CHAPTER 1

INTRODUCTION

The recombinant plasmid pCSBC14 was constructed by Tanunat (1995). It was a clone of fragment from *Bacillus subtilis* TISTR25. Partially *Sau*3AI-digested chromosomal DNA from *B. subtilis* TISTR25 was ligated to *Bam*HI-digested pUC18 and transformed into *E. coli* JM109. The pCSBC14 was isolated using skim milk plate. The clear zone around the colony indicated the presence of the protease gene. The insert fragment of pCSBC14 was analysed by agarose gel electrophoresis. It was approximately 4 kb. The restriction mapping of the DNA insert was reported as shown in Figure 1.1. Protease activity was assayed by casein hydrolysis albeit it was very low. Characterization of protease gene type was studied by hybridization technique, using pNC3 and pKWZ (plasmids containing neutral and alkaline protease genes, respectively) as DNA probes. By comparison of the hybridization signals, the insert fragment was shown to be faint signal which was similar to both neutral and alkaline protease genes. The insert fragment of pCSBC14 was suspected to be a clone of protease gene.

DNA sequencing analysis in this study indicated that the insert fragment of pCSBC14 was not a protease gene but a L-glutamine D-fructose-6-phosphate amidotransferase (*gcaA*) gene. The *gcaA* gene encodes L-glutamine D-fructose-6-phosphate amidotransferase or glucosamine-6-phosphate synthase (EC 2.6.1.16). This enzyme catalyzes the formation of glucosamine-6-phosphate, and is the first and rate-limiting enzyme of the hexosamine biosynthetic pathway (Figure 1.2). It belongs to the family of amidotransferases that catalyse transfer of an amide group from glutamine to a substrate to form a new C-N bond. This enzyme is inactivated by the glutamine analogue, 6-diazo-5-oxo-L-norleucine (DON), and by iodoacetamide (Badet *et al.*, 1987). The eukaryotic glucosamine-6-phosphate synthase is subject to feedback inhibition by uridine 5′-diphosphate *N*-acetylglucosamine (UDP-GlcNAc),

the substrate for chitin synthase in fungi, some protozoon, and most invertebrates (Kornfeld, 1967). UDP-GlcNAc is a noncompetitive inhibitor with respect to both glutamine and fructose-6-phosphate, suggesting that the amidotransferase has a separate "allosteric" binding site for UDP-GlcNAc (Endo *et al.*, 1970). In mammalian cells, this enzyme is an insulin-regulated enzyme which controls the flux of glucose into the hexosamine pathway.

The product, glucosamine-6-phosphate ($GlcNH_2$-6-P), undergoes sequential transformations leading to the formation of UDP-GlcNAc, the major intermediate in the biosynthesis of all amino sugar containing macromolecules such as glycoproteins and chitin (β1-4 homopolymer of N-acetylglucosamine) both in prokaryotic and eukaryotic cells. Glucosamine is a common component of many macromolecules in the cell envelopes of both bacterial and animal cells, including peptidoglycan, lipopolysaccharide, and teichoic acid in bacterial cell envelopes, and glycolipids and glycoproteins in animal cell membranes. In higher eukaryote, glucosamine can help the body repair eroded and damaged cartilage. Since glucosamine stimulates the body's manufacture of collagen, the protein portion of the fibrous substance that holds joints together, it is used to repair damaged joints for Osteoarthritis (Crolle and D'Este, 1980; D'Ambrosio *et al.*, 1981). Furthermore, glucosamine can be used as a precursor for the synthesis of modified nucleosides in medicine treatment. For example, the *C*-nucleosides tiazofurin, showdomycin, pseudouridine, formycin, *etc.*, show therapeutically useful antitumour agents. In addition, certain structurally modified nucleosides such as 5-fluorouracil, ribovirin and AZT (3'-azido-3'-deoxythymidine) are strong drugs for antiviral agents (Jung *et al.*, 1990).

