Microbiological Research 208 (2018) 99–112

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Marine microbial L-asparaginase: Biochemistry, molecular approaches and applications in tumor therapy and in food industry

Fatemeh Izadpanah[a], Ahmad Homaei[b,*], Pedro Fernandes[c,d], Sedigheh Javadpour[e]

[a] Department of Marine Biology, Faculty of Sciences, University of Hormozgan, Bandar Abbas, Iran
[b] Department of Biochemistry, Faculty of Sciences, University of Hormozgan, Bandar Abbas, Iran
[c] Department of Bioengineering and IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
[d] Faculty of Engineering, Universidade Lusófona de Humanidades e Tecnologias, Av. Campo Grande 376, 1749-024 Lisboa, Portugal
[e] Department of Microbiology, Hormozgan University of Medical Sciences, Banddar Abbas, P.O. Box 13185-1678, Iran

ARTICLE INFO

Keywords:
L-asparaginase
Marine bacteria
Biotechnological application

ABSTRACT

The marine environment is a rich source of biological and chemical diversity. It covers more than 70% of the Earth's surface and features a wide diversity of habitats, often displaying extreme conditions, where marine organisms thrive, offering a vast pool for microorganisms and enzymes. Given the dissimilarity between marine and terrestrial habitats, enzymes and microorganisms, either novel or with different and appealing features as compared to terrestrial counterparts, may be identified and isolated. L-asparaginase (E.C. 3.5.1.1), is among the relevant enzymes that can be obtained from marine sources. This amidohydrolase acts on L-asparagine and produce L-aspartate and ammonia, accordingly it has an acknowledged chemotherapeutic application, namely in acute lymphoblastic leukemia. Moreover, L-asparaginase is also of interest in the food industry as it prevents acrylamide formation. Terrestrial organisms have been largely tapped for L-asparaginases, but most failed to comply with criteria for practical applications, whereas marine sources have only been marginally screened. This work provides an overview on the relevant features of this enzyme and the framework for its application, with a clear emphasis on the use of L-asparaginase from marine sources. The review envisages to highlight the unique properties of marine L-asparaginases that could make them good candidates for medical applications and industries, especially in food safety.

1. Why marine L-asparaginase?

Marine biosphere is one of the richest Earth habitats, accounting for 70% of Earth's surface. Yet is one of the least well characterized, although it provides the largest inhabitable space for organisms, hence for the production of secondary metabolites such as enzymes (Mohapatra et al., 1998; Homaei et al., 2013a; Homaei, 2015b; Homaei et al., 2016a; Homaei et al., 2016b; Parte et al., 2017).

Enzymes are proteins that catalyze biochemical reactions. As such, they are the catalytic cornerstone of metabolism and have a key role in general health but also in many manufacturing processes in a pattern that dates back millennia (Homaei et al., 2014; Homaei, 2015a, 2015b, 2015c; Homaei and Etemadipour, 2015; Abd El Baky and Baroty, 2016; Singh et al., 2016; Homaei, 2017). Accordingly, enzymes are the focus of intense and multidisciplinary research worldwide, involving not only the biological community, but also process designers/engineers, chemical engineers, and researchers working in other scientific fields (Bornscheuer et al., 2012; Woodley et al., 2013; Homaei and Saberi, 2015; Homaei and Samari, 2017; Homaei et al., 2017; Sheldon and Woodley, 2017). Similar to chemo-catalysts, enzymes speed up the rate of reactions without altering the thermodynamics. Unlike chemo-catalysts, enzymes display high chemo-, enantio- and regioselectivity, therefore minimizing the risk of side-reactions and formation of by-products, thus easing downstream processing. Additionally, enzymes can be easily produced in large-scale fermentations; they operate under mild temperature and atmospheric pressure, which rules out high energy requirements and complex equipment typical of chemo-catalysis; and are biodegradable. Given these features enzyme-based processes are considered eco-friendly (Tyler et al., 2006; Homaei et al., 2010; Homaei et al., 2013b; Grayson, 2016). Terrestrial organisms have been typicaly used as enzyme sources, yet in the last decades the vast diversity of marine organisms has been tapped for enzymes with novel or improved properties. Given the features of marine habits, enzymes produced by marine organisms, e.g. algae, bacteria, fungi, and sponges,

