on ZM agar. The absolute and pure bacterial cultures thus obtained are then preserved and protected on ZM agar slants at 4 °C under mineral oil. The identification tests that help to generate pure culture are done via microscopic screening and a series of biochemical evaluations. After the identification of bacteria numbers, wet weight per g is measured, which is used to gain a total viable number of each bacterial clone. One of the ways for harvesting the separated bacteria is centrifugation (5500 g, 30 min) at low temperature like 4 °C. The cell suspension is in 10 mmol/l Tris-HCl buffer (pH 7.2). Cell disruption is done through freezing and thawing and continued by grinding with glass powdered in an ice bath. After another centrifugation (5500 g, 30 min), enzyme preparation starts, using the supernatant fluid. In the next level, after providing the metagenomics library, cloning in the host cell (for example, *E.coli)* helps in the extraction of bioactive metabolites and enzymes through sequence and functional based screening[17].

Marine Enzyme Applications

Polysaccharide-degrading enzymes

The polysaccharolytic enzymes find application in several stages of the process of food, increasing the efficiency of detergents, and bleaching of paper and fabrics. Furthermore, their intensive role in the production of renewable energy and in the management of soil, water, and air pollution cannot be ignored. In food industry, cellulase and amylase is generally used in the baking process to avoid the use of artificial sweeteners. These two enzymes are also used in brewing industries. Moreover, amylase is used in several laundry detergent compositions. There are several studies related to plant-sourced biofuel degradation, involving cellulolytic and lipolytic enzyme application to convert complex polysaccharides in plant biomass into sugar with fast fermentation for bio-ethanol production. Hemi-cellulose, cellulose, lignocellulose and xylene are polysaccharides that make the compositions of the plants, bio-waste, and some industrial waste. In many cases, marine enzymes, by degradation of these kinds of materials, help scientist to take a big step toward dealing with one aspect of pollution problem. The marine habitat is one of the natural sources of enzymes found in microorganisms, fungi, seaweeds or animals. However, extremophiles and symbiotic microorganisms in particular have introduced a great spectrum of the useful marine enzymes. Besides, fish, prawns, crabs, snakes, plants and algae have also displayed abundant sources of enzyme biodiversity[18].

Amylase

Amylase is a digestive enzyme that catalyzes the hydrolysis of starch molecules and converts them into polymers composed of glucose units (sugars). There are adaptable and functional amylases extracted from marine organisms. From 269 thermophilic enzymes isolated from deep-sea hydrothermal vents, Lignin and colleagues screened 70 microorganisms for amylase production. Genus *Thermococcus* is rich in thermostable enzymes. Deep-sea sediments of Antarctica contain psychrophilic bacteria generating amylase. Out of three *Pseudomonas* strains isolated from deep-sea sediments of Prydz Bay, Antarctica, two strains, 7193 and 7197, showed the highest amylolytic activity at 40 °C and pH 9.0 and maintained 50% activity at 5 °C [19]. Among these assemblage bacteria, a variety contains low molecular weight compound which is biologically active. Molecular phylogeny studies revealed that bacterial strains of *Pseudomonas, Rhodococcus* and *Nocardiopsis* are helpful in the production of amylase[20]. In the past decades, a small number of enzymes were discovered from bacteria and fungi living in symbiosis with marine sponges and algae, including acetylcholinesterase, amylase, urethanase, cellulase, alginate, and pectinlyases[21]. In 2003, it was reported that the Gram-positive bacteria is mainly found in all six marine sponges, except for *Azorica sp* (Table 1). In contrast, Gram-negative bacteria was detected majority in soft coral that cause of their different productivity. Fifty-six bacteria discovered and separated from the samples were evaluated for their potency to produce amylase, carboxymethylcellulose and protease (Table 2).

In marine environment, the extracellular enzymes production for hydrolysis of tenacious macromolecules occurs in free-living bacteria in contrast to the enzymes from those bacteria which

live symbiont and attached to the sedentary marine organisms where they are forcefully restricted to their cells. The behavior of cells of attached bacteria is slow secretion and continual discharge of enzyme in to the environment. Therefore, they are major contributors in wastewater treatment processes. Being attached to the membrane of the bacterial cell has several privileges in the conversion of the waste to the reusable material, such as prevention of losing the enzyme in discarded liquid after settling and activated sludge plants system. In another research on the potential of *Bacillus aquimaris MKSC 6.2*., it was this bacterium attached to a soft coral *Sinularia* sp. has shown an ability to degrade raw corn, rice, sago, cassava, and potato starches with adsorption percentage in the range of 65-93 %. Moreover, marine fish pathogens *Aeromonassal monicida*, *Photobacterium profundum*, *Vibrio splendidus* and *Hahellache juensis* all contain glycosidase which under genome sequencing of marine bacteria make a subfamily of glycoside hydrolyze GH13 closely related to the α-amylase[22].

