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Review

Potential biocatalysts originating from sea environments

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ABSTRACT

This review is intended to give an account of the knowledge about known enzymes of marine origin described in literature thus stimulating future applications in biocatalysis that these biocatalysts can offer to a large spectra of end-users. The uniqueness of marine biocatalysts is not only based on habitat-related properties such as salt tolerance, hyperthermostability, barophilicity, cold adaptivity, etc. A marine enzyme in fact may carry more, e.g. novel chemical and stereochemical properties. This "chemical biodiversity" increases interest in this field; substrate specificity and affinity are evolved properties linked to the metabolic functions of the enzymes and to ecological asset related to the natural source and this is an important aspect in the bioprospecting for new biocatalysts. The importance of all examples reported should be sufficient to trigger the attention of the biocatalytically oriented scientific community towards marine environment as source of biocatalysts, and this could in turn enhance both new discovery and improvement of marine enzymes.

Contents

1. Introduction

Many excellent summarizing reports dealing with the topic of the biocatalysis have been disseminated during last decade in scientific literature. Any simple search for this kind of articles can give an exhaustive and detailed account of historical developments in the field, an aspect which is not included within this introductory part of the present review for the sake of space.

Generally speaking some specific topics in biocatalysis acquired great renown through the years, often due to the natural technological evolution, but soon they have been replaced by others, paralleling the wavering way new terms are coined in this field. Alternatively, one of the basic, evergreen topic is the search for novel biocatalysts, a research bioprospecting activity which has not

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been completely substituted but has incorporated in itself the new technological system of knowledge from different fields, acquiring new potency and effectiveness. Novel biocatalysts have been discovered by extensive screening of a large number of microorganisms from Nature thus securing wide biological diversity [1] and fruitful establishment of collections of biocatalysts as toolboxes has been possible [2]. Metabolomic based high-throughput approaches have become attractive in the discovery of enzyme's function in this context [3].

With respect to traditional screening procedures ignoring microorganisms which cannot be cultivated, taking full advantage of the enormous naturally occurring microbial resources implies the use of an approach based on direct cloning of environmental DNA, a cultivation-independent methodology greatly enhancing the effectiveness of the biocatalyst discovery. In these methodologies DNA is directly isolated from environmental samples and cloned into suitable vectors to construct complex genomic libraries. These libraries can be analyzed for novel genes or pathways using sequence-based techniques or through screening of the enzymatic activities produced in surrogate hosts [4].

Database information on enzymatic activties and stereochemistry has been of great help in the development of biocatalytic protocols for new and existing enzymes, during these years [5].

The uniqueness of marine biocatalysts characterizes their bioprocesses taking advantages by properties such as salt tolerance, hyperthermostability, barophilicity, cold adaptivity, which are habitat-related characteristics of the isolated proteins. Moreover a marine enzyme may carry more, e.g. novel chemical and stereochemical properties. This "chemical biodiversity" increases interest in this field, in fact substrate specificity and affinity play somehow leader roles. Both are evolved properties that are linked to the metabolic functions of the enzymes and to the ecological asset related to natural sources.

Marine sources are represented by microorganisms and fungi, plants and animals, but great efforts are directed towards extremophiles and symbiotic microorganisms and are specially tending to molecular biology tools for production and modification.

This review is intended to give an account of the knowledge about known enzymes of marine origin described in literature thus stimulating future applications that these biocatalysts can offer.

2. The sea, the disregarded source of biocatalysts

Comparing the amount of scientific hits (articles, reviews, patents, etc.) containing the three concepts of "marine enzymes", "marine natural products" and "biocatalysis", during last decades (1961–2009), as shown in Fig. 1, is a bit impressive. As can be immediately seen, the well known exploit for biocatalysis-related articles starting around 1980, cannot be recognized for the concepts of "marine natural products" or "marine enzymes" both showing indeed a similar slow-increasing trend over the whole half century (1961–2009). Analysis of data of Fig. 1 should be done in the light of intimate details about proprietary construction of scientific databases (i.e. relative value of concepts described by single or many words in CAPLUS and MEDLINE, and detailed knowledge of tag assignments, during insertion of articles), however the success of biocatalysis during these decades clearly resulted. Moreover the data in Fig. 1 are validated also by their correlation with data on discovery of new enzymes as judged by new EC numbers over the past decades [6]. Considering the above examination it can be derived that marine enzymes have been mostly neglected by organic chemists practitioning biocatalysis, otherwise a major increase should be evidenced for this concept by the data in Fig. 1, as inducted by the strong increase of hits related to biocatalysis.

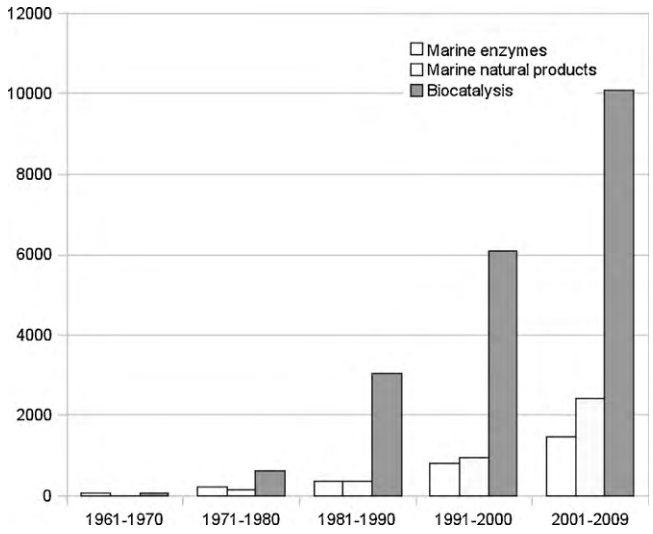


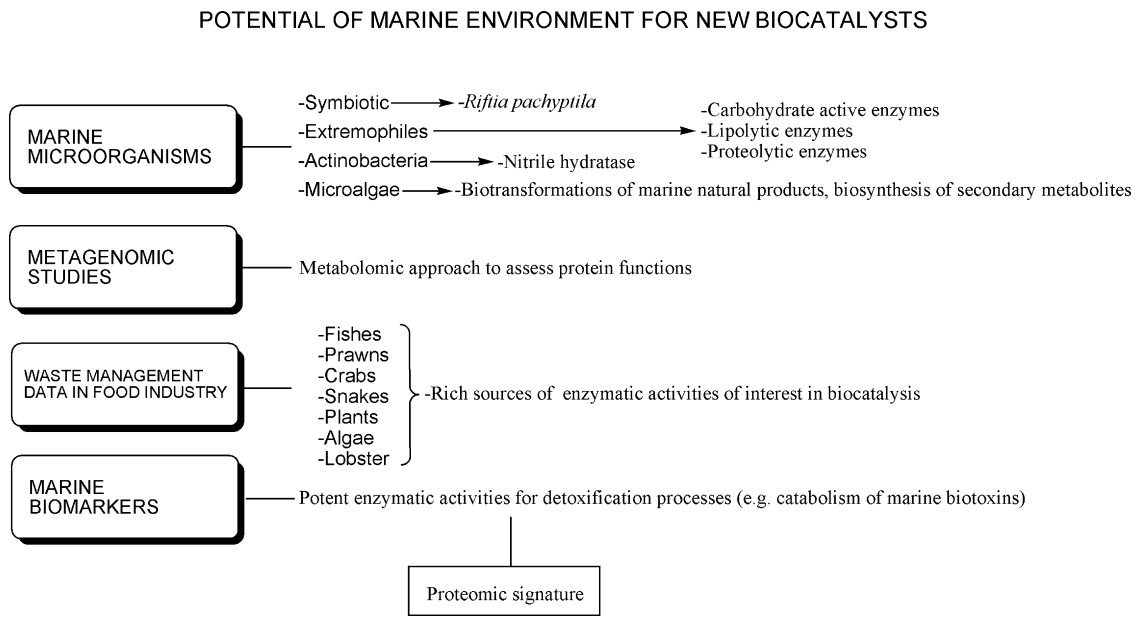
Fig. 1. Scientific hits (articles, reviews, patents, etc.) containing the concepts of "marine enzymes" (white), "marine natural products" (light-gray) and "biocatalysis" (dark-gray), during last decades as assessed by CAPLUS and MEDLINE searches conducted at the end of 2009. e-Alerts for marine enzymes are also used to add "hot of press" articles to the whole body of the review up to submission.

The reasons for the omission of marine enzymes by practitioners are diverse as indicated by the fact that they are not considered an easily accessible "reagent" such as commercial baker's yeast in the laboratories of organic chemistry for many years.

3. Marine enzymes as potential biocatalysts

The marine habitat has been and continues to be a source of unique natural products used as pharmaceuticals or possessing useful characteristics for biotechnological applications. Enzymes are among these products and the marine source can be considered almost unexplored in relation to the presence of enzymatic activities which can be found. These enzymes can offer properties related to the habitat which are greatly appreciated under a general biotechnological perspective. But novelty brought out by marine enzymes is more pervasive, in fact new characteristics can be discovered at molecular level of catalysis specially concerning the stereochemical asset of products. The analysis of marine bio-processes from chemical and stereochemical viewpoints of catalysis could better reveal the potential of marine sources in biocatalysis.

The primary source of marine enzymes can be considered the general category of marine microorganisms including symbiotic and deep-sea microorganisms and extremophiles (Scheme 1) [7,8]. The latter can play important roles as source of high (bio)-chemical diversity. Specific classes of marine bacteria have been also selectively investigated; in a recent review the current state of research on the biology and biotechnology of *Actinobacteria* in the marine environment is evidenced [9]. From these studies genes from marine sediments encoding the very interesting nitrile hydratases with novel activities were recovered [10]. Marine microorganisms can be found as intracellular or extracellular symbionts, and their hosts are mostly marine animals (vertebrates or invertebrates). These symbiotic microorganisms must possess arsenal of biomolecules (e.g. enzymes) and pathways to fulfill requests of the host organisms [11]. A particular interesting case of symbiotic relationship concerns *Riftia pachyptila* (*Vestimentifera*) which is a giant tubeworm living around the volcanic deep-sea vents of the East Pacific Rise. The animal does not possess a digestive tract and lives in an intimate symbiosis with a sulfur-oxidizing chemoautotrophic bacterium localized in the cells of the tropho-



Scheme 1. Schematic representation of the potential of marine environment in biocatalysis.

some, an organ of the animal. These organisms are adapted to their extreme environment and their organization constitutes a very interesting system to study the molecular and metabolic basis of biological adaptation [12]. Moreover entire communities of shrimps and crabs have been found living around these giant tubeworms and could possess metabolic tools of interest in biocatalysis as well.

