



Insight of Chitinolytic Cascade of Marine Bacteria: A Vista for Material Cycling and Valorization

Suman Kumar Halder*

Department of Microbiology, Vidyasagar University, West Bengal, India

*Corresponding Author: Suman Kumar Halder, Department of Microbiology, Vidyasagar University, West Bengal, India.

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Polysaccharides are simple and utilizable precursors in nature which microbes can readily metabolize to maintain their physiological carbon balance. Polysaccharides and its fragmented byproducts play the central role in energy flux, and for their metabolism a wide range of enzymes have evolved [1]. In nature a vast amount of enzyme produced by microbes depending upon the abundance of utilizable substrates, which gained importance day to day in various sectors. Efficiency and quantity of enzyme production can be improved several fold by enriching the cultural conditions, or by genetic/metabolic manipulation of the wild type strains.

Chitin is one of the most widespread natural structural bio-polymers (after cellulose) composed of repetitive N-acetylglucosamine unit linked by β (1,4) glycosidic bond [2,3]. It is water insoluble, nitrogen-bearing carbohydrate and often naturally found in composite form with other glucans, proteins, minerals etc [4,5]. Chitin as biomolecules found in fungi, arthropods, insects and molluscs [6,7]. Approximately 3/4 of the total weight of shellfish such as shrimp, prawn, crab and krill is considered as waste of which 20 - 58% is chitin [8]. Chitin is ubiquitous in the marine environment and it has estimated that, annually about 10^{11} metric tons of chitin is generated in the aquatic biosphere. Nevertheless, no substantial accumulation of chitin takes place in the ocean sediments as the chitinolytic microbes of the habitats essentially biodegrade and mineralize the chitin [7,9]. Chitinolytic enzymes have wide spectrum of distribution in nature including bacteria, fungi, nematodes, plants, insects, fish and human where they take parts in a variety of physiological processes including nutrition, pathogenesis, morphogenesis, quorum-sensing, parasitism, cell wall synthesis, growth regulation, cell division, defense and immunity [10].

Chitinolytic enzymes or chitinases can be classified into two major categories; exochitinases and endochitinases. Endochitinases (EC 3.2.1.14) cleave chitin at internal sites randomly, generating N-acetylglucosamine (GlcNAc) of low degree of polymerization, viz. chitotetraose, chitotriose and chitobiose. Exochitinases can be divided into two subcategories: chitobiosidases (EC 3.2.1.29, catalyzes the release of diacetylchitobiose starting at the non-reducing end of chitin microfibril), and β -(1, 4) N-acetylglucosaminidases (EC 3.2.1.30, cleaves the products of endochitinases/chitobiosidases

and generate GlcNAc) [11-13]. An alternative pathway involves the deacetylation of chitin to chitosan by chitin deacetylase (EC 3.5.1.41), which is finally converted to glucosamine residues by the action of chitosanase (EC 3.2.1.132) [12]. Analysis of the hydrolytic specificity suggested that chitinases belongs to the glycosyl hydrolase family 18 and 19 [7,14].

In the last two decades, exploration of the chitinolytic mechanisms of bacteria was extensively carried out especially on Gram negative *Vibrionaceae* [15-19]. In general, marine bacteria possess complex signal transduction systems for sensing chitin, adhering to chitinous substrate, degrading the chitin to chitosaccharides, transporting the latter to the cytoplasm, and catabolizing the transported products. Chitooligosaccharides in the surrounds probably act as a chemo-attractant that triggers adhesion of the bacteria on chitinous substratum. Secretion of endochitinases (chitin depolymerase) leads to fractional degradation of chitin to GlcNAc, chitobiose and chitooligosaccharides from the polymer outside the bacterial cell. After chitin digestion, the digested products presumably permeate the outer membrane of the bacteria through non-specific porin (for monomer and dimer) and substrate-specific porin termed 'chitoporin'. In the periplasm, β -N-acetylglucosaminidase and chitodextrinase act on translocated chitosaccharides to form a pool of GlcNAc and lesser extent chitobiose. Chitobiose bind with chitin binding protein (CBP), dissociate the latter from the ChiS sensor, activate the ChiS sensor which in turn up-regulates expression of the genes that comprise the chiS regulon. GlcNAc is transported across the inner membrane by the phosphoenolpyruvate transferase system and get phosphorylated. Chitobiose is transported via chitobiose ATP-binding cassette (ABC)-type permease. In cytoplasm, individual dimers and GlcNAc are phosphorylated by the cytoplasmic phosphorylase and kinase respectively to form N-acetylglucosamine-6-phosphate. The latter is converted into fructose-6-phosphate via the action of an N-acetylglucosamine-6-phosphate deacetylase and a glucosamine-6-phosphate deaminase, along with acetate and NH_3 as byproducts, respectively. The fructose-6-phosphate utilized for energy earning as it is intermediate of many carbohydrate metabolic pathway, whereas NH_3 utilized for production of amino acids [15-19].

One of the structural features of most chitinases is multidomain architecture; contain auxiliary carbohydrate-binding module (CBM) domains [20,21]. Chitin-binding properties have been reported in CBMs belonging to families 1, 2, 3, 5, 12, 14, 18, 19, 33, 37 and 50 [21]. CBM 5 is related to 12, and will hence be referred to as CBM 5/12. In bacteria, chitin-binding modules mainly belong to CBM33 and CBM 5/12. Chitinases often work synergistically with CBM. The CBM enhances the activity of the chitinolytic enzymes towards insoluble substrates by two mechanisms: (i) by anchoring the enzyme to the substrate and disrupting the crystalline structure of the substrate resulting in the formation of free chain ends, and (ii) help to act the enzyme in a processive manner by threading the attached chitin chain through their narrow catalytic cleft and performing several hydrolytic cuts instead of releasing the substrate after each cleavage [22].

Since the previous decade, chitinases have received great attention because of their multifaceted applications. So far, a number of chitinases produced by different types microorganisms were reported which shows expanded properties and multitude of applicability like isolation of protoplast of fungi and yeast, preparation of single cell protein, control of pathogenic fungi, production of bio-pesticides and control of mosquito propagation [3,12,13,23]. Moreover, the chitinolytic products (chitosaccharides and N-acetylglucosamine) have colossal therapeutic, medical, health promoting and other versatile applicabilities [7].

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