CHAPTER 1

Introduction

Copra meal is the by-product of coconut oil extraction. On a dry matter basis, copra meal contains 25-30% β-mannan. The non-starch polysaccharides (NSPs) of copra meal are in the form of galactomannan (61%), mannan (26%) and cellulose (13%). The galactomannan in legumes, such as locust bean, guar bean and soybean, is found to be anti-nutritional polysaccharides (Sundu *et al*., 2006). Due to the anti-nutritive factor (ANF) property, copra meal cannot be fully utilized as feed ingredient for monogastric animals. Therefore, β-mannan in copra meal must be hydrolyzed by β-mannanase. Mannooligosaccharide (MOS) derived from copra meal β-mannan hydrolysis was reported to function as prebiotic that give beneficially effects on fecal bacteria of broiler (Khanongnuch *et al*., 2006). The same effects were also expected on human health (Yamabhai *et al*., 2014).

β-Mannanase or 1,4-beta-D-mannan mannanohydrolase (EC 3.2.1.78) is the enzyme that hydrolyses β-1,4 mannosidic likage in β-mannan molecule (Moreira, 2008). The enzyme is applied in various applications such as biobleaching of pulp and paper, textile, improvement of animal feed, and production of potentially health-promoting MOS (Yamabhai *et al*., 2014). Crude β-mannanase from *Bacillus subtilis* 5H was used to hydrolyze copra meal for MOS production (Khanongnuch *et al*., 2006).

Bacillus subtilis MR10 was previously reported as the β-mannanase producer (Wongputtisin *et al*., 2012). Furthermore, this bacterium also produces lipase during copra meal hydrolysis for MOS production, however, products from lipase activity in crude enzyme causes the unpleasant odor regarding the lipid hydrolysate which is an important limitation for food application.

Therefore, the mutagenesis of *Bacillus subtilis* MR10 by X-ray irradiation was conducted and a lipase defective mutant, *B. subtilis* M7, was obtained. The M7 strain maintained the same β-mannanase production but lipase activity was produced lower

(unpublished data). The β-mannanase produces by this mutant strain is expected to be used in copra meal hydrolysis for obtaining non-smelly MOS for food application.

The objectives of this study are:

1. Finding the optimal medium for β-mannanase production by *Bacillus subtilis* M7.
2. Purification and characterization of β-mannanase produced by this bacterial strain.