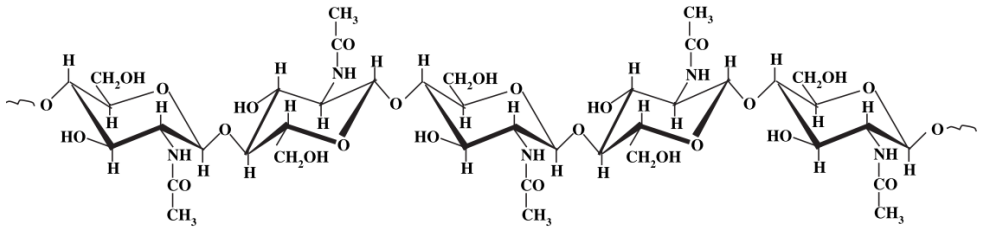
CHAPTER 2
LITERATURE REVIEWS

2.1 Chitin and Chitosan

Chitin is the second abundant biopolymer after cellulose, with a production of approximately 10^{10}–10^{12} tons annually (Zainol Abidin, Kormin, Zainol Abidin, Mohamed Anuar, & Abu Bakar, 2020). It is a glycan of β(1 ⟶ 4)-linked N-acetylglucosamine units, and it is widely distributed in crustaceans and insects as the protective exoskeleton and cell walls of most fungi (Ngo & Kim, 2014). Chitin is insoluble in water because of the highly extended hydrogen bonded semi-crystalline structure (Pillai, Paul, & Sharma, 2009). Chitin is arranged in three different microcrystalline structures; antiparallel (↑↓↑) sheets (α-chitin), parallele (↑↑↑) sheets (β-chitin) and a combination of both (γ-chitin), consist of two parallel strands which alternate with a single parallel strand (↑↑↓) (Rudall, 1963). The α-chitin is found in exoskeleton of arthropods, insects and fungal and yeast cell walls, while the β-form is mainly obtained from squid pen. The molecular arrangement of α-chitin is strongly packed with both inter- and intra-molecular hydrogen bonding, and it is the most stable form of the three crystalline variations, while β-chitin has weak intramolecular hydrogen bonding (Hackman & Goldberg, 1965). The degree of acetylation of chitin is >90%, the degree of polymerization is about 5,000–10,000, and its molecular weight can be 1,000–2,500 kDa (Kaczmarek, Struszczyk-Swita, Li, Szczęsna-Antczak, & Daroch, 2019).

a



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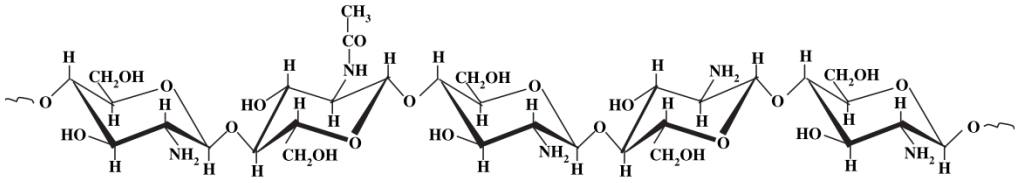

Figure 2.1 Primary structure of (a) chitin (b) chitosan (Harish Prashanth & Tharanathan, 2007).

Chitosan, a heteropolymer of D-glucosamine polymer (GlcN; D) and N-acetylD-glucosamine polymer, is a completely or partially deacetylated derivative of chitin (Harish Prashanth & Tharanathan, 2007). Chitosan can be classified according to degree of N-acetylation (DA) or fraction of N-acetylated residues (FA), the degree of polymerization (DP) or molecular weight (MW), the molecular weight distribution (PD) and the pattern of N-acetylation (PA) (Aam et al., 2010). The molecular weight of chitosan can be 10–1000 kDa (Moura, Moura, Soares, & Pinto, 2011). Chitosan is soluble in acid solutions such as acetic, formic, lactic, citric acids, and solvents such as dimethyl sulfoxide by structural modification; therefore, chitosan is a more suitable substrate for enzymatic bioconversion into chito-oligosaccharide (chitosan oligomers, COS or CHOS) than chitin, which must be dissolved in harsh acidic condition (Jagadish, Fabien, Stéphane, & Ada, 2017).

