CHAPTER 1
INTRODUCTION

1.1 Significance of this study

Chitosanase (CsnA) or chitosan N-acetylglucosaminohydrolase (EC 3.2.1.132) is an enzyme which catalyzes the hydrolysis of β-1,4 glycosidic bond of chitosan, a recalcitrant waste from the seafood industry, to chito-oligosaccharides (COS/CHOS) (Aam et al., 2010; Dahiya, Tewari, & Hoondal, 2006; Khoushab & Yamabhai, 2010; Liu et al., 2009; Pechsrichuang, Yoohat, & Yamabhai, 2013; Thadathil & Velappan, 2014) which have a diverse range of biological activities such as inhibition of fungi and bacteria growth (Aam, 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, Cong, Zhou, Zhang, & Wang, 2024). Enzymatic bioconversion of chitosan to CHOS is superior to chemical or physical methods by its low cost, environmental compatibility, reproducibility, and production of well-defined CHOS (Jitprasertwong et al., 2021; Pechsrichuang et al., 2013; Sak-Ubol et al., 2016; Zhou et al., 2015) and so, chitosanase has a high demand across agricultural, food, medical, pharmaceutical, and cosmeceutical industries.

Recently, chitosanase production has been improved by recombinant DNA technology in which microbial chitosanase gene is cloned and expressed in a production host, leading to improvement in purity and yield of chitosanase (Sinha, Chand, & Tripathi, 2016). This approach leads to enhanced enzyme activity per unit as well as high specific activity (Sinha et al., 2016). We used recombinant 10xhistidine-tagged *Bacillus subtilis* chitosanase (*Bs*CsnA-10xHis) for both small scale and larger scale CHOS production and the anti-inflammatory activity of obtained CHOS mixtures were well-documented (Jitprasertwong et al., 2021; Yamabhai et al., 2024). Studies

have been done to improve the production of chitosanase in terms of expression, and purification (Table 1.1). For example, the chitosanase gene (*Bs*CsnA) from *B. subtilis* 168 has been expressed in *Escherichia coli* (Pechsrichuang et al., 2016; Pechsrichuang et al., 2013) as well as in *Lactobaciulus plantarum* (Sak-Ubol et al., 2016).

Table 1.1 Recombinant protein engineering for chitosanase production

Microorganism	Expression system	Secretion system by signal peptide	Selection marker	Epitope tag/ Fusion protein	Yield (mg/L)	Enzyme activity (U/mg)	Reference
Bacillus subtilis 168	*E. coli* TOP10	OmpA	amp^R	10x His tag	18.5	650	(Pechsrichuang et al., 2016)
Bacillus subtilis 168	*E. coli* TOP10	Native *Bacillus*	amp^R	10x His tag	0.4	650	(Pechsrichuang et al., 2016)
Bacillus subtilis 168	*E. coli* TOP10	OmpA	amp^R	10x His tag	14	904.7	(Pechsrichuang et al., 2013)
Bacillus subtilis 168	*Lactobacillus plantarum* WCFS1	Native *Bacillus*	amp^R	6x His tag	25	195	(Sak-Ubol et al., 2016)
Bacillus subtilis 168	*Lactobacillus plantarum* WCFS1	OmpA	amp^R	6x His tag	12	90	(Sak-Ubol et al., 2016)
Bacillus subtilis 168	*Lactobacillus plantarum* WCFS1	Native *Bacillus*	emr^R	6x His tag	79	800	(Sak-Ubol et al., 2016)
Bacillus subtilis 168	*Lactobacillus plantarum* TGL02	Native *Bacillus*	*alr*	6x His tag	39	800	(Sak-Ubol et al., 2016)

Commonly, recombinant chitosanase in expression medium is purified through IMAC (Immobilized Metal Affinity Chromatography) via 10xHis-tag (Pechsrichuang et al., 2016; Pechsrichuang et al., 2013; Sak-Ubol et al., 2016) which can be affected by the presence of strong reducing and chelating agents such as EDTA in the buffer system (Costa, Almeida, Castro, & Domingues, 2014). Alternatively, fusion of protein-of-interest to maltose-binding protein (MBP) followed by one-step purification using amylose beads is a safer method that may increase enzyme productivity (Lebendiker, 2011; Riggs, 2000). Maltose-binding protein (MBP) is a large (43 kDa) periplasmic protein of *E. coli* that can be used as a solubility enhancer tag (Fox, Kapust, & Waugh, 2001). In the present study, we engineered MBP-*Bs*CsnA, expressed it in *E. coli*, and used it to produce CHOS from chitosan.

1.2 Research objectives

1.2.1 Main objective

The main objective is to investigate the efficiency of recombinant *Bacillus subtilis* chitosanase-maltose binding protein fusion (MBP-*Bs*CsnA), which produce pure chitosanase as bioconversion of chitosan into chito-oligosaccharide (CHOS).

1.2.2 Specific objective

The main objective can be divided into 4 specific objectives as follows.

1.1. To investigate the recombinant MBP-*Bs*CsnA expression by *E. coli* TOP10

1.2. To obtain pure chitosanase by fusion of protein-of-interest to maltose-binding protein (MBP) followed by one-step purification using amylose beads

1.3. To characterize the property of recombinant MBP-*Bs*CsnA fusion which was expressed from *E. coli* TOP10

1.4. To investigate its potential application for the bioconversion of chitosan into CHOS

1.3 Scope of this study

This study focuses on the development of an efficient recombinant *Bacillus subtilis* chitosanase-maltose binding protein fusion (MBP-*Bs*CsnA), which is used for the bioconversion of chitosan into chito-oligosaccharide (COS). The recombinant chitosanase was fused with Maltose Binding Protein (MBP) to avoid using 6xHis or 10xHis, followed by one-step purification using amylose beads. In addition, chitosan from 2 companies, Morena and Marine Bioresource, Thailand, were used as a substrate to produce CHOS. Finally, the biological assay of CHOS was done in vitro.