CYCLODEXTRIN GLYCOSYL TRANSFERASE

Cyclodextrin glycosyl transferase (CGTase), EC 2.4.1.19, is an extracellular enzyme that converts starch into non-reducing, cyclic malto-oligosaccharides called cyclodextrins (CDs). It is an important hydrolytic enzyme that carries out reversible intermolecular as well as intramolecular transglycosylation and performs cyclization, coupling and disproportionation of maltooligosaccharides (Biwer et al., 2002). Cyclodextrins have their systematic names of cyclic α-d-(1,4)-linked d-glucose oligosaccharides consisting of 6–8 glycosyl units, well known as α-, β- and γ-CDs (Bender, 1986). CD molecules have the ability to form inclusion complexes with a variety of compounds therefore they are used in a wide range of application in food, pharmaceutical, cosmetic and agricultural industries (Szejtli, 1992; Hedges, 1992).

CGTase is produced by species of Bacillus, Brevibacterium, Clostridium, Corynebacterium, Klebsiella, Micrococcus, Pseudomonas, Thermoanaerobacter and Thermoanaerobacterium (Szejtli, 1992): All the CGTase enzymes produce α-, β- or γ-CDs from starch in different ratios. Enzymes that can synthesize predominately one type of CD are preferred for industrial applications because separation of individual CDs from their mixture is expensive (Parsiegla et al., 1998). In most organisms studied, the enzymes are extracellular enzymes, which differ from one another not only in the properties but also in the relative amounts of products formed from starch. The β form of cyclodextrins is reported to be more suitable for industrial use because the inclusion complexes are easily prepared and very stable due to the low solubility in water of β-cyclodextrin. In addition, the yield of β-cyclodextrin from starch is usually higher than that of other cyclodextrins. Thus, high CGTase activity and a good ratio of products
formed are desired (Piamsook and Mitsuo, 1978). The enzyme has been studied by many workers, in order to elucidate the reaction mechanism for the production of cycloamyloses (Shoicki et al., 1978).

French and his co-workers worked extensively on the mechanism of the enzyme to learn, e.g., the co-substrate for the coupling reaction, the action pattern of disproportionation, and the conversion of cyclohexamylose into cycloheptaamylose. The action of this enzyme was considered to be complex, because the enzyme seems to catalyze at least three reactions, namely cyclization, coupling, and disproportionation. There have been very few reports on the action pattern and mechanism of the purified enzyme (Shoicki et al., 1978).

Types of Reactions

Cyclomaltodextrin glucanotransferase is a unique and a multifunctional enzyme, which catalyzes at least three different reactions:

- Intra-molecular transglycosylation (cyclization and coupling)
- Intermolecular transglycosylation (disproportionation) and
- Ring opening of cyclodextrins

Although the enzyme has been studied by many workers in order to elucidate the reaction mechanism for these reactions, there is a report on the reaction mechanism of the enzyme. Recently, the genes for CGTase from the chromosomal DNA of some species of Bacillus were cloned. The site-directed mutagenesis and crystallographic study will make it possible to elucidate the reaction mechanism of CGTase (Kunihiro et al., 1991).

The characteristics of CGTase to transglycosylate various aglycone molecules like stevioside, rebaudioside, etc. are gaining attention of researchers in the field of
biotransformation (Kochikyan et al., 2006; Park et al., 1998; Sato et al., 1992; Riva, 2002).

Mechanism of action of CGTase

CGTases catalyse mainly two intermolecular transglycosylation reactions: coupling, in which a cyclodextrin ring is cleaved and transferred to an acceptor maltooligosaccharide substrate and disproportionation, in which a linear maltooligosaccharide is cleaved and the new reducing end sugar is transferred to an acceptor maltooligosaccharide substrate. In addition the enzyme has a weak hydrolyzing activity (Penninga et al., 1995; Van der Veen et al., 2000) (Figure 1.1).

Applications of CGTases

The most important application of CGTases is for synthesis of cyclodextrins (Szejtli, 1988). Cyclodextrins are cyclic oligosaccharides consisting of six α-cyclodextrin, seven β-cyclodextrin, eight γ-cyclodextrin or more glucopyranose units linked by α-(1,4) bonds. Figure 1.2a, 1.2b and 1.2c represents the structure of different types of cyclodextrins. α-, β- and γ-cyclodextrins are composed of six, seven and eight α-(1,4)-linked glycosyl units, respectively (Villiers, 1981). They are also known as cycloamyloses, cyclomaltoses and Schardinger dextrins (Villiers, 1981; Eastburn and Tao, 1994). Among the three types of cyclodextrin the β-Cyclodextrin is the most accessible, the lowest-priced and generally the most useful. It is of high interest due to the size of its non polar cavity which is suitable to accommodate many molecules such as aromatics and drugs; its low solubility in water which facilitate its separation from the reaction mixture (Sonia Jemli et al., 2007).
The circles represent glucose residues; the white circles indicate the reducing end sugars.
(a) Cyclization, (b) coupling, (c) disproportionation, (d) hydrolysis