Interest for extremophiles that are adapted to survive in ecological niches such as high or low temperatures, extremes of pH, high salt concentrations and high pressure is based upon this ability to thrive in such environments and robustness shown by their biocatalysts. A number of these unique microorganisms have been isolated from marine environments [13]. Different classes of enzymes have been identified from these extremophiles including carbohydrate active enzymes, proteolytic and lipolytic activities and different alcohol dehydrogenases of interest.

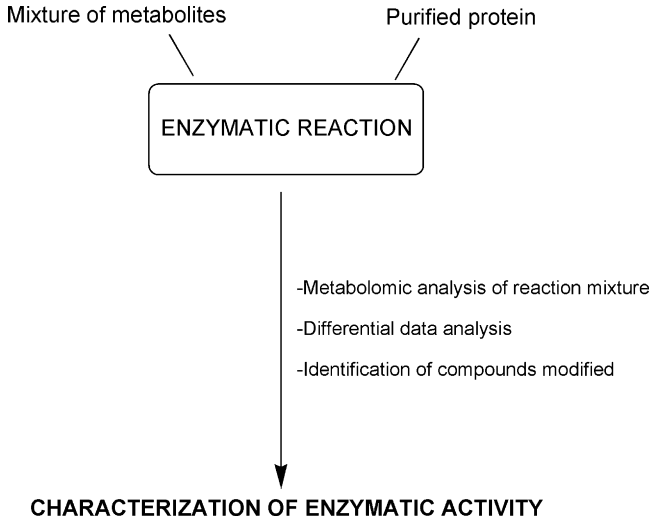
Marine microalgae are also used for biotechnological applications as biocatalysts performing biotransformations of natural products, biosynthesis of secondary metabolites and as test organisms in ecotoxicology. Interest for immobilization of such organisms is reported [14].

The powerful cultivation-independent approach of metagenomics can be applied to gain access to the biocatalysts from uncultured marine microorganisms. Both sequence-based and function-based screening methods are in use to identify genes for novel biocatalytic activities. Metagenomic strategies hold great potential to study and exploit the enormous microbial biodiversity which is present within these marine environments [15,16] and this hold true specially for symbiotic bacteria associated to sponges [17].

The information on genome sequence and the possibility to express clone libraries for all ORFs made possible to use recombinant proteins trying to associate them to specific activities. A metabolomic approach for this enzyme-function searching is very interesting in this respect [3]. A mixture of metabolites is incubated with the expressed enzyme and the reaction mixture is subsequently analyzed by mass spectrometry. Specific changes in the metabolite composition can directly suggest the presence of an enzymatic activity and the identification of the compounds whose level changed specifically can indicate the substrate(s) and product(s) of the reaction (Scheme 2).

Despite most general current biotechnological applications are founded on microbial products, marine organisms such as fishes, prawns, crabs, snakes and plants and algae can represent rich sources of biodiversity. Specifically in this context, waste management research concerning fisheries and seafood related industries [18], contains lots of data about intelligent marine enzymes exploitation in waste management and in fields generally related to human need and great commercial importance (Scheme 1). Interesting examples are: (i) scallop mantle which is not consumed as food, is used to produce collagen and collagen peptides substances useful in cosmetic, as improved hair damage restoring product with respect to the known collagen peptide of bovine origin; (ii) biomedical application of squid β-chitin obtained from squid pen; (iii) digestive juice of abalone and scallop rich in digestive enzymes may be applicable to degrade crystalline cellulose from plant source by-products into glucose [18].

An additional illustrating case of application in food industry is a study concerning the use of marine enzymes extracted from the crustacean *Munida* (lobster) as adjuvants for milk coagulation and



Scheme 2. Metabolomic approach for enzyme-function screening.

Table 1
Selected articles reporting on oxidoreductases of marine origin.

Enzyme	Source	Notes	References
Alcohol dehydrogenase	*Pyrococcus furiosus*	Marine hyperthermophilic strain	[29]
Hydrogenase	*Pyrococcus furiosus*	Marine hyperthermophilic strain	[30]
Thermostable glutamate dehydrogenase	*Thermococcus litoralis and Pyrococcus furiosus* (Pfu)	Marine hyperthermophilic strain	[31]
Glutamate dehydrogenase	*Loligo pealeii*	Squid	[32]
Alcohol dehydrogenase	*Chaetoceros gracilis, Chaetoceros sp., Nannochloropsis sp., Pavlova lutheri*	Marine algae	[33,34]
Alcohol dehydrogenase	*Acanthopagrus latus, Mugil cephalus and Lateolabrax japonicus*	Yellowfin porgy, gray mullet, Japanese sea perch	[35],[36]
Alcohol dehydrogenase	*Hyalinoecia tubicola*	Marine polychaete	[37]
Alcohol dehydrogenase	*Cloned from marine teleost Sparus aurata*	Fish	[38]
Alcohol dehydrogenase, malate dehydrogenase, lactate dehydrogenase, glucose dehydrogenase, malic enzyme, superoxide dismutase	*A. maculatus and O. militaris*	Marine catfish	[39]
Methanol dehydrogenases	*Methylophaga sp. strain 1*	Marine methylotroph	[40]
Lactate dehydrogenase	*Meganyctiphanes norvegica*	Shrimp	[41]
Alanine dehydrogenase	*Vibrio proteolyticus*	Marine bacterium	[42]
Vanadium bromoperoxidase (V-BrPO)	*Plocamium cartilagineum, Laurencia pacifica, Corallina officinalis*	Enzymes isolated and cloned from marine red algae that produce Halogenated compounds	[44,45]
Vanadium bromoperoxidase (V-BrPO)	*Ascophyllum nodosum*	Marine brown alga	[46]
Lipoxygenase	*Ulva conglobata*	Marine green alga	[47]
Lipoxygenase	*Pseudonitzschia delicatissima*	Pennate diatom	[48]
Superoxide dismutase EC 1.15.1.1	*Mytilus galloprovincialis*	Bivalve mollusk	[49]
Catalase EC 1.11.1.6	*Mytilus galloprovincialis*	Bivalve mollusk	
Glutathione peroxidase EC 1.11.1.9	*Mytilus galloprovincialis*	Bivalve mollusk	
Glutathione reductase EC 1.6.4.2	*Mytilus galloprovincialis*	Bivalve mollusk	
Antioxidant enzymes	*Perna viridis*	Marine mussel	[50]
Peroxisomal enzymes	*Mytilus galloprovincialis, Chelon labrosus*	Marine mussel and fish	[51]
Dihydroxylating dioxygenases	PAH utilizing marine bacteria	Enzymes expressed in *E.coli*	[52]
Alkane hydroxylases	Clone libraries	–	[53]
Fatty acid desaturases	*Pseudoalteromonas sp. MLY15*	Cloned from the isolated marine bacterium in *E. coli*	[54]
Glucose-3-dehydrogenase	*Halomonas* sp.	Marine bacterium	[56]
Lignin peroxidase, manganese-dependent peroxidase, laccase	*Aspergillus sclerotiorum* CBMAI 849, *Cladosporium cladosporioides* CBMAI 857, *Mucor racemosus* CBMAI 847	Marine fungi	[57]
Reductive dechlorination of 2-chlorophenol	*Desulfovibrio dechloracetivorans*	Marine dechlorinating bacterium	[58]
NAD(P)-dependent formaldehyde dehydrogenase	*Methylobacter marinus* A45	Marine methanotroph	[59]
Sulfite oxidase	*Sulfitobacter pontiacus*	A Gram-negative heterotrophic bacterium isolated from the Black Sea	[60]

cheese ripening [19]. The article gives a survey of the biophysical and biochemical properties of the *Munida* enzymes and of their innovative role in cheese production.

Another field of knowledge which can greatly concur to the appreciation of marine sources for enzymes to be used in biocatalysis, is the study of marine biomarkers and their significance and application in pollution monitoring (Scheme 1) [20]. The general increasing awareness for the potential long-term adverse effects of chemicals and risks for aquatic and terrestrial ecosystems have greatly enhanced the knowledge in this field. As a change in biological response (molecular, cellular, physiological, behaviorial modifications), a biomarker is related to the presence of toxic substances and the sub-organismal biomarkers indicated the presence of enzymatic activities which are of great interest for biocatalysis [21–23]. Obviously the gap between these analytical measurements and preparative applications must be overcome by applied research after recognizing biocatalysts of interest. However it is of high significance that developments in this field secured the identification of proteomic signatures of exposure to marine pollutants in mussels (*Mytilus edulis*). A unique set of protein expression (signature) for exposure to different chemical compounds has been recognized and the expressed proteins identified to participate in α- and β-oxidation pathways, in xenobiotic and amino acid metabolism, in cell signalling, and in oxyradical metabolism [24]. A particular case of study in the context of marine biomarkers is the collection of information aimed to discover the potential of bivalve mollusc (or microorganisms associated to them) to catabolize marine biotoxins specially the paralytic shellfish toxins for which commercial detoxification methods are difficult or do not exist. An algal extract containing a mixture of different toxins (saxitoxin, neo-saxitoxin, gonyautoxins and others) was used for the analysis and after 5 days of incubation the reduction of overall toxicity was verified by mouse bioassays and HPLC. Novel strains of *Pseudoalteromonas haloplanktis* were identified capable of catabolizing the biotoxins analyzed [25]. Enzymes involved in such reactions could be of extreme interest in semi-synthetic steps adopted for biotransformation of bioactive natural products.

Finally the attention of scientific community for marine enzymes as potential biocatalysts is witnessed also by the publication in due course of a special issue of Marine Drugs (an Open Access journal on the research, development and production of drugs from the sea), entitled “Enzymes from the Sea: Sources, Molecular Biology and Bioprocesses” guest-edited by the author of this review [26] (URL: http://www.mdpi.com/journal/marinedrugs/special_issues/sea-enzymes/).

In the following paragraphs of this review, which are entitled using the name of related enzyme classes, most of original articles will be discussed or presented in tabulated form for the sake of reporting a sufficient number of original research data. However the effort will be more selective than exhaustive and signalling of outstanding comprehensive reviews in the field as collection of additional research articles, will not avoided. The discussion will mainly focus on convincing examples, in which specific diversity of biocatalysis can be recognized with respect to terrestrial counterparts.

