CHAPTER I

INTRODUCTION

1.1 Oligosaccharides

1.1.1 Chemical composition of oligosaccharides

Oligosaccharides consist of short chains of monosaccharide units (typically 2-20 units) joined by characteristic linkage called glycosidic bonds (Nelson and Cox., 2000). Since monosaccharides have multiple hydroxyl groups, various glycosidic linkages are possible. Complex carbohydrates are information-rich molecules due to wide array of these linkages in concert with the wide variety of monosaccharides and their many isomeric forms (Berg *et al.*, 2002).

Various types of oligosaccharides have been found as natural components in many common foods including fruits, vegetables, milk and honey (Nakakuki, 2002). Three abundant and well known disaccharides are sucrose, lactose and maltose. Sucrose (common table sugar) is commercially produced from cane or beet. In this disaccharide, the anomeric carbon atoms of a glucose unit and a fructose unit are joined so that the configuration of this glycosidic linkage is α for glucose and β for fructose. Lactose, the disaccharide of milk, consists of galactose joined to glucose by a β-1,4-glycosidic linkage. In maltose, two glucose units are joined by an α-1,4-glycosidic linkage (Berg *et al.*, 2002).

Several oligosaccharides such as starch-related, sucrose-related and lactose-related oligosaccharides have been commercially developed (Table 1.1). In addition, xylo-oligosaccharides, manno-oligosaccharides and chitin/chitosan oligosaccharides have been produced from various polysaccharides such as xylan, agar, mannan, chitin and chitosan as the raw materials. At present, these oligosaccharides have been widely utilized in foods, beverages and confectioneries due to their various useful properties (Table 1.2).

1.1.2 Properties of oligosaccharides

Oligosaccharides are widely known are functional food ingredients that have a great potential to improve the quality of many foods. In addition to providing useful modifications to physicochemical properties of foods, oligosaccharides have various physicological functions such as the improvement of intestinal microflora based on the selective proliferation of bifidobacteria, stimulation of mineral absorption, anticariogenicity, and the improvement of both plasma cholesterol and blood glucose level (Nakakuki, 2002). Functional properties evaluated until now are as summarized in Table 1.2.

Oligosaccharides are water soluble, they are typically 0.3–0.6 times as sweet as sucrose. The sweetness of sugar depends on chemical structure, the degree of polymerization and the levels of mono- and disaccharides in the mixture (Crittenden and Playne, 1996 ; Voragen, 1998). The sweetness decreases with the longer oligosaccharide chain length. Sugars with low sweetness property are useful in various kinds of foods where the use of sucrose is restricted by its high sweetness property (Roberfroid and Slavin, 2000). Low sweet sugars may be used as bulking

agents in conjunction with artificial sweeteners such as aspartame or sucralose, for example, with the advantage to mask the aftertastes produced by some of these intense sweeteners. In addition, when compared with mono- and disaccharides, the bigger size oligosaccharides provide an increase in viscosity, leading to improved body and mouthfeel properties. The oligosaccharides can also be used to alter the freezing temperature of frozen foods, and to control the intensity of browning due to Maillard reactions in heat-processed foods. They also provide a high moisture-retaining capacity, preventing excessive drying, and a low water activity, which is convenient in controlling microbial contamination (Crittenden and Playne, 1996).

For various classes of oligosaccharides, stability can greatly differ, depending on the sugar residues present, their ring form and anomeric configuration and linkage types. Generally β-linkages are stronger than α-linkages, and hexoses are more strongly linked than pentoses. Nevertheless, at pH < 4.0 or at high temperature treatments or prolonged storage, oligosaccharides present in food can be hydrolyzed resulting in loss of nutritional and physicochemical properties (Voragen, 1998).