Table 1. — The Enzyme production from marine organisms:-

Name	Class	Order	Family
Aaptos s.	Demospongiae	Hadromerida	Tethyidae
Spirastrella sp.	Demospongiae	Hadromerida	Spirastrellidae
Ircinia sp.	Demospongiae	Dictyoceratida	Spongiidae
Phyllospongia sp.	Demospongiae	Dictyoceratida	Spongiidae
Axinella sp.	Demospongiae	Axinellida	Axinellidae
Azorica sp.	Demospongiae	Lithistida	Siphonidiidae
Lobophytum sp. (coral)	Anthozoa	Alcyonacea	Alcyoniidae
Sargassum sp. (algae)	Phaeophyceae	Fucales	Sargassaceae

Table 2. — The list of sponge used in Enzyme production

Name	Class	Order	Family
Aaptos s.	Demospongiae	Hadromerida	Tethyidae
Spirastrella sp.	Demospongiae	Hadromerida	Spirastrellidae
Ircinia sp.	Demospongiae	Dictyoceratida	Spongiidae
Phyllospongia sp.	Demospongiae	Dictyoceratida	Spongiidae
Axinella sp.	Demospongiae	Axinellida	Axinellidae
Azorica sp.	Demospongiae	Lithistida	Siphonidiidae
Lobophytum sp. (Coral)	Anthozoa	Alcyonacea	Alcyoniidae
Sargassum sp. (Algae)	Phaeophyceae	Fucales	Sargassaceae

Production and activity of α-amylase investigated in *Bacillus subtilis JS-2004* strain showed that potato starch is the main nutrient. The optimum activity of ά amylase is reported at 70 °C and pH 8.0. The stability of enzyme maintained for 1 h at 60-70 °C and increased in optimal conditions. Therefore, the marine enzyme is appropriate for use in food and starch processing. In contrast, there is another type of bacillus active in lower temperature as well. A hard coral, *Acropora sp.* contain the associated bacterium, known as *Bacillus amyloliquefaciens ABBD,* which produce ά amylase by its capability of degradation of raw starch, collected from Bandengan Water, Jepara, North Java, Indonesia. This enzyme showed the ability to degrade various raw starch granules from corn, rice, cassava and sago at room temperature[23]. Two thermo-stable alkaliphilic amylases isolated from *Streptomyces sp. D1,* a marine microbe, displayed extreme constancy while it was exposed to the surfactants and chemical detergents. This enzyme causes a maltose-foaming system increasing the volume of bread in bread baking process and retained bread softness for a long time[24]. In Nicobar Island, *Halobacillus pumilus* was examined for amylase enzyme activity of bacteria harvested in different temperatures (25 to 45°C), pH (6 to 8), spectrum of carbon sources (starch, glucose, sucrose, fructose, xylose and lactose), different organic nitrogen sources (yeast extract, meat extract, beef extract, nutrient broth, urea and casein) and varying concentration of NaCl (Table 3). Maximum enzyme activity was obtained at 40 ºC, pH 8.0 and 1.0 % of substrate concentration which has several industrial applications because of its relative heat-sensitive and moderately alkalophilic derivatives[25].

Cellulase

Cellulolytic enzymes catalyze the cellulose and its derivatives such as hemicellulose, lichenin, and cereal. β-D-glucans is most of the time found in marine organisms, fungi, bacteria, and protozoans. A *Saccharophagus degradans* is a Gram-negative marine bacterium helpful to digest several types of complex polysaccharides, such as cell wall polymers, as an energy source from spineless animals, plant, and