3.1. Oxidoreductases

During the periods covered by two key reviews 1988–1991 [27] and 2003–2008 [28], the attention of chemists towards biocatalysts for oxidoreductions seems to be changed as can be easily seen just by the physical space devoted to baker's yeast applications in the Santaniello's report (ten pages) compared to that in the updated review of 2009 (eight lines). The latter further indicates that many putative genes of dehydrogenases in *Saccharomyces* have been expressed in *E. coli*, and the recombinant *E. coli* cells or the isolated enzymes have been used for asymmetric reductions. Hyperthermophilic enzymes for oxidation-reduction reactions are also mentioned by Japanese authors of the most recent account, as dehydrogenases with such properties are judged important.

Selected articles [29–42,44–54,56–60] reporting on oxidoreductases of marine origin are listed in Table 1.

Biocatalytic characteristics such as substrate specificity and enantioselectivity of an alcohol dehydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus* have been fully evaluated. This enzyme catalyzes the reduction of various ketones including alkyl and aryl ketones, and α- and β-ketoesters. Aryl ketones with phenyl group next to the carbonyl group were reduced to the corresponding chiral alcohols in an enantiomerically pure form. Ketone substrates lacking phenyl groups were reduced with a moderate enantioselectivity. α-Ketoesters behave in a similar way while β-ketoester as ethyl 3-oxo-butyrate was reduced to ethyl (*S*)-3-hydroxybutyrate in a high enantiomeric purity. As the size of the chain linked to carbonyl group increased, the enantioselectivity of the reductions decreased, with the exception of a phenyl group, in which the product was obtained in 99% *e.e.* The reaction temperature increased the enzyme activity, but exerted no effect on the enantioselectivity. This alcohol dehydrogenase showed also an interesting high tolerance towards organic solvents such as dimethyl sulfoxide, iso-propanol, methyl *tert*-butyl ether, and hexane, a useful feature for working with ketones possessing low solubility in aqueous buffers [29].

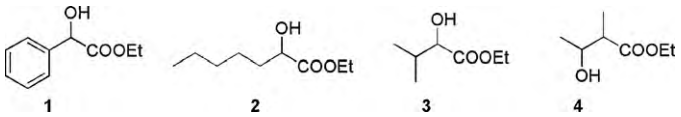


Fig. 2. Products of enzymatic reduction of different α- and β-ketoesters by four selected marine algae *Chaetoceros gracilis*, *Chaetoceros* sp., *Nannochloropsis* sp., *Pavlova lutheri*. Stereochemistry and *e.e.* are detailed in the text and in ref. [34].

The same marine microorganism, *Pyrococcus furiosus*, was shown to possess both a marine hydrogenase [30] which can be used as biocatalyst in the enzymatic production and regeneration of NADPH, utilizing cheap molecular hydrogen and forming protons as the only side-product, and a glutamate hydrogenase [31] as well. This latter biocatalyst showed substantial enhancement of activity as well as thermostability at increasing salt concentrations. Salt availability and the overall effects on structural integrity and thermostability of marine enzymes are considered partially responsible of the higher thermal stability in marine species than in related freshwater species.

Interesting results by Japanese scientists in 2003 reported the reduction of different α- and β-ketoesters by four selected marine algae (*Chaetoceros gracilis*, *Chaetoceros* sp., *Nannochloropsis* sp., *Pavlova lutheri*). All marine species reduced ethyl benzoylformate to the corresponding alcohol (**1**, Fig. 2) with a high conversion ratio, however, the enantioselectivity of the reaction is poor. The reduction of ethyl 2-oxoheptanoate by *Chaetoceros gracilis* gave the corresponding alcohol (*S*)-**2** (Fig. 2) in high *e.e.* Ethyl 3-methyl-2-oxobutanoate was reduced by *Nannochloropsis* sp. to (*R*)-**3** (Fig. 2) in high *e.e.* (98%) with a high conversion ratio (99%). The reduction of ethyl 2-methyl-3-oxobutanoate by the microalgae gave the corresponding anti-hydroxy ester anti-**4** Fig. 2 in low conversion ratios (25–68%). In particular, *Nannochloropsis* sp. reaction had an excellent diastereo- (syn:anti = 1:99) and high enantioselectivity (>99%), when compared with the selectivity expressed by *S. cerevisiae*, *Chlorella*, and *Glycine max*, which produced predominantly the syn-hydroxy ester of this product [33,34].

Alanine dehydrogenase is an enzyme useful in different applications: as catalyst for the analysis of L-alanine, or for the production of L-alanine labelled representative, etc. Versatile aminoacid dehydrogenases with broad substrate specificity are also required for the synthesis of non-protein artificial aminoacids. Alanine dehydrogenase of a marine bacterium, *Vibrio proteolyticus* DSM30189, shows a high activity toward β-hydroxypyruvate, thus the enzyme

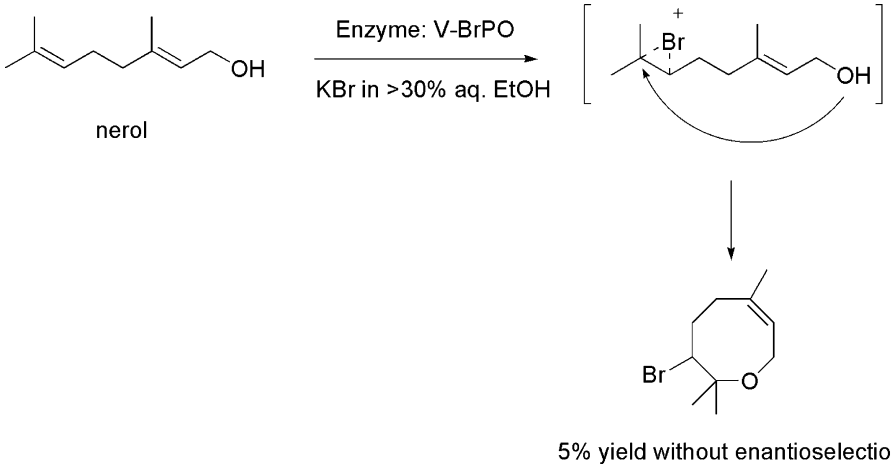


Fig. 3. Reaction of V-BrPO using nerol and forming a cyclic ether [44].

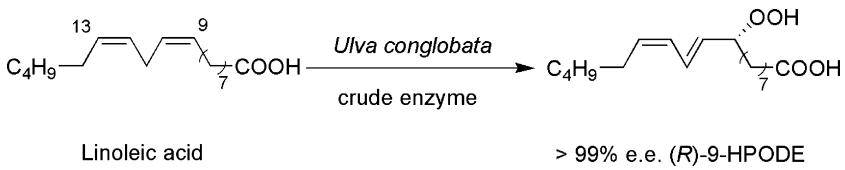


Fig. 4. *Ulva conglobata* crude enzyme reaction forming (*R*)-9-hydroperoxy-(10E,12Z)-10,12-octadecadienoic acid ((*R*)-9-HPODE).

is applicable to the production of L-serine [42]. The application of such enzymes in asymmetric synthesis is of interest in that they do not require nicotinamide cofactors [43].

Vanadium bromoperoxidase (V-BrPO) [44–46] isolated from the marine brown alga, *Ascophyllum nodosum* was known to catalyze the chlorination of phenolsulfonephthalein (phenol red) and taurine [45]. Other marine algal haloperoxidase enzymes are also known [46]. Functioning of these enzymes is exerted by coordination of hydrogen peroxide to the metal V(V), subsequent oxidation of halides producing a two-electron oxidized halogen species (e.g., "Br^+"), followed by electrophilic halogenation of the organic substrate. Recently from marine red algae (*Plocamium cartilagineum*, *Laurencia pacifica*, *Corallina officinalis*) which produce halogenated compounds, a vanadium bromoperoxidase (V-BrPO) has been isolated and cloned. The biocatalyst can catalyze the bromination and cyclization of terpenes and terpene analogs [44]. This article summarize on the first V-BrPO-catalyzed brominative cyclization reactions of monoterpene substrates. Terpene binding in the active site channel of V-BrPO may direct the brominative cyclization reaction in aqueous solution and the hydrophobic nature of the active site could promote the bromonium ion induced cyclization (Fig. 3 for nerol) [44].

An essential oil which could be prepared by distillation of marine alga *Ulva conglobata*, contains different long- and short-chain unsaturated aldehydes which are formed from long-chain unsaturated fatty acids linoleic (LA) and linolenic acid (LNA) [47]. Lipoxygenases (EC 1.13.11.12, LOX) catalyze the oxygenation of fatty acids containing a (1Z, 4Z)-pentadiene moiety in a regio- and stereoselective manner involving the corresponding hydroperoxides converted into aldehydes. It has been reported that when LA and LNA were incubated with a crude enzyme of *U. conglobata*, the corresponding hydroperoxides (*R*)-9-HPODE and (*R*)-9-HPOTrE were formed with a high *e.e.* (>99%), respectively (Fig. 4 for LA). This regio- and stereoselective way differs from those found in plants and in other organisms and this is of interest in the applicative aspect of lipooxygenases [47]. From the synthetic chemist's point of view, the asymmetric oxygenation reaction of unnatural substrates has a tremendous potential.

Data from researchers involved in Environmental Risk Assessement (ERA) can be useful information about existence and activity of marine enzymes [20]. A pool of antioxidant enzymes present in *Mytilus galloprovincialis* and other organisms, are actively studied [49–51].

Polycyclic aromatic hydrocarbons (PAHs) is a class of organic pollutants present in the marine environment. They are composed of two or more fused aromatic rings. Persistence, ability to bioaccumulate and carcinogenicity of PAHs are all important aspects of interest concerning biotransformation of these compounds [21]. Results of experiments using various mono- or di-substituted naphthalenes such as dimethylnaphthalenes using the cells of *Escherichia coli* expressing aromatic dihydroxylating dioxygenase genes of marine bacteria, *Nocardioides* sp. KP7 and *Cycloclasticus* sp. A5, respectively, were reported. Authors shed the light about the broad substrate preference of enzymes which often were able to hydroxylate methyl groups. Specifically, 1,4-dimethylnaphthalene was predominantly bioconverted into 1,4-dihydroxymethylnaphthalene [52] possessing applications as starting materials for the synthesis of industrially useful chemicals.