Table 1.1 Various kinds of commercially available oligosaccharides (Nakakuki, 2002)

Raw material	Product
Starch	Malto-oligosaccharides: maltose ~ maltoheptaose Isomalto-oligosaccharides: isomaltose, panose, isomaltotriose Cyclodextrins (CDs): α-CD, β-CD, γ-CD, HP-β-CD, branched CDs Others: maltitol, gentio-oligosaccharides, trehalose, nigerose
Sucrose	Glycosylsucrose, fructo-oligosaccharides, palatinose (isomaltulose), lactosucrose, xylosucrose, raffinose, stachyose, trehalulose
Lactose	Galacto-oligosaccharides, lactosucrose, lactulose, lactitol
Xylan, agar, mannan, chitin, chitosan, etc.	Xylo-oligosaccharides, agaro-oligosaccharides, manno-oligosaccharides, chitin/chitosan oligosaccharides, etc.

Table 1.2 Properties of oligosaccharides (Nakakuki, 2002)

Physicochemical property	Sweetness, bitterness, hygroscopicity, water activity, reinforcement agent for drinks, stabilization of active substances (protein, flavor, color, etc.), inclusion capability, etc.
Biological property	Digestibility, nondigestibility, noncariogenicity, anticariogenicity, bacteriostatic action, selective proliferation of bifidobacteria, improvement of serum lipids, and blood glucose, etc.
Other properties	Specific substrate for enzyme, enzyme inhibitors, elicitors, etc.

Most oligosaccharides are hydrolyzed in the upper part of the gastrointestinal tract. The resulting monosaccharides are then transported via the portal blood to the liver and, subsequently, to the systemic circulation. Such carbohydrates are essential for health as they serve both as substrates and regulators of major metabolic pathways. However, some oligosaccharides have specific physicochemical properties and resist to the normal digestive process, they then reach the caeco-colon intact and utilized by probiotic microorganisms (see section 1.3).

1.2 Prebiotics

"Prebiotic" is defined as a dietary ingredient that reaches the large intestine in an intact form and has a specific metabolism directed towards advantageous (beneficial) rather than adverse (pathegenic) bacteria. This would lead to a marked change in the gut microflora composition. At present, food component which seems to exert the best prebiotic effect are the non digestible oligosaccharides (NDOs). These are oligomeric carbohydrates, the osidic bond of which is in a spatial configuration that allows resistance to hydrolytic acitivities of intestinal digestive enzyme, but are sensitive to metabolic effects of colonic bacteria (probiotic see section 1.3) These microorganisms can then ferment the carbohydrate to produce short chain carboxylic acids and gases, as well as increase metabolic energy, growth and proliferation (Roberfroid, 1997). NDOs that present bifidogenic function, and are commercially produced were grouped into 13 classes (Table 1.3) (Sako *et al.*, 1999).

Table 1.3 Non-digestible oligosaccharides with bifidogenic functions commercially available (slightly modified from Crittenden and Playne, 1996 and Sako *et al.*, 1999)

Class of oligosaccharide	Estimated[a] production in Japan in 1995 (ton)	Molecular structure[b]
Cyclodextrins	4,000	$(Gu)_n$
Fructo-oligosaccharides	12,000	$(Fr)_n$-Gu
Galacto-oligosaccharides	15,000	$(Ga)_n$-Gu
Gentio-oligosaccharides	400	$(Gu)_n$
Glycosylsucrose	4,000	$(Gu)_n$-Fr
Isomalto-oligosaccharides	11,000	$(Gu)_n$
Isomaltulose (or palatinose)	5,000	$(Gu\text{-}Fr)_n$
Lactosucrose	1,600	Ga-Gu-Fr
Lactulose	20,000	Ga-Fr
Malto-oligosaccharides	10,000	$(Gu)_n$
Raffinose	200	Ga-Gu-Fr
Soybean oligosaccharides	2,000	$(Ga)_n$-Gu-Fr
Xylo-oligosaccharides	300	$(Xy)_n$

[a] Data were obtained by surveying major manufacture of food-grade NDOs.
[b] Ga, Galactose; Gu, Glucose; Fr, Fructose; Xy, Xylose.