Table 3 — Chitinase production parameters and its Industrial Applications

Marine Enzyme	Source	Optimum pH	Optimum Temperature	Industrial Applications
Chitinase C	*Streptomyces sp.*	8.0	50ºC	Antifungal activity
Chitinase B	*Alteromonas sp.*	6.0	30ºC	Cold resistance enzyme
Chitinase	*Beauveria bassiana*	9.2	-	Marine industry
Chitinase	*Beauveria bassiana BTMF S10*	9.5	-	Shellfish processing industry

algae sources. They have degraded parts including xylanase, arabinoxylanase, β-mannanase, β-1,3-glucanase, and pectinase[26]. In another experiment performed by Suvorov and his group, derivatives from *Saccharophagus degradans* have shown degradation of whole plant material by carbohydrases followed by release of sugars. The fuel-producing organism converts cellulosic biomass directly into the fuel . Nowadays, production of some advanced biofuels is doing by insufficiencies, but they are still under improvement process. *S. degradans* is an ideal and suitable organism for this enhancement[27]. A strain of marine bacterium *Bacillus aquimaris* secretes extracellular marine cellulase which is stable in alkaline environment and is an organic solvent-tolerant bacteria that helps to decontaminate the organic pollutants. Nitin Trivedi and his co-workers isolated 19 marine bacteria for experimentation to obtain their solvent tolerance feature at 10 % concentration. Out of these, only one exhibited significant tolerance and displayed a relative growth yield of 86 % for acetone, 71 % for methanol, 52 % for benzene, 35 % for heptane, 24 % for toluene and 19 % for ethyl acetate. Optimum enzyme activity at pH 11 and 45 ºC was reported in this experiment. Thus, the enzyme produced by *B. aquimaris* with its organic solvent tolerance and stability in alkaline environment can be a useful substance in industrial processes involving biphasic organic-aqueous fermentation and bioremediation of saturated hydrocarbons. Moreover, it can be utilized as a decomposer of industrial and agricultural wastes[28]. An interesting research was accomplished in 2014 to evaluate a microalgal pretreatment method using cellulolytic bacteria that naturally degrades microalgae *Botryococcus braunii and Nannochloropsis gaditana* in their native habitat. Bacterial strains were obtained from two marine mollusk species. Bacteria belonged to the genera *Aeromonas, Pseudomonas, Chryseobacterium,* and *Raoultella* with endoglucanase activity and whole cell wall degradation. The result showed that because of the low-temperature environment that the bacteria were isolated at 30 °C, energy costs were lower than with the commercial enzymes that are available with maximal activity over a temperature range of 50 to 55 °C [29]. Kalaiselvi and her co-workers conducted a study on biofuel production activity of marine microbes and marine yeast such as *Klebsiella ozeanae* and *Pseudomonas aeruginosa* on the wood powder which are cheaper sources of agricultural residues such as corn stalk, paddy straw, ragi stalk, millet stalk and sugarcane stalk. The results showed an extended area of lyses. Therefore, it can be concluded that cellulose decomposing marine bacteria play a serious role in mineralizing organic matter also influencing the productivity of the marine environment[30] (Table 4).

Chitinases

Chitin comprises a majority of the biopolymer in the cell walls of fungi, the rigid external body cover of arthropods such as shrimps, lobsters, crabs, mollusks (crustaceans) and insects as well as hard protective internal case of cephalopods, such as squids and octopuses which are almost resistant to degradation. Most of these organisms belong to the marine ecosystem and for as much as chitin cannot be digested by animals, chitin hydrolytic enzymes (chitinase) is expected to be plentiful in the marine environment. Chitinivorous contains bacteria species of *Aeromonads, Bacillus, Vibrio*, which play a pathogenic role in attacking living arthropods, zooplankton or fungi or degrading the remains of these organisms. In the field of pest management and

Table 4. — Protease production parameters and Industrial Applications

Marine enzyme	Source	Optimum pH	Optimum Temperature	Industrial Applications
Alkaline protease	Alcaligenesfaecalis	9.0	55ºC	Detergent Industry
Chitin-binding protease	Alteromonas sp. Strain O-7	11.5	35ºC	Promote chitinase activity
Extracellular Serine Protease	*Pseudoalteromonas sp. strain A28*	8.8	30ºC	Algicidal activity, kill the diatom Skeletonema costatum strain NIES-324
Protease 1	*Bacillus sp.*	11-12	50ºC	Surfactant, bleaching and detergents
Protease 2	*Bacillus sp.*	11-12	55ºC	Surfactant, bleaching and detergents
Serine protease	*Bacillus clausii*	11.5	80ºC	Oxidative reactions, SDS stable
Protease	*B. Mojavensis*	8.5	60ºC	Solid and liquid detergents
Protease	*B. licheniformis*	9.0	70ºC	Halotolerant activity
Protease	*Engyodontium album*	11.0	60ºC	Detergent industry
Protease	*Marine proteobacteria*	9.0	30-70ºC	Food processing. Leather industry,