Alkane biodegradation potential focused interest for the difficulty which characterize chemical routes suitable for this process. The diversity and distribution of alkane hydroxylase genes in sediments of the Timor Sea was studied. Protein sequences derived from clone libraries suggest that Timor Sea may be a rich reservoir for novel alkane hydroxylase enzymes [53].

A very recent basic research regarding molecular mechanism for cold adaptation has attracted interest for the key role of unsaturated fatty acid for the maintenance of appropriate membrane fluidity and correct function. In a marine bacterium belonging to the genus *Pseudoalteromonas*, a gene encoding a $\Delta 9$ fatty acid desaturase involved in monounsaturated fatty acid biosynthesis has been found and expressed in *E. coli*. Enzymatic activity demonstrates effectively catalysis in the desaturation of both palmitic acid and stearic acids [54]. Although these enzymes have been regarded as too restrictive in terms of substrate specificity to be of general interest in biocatalysis, new insights from new enzymes can lead to the discovery of novel catalytic behaviour capable of generating renewed enthusiasm [55].

Biosensor systems for detection of sulfite in food and beverages are of interest. *Sulfitobacter pontiacus* produces a sulfite oxidase of high activity [60].

An interesting study regarding biotransformations conducted by five species of marine microalgae on aliphatic and aromatic ketones, including several monoterpenoid ketones also appeared [61].

3.2. Hydrolases

Marine enzymes belonging to this large category of biocatalysts are listed in Table 2 using a practical subdivision regarding the type of the substrate. Inclusion in the list has been ensured for those examples in which chemical details of biocatalysis are reported and/or briefly discussed, or enzymes are not reported before in previous reviews.

3.2.1. Carbohydrate active hydrolases

The potential of marine sources for this category of biocatalysts has been demonstrated by various examples found in literature for the synthesis and hydrolysis of glycosidic bonds. Further aspects to be considered are the applications of marine carbohydrate active hydrolases for vegetal waste treatment in recovering useful materials, for structural identification and for preparation of target materials from new purified polysaccharides (see below).

Complete genome sequence of the marine bacterium *Saccharophagus degradans* strain 2-40 has been reported and an unusual large number of enzymes degrading complex polysaccharides has been found. The authors claim that it is not only an extraordinary range of catabolic capability, but many of the enzymes exhibit unusual architecture including novel combinations of catalytic and substrate-binding modules which can be highly interesting for biocatalysis specially in the enzymatic synthesis of glycoconjugates [62].

Aplysia is a genus of sea hares belonging to the family *Aplysiidae*, containing different species of organisms. *Aplysia fasciata* Poiret,

Table 2
Selected articles reporting on hydrolases of marine origin.

Enzyme	Source	Notes	References
Carbohydrate active hydrolases			
β-mannosidase	*Aplysia fasciata*	Sea hare	[64]
α-glucosidase	*Aplysia fasciata*		[65]
β-galactosidase	*Aplysia fasciata*		[66,67]
α-amylase	*Streptomyces* sp. D1	Marine microorganism	[71]
amylase	*Pseudoalteromonas undina* NKMB 0074	Marine microorganism	[72]
α-L-arabinofuranosidase	*Rhodothermus marinus*	Extreme thermophilic eubacterium	[74]
α-L-fucosidase	*Pecten maximus*	Marine mollusk	[75,76]
Fucoidan degrading activity	*Sphingomonas paucimobilis* PF-1	Marine microorganism	[83]
laminarinases	*Spisula sacchalinensis, Chlamys albidus*	Marine bivalvia	[84]
β-1,3-glucanases	*Aplysia kurodai*	Sea hare	[85]
β-glucosidase	*Thermotoga maritima*	Extreme thermophilic eubacterium	[86]
Xylanase	*Thermotoga maritima*	Extreme thermophilic eubacterium	[87]
Xylanase, glycosidases	*Thermotoga neapolitana*	Marine hydrogen producing bacterium	[88]
Xylanase	*Geobacillus* sp. MT-1	Deep-sea thermophilic microorganism	[89]
β-1,4-xylanase, cellulase, β-1,3-xylanase, porphyranase, and β-1,4-mannanase	*Pseudomonas* sp ND 137	Encoding genes	[90]
β-N-acetylglucosaminidase	Eukariotic marine phytoplankton		[91]
β-N-acetylglucosaminidase	*Scylla serrata*	Green crab	[92]
β-N-acetylglucosaminidase	*Penaeus vannamei*	Prawn	[93]
Agarase	*Agarivorans albus* QM38	Marine bacterium	[94]
Inulinase	*Pichia guilliermondii*	Marine yeast	[96]
Inulinase	*Cryptococcus aureus* G7	Marine yeast	[97,98]
Hyaluronidase	*Synanceia horrida*	Stonefish	[99]
Lipid active hydrolases			
Wax esterase	Marine fishes	7 species	[103]
Lipid digestive enzymes	Turbot	Edible fish	[104]
Lipases	427 yeast strains	From seawater, sediments, mud of salterns, guts of the marine fish and marine algae	[105]
Esterase	*Bacillus* sp.	Marine bacterium	[106]
Lipase	*Bacillus pumilus* B106	Marine bacterium associated to the sea sponge Halichondria rugosa	[107]
Lipases	*Alteromonas macleodii*	Ubiquitous marine bacteria	[108]
Esterases	Metagenomic library	–	[109]
Lipase	Metagenomic library	–	[110]
Cold active lipase	Metagenomic library	–	[111]
Lipoprotein lipase LPL	*Dicentrarchus labrax* L	Edible fish	[112]
Steroid fatty acid ester conjugation	*Mytilus edulis*	Bivalve mollusk	[113]
Esterase activity	*Scylla serrata*	Crab	[114]
Lipolytic activity	112 strains	Cold adapted Antarctic marine bacteria	[115]
Lipase	*Vibrio fischeri*	marine microbe	[116]
Alkaline lipase	*Pseudomonas* sp. (MSI057)	From marine sponge *Dendrilla nigra*	[117]
Lipolytic activity	*Yarrowia lipolytica* CL180	Marine yeast	[118]
Extracellular lipolytic enzymes	*Psychrobacter* sp.	Antarctic sea-water bacteria	[119]
Cold active lipase	*Pseudoalteromonas haloplanktis* TAC125	Antarctic bacterium	[120]
Lipase activities	*Perkinsus marinus*	Oyster protozoan parasite	[121]
Extracellular lipase	*Geotrichum marinum*	Marine fungus	[122]
Phospholipase A2	*Hexaplex trunculus*	Sea snail also known as *Murex trunculus*	[123]
Phospholipase A2	*Hydrophis cyanocinctus*	A sea snake	[124]
Phosphate active hydrolases			
Alkaline phosphatase	*Pinctada fucata*	Pearl oyster	[130]
Alkaline phosphatase	*Cobetia marina* KMMMC296	Marine bacterium isolated from the mussel *Crenomytilus grayanus*	[132]
phytase	*Kodamea ohmeri* BG3	Yeast strain isolated from gut of marine fish *Hexagrammes otakii*	[134]
Protein active hydrolases			
Endopeptidases	*Cancer pagrus*	Marine crab	[139]
Protease	*Geodia cydonium*	Marine sponge	[141]
Alkaline protease	*Aureobasidium pullulans* HN2-3	Marine yeast	[144]
Others			
sulfoesterase	*Pecten maximus*	Marine mollusk	[145]
arylsulfatase	*Sphingomonas* sp. As6330	Marine bacterium	[146]
acetylcholine esterase	*Mytilus edulis*	Bivalve	[147]
epoxide hydrolase	*Mugil cephalus*	Marine fish	[148]
epoxide hydrolase	*Sphingomonas echinoides*	Marine microorganism	[149]
epoxide hydrolase	*Danio rerio*	Tropical freshwater zebra fish	[150]
AMP aminohydrolase	*Scorpaena porcus, Sciena umbra*	Sea scorpion, marine fish	[152]

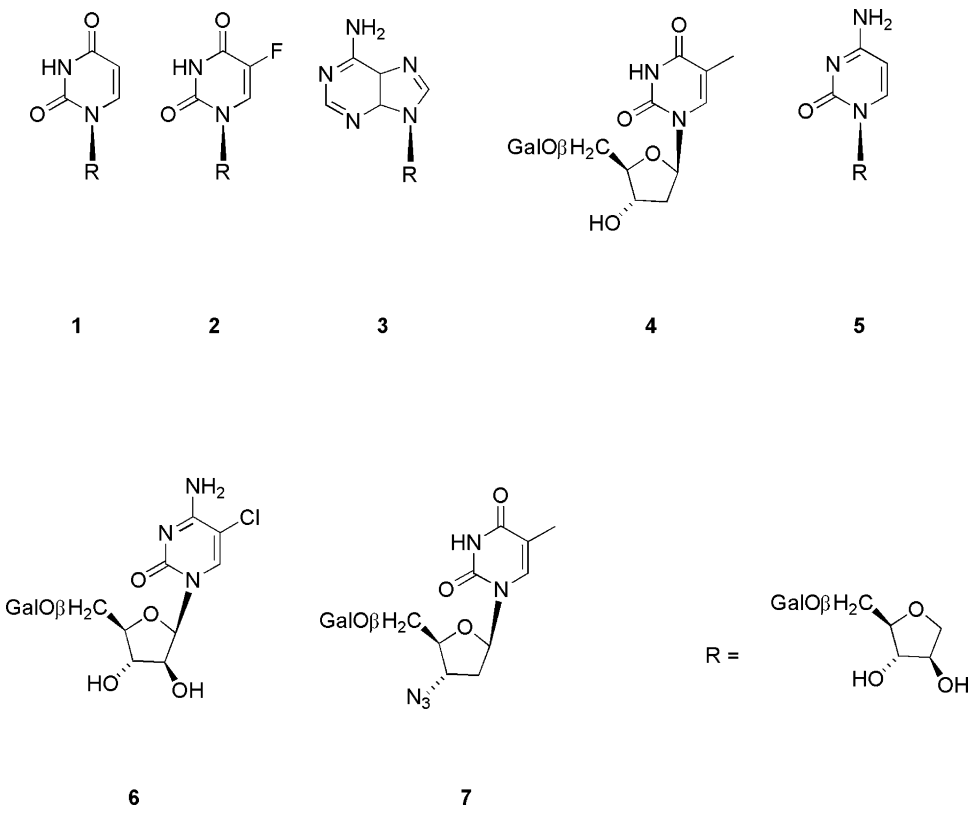


Fig. 5. Nucleoside derivatives syntesized in high yield using β-galactosidase purified from *A. fasciata*.