The L-glutamine D-fructose-6-phosphate amidotransferase (glucosamine-6-phosphate synthase) encoded by *gcaA* gene for *B. subtilis* 168, *glmS* gene for *Escherichia coli* (Walker *et al.*, 1984), *nodM* gene for *Rhizobium melliloti* (Baev *et al.*, 1991), *GFA1* gene for *Saccharomyces cerevisiae* (Watzele and Tanner, 1989) and *Candida albicans* (Smith *et al.*, 1996), and, *GFAT* gene for human (McKnight *et al.*, 1992) and mouse (Sayeski *et al.*, 1994; Sayeski *et al.*, 1997).

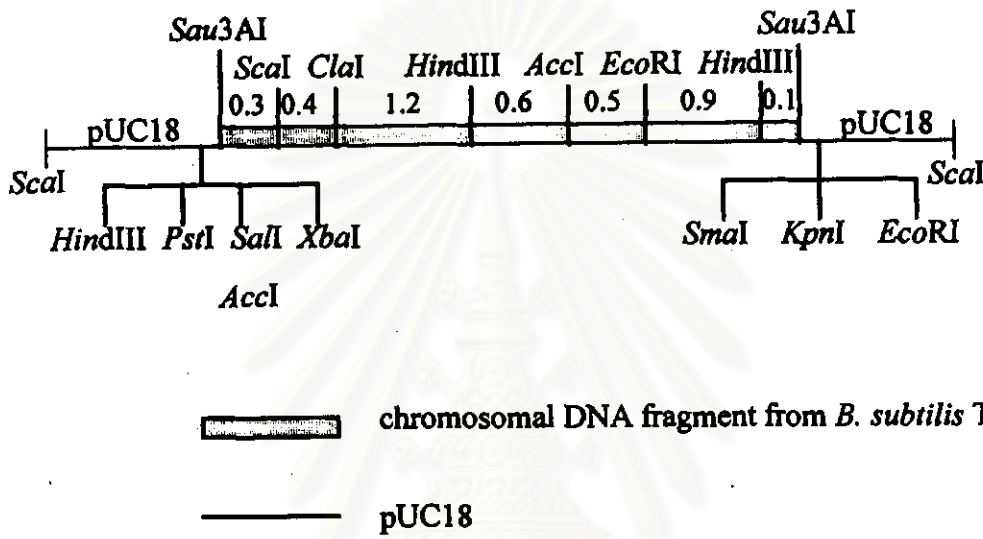

Figure 1.1 Restriction map of pCSBC14 (Tanunat, 1995)

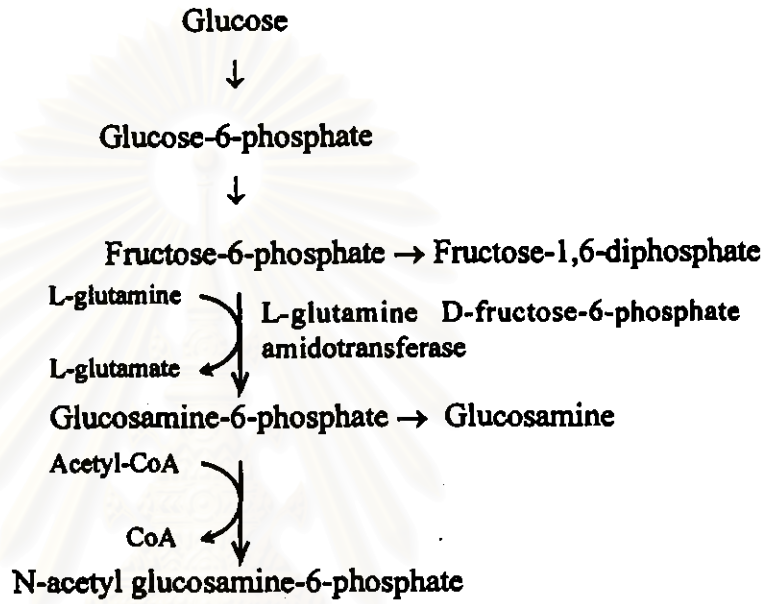

Figure 1.2 Hexosamine biosynthetic pathway