control and also in formulation and construction of medicine, marine chitinase can be a useful inter mediate. Infectious fungi, protozoa such as plasmodium causing malaria and helminths (parasitic worms, especially intestinal worms) have a cell wall made of chitin and chitin-related material and microbial chitinase enzymes are able to hydrolyze them. Therefore, treatment of these microbial infections using this enzyme is promising. For the first time, a study was done on sponge-associated microbial chitinase in 2008 by Han. Chitinase extracted from *Streptomyces sp.* having optimal pH, temperature, and salinity in 8.0, 50 °C, and 45% psu (power supply unit) respectively, was studied for its antifungal activity opposed to *Candida albicans* and *Aspergillus niger.* This bacteria is found in symbiont with *Craniella australiensis* which is a sponge from South China. These bacteria showed that the chitinase belongs to ChiC type with antifungal activities . The chitinase activity increased by Mn^{2+}, Cu^{2+}, and Mg^{2+}, while strongly inhibited by Fe^{2+} and Ba^{2+}. The symbiotic life of this sponge by chitinase is may be because of chitin degradation and antifungal defense[31]. From *Alteromonas sp.* strain O-7, a marine enzyme in the name of chitinase B (ChiB) was isolated in optimum pH 6.0 and temperature at 30 °C, which was found to have activity even in lower temperature close to 0 °C in contrast to mesophilic chitinases ChiA and ChiC. Therefore, ChiB easily does digestion and extraction of nutrients from chitin material to survive in cold environment. In a study, *Beauveria bassiana* a marine fungus found from marine sediments showed an alkalophilic chitinase by 9.20 in pH isolated by solid-state fermentation utilizing wheat bran. The solid-state fermentation produced chitinase on industrial scale from prawn waste in shellfish processing from marine fungus *Beauveria bassiana* BTMF S10. Optimize yield of this product occurred at 27 ºC and initial pH 9.5[27] (Table 5).

Pectinase

Pectinase has application in the industrial processing of fruit juices because of its ability to break down pectin. Pectic enzymes include pectolyase, pectozyme and polygalacturonase. Polygalacturonase is a pectinase involved in degradation of plant materials and speeding up the extraction of fruit juice for fruit and wine production. In brewing, pectinase works in two steps: First, it helps break down the fruit followed by extraction of flavors from the mash and secondly, it provides clear wine without haze due to degradation of pectin. *Aspergillus niger is* one of the good sources for pectinase, but there are some marine sources as well. A study was conducted to determine the efficacy of pectinase in *Bacillus subtilis*. Pectinase extracted from these bacteria was evaluated for eliminating dirt and pollution from cotton fiber to improve its hydrophilicity for further wet processes. One of the most economical approaches to produce pectinase is the application of *Citrus limetta* peel powder, as it contains pectin substrate. Pectinase showed it effectively with a dose of 10 % (2.8 IU/g of the fabric). The suitable condition for such production is pH 7.0, 60 °C for 120 min. The characteristic of pectinase as a scour agent helps to conserve the structure of fibers and avoid degradation as marked from elastic stability[32]. In another study, , production of extracellular pectinase from *Bacillus subtilis* in presence of pectin-rich *Citrus limetta* in submerged fermentation (SmF) was evaluated. The marine *Bacillus subtilis* was collected from sediment sample from Chinchani beach, India. The maximum enzyme activity, in medium containing *citrus limetta* peels, is pH 5.0 at 40 °C and maximum production of this enzyme is in the the late exponential phase of the growth[32].