1789 which is one of them, is very common in Mediterranean habitats. The *Aplysiidae* are herbivorous, eating a variety of red, green or brown algae. They have been revealed to be potent producers of a library of glycoside hydrolases applied in the synthesis of glycosidic bonds [63]. A β-mannosidase with catalytic efficiency significantly higher than those reported for β-mannosidases from other sources, has been reported in this marine organism [64]. It possesses exo-acting activity and when the enzyme is incubated in the presence of *p*-nitrophenyl β-D-mannopyranoside, self-transfer of the mannosyl group is observed, and a 10–15% yield of a β-1-4 disaccharide was obtained. In the presence of heteroacceptor such as *o*-nitrophenyl α-D-2-deoxy-N-acetyl glucopyranoside, two regioisomers (85:15, 12% yield) due to the β-mannosylation in 4 and in 6 positions were formed.

In the same organism an α-glucosidase and a β-galactosidase were detected, purified and used for different transglycosylation reactions with various acceptors [65,66]. Results of transgalactosylation reactions indicate a clear preference of the *Aplysia* enzyme for the galactosylation of polar acceptors. Owing to the specificity of the acceptor site of most terrestrial galactosidases for compounds with phenyl groups, the yields obtained in the reactions using free or methyl derivative of xylose and methyl β-galactopyranoside and D-galactose, are interestingly high for this marine enzyme. Another exciting characteristic of the enzyme from *Aplysia* is the uncommon β-1,3 selectivity in the transgalactosylation reactions with most of the acceptors. Enzyme regioselectivity was absolute in reactions for the synthesis of nucleoside derivatives. In these cases only the product of galactosylation at the 5′ position of the nucleosides

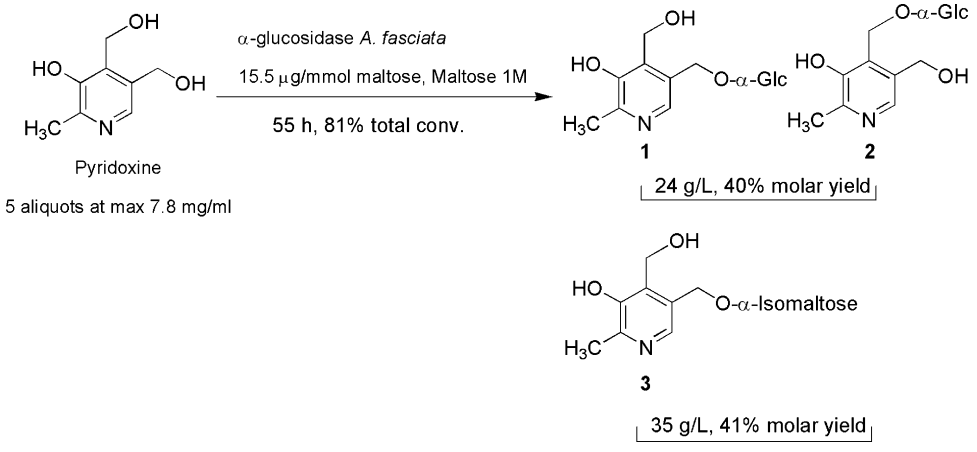


Fig. 6. Glucosylation of pyridoxine using α-glucosidase of *Aplysia fasciata* and maltose as donor.

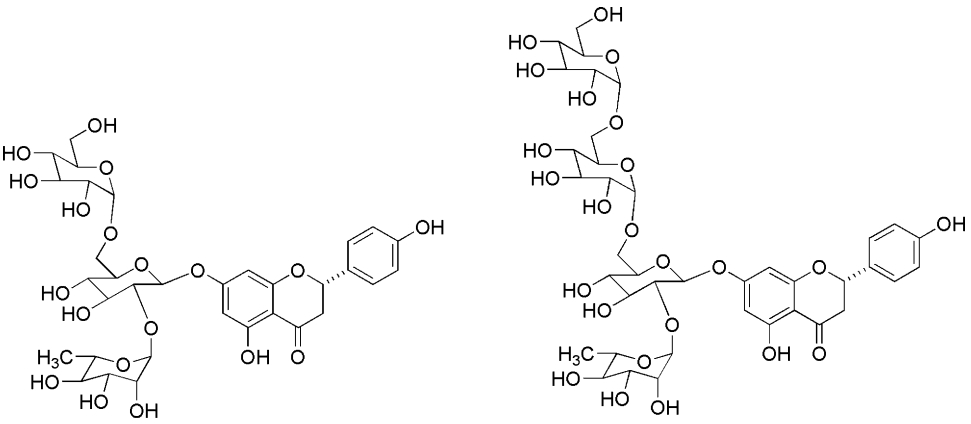


Fig. 7. Glucosylated derivatives (1 and 2) obtained by enzymatic glucosylation performed by α-glucosidase of *Aplysia fasciata*.

was observed (Fig. 5). Reaction yields were satisfactory in most cases, and very high for uridine derivatives. In particular, 5′-O-β-galactosyl-5-fluorouridine (2, Fig. 5), the galactosylated derivative of the anticancer drug fluorouridine, was synthesized with a 60% yield, and 5′-O-β-galactosyl-3′-azido-3′-deoxythymidine (7, Fig. 5), the derivative of the anti-HIV drug, was obtained in 43% yield [67]. This was the first report dealing with a glycoside hydrolase used for the modification of nucleosides with such convenient yields.

Characterization of performances of the α-glucosidase from *Aplysia* in transglycosylation reactions indicated the preferential enzymatic formation of α-1-4 linkages in the early stages of reaction and the accumulation of α-1-6 products which were confirmed by time course experiments [65]. The reactions with cellobiose, saccharose, pyridoxine, naringin and 9-fluorenone derivatives as acceptors have been studied in detail. High-yielding enzymatic α-glycosylation of pyridoxine using this marine enzyme were obtained after optimization of reaction conditions, reaching ca. 80% molar yield of products (pyridoxine monoglucosides 24 g/l; pyridoxine isomaltoside 35 g/l). High selectivity toward the 5′ position, in both mono- and disaccharic material, was observed (Fig. 6) [68]. The enzymatic glucosylation of naringin has been also possible. The regioselective formation of both the β-gluco-C6 α-glucosyl derivative and of the corresponding isomaltosyl diglucoside of naringin is observed in high yield and efficiency of reaction: suspensions of insoluble naringin can be used up to ca. 90 mg/ml initial acceptor concentration. In different experiments it was demonstrated that one of diasteromers of the naringin is preferred by the enzyme from *A. fasciata* during glucosylation/deglucosylation enzymatic steps. Finally, the feasibility of efficient naringin glucosylation directly within grapefruit juice is also demonstrated at low maltose concentrations but optimal pH of the enzyme (Fig. 7) [69]. Other acceptors belonging to 9-fluorenone derivatives were used providing several α-O-polyglucosides which were useful for a quick screening of the pharmaceutical profile as modified by carbohydrate(s) moieties with respect to parental antiviral aglycones [70]. The tendency about polyglycosylation is a useful asset of the protein for the easy access to a series of glycosides and is a characteristic in common to other enzymes of marine origin (see also below).

Interest for marine starch-acting enzymes is very high, in fact a novel α-amylase from marine *Streptomyces* sp. D1 has been very recently reported. The authors did not mention about transglycosylation activity but interestingly the hydrolysis pattern indicated that it has α-1-4, α-1-6 (debranching) activity [71]. Moreover this is the first report for a *Streptomyces* enzyme having a wide range of pH stability and therefore widespread application in detergent industry is conceivable. An amylase-producing bacterium, *Pseudoalteromonas undina* NKMB 0074 was also isolated and identified, it can be used in saccharification of marine microalgae producing ethanol, in saline conditions. Terrestrial amylase and glucoamylase were inactive in saline suspension. Therefore, marine amylase is necessary in saline conditions for successful saccharification of marine microalgae [72].

Arabinofuranosidases are enzymes of xylanolytic systems used in the biobleaching allowing reduction of the amounts of bleaching chemicals in the processes. Moreover these biocatalysts may be applied for increasing the aroma of wines and fruit juices as well as the production of arabinose in food industry. A thermostable α-L-arabinofuranosidase was also reported for its ability to perform transarabinosylation reaction [73]. An optimisation study for the best culture medium and conditions for α-L-arabinofuranosidase production by the extreme thermophilic eubacterium *Rhodothermus marinus* has been evidenced [74]. This marine thermophilic eubacterium, has been reported to produce also highly thermostable xylanases and endoglucanases.

L-fucose is one of the most common monosaccharides at the nonreducing end of many glycans and it is thus an important biological determinant. Its terminal location on glycoconjugates makes it sensitive to the action of α-L-fucosidases which are thus involved in many important biochemical processes such as plant defense, inflammation, metastasis and the genetic disease fucosidosis. An interesting α-L-fucosidase has been identified in the digestive glands of the common marine mollusk *Pecten maximus*. The authors appreciated the high catalytic activity compared with other known fucosidases and the fact that their report seems to be the first about an enzymatic activity of fucosidase type that can efficiently release fucose from fucoidan (exo-acting activity) [75]. This enzyme has an interesting transfucosylase activity and is able to synthesize oligosaccharides higher than disaccharides by transglycosylation processes. The produced disaccharides, despite their low concentrations can undergo further transfucosylation events even in the presence of high concentrations of acceptor, leading to the formation of trisaccharides and in turn of tetrasaccharides. All new glycosidic linkages are of α-L type and highly branched products are formed [76]. This capability to synthesize glycooligosaccharides up to four units despite the high concentration of acceptor substrate present in the reaction, is also a characteristic of the α-glucosidase from *Aplysia fasciata* [65] (see above). Other α-L-fucosidases from marine environment were found: in *Charonia lampas* [77], *Venus mercenaria* [78], *Littorina littorea* [79], abalone liver [80], *Octopus vulgaris* [81], *Aplysia kurodai* [82] but none of

them was used to investigate any transfucosylation property as it would have deserved.