Fig 1.1 Schematic representation of the CGTase catalyzed reactions
Cyclodextrins have many applications particularly in food (Fujishima et al., 2001), pharmaceuticals (Bhardwaj et al., 2000), cosmetics (Holland et al., 1999), environment protection (Lezcano et al., 2002), bioconversion (Dufosse et al., 1999), packing and the textile industry (Hedges, 1998). The potential guest list for molecular encapsulation in cyclodextrins is quite varied and includes such compounds as straight or branched chain aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gases, and polar compounds such as halogens, oxyacids and amines (Nash, 1994).

The major advantages of these applications are as follows:

- Protection of the active ingredient(s) against oxidation, light induced reactions, decomposition and thermal decomposition.
- Elimination (or reduction) of undesired tastes or odours, microbiological contaminations.

The applications of cyclodextrins include

- Cyclodextrins are mainly used in cosmetic preparation to suppress the volatility of perfumes, room fresheners and detergents by controlled release of fragrances from inclusion compounds.
- They are used in food formulations for flavour protection or flavour delivery. They are also used as process aids to remove cholesterol from milk, butter and eggs.
- In pharmaceuticals they have their ability to enhance drug delivery through biological membranes. They have been used successfully in aqueous dermal formulations (Uekama et al., 1992), aqueous mouth wash solutions (Kristmundsdottir et al., 1996), nasal drug delivery systems (Kublik et al., 1996)
and several eye drop solutions (Loftson and Stefansson, 1997; van Dorne, 1993; Jarho \textit{et al.}, 1996).

- In agriculture industries they can be applied to delay germination of seed.
- In the chemical industry they are widely used to separate isomers and enantiomers, to catalyse the reaction to aid in various processes and to remove or detoxify waste materials.
- They also increase the tackiness and adhesion of some metals and adhesives. They also make additives and blowing agents compatible with hot melt systems.

CGTases are also used to synthesize linear oligosaccharides and their derivatives by its coupling and disproportionating reactions (Kobayashi, 1996). A new transglucosylated derivative of thiamin has also been synthesized by the reaction of CGTase and a glucoamylase with dextrin and thiamin (Uchida and Suzuki, 1998).
Fig 1.2a. Structure of $\alpha$-Cyclodextrin
Fig 1.2b. Structure of β - Cyclodextrin
Fig 1.2c. Structure of $\gamma$ - Cyclodextrin
**Aims and objectives**

The demand for cyclodextrin glycosyltransferase grew because of their widespread use in food, cosmetic, plastic, paint and pharmaceutical industries. The cost of CGTase and hence that of cyclodextrin are quite high. And there is a lack of availability of suitable high yielding indigenous industrial strains. To overcome such limitations attention has been devoted for studies on CGTase to tackle the problem. Recent approaches for increasing CGTase yield including screening for naturally occurring enzymes with intrinsic stability or to produce stable enzymes by means of protein engineering and optimization of fermentation media through a statistical approach (Prakasham et al., 2005) are some of them. The simplest approach to obtain a stable enzyme is to look for the desired enzyme in a readily available organism. Hence great interest has been generated in the search for new *Bacillus* strains and their fermentation conditions optimization by using statistical methods to get more economical yields for industrial applications.

Keeping the above mentioned points in view, the present investigations have been undertaken to isolate a novel CGTase producing microbial strain and subsequent development of an ecofriendly, efficient and economically suitable fermentation process to produce high productivity titers by applying statistical methods.
Chapter I

Introduction

Objectives

The present investigation was carried out to isolate a novel CGTase producing strain and to develop suitable fermentation process to yield high productivity by applying statistical methods.

- Screening and isolation of CGTase producing bacteria from soil samples.
- Characterization of isolated species by biochemical methods and using 16S rRNA analysis.
- Optimization of physiological and nutritional conditions for the production of CGTase by conventional method in submerged fermentation.
- Strain improvement studies to increase the productivity of CGTase.
- Optimization of nutritional and environmental parameters employing statistical methods like Taguchi method, Placket-Burmann Design and Response Surface Methodology.
- Production of CGTase by immobilization of whole cells.
- Purification and characterization of CGTase.