Peroxidase

Peroxidases are enzymes with large and complex molecules and complicated shapes involving multiple folds. They act as catalysts in biological and non-biological processes. For many of peroxidase enzyme groups, the optimal substrate is hydrogen peroxide (H_2O_2), which after reduction, forms harmless substrates such as water. Peroxidases are found in humans, animals, plants and marine organisms. They increase a plant's defense against pathogens. Glutathione peroxidase found in the cytoplasm of

Table 5. — Lipase production parameters and Industrial Applications

Marine enzyme	Source	Optimum pH	Optimum temperature	Industrial application
Lysophospholipase	*Pyrococcusfuriosus*	-	85ºC	Novel drug intermediates
Lypase	*EhV–86*	-	-	-
Psychrophilic alkaline lipase	*Pseudomonas sp. (MSIo57)*	9.0	37ºC	-

nearly all mammals, uses glutathione as a donor to protect the organism from oxidative damages in humans and mammals. Peroxidase enzymes are used as histological markers in medical laboratories as well as in waste water treatment for industrial purposes. Phenols as important pollutants can be oxidized to phenoxy radicals by the enzymatic activity of horseradish peroxidase (extracted from horseradish's root). Phenoxy radicals are of less toxic than phenol. The application of peroxidase is under investigation; for example, in the probable use of this enzyme in many manufacturing processes like adhesives, computer chips, carparts, and linings of drums and cans and also polymerization of anilines and phenols in organic solvent matrices. Peroxidase enzyme is in the marine world and extraction of this enzyme and its derivatives has several applications in the industrial field. Four marine invertebrates, namely, a blue mussel, *Mytilus edulis* which is physiologically adapted to use marine plant material; a scallop, *Pectenmaximus* belonging to the family of pectinoidae same as *M. edulis* feed on plant material; a crab, *Carcinus maenas* which feeds both animals and plants; and a starfish, *Asterias rubens* which only feeds on flesh have been studied to understand the function of selenium-dependent glutathione peroxidase (Se-GPX) and antioxidant enzyme. This enzyme was generally highest in the digestive gland, hepatopancreas or pyloric caeca of each species (digestive detoxication tissues) to produce oxiradial. Through this research, it was found that exogenous sources of oxyradical production are important in determining levels of antioxidant defenses. Another investigation on erythrocytes and liver of freshwater and marine fish species with a view to evaluate activities of superoxide dismutase, catalase, and peroxidase in the creature by Wdzieczak and his group indicated that these antioxidative defense enzymes are responsible for interspecies differences and also seasonal variations. The results showed that peroxidase activities at the high level in freshwater and marine source fish erythrocytes caused protection of polyunsaturated acids against uncontrolled oxidative processes. A very unusual peroxidase enzyme (dehalogenation peroxidases) was detected interebellid polychaete *Amphitrite ornata* produces. Dehaloperoxidase, DHP I, contains amino acids aspartic acid (+ asparagine) and glutamic acid (+ glutamine) as a heme enzyme. This enzyme performs its effect by converting gtrihalogenated phenols, such as 2,4,6–tribromophenol, into dihalgenatedquinones with optimum pH 5.0. DHP I also oxidizes bromo-, chloro-, and fluoro-phenols. In 2009, production of manganese peroxidase, lignin peroxidase and laccase was done from three marine fungi, viz., *Aspergilluss clerotiorum* CBMAI 849, *Cladosporium cladosporioides* CBMAI 857, and *Mucor racemosus* CBMAI 847. These fungi produced a high value of this enzyme when they were cultured in malt extract. Statistical analysis showed that under 12.5 and 23% (w/v) salinity, the manganese peroxidase production increased. All these results concluded these fungi have potential applications for industrial applications including bioremediation of contaminated sites with high salt concentrations[33]. For the first time, a research was done on marine fungi and their effect on BPE (bleach plant effluent) decolonization or lignin degradation. Raghu Kumar and colleagues reported the ability of three marine fungi to produce the lignin modifying enzymes known as laccase, manganese peroxidase (MNP) and lignin peroxidase (LIP)[10].

Proteolytic enzyme

Protease

Proteolytic enzymes catabolize proteins by hydrolysis of the peptide bonds in the construction of polypeptides. The use of proteinase in industry is very common since they cover 60% of the global enzyme market. They are involved in all organisms, even in viruses, to break down a complete peptide in to amino acids to activate a function or produce a signaling pathway. This powerful enzyme has applications in leather processing and detergent, healthcare and food industries and also for research biotechnology . As in case of cellulose, proteinase also finds application in bioremediation and waste management. About 70% of industrial proteases come from microbial sources; at the same time, acid proteases can be found in fungi abundantly. Proteases isolated from marine microbials are heat-, cryo-, pH - and metal-tolerant and are stabile in the presence of a broad spectrum of chemicals and hence have attracted the interest of researchers. A protease with optimizing pH 11.0 and temperature 60 ºC used in detergent industry as a favorable enzyme with high alkaline pH and temperature was extracted in the west coast of India. This marine enzyme isolated from *Engyodontium album* BTMFS10 strain lives in sediment[34]. This protease is an extracellular enzyme and can be generated by solid-state fermentation in an optimized condition at 25 ºC for 120 h. The enzyme is active over a broad range of pH values (6.0-12.0) and