Enzymes endo-acting on fucoidans attracted also recent interest: these polysaccharides are known to exhibit a wide range of physiological and biological activities, including therapeutically useful actions (anti-inflammatory, antiviral, anticoagulant, antitumor, antiangiogenesis) [83 and references cited therein]. A marine microorganisms growing on fucoidan (*Sphingomonas paucimobilis*, PF-1) depolymerized the polymer into small fucose-containing oligosaccharides, ranging from 2 to 20 fucose units. It has been suggested the possibility that the enzyme is located on the surface of the cells. Enzyme preparation neither released the monomer L-fucose from the fucoidan nor hydrolyzed the chromogenic substrate *p*-nitrophenyl-α-L-fucoside, indicating the endo-acting fucoidanase functioning rather than an α-L-fucosidase activity. No mention about transfucosylation activity has been reported [83].

Among endo-acting enzymes, laminarinases from *Spisula sacchalinensis* and *Chlamys albidus* are of great interest to biotechnological applications. These enzymes catalyze glucanosyl transferase reactions providing the synthesis of poorly available β-glucooligosaccharides (β-1,3) and glycosides as well as branched glucans (β-1,3-β-1,6) such as translam, that in contrast to the initial laminaran possesses documented immonostimulating and anticancer activities. Efficient immobilization and action of these enzymes are also studied in novel hybrid polysaccharide silica nanocomposites [84].

Research interest for these endo-acting enzymes is very high as demonstrated by a very recent publication about isolation and characterization of two types of β-1,3-glucanases from a different marine organism, the common sea hare *Aplysia kurodai* [85].

Thermophilic marine microorganisms belonging to *Thermotoga* sp. possess interesting glycoside hydrolases often used in transglycosylation reactions [86–88]. A thermostable xylanase has been also found in *Geobacillus* sp. MT-1 a deep-sea microorganism [89] as well as an array of different polysaccharide degrading enzymes have been signalled in marine bacterium *Pseudomonas* sp. ND137 [90].

Mentioning of enzymes about chitin recycling is important. The polymer (chitin) is, after cellulose, the most abundant polymer in nature; it is an insoluble polysaccharide composed by β-1,4-linked N-acetylglucosamine units. All enzymes of the chitinolytic system are important and β-N-acetylglucosaminidase is a terminal enzyme of chitin degradation disassembling dimers or trimers to monomers of N-acetylglucosamine. These enzymes are widespread biocatalysts in marine environment but the presence in various taxa of marine phytoplankton seems to be worth to mention. In fact from an ecological point of view it suggests a relevant action of marine phytoplankton in chitin degradation and cycling in marine systems [91]. Enzymes of this type are known in crustaceans, crabs, prawns and other marine organisms [92,93].

Agarases are important enzymes in applications for food, cosmetics and medical industries and for the production of oligosaccharides from agar. Agar is the main matrix of various polysaccharides and consists of a linear backbone of alternating L- and D-galactose linked by α-1,3 and β-1,4 linkages respectively with various substituents such as sulfonic groups, methyl ethers and pyruvic acid. Agarases are known from a number of marine organisms. Interest for this class of enzymes is shown by recent reports of an enzyme capable of hydrolyzing jelled agar yielding simple low molecular weight products [94,95].

Fructose is a safe sweetener widely used in many foods and beverages instead of sucrose. Since fructose metabolism bypasses the metabolic pathway of glucose it does not require insulin, moreover fructose can be fermented into fuel ethanol. This monosaccharide can be obtained by acid or enzymatic hydrolysis of inulin. Inulinase (β-2,1-D-fructan fructanohydrolase, EC 3.2.1.7) attacks the β-2,1 linkage of inulin and hydrolyzes it into fructose. Complete hydrolysis of inulin using inulinase produces 95% pure fructose, thus these biocatalysts can be used for production and, at present, it is very important to obtain inulinases with high activity. Marine yeast *Pichia guilliermondii* mutated by UV light and lithium chloride, produced the highest inulinase activity reported up to now for the yeast [96]. Additionally the marine yeast *Cryptococcus aureus* isolated from sediment of China South Sea was found to secrete a large amount of inulinase into the medium [97]. Response surface methodology has been used for optimizing process parameters for high inulinase production [98].

An almost ubiquitous polymer such as hyaluronan composed by alternating units of N-acetylglucosamine and glucuronic acid is hydrolyzed by hyaluronidase. The importance of this polymer in medical fields and biomedical applications is due to the ability to form a highly viscous solution influencing the properties of extracellular matrices of vertebrates and as drug carrier. This importance has as consequence that the isolation of hyaluronan related enzymes has been reported from diverse sources as mammalian testis, venom of snakes, bees, scorpions and salivary glands of leeches. The first marine source documented is the venom of the stonefish *Synaceia horrida* [99]. The stonefish hyaluronidase is specific for hyaluronate but has no enzymic activity towards chondroitin sulfate or dermatan sulfate. This interesting enzyme which may have antitumor potential has been expressed in insect cells but not in *E. coli*.

3.2.2. Lipid active hydrolases

In this section hydrolytic enzymes of marine origin acting on lipid molecules (esterases, lipases, etc.) will be listed. This is a wide class of biocatalysts known for a long time and fruitfully adopted in solving many problems in organic synthesis (enantioselective hydrolysis and acylation reactions) at both lab scale and industrial processes. Lipases have emerged as key enzymes in food, chemical, pharmaceutical, cosmetic and detergent productions, leather processing, and biodiesel production since many years [100].

Marine originating enzymes of this type are hardly used although scientific interest has been present at least since four decades [101] and a review on production, characterization and gene cloning of the extracellular enzymes, including lipases, from marine-derived yeasts very recently appeared [102].

In an old study, marine fish species were recognized as possessing a general facility for hydrolysis of wax esters, followed by oxidation of the released alcohol and incorporation of the resulting fatty acid into acyl lipids [103]; this kind of study posed also the problem of localization of such enzymes in various segments of digestive tracts of marine organisms and of the contribution of highly lipolytic bacteria such as *Vibrio* sp. to fish digestive capability [104].

Lipases from marine yeast strains actively hydrolyze different oils, indicating that they may have potential applications in industry [105] and the same can apply for esterase from marine *Bacillus* sp. [106]. The production of a lipase has been optimized in another *Bacillus* species (*Bacillus pumilus* B106) which is associated to the south China sea sponge *Halichondria rugosa*. This enzyme was recognized active in high salinity, high temperature and at pH 8. Methanol (10–20%) exhibited a stimulatory effect on the lipase activity, while increasing amount of different solvents including methanol showed inhibitory action [107]. According to the listed characteristics, this marine lipase from the sea sponge associated *Bacillus pumilus* B106, may have great potential in marine biotechnology industry. Based on this and other study, sponge-associated microorganisms represent important sources for novel marine enzymes.

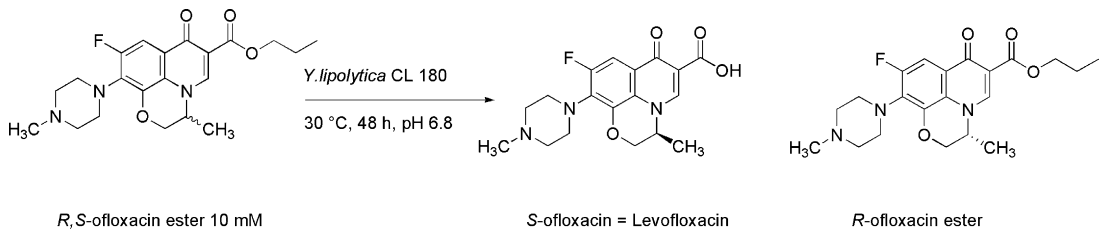


Fig. 8. Reaction scheme for the enantioselective resolution of *R,S*-ofloxacin propyl ester by *Y. lipolytica* CL180 esterase; the *S*-isomer called Levofloxacin is obtained as free acid in ca. 54% *e.e.*

Dissolved organic matter in aquatic environment is composed by lipids derived from biological production in 3–55% amounts. Lipid hydrolysis, by lipases produced by heterotrophic bacteria, is essential for lipid assimilation. With the aim of obtaining a better understanding of lipid–lipase interactions in these bacterioplankton communities in oceans, different interesting methods for measuring lipase activities in pure cultures of the marine strain *Alteromonas macleodii* were studied [108].

Two novel esterase genes were isolated from a marine microbial metagenomic library by functional screening, and the corresponding esterases were biochemically characterized. While one enzyme exhibited habitat-specific characteristics (high level of stability in the presence of various divalent cations and at high concentrations of NaCl), the other displayed remarkable activity against *p*-nitrophenyl esters and was highly stable in organic solvents such as methanol, ethanol, dimethylformamide, and dimethyl sulfoxide. The latter is a useful characteristic for industrial applications in fine chemical industry [109].

In a recent study on the uptake of estrogens by the blue mussels (*Mytilus edulis*), it has been found that after 13 days of exposure to radiolabeled 17-β-estradiol, in soft tissues of *M. edulis* the amounts of radiolabeled residues were 2428-fold higher compared with the ambient water concentration of these compounds. All the estrogen residues were present as lipohilic ester of C16 fatty acids indicating an high activity of an acyl transferase and other lipid-acting enzymes in the metabolism of these molecules by the mussels [113]. Esterase activity have also been signalled in crab *Scylla serrata* [114]. Isolation and characterization of bacteria that are able to efficiently remove lipids at low temperatures will provide insight into the possibility to use cold-adapted bacteria as a source of exploitable enzymes. The lipolytic activity of cold adapted antarctic marine bacteria was studied [115].

Levofloxacin, the *S*-isomer of ofloxacin, shows a broad spectrum of antibacterial activity against both gram-positive and gram-negative bacteria and the activity is doubled with respect to racemic ofloxacin. A recent study presents screening of marine organisms, from a variety of marine environments such as cold sea, hydrothermal vent area, sediment, tidal flat area, arctic sea. Cloning, overexpression, and biochemical characterization of a novel esterase from *Yarrowia lipolytica* CL180 was possible and the enantioselective resolution of racemic ofloxacin propyl ester using the recombinant enzyme (Fig. 8) was settled up [118].

In the field of seafood production amount of studies reported on the content of lipase activites in different parts of marine organisms and should not be neglected [125–127].

The sea squirt *Ciona intestinalis*, a marine tunicate, is of a particular interest among marine hydrolytic enzymes acting on lipids. The purpose of a study dated 2005 was to look for the presence and role of a complete system of cannabinoid receptors and presence of endogenous ligands (endocannabinoids), such as anandamide and 2-arachidonoylglycerol, binding to cannabinoid receptors. However endocannabinoids are metabolized by specific enzymes, such as fatty acid amide hydrolase, cyclooxygenase 2 and monoacylglycerol lipase. Indeed authors identified a fatty acid amide hydrolase and an amidase enzyme which could possess interesting new catalytic properties suitable for biocatalytic applications [128].