2.2 Chitosanase

Chitosanase (EC 3.2.1.132) is glycosyl hydrolases that catalyze the endohydrolytic of β-1,4-glycosidic bonds of partially acetylated chitosan to release chito-oligosaccharides (COS) (Thadathil & Velappan, 2014). Chitosan is a poly cationic natural polymer, an unbranched copolymer consisting of β-(1⟶4)-2-acetamido-D-glucose (N-acetyl-D-glucosamine, GlcNAc) and β-(1⟶4)-2-amino-D-glucose (D-glucosamine, GlcN), which can be found in nature as a structural component mostly in the cell wall of Zygomycetes fungi, Chlorophycean algae *Chlorella sp*., and in insect cuticle (Zitouni et al., 2013). Practical applications of chitosanase include the preparation of bioactive COS (Ming, Kuroiwa, Ichikawa, Sato, & Mukataka, 2006), preparation of fungal protoplasts mainly for Zygomycetes, a biocontrol agent to increase the resistance of plants against pathogenic fungi (Hsu, Chung, Chang, & Sung, 2012), chitosan mediated gene delivery and the bioconversion of marine crustacean chitinous bio waste (Wang, Tseng, & Liang, 2011). Enzymatic bioconversion of chitosan to COS is superior to chemical or physical methods by its low cost, environmental compatibility, reproducibility, and production of well-defined COS (Jitprasertwong et al., 2021; Pechsrichuang et al., 2013; Sak-Ubol et al., 2016), and so, chitosanase has a high demand across agricultural, food, medical, pharmaceutical, and cosmeceutical industries.

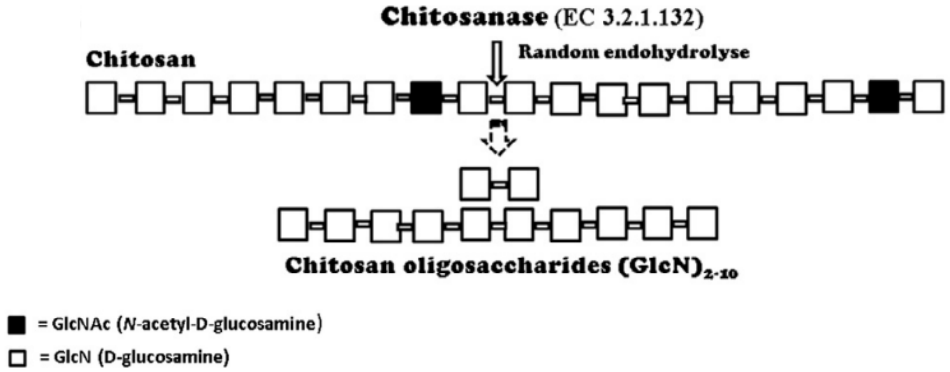

Figure 2.2 Chitosanase specificity (Thadathil & Velappan, 2014)

2.3 Chito-oligosaccharide

Chito-oligosaccharides (chitosan oligomers, COS or CHOS) are short chitosan polymers which has degree of polymerization varies from 2 to 20 units and the average molecular weight is <3.9 kDa (Liaqat & Eltem, 2018). In general, COS is fully soluble in water, partially soluble in methanol and dimethyl sulfoxide, and insoluble in acetone, butanol, ethanol, ethyl acetate, propanol, and pyridine (Phil, Naveed, Mohammad, Bo, & Bin, 2018). The properties of COS such as degree of deacetylation (DDA), degree of polymerization (PA), charge distribution, and nature of chemical modification are important factors influencing the biological activities of COS (Muzzarelli, 1996). COS have a diverse range of biological activities such as inhibition of fungi and bacteria growth (Aam et al., 2010), anti-tumor and immunity-enhancing effects (Naveed et al., 2019), enhancement of phytoalexin production in higher plants (Zhou et al., 2015), and food additive properties (Fang et al., 2024).