temperature ranges (15-65 ºC). *Sana* et al. reported a bacteria from gamma-Proteobacteria family from the littoral zone in the Bay of Bengal that produces alkaline serine protease. The enzyme was tolerant to bleaching agents, solvent, salt, and detergent. Hence, it performed a complete removal of recalcitrant blood and egg stains in both wet and dry wash operations. Also, it has potential to be used in soy protein and gelatin hydrolysis in the food processing industry, hair removal process in the leather industry, and as a catalyst in hydrolysis reactions at high salt concentrations. The optimum condition of pH is 9.0 and temperature activity range from 30 to 70 °C and in presence of up to 30% NaCl, its activity increased. Berela *et al.* by a combination of ion-exchange and size exclusion chromatographic methods purified extracellular alkaline protease from the alkaliphilic bacterium *Alcaligenes faecalis*. Its optimal pH and highest temperature activity were pH 9.0 and 55 °C, respectively[35]. An interesting research done on *Alteromonas sp.* strain O-7 showed that secretion of protease increases with increase in chitin concentration. A protease known as anti-protease-activated receptor-4(AprIV) works as an achitin-binding protease in chitinolytic reactions. Production of this enzyme, can be regulated by the existence of chitin, N-acetylglucosamine or N-acetylchitobiose. The most favorable condition for anti-protease-activated receptor (aprIV) production is pH 11.5 and temperature 35 ºC.A protease with optimum pH and temperature at 8.8 and 30 ºC, respectively, has been extracted from marine bacterium *Pseudo alteromonas sp*. strain A28 which is poisonous for diatom *Skeletonema costatum* strain NIES-324. Ion-exchange chromatography followed by preparative gel electrophoresis were applied to purifiy protease inhomogeneity from A28 culture supernatants. This protease has algicidal activity, discovered when Paper-disk assays were used to purify it. In another experiment, marine bacterium *Bacillus sp.* was isolated from the eastern harbor of Alexandria. After culturing the bacterium on wheat bran, two alkaline proteases as Pro1 and Pro 2 by optimal activities at pH 11 and 12 and temperature of 50 and 55 ºC, respectively, extracted inhomogeneity, using exchange chromatography on CM-Sepharose CL-6B followed by Sepharose G-75superfine. The enzyme demonstrated stability in presence of surfactant and bleaching agent and therefore is considered a proper offer for commercial detergent industries. In another study, a marine halo alkalophilic *Bacillus clausii* isolated from wet coast of the Korean Yellow Sea near Inchon City produces an oxidative and SDS-stable alkaline protease. In 2004, Ganesh Kumar et al. extracted this enzyme by culturing it in batch fermentation in shake flasks and in a bioreactor. The optimal pH and temperature are 11.5 and 80 °C, respectively . In 1998, after a protease secreted from the halotolerant strain of *Bacillus licheniformis* was isolated from marine sediments cultured in the sea-water fermentation medium, an alkaline serine protease was obtained by Manachini with optimal activity at pH 9.0 and temperature at 70 °C[36].