* Corresponding author at: Department of Biochemistry, Faculty of Science, University of Hormozgan, Bandar Abbas, P.O. Box 3995, Iran.
E-mail address: a.homaei@hormozgan.ac.ir (A. Homaei).

https://doi.org/10.1016/j.micres.2018.01.011
Received 22 December 2017; Received in revised form 23 January 2018; Accepted 28 January 2018
Available online 02 February 2018
0944-5013/

exhibit unique physiological properties such as hyper-thermal stability, barophilicity, salt and pH tolerance, adaptability to extreme cold conditions, and novel chemical and stereochemical properties. Moreover, most halobacterial enzymes, often found in marine organisms, are considerably thermotolerant and remain stable at room temperature over long periods (Mohapatra et al., 1998; Shojaei et al., 2017). These features enable marine enzymes to catalyze chemical reactions under extreme conditions, that prove deleterious to most of their terrestrial counterparts and confer upon marine enzymes a unique potential for relevant biotechnological applications, both in the manufacture of commercial products and in the health sector (Wang et al., 2016; Beygmoradi and Homaei, 2017; Parte et al., 2017; Sharifian et al., 2017b; Sharifian et al., 2017a; Trincone, 2017).

L-asparaginase (L-asparagine amidohydrolase, E.C. 3.5.1.1) is a hydrolase that primarily catalyzes the conversion of L-asparagine to L-aspartic and ammonia. This enzyme has also some L-glutaminase activity (El-Bessoumy et al., 2004; El-Sharkawy et al., 2016; Labrou and Muharram, 2016). Microbial L-asparaginase formulations for biomedical applications currently contribute to one-third of the global requirements for antileukemia/antilymphoma agents. This is far more than other therapeutic enzymes and L-asparaginases have been extensively used for anti-leukemia chemotherapy in acute lymphoblastic leukemia (ALL) disease, particularly in children (Fig. 1). As a result of the effective role of L-asparaginase as an antineoplastic agent in the treatment of ALL, it has been used as anti-tumor agent in non-Hodgkin's lymphoma, pancreatic carcinoma, and bovine lymphoma sarcoma, acute myelomonocytic leukemia, acute myelocytic leukemia, chronic lymphocytic leukemia, reticulum sarcoma, lymphosarcoma, and melanoma sarcoma (Broome, 1963; Ghoshoon and Raee, 2008; Ebrahiminezhad et al., 2014; Abd El Baky and Baroty, 2016; Kamala and Sivaperumal, 2017).

L-Asparaginase is also used to reduce the formation of acrylamide in cooked foods. Although his application is within the scope of food safety it is still cancer related, as acrylamide is a suspected carcinogen for humans (Cachumba et al., 2016; Xu et al., 2016).

Research work in the 1920s pointed out the potential of L-asparaginase in anticancer therapy (Clementi, 1922), yet the focus on L-asparaginase mostly traces back to the 1960s, when it was observed that this enzyme is the antitumor principle in guinea pig serum and that L-asparaginase purified from *Escherichia coli* had an antitumor activity similar to that of guinea pig serum (Broome, 1963; Mashburn and Wriston, 1964; Ghoshoon and Raee, 2008; Ebrahiminezhad et al., 2014). The later finding sparked a considerable interest in L-asparaginase from microorganisms (Ebrahiminezhad and Rasoul-Amini, 2011). Although this enzyme can be obtained from a wide array of natural sources, e.g. bacteria, yeasts, molds, plants, and vertebrates, microorganisms are preferred for the large-scale production of L-asparaginase and its use in clinical and industrial applications (Gupta et al., 2009a; Bonugli-Santos et al., 2015). Microorganisms known to produce this enzyme include *E. coli*, *Erwinia carotovora* (Warangkar and Khobragade, 2010), *Streptomyces* (Dejong, 1972), *Vibrio* spp. (Kafkewitz and Goodman, 1974), *Aerobacter* spp., *Bacillus* spp., *Photobacterium* spp., *Serratia* spp., *Xanthomonas* spp. (Peterson and Ciegler, 1969), *Pseudomonas aeruginosa* (El-Bessoumy et al., 2004) and *Aspergillus tamari* (Sarquis et al., 2004).