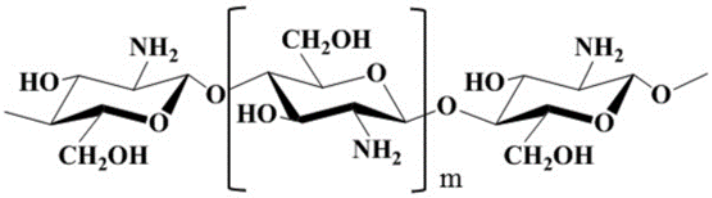

Figure 2.3 Chemical structure of chito-oligosaccharide (Vo, Ngo, Kang, Jung, & Kim, 2015)

2.4 Maltose Binding Protein

Maltose-binding protein (MBP) is a large (43 kDa) periplasmic and highly soluble protein of *E. coli* that acts as a solubility enhancer tag (Fox, Kapust, & Waugh, 2001; Kapust & Waugh, 2000). It has a native affinity property to handle target protein purification (Costa et al., 2014). MBP fusion vector, such as pMAL vectors by New England Biolabs, are available for cytoplasmic or periplasmic expression in all three reading frames, with factor Xa, enterokinase, or genenase I protease cleavage sequences (Kimple, Brill, & Pasker, 2013). In the study of MBP application, protein production can increase in comparison to commonly used tags e.g., the Fc, Glutathione

S-transferase (GST), SlyD, and serum albumin (ser alb) tag (Reuten et al., 2016). Moreover, the traditional antigen for immunological detection of deoxynivalenol in food and feed can be replaced by MBP fusion protein (Xu et al., 2018).

MBP allows one to use a simple capture affinity step on amylose-beads column, resulting in a protein that is often 70-90% pure (Lebendiker & Danieli, 2017). The MBP tag can enhance the solubility and expression of several difficult to express protein because of its large hydrophobic cleft that is able to alter its shape to accommodate different target proteins, promoting the latter's proper folding (Costa et al., 2014). The MBP is one of the most frequently used protein tags due to its capacity to stabilize, solubilize, and even crystallize recombinant proteins that are fused to it (Momin, Hameed, & Arold, 2019). Given that MBP is thought to be a highly stable monomeric proteins with known characteristics, fused passenger proteins are often studied without being cleaved from MBP (Momin et al., 2019).

2.5 Food Grade Expression System

Food grade expression systems are potential platforms for safety and efficient production of enzymes. There is some food grade expression system available based on FDA regulations, GRAS affirmation petitions, and GRAS notices (Z. Olempska-Beer, R. Merker, M. Ditto, & M. Dinovi, 2006). Those food grade expression systems are listed in Table 2.1. Nevertheless, these systems are still in a developmental process for the expression of enzyme from heterologous source in order to enable the reliable, efficient, and inexpensive production of high yields of enzymes (Wenzel, Müller, Siemann-Herzberg, & Altenbuchner, 2011).

Table 2.1. Different food-grade expression system available (based on FDA regulations, GRAS affirmation petitions, and GRAS notices) (Z. S. Olempska-Beer, R. I. Merker, M. D. Ditto, & M. J. DiNovi, 2006).

Source microorganism	Enzyme	Reference[a]
Aspergillus niger	Phytase	GRASP 2G0381
	Chymosin	21 CFR 184.1685
	Lipase	GRN 158
Aspergillus oryzae	Esterase–lipase	GRASP 7G0323
	Aspartic proteinase	GRN 34
	Glucose oxidase	GRN 106
	Laccase	GRN 122
	Lipase	GRN 43; GRN 75; GRN 103
	Pectin esterase	GRN 8
	Phospholipase A1	GRN 142
Bacillus licheniformis	α-amylase	GRASP 0G0363; GRN 22; GRN 24; GRN 79
	Pullulanase	GRN 72
Bacillus subtilis	α-acetolactate decarboxylase	21 CFR 173.115
	α-amylase	GRASP 4G0293; GRASP 7G0328
	Maltogenic amylase	GRASP 7G0326
	Pullulanase	GRN 20
Escherichia coli K-12	Chymosin	21 CFR 184.1685
Fusarium venenatum	Xylanase	GRN 54
Kluyveromyces marxianus var. *lactis*	Chymosin	21 CFR 184.1685
Pseudomonas fluorescens Biovar I	α-amylase	GRN 126
Trichoderma reesei	Pectin lyase	GRN 32

[a]GRASP, GRAS affirmation petition.