Fat hydrolyser enzyme

Lipase Esterase is a hydrolase enzyme which digests esters into acid and alcohol in a chemical reaction. Lipase, a sub-class of esterase, catalyzes the hydrolysis of lipids or carboxylic ester bonds. The physiologic role of lipase is to hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. In addition, its application in bioprocesses is due to its availability and stability in organic as well as in aqueous media. In non-aqueous conditions, lipase can catalyze esterification, interesterification, and transesterification reactions which are known as reverse reactions to produce glycerides. They are found in marine and terrestrial environment amongst animal, plant, molds and bacteria. According to its habitat-related features in marine environment, this enzyme has affected food, detergent, pharmaceutics, leather, textile, cosmetic, and paper industrial processes[37]. The most significant application of lipase is in the food industry. Compared with other enzymes, lipases are more enduring in organic solvents. Lipases choose triglycerides containing long chain fatty acids because they have lipophilic substrates, typically. Marine lipase attracted interests of scientists because of its adaptation to the extreme environment and has application in biopolymers and biodiesel synthesis, enantiomerically pure pharmaceutical compounds, agrochemistry like pesticides, flavor compounds, and cosmetic industry[33]. A hyperthermophilic from the profound area in marine isarchae, *Pyrococcus furiosus* contains a lipase enzyme that has lysis activity on p-nitrophenylesters (*p*-NPE) and can incise it from mid-chain length. Moreover, it has shown higher reactivity to *p*- nitrophenylcaproate (C6), a raw material for industrial production. Actually, this enzyme is a lysophospholipase which demonstrates paramount reaction at 80 °C. Therefore, in the process of yielding of novel drug intermediates, this enzyme can contribute as a bio-catalysit. Marine virus, EhV–86

is a lytic virus with giant double-stranded DNA and molecular size of 160-200 nm. This virus is a *Coccolitho viridae* belonging to the family *Phycodnaviridae* and infects *Emiliania huxleyi* a photosynthetic plankton forming the basis of nearly all marine food webs which freely swims in the euphotic zone of the ocean. EhV-86 contains 472 protein-coding, a unique feature amongst all viruses making it a larger marine virus by genome until now. It is viral genome contains genome parts that code for esterase's and lipases[38]. Seghal Kiran and his group introduced an endosymbiotic *Pseudomonas sp. (MSI057)*, in 2008, collected from the peninsular coast of India. This bacteria is able to generate high yields of psychrophilic alkaline lipase. They have found it generally in a sponge known as *Dendrillanigra*. In minimal medium supplemented with 1% tributyrin, it showed the maximum productivity of enzyme. This enzyme exhibited optimum activity at pH 9.0 and optimum temperature activity at 37 °C; the enzyme activity decreased intensely above 50 °C. The marine strain of *Alteromonas macleodii*, a flagellated proteobacteria, has been used to study the lipids-lipases interactions in bacterioplankton communities. The lipase activity strain has been investigated through its enzymatic effect on fluorogenic lipid analogs such as MUF-palmitate (4-Methylumbelliferyl) and ELF-palmitate (enzyme-labeled fluorescence-palmitate). When hydrolysis occurs by lipase, the non-fluorescent substrates release MUF and ELF alcohol (ELFA) which are fluorescent. ELFA is water-insoluble and because of the hydrolysis, theoretically, it should precipitate the external membrane of bacteria, but in epifluorescence microscopy, no accumulation of ELFA was reported. The relation of hydrophobic/hydrophilic conditions required for precipitation and activity of enzyme should be considered[39].

Conclusion

Marine organisms and microorganisms contain an exceeding amount of enzymes with a unique standard which is mostly distinctive from their counterparts on the land. Moreover, according to several evaluations, extremophile enzymes can be beneficial in a vast range of industrial processes. In addition to the extremophile bacteria, cryophilic microorganisms with their spectrum of activities at low temperatures can be considered to be useful in laundry processes to retain the energy. For example, cryophilic lipases or proteases can be considered for production of detergent to bleach. Some other marine enzymes can be utilized in food industry, such as amylase due to their ability to lead the process in the low temperature. Moreover, controlling of cryophilic enzymes is easier because of their sensitivity to temperature. Therefore, they can be deactivated by changing the temperature. Another enzyme such as cellulase is able to perform saccharification and fermentation used in biofuel and ethanol production at low temperature as well as to remove agricultural wastes. Chitinase as antifungal can be used in pharmaceutical and fishery. Many other enzymes such as peroxidase and pectinase though difficult to find in the marine environment have applications in pharmaceutical and food productions. On the other side, in some industrial processes where mass transfer and viscosity is a crucial point, some marine enzymes can act as bio-catalytics through elevation of temperature to make a favorable condition. Therefore, thermal or hyperthermophilic marine enzymes are valuable in these processes. Although, cultivation, growth, and harvesting of natural products from marine strains is usually faced with difficulties, but novel cultivation techniques developing on lab scale are promising. This review covered some enzyme characteristics and their applications, but until now only a small part of the marine diversity of bacteria and organisms has been recognized. Through the metagenomics and molecular biology, a lot more can be identified to provide for vast number of enzymes applicable for food, feed, cosmetic, medicine, etc.

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