3.2.3. Phosphate active hydrolases

Alkaline phosphatase from shrimp *Pandalus borealis* is a well known enzyme used in molecular biology studies [129]. These enzymes act through a phosphoseryl intermediate producing free inorganic phosphate or transferring the phosphoryl group to other alcohols. More recently an enzymatic activity of the same type from *Pinctada fucata* has been reported [130] by the same group studying the enzyme from *Scylla serrata* [131]. A comparison between enzyme characteristics mostly related to optimal temperature and pH and to inhibitory effects of various elements, is very instructive for the employment of such enzyme for biocatalysis.

An highly active alkaline phosphatase was isolated from the marine bacterium *Cobetia* associated to the mussel *Crenomytilus grayanus*. Properties of the enzyme, such as a very high specific activity, no activation with divalent cations, resistance to inhibition by high concentrations of inorganic phosphate, as well as substrate specificity toward 5′ nucleotides are very interesting [132].

Bacterial alkaline phosphatases are important in organophosphate utilization in the ocean and a study on the subcellular localization of APases has been recently published [133].

Phytases represent another interesting type of enzymes belonging to the class of phosphate active hydrolases. A marine example has been reported in 2008. Phytases are phosphohydrolases catalyzing the release of phosphate from phytate (myo-inositol hexakisphosphate) which is the most abundant phosphorus form present in legumes, oilseeds and cereal grains. Incorporating the enzyme in animal food improves the quality in terms of availability of mineral, aminoacid and contribute significantly to environmental protection. Phytases can be added to fish diet in maricultural industry leading to significant improvement of wastewater treatment from these industries. After a large phytase screening of 327 marine yeast strains the authors indicated *Kodamea ohmeri* as the best producer and that phytase-producing marine yeasts as maricultural additives are more suitable than the added phytase from terrestrial counterparts [134]. Phytases can also have non-specific phosphate monoesters hydrolyzing activity [100] thus they could be used to solve different problems in fine chemicals. They could be able to serve as potential enzymes that can produce versatile lower myo-inositol phosphates of pharmaceutical importance [100,135].

3.2.4. Protein active hydrolases

Among hydrolases from the sea numerous articles dedicated to biocatalysts active on proteins appeared [11]. Crustaceans in particular are seen possessing immeasurable diversity in their digestive enzymes due to the adaptive response to many environmental factors. Proteolytic enzymes from crustaceans are noted for collagenolytic activity and raised attention in dermatological field. Marine fungi as sources of proteases have also been firmly established [136]. Many proteases from extremophililc organisms are also very well known [13].

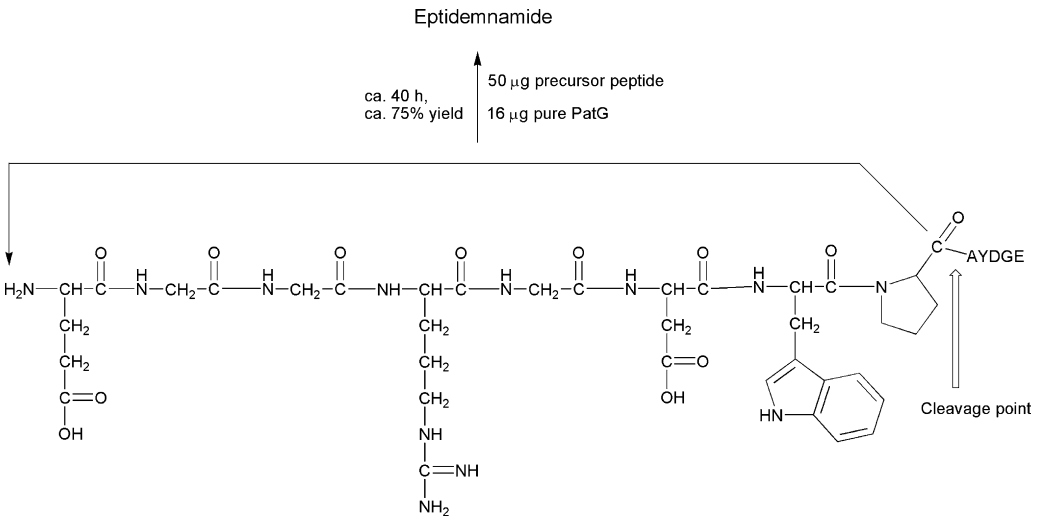


Fig. 9. PatG catalyzing the formation of eptidemnamide cleaving the source peptide into a cyclic compound and producing the small AYDGE peptide representing the recognition sequence.

Proteases are known since long time as useful catalysts in organic synthesis [27 and references cited therein], have also found many industrial applications and as anti-inflammatory and digestive agents in pharmaceutical field. Marine proteases differ from their counterparts in terrestrial cold-adapted organisms in being more active at lower temperature but less resistant to thermal denaturation. They are useful in detergent market and used also in the industry for the ripening of salted fish, fish sauces and marinades, modifying fish protein concentrations, and deskinning. Proteolytic preparations from different marine sources are used successfully in food processing [137].

Recombinant expression of three proteases, one xylanase and one lipase, from different strains of Antarctic sea water microorganisms in *E. coli* cells was reported [138].

In our Table 2 selected additions to the extensive list of known enzymes of this type are registered.

The stability and the effects of organic solvents on endopeptidases contained in the gastric fluid of the crab *Cancer pagurus* were studied thus assessing the positive enhancement of trypsin activity in the presence of 2-propanol with respect to vertebrate trypsin. Organic solvents can be advantageous in various industrial enzymatic processes. The use of organic solvents can increase the solubility of non-polar substrates, increase the thermal stability of enzymes, decrease water-dependent side reactions, or eliminate microbial contamination [139]. Enzymes remained stable for 127 days at 5 °C and seem suitable for different biotechnological application at neutral pH.

Interest in proteolytic sponge enzymes has been present for a long time [140]. More recently a protease from the marine sponge *Geodia cydonium* probably involved in the catabolism of collagen in the sponge, was purified from the aqueous extract. The enzyme was able to degrade casein, bovine collagen, and synthetic substrates and showed an extraordinary heat resistance [141].

Cyclic peptides are natural products known from marine ascidians, sponges and different genera of cyanobacteria. The biosynthesis of these diverse peptides, called cyanobactins, has been intensively studied and a recent report about proteins responsible for cyclization appeared. Two proteases have been recognized for cyclization process and no addition of energy (ATP) is required. Interestingly one of the two enzymes (PatG) is highly tolerant of diverse substrate sequences and more than 30 natural peptide sequences appeared to be cleaved and cyclized by this enzyme (eptidemnamide case in Fig. 9). This study is very important for the possibility that PatG could serve as a useful general biocatalyst for the cyclization of peptides [142].

Aquaculture could be very important as sources of enzymes for biocatalytic use at least for lab-scale testing. In this context it seems interesting to note as an example a study about the modification of digestive enzymes including proteases in trout in response to modification of diet using plant proteins [143].

3.2.5. Additional studies of interest

Other hydrolytic enzymes are of various type and are listed at the end of Table 2 [145–150,152]. Marine animals feeding on marine plants are known to secrete carbohydrate sulfatases as digestive enzymes that cleave the sulfate ester bonds in dietary polysaccharides. A sulfoesterase from the marine mollusk *Pecten maximus* has been applied to the study of fucoidan structure present in the brown algae *Ascophyllum nodosum*. This enzyme, present in the digestive glands of the marine mollusk, performed a desulfation reaction of sulfated L-fucopyranoside moieties of fucoidan. The results demonstrated a high regioselectivity of this sulfoesterase, for the hydrolysis of sulfate group at the 2-O position of the fucopyranosides. It can serve as a helpful tool in the structure-activity relationship of the fucoidan, where 2-O sulfation degree has been suggested to play a central role in the biological properties of the polysaccharide [145].

Arylsulfatases (E.C. 3.1.6.1) are enzymes hydrolyzing arylsulfate ester bonds producing the corresponding free phenols and sulfate; they are distributed in a wide range of organisms from mammals to bacteria. In the marine environment, arylsulfatase activities have been reported in algae but the digestive glands of various mollusks have also been found to be a rich source of arylsulfatase. The increasing demand for agar in the food and pharmaceutical industries is favouring many studies focused on the elimination of sulfate groups for improving the gel strength. The enzyme from marine bacterium *Sphingomonas* sp. AS6330 showed higher activity towards agar than other sulfated marine polysaccharides such as porphyran, fucoidan and carrageenan [146].

Epoxides and diols in pure stereochemical forms are of considerable importance in chiral synthesis of drugs and as general synthetic intermediates, thus great efforts in the study of catalysis by epoxide hydrolases have been carried out. Adopting the so called enantioconvergent process (Fig. 10), two different enzymes can be used each possessing different specificity for the attachment to the α- or β-carbons of an epoxide ring. In this method

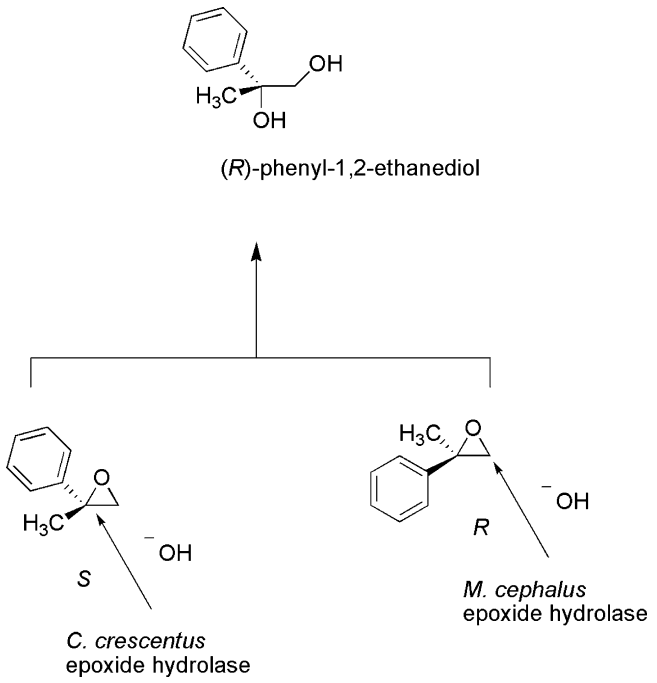


Fig. 10. Enantioconvergent process using two different enzymes (*C. crescentus* and *M. cephalus* epoxide hydrolases) with different specificity for the attachment to the α- or β-carbons of an epoxide ring (see text for details).

one enzyme acts on a specific carbon while the complementary biocatalyst hits the opposite, resulting in theoretically 100% yield of a diol with specific stereochemistry. It is clear that for this enantioconvergent application different sources of epoxide hydrolases to be used in combination are of extreme interest as they can possess different specificities. A recent success in the synthesis of (*R*)-phenyl-1,2-ethanediol from racemic styrene oxide involves the use of an epoxide hydrolase from the marine fish *Mugil cephalus* [148]. Combined use of plant and bacterial enzymes (*Solanum tuberosum* and *Agrobacterium radiobacter*) were experienced; in addition, *Caulobacter crescentus* enzyme has been also used [148 and ref. cited therein]. Enantioselective epoxide hydrolases activities were also found in the isolated microorganism *Sphingomonas echinoides* [149] and from another fish *Danio rerio* [150].