Clinical results have shown that currently used L-asparaginases from terrestrial microorganims cause toxicity and immunosuppression in addition to the development of resistance. Recently, L-asparaginase from marine bacterial sources have been suggested as promising candidates for application in the clinical and food areas due to features such as novel structures, lower molecular weights, and high substrate specificity (Sahu et al., 2007; Sun et al., 2016). Accordingly, dedicated research is underway towards the identification of asparaginases from marine microbial sources with such features, together with low toxicity. In the present review, the different marine sources of L-asparaginase and their classification, the structures of the enzymes isolated and their

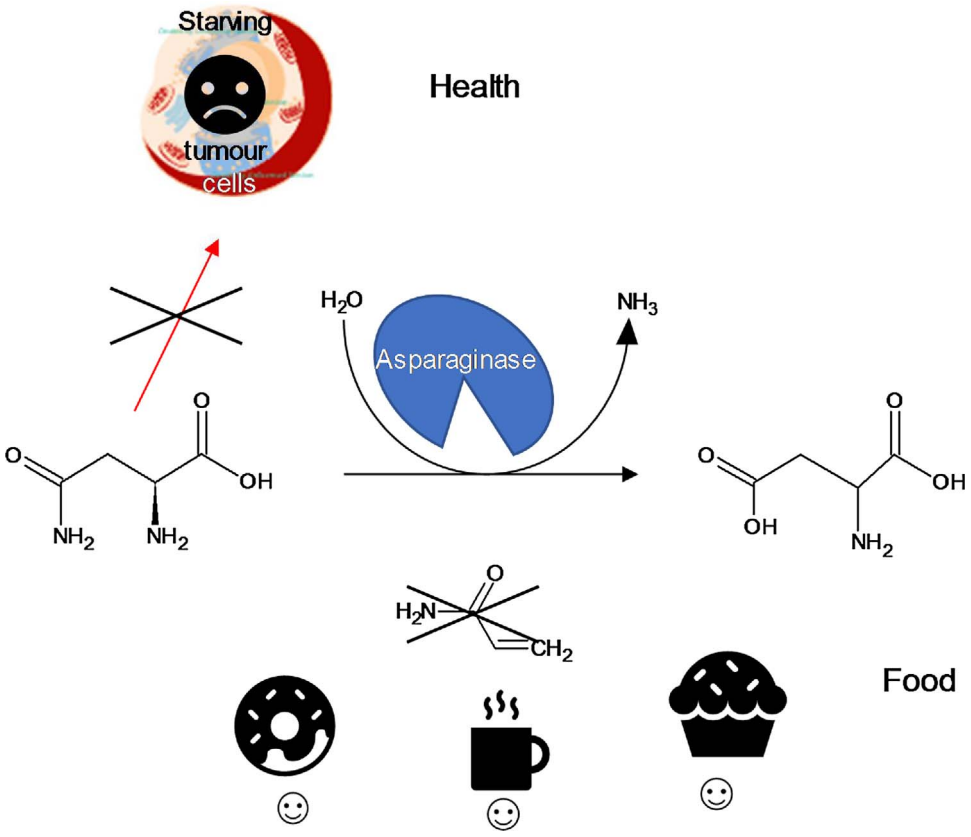

Fig. 1. L-Asparaginase hydrolyzes asparagine to aspartic acid, thus preventing feeding of asparagine to tumor cells and formation of acrylamide in heat-processed foods.