There are other enzymes of hydrolase family intensively studied such as acetylcholinesterases in bivalves, which are a useful biomarker of exposure to organophosphates (OP) in the marine environment [147]; these studies can be useful for developments of enzyme-based biosensor electrodes for organophosphate pesticide detection [151]; and the AMP-deaminase (AMP aminohydrolase, EC 3.5.4.6), the study of which, in different species, may help to elucidate the mechanism of ammoniogenesis and its coordination with other metabolic pathways [152].

3.3. Transferases

Among marine bacterial transferases attention in this section is for those functioning in the carbohydrate domain which are of a particular interest in synthesis. A review recently appeared on sialyltransferases from marine bacteria. In particular because enzymatic sialylation by sialyltransferase is a single-step process with excellent stereochemistry (high positional and anomer selectivity) and high yield under mild reaction conditions, these biocatalysts are believed to be among the most important enzymes in glycosynthesis and powerful tools for glycobiology studies. N-acetylneuraminic acid (Neu5Ac) is transferred from CMP-Neu5Ac to different positions of glycans or glycoconjugates including glycoproteins and glycolipids. The acceptor specificity of marine bacterial sialyltransferases is broad and this property is considered to be useful when various sialylated glycans are to be prepared [153]. Sialyltransferases of marine bacteria efficiently utilize glycosphingolipids. Sialyltransferases have unique features, including substrate specificities restricted to lacto- and neolactoseries of glycosphingolipids, as well as catalytic potentials for ganglioside synthesis [154].

A trehalose analog containing galactose has been synthesized using a trehalase synthesizing enzyme, a glycosyltransferase found in *Pyrococcus horikoshii*. The yield can be superior to 30% and it is function of the donor concentration (NDP-Glc) which can react with galactose forming the analog disaccharide α-D-glucopyranosyl α-D-galactopyranoside, used as inhibitor of trehalases [155].

Transglutaminases are transferases catalyzing the acyltransfer reaction in which the γ-carboxyamide groups of glutamine residues act as acyl donors and primary amino groups including ε-amino groups of lysine residues, either as peptide-proteins bound or free lysine, act as the acyl acceptors.

Linkages occur both intra- and inter-molecularly introducing covalent cross-linkages between the ε-amino groups of lysine residues and the γ-carboxyamide group of a glutamine residue in a protein molecule. These bonds are stable and resistant to proteolysis and these enzymes have found applications in functional improvements of various food proteins. Many examples of transglutaminases from marine organisms including fishes are known and high content of this enzyme is found in muscles of the tropical fish tilapia (*Orechromis niloticus*) [156].

Different transferases are part of biotransformation enzymes which are induced after long term exposure to polychlorynated biphenyls. The effects on UDP-glucuronyltransferases and other enzymes have been studied since long time in rainbow trout livers [157].

3.4. Isomerases

Just two examples will be mentioned for this type of enzymes from marine environments. D-Alanine, present as component of peptidoglycans, was also found in specialized peptides and antibiotics and is involved in the response to osmotic stress in several marine invertebrates. Indeed alanine racemase activity was detected in the homogenates of the tissues of these organisms. This enzyme has been purified to homogeneity from the hepatopancreas of the black tiger prawn, *Panaeus mondon*. This prawn enzyme is activated and stabilized by the presence of monovalent anions including chloride. This is consistent with the previous hypothesis that D-alanine serves as an osmoregulator in marine animals [158].

The conjugated olefinic system is present in many natural products including marine algae and the bioproduction of this structural characteristic, in the frame of secondary metabolism of fatty acids, has been of intense interest to research; a novel enzyme, which is a fatty acid isomerase from marine alga *Ptilota filicina*, was recognized. This enzyme is different from a mechanistic point of view from oxidative enzymes performing isomerizations in other organisms. The results of enzyme-catalyzed isomerizations using a broad range of substrates indicated that the enzyme orients the substrate in the catalytic pocket with respect to the methyl terminus and that it likely reacts, with the protonated form of the substrate [159].

3.5. Ligases

Polyamides are interesting compounds present in different marine organisms and enzymes related to their biosynthesis are grouped in the class of nitrogen-carbon binding enzymes belonging to ligases. Polyglutamic acid, polylysine and cyanophycin are

Table 3
Selected patents involving marine biocatalysts.

Patent	Comment	References
EP2103686A1	Isolation of hepatocytes from marine organisms. Useful in culture modeling in vitro and in biotechnological applications: proteomics and functional genomic studies.	[162]
WO91/19791	Protease from *Staphylothermus*. Potential applications in detergent industry.	[163]
WO2009108069	Hydrolases from fish offal. Applications in seafood farming.	[164]
EP1813678A1	Marine branching enzymes for glycogen production from amylose. Applications in food industry, in medicine, and in cosmetic.	[165]
EP1514923A1	F. marinus sulfatase and endo-type of α-L-fucosidase activity. Applications in biocatalysis: coupling reaction of small oligomers obtained to fluorescent material thus preparing useful molecular tools in glycotechnology	[166,167]
US2002/0156240A1 JP 2005192492	Processes for biocatalyzed production of an agarooligosaccharide	[168,169]
JP10295372	β-1,3-xylanase from *Alcaligenes* strain XY-234 for production of xylooligosaccharides from β-1,3-xylan	[170]
WO200814781A2	Enzymes producing hydrocarbons in the field of alternative source of biofuels	[171]
WO2007/093776	D9-elongase. D8-desaturase and D5 desaturase activities for processes for obtaining oils, lipids or free fatty acid	[172]
US6162626/1999	Reagent kit for ammonia determination by GDH from *Pyrococcus furiosus*	[173]
WO96/19569	Conversion of cephalexin to cephachlor with enzyme from *Rathayibacter biopuresis* isolated from the gut of the marine worm *Notomastus lobatus*	[174]
WO9804676 A2	Biocatalytic enzymes from the gorgonian *P. americana* or *P. elisabethae* efficiently transforming a variety of sterols, to their corresponding metabolites, e.g., 9(11)-secosteroid in high yield.	[175]

the most important polyamides and their stereochemistry plays a great role in relation to the polymer function. Thermoplasticity is significantly influenced by the homogeneity of stereochemical composition and enzymatic formation of these molecules is of great importance [160].

Current prospects for the synthesis of polyhydroxyalkanoates and production with enzymes from bacteria or archea (halophiles) found in marine-related niches have also been reviewed [161].

3.6. Lyases

Though not specific for a particular enzyme, an interesting study is present in literature concerning the nitrile modifying activity in deep-sea and terrestrial actinomycetes. Metabolic profiling and activity assays confirmed that all strains catalysed the hydrolysis of nitriles by a nitrile hydratase/amidase system [10]. These nitrile manipulating enzymes are of great interest in biocatalysis for productions of specialty chemicals in fine chemical industry and new enzymes with wide substrate specificity are particularly requested for this class of biocatalysts.

4. Patents

Many patents were found concerning use and applications of marine catalysts. From a scientific point of view, as opposed to technological perspective, the analysis of this material (Table 3) is of a certain interest in that it may show trends and characteristics of enzymes of marine origin convincing about their great potential in applicative biocatalysis.

5. Conclusion

The enormous pool of biodiversity which can be found in marine ecosystems is an excellent natural reservoir for acquiring an inventory of enzymes with potential for biotechnological applications as also reported in a “hot of press” review, mainly dedicated to new approaches of enzyme discovery [176].

As shown in the present review novel chemical and stereochemical properties found in examples of marine biocatalysts should be appended to the list of habitat related characteristics possessed by marine enzymes. Important examples have been found among oxidoreductases and carbohydrate active enzymes. In the first class of enzymes different stereoselectivity could be observed with respect to terrestrial counterparts adding value to the often observed usual resistance to high salt concentration and/or organic solvent resistance. In the realm of enzymes used by marine organisms to face environmental pollution, very interesting examples could be noticed which are characterized by a potent chemical action on non-activated carbon atoms difficult to manage by pure chemical routes. Also in these cases substrate preferences and positions of functionalization could differ from known examples.

Among enzymes acting on carbohydrates, potency of catalytic activity, in terms of large number of enzymes, and particular catalytic characteristics should be outlined. Among others the tendency about polyglycosylation is an interesting common quality of *Aplysia* α-glucosidase and other enzymes of marine origin although this aspect deserves in-depth examination.

As far as lipid active hydrolases are concerned, interesting unusual characteristics could be found among enzymes acting on wax esters coupled to cold adaptivity. The case of the novel esterase from *Yarrowia lipolytica* CL180 for the enantioselective resolution of racemic ofloxacin propyl ester is very important in this respect. Other hydrolytic activities specific for different substrates (proteins, phosphate esters, etc.) include very interesting examples above detailed. The spectacular examples of PatG, serving as a useful general biocatalyst for the cyclization of peptides, is remarkable. Great advantage from marine enzymes has been demonstrated by studies on epoxide hydrolase with the possibility of setting up an enantioconvergent application for the production of interesting diol with specific stereochemistry.

Details reported above on the characteristics of marine representatives belonging to other class of enzymes, further support the view that marine catalysts are just waiting to be discovered.

The importance of all examples reported and the many patents found concerning use and applications of marine catalysts in various technological fields remind us of the marine environment.

The potential of this habitat should be thoroughly known and possibly the way for access to an useful biocatalysts should avoid destructive large-scale collections of marine biomass for enzyme production. These two aspects are day by day becoming of interest and a future increase in the use of marine enzymes in biocatalysis should be expected.

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