It is difficult to evaluate of an acceptable dose of prebiotics because each individual has his own feeling about acceptable and non-acceptable intestinal discomfort. Excessive consumption doses of prebiotics may cause intestinal discomfort, flatulence or even diarrhea because of their osmotic effect, which may transfer water into the large bowel, and because of their high fermentation rate and production of gases (Roberfroid and Slavin, 2000). For example, galacto-oligosaccharides consumption higher than 20 g/day, and fructo-oligosaccharides consumption higher than 40 g/day are reported to cause diarrhea (Sako *et al.*, 1999).

1.3 Probiotics

Probiotics are living microorganisms (mostly bacteria and yeasts) in human and mammalian gut that are beneficial to health (Holzapfel and Schilinger, 2002). At present, they are widely used as dietary supplements. Commonly used probiotics include lactic acid bacteria (LAB) (such as *Lactobacillus acidophilus*, *L. casei*, *L. lactis*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. salivarius* and *L.johnsonii*) as well as various bifidobacteria (such as *Bifidobacterium animalis*, *B. infantis*, *B. lactis*, *B. longum* and *B. breve*), non-pathogenic strains of *Escherichia coli* or *Enterococcus* spp. (Fig 1.1), and *Saccharomyces* spp (Vouloumanoua et al., 2009).

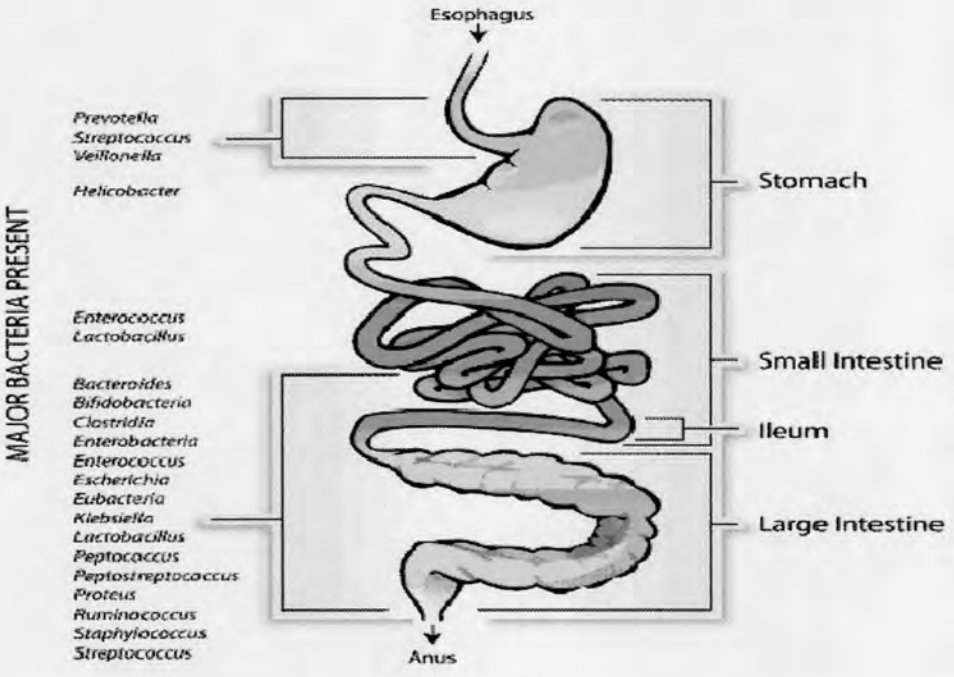


Figure 1.1 Showing the beneficial bacteria in different parts of human and mammalian gut (Madigan and Martinko, 2005)

In small intestine, a larger bacterial load that consists of facultative anaerobes such as lactobacilli, streptococci, and enterobacteria as well as anaerobes such as *Bifidobacterium* spp., and clostridia at level ~10^4-10^8 CFU/ml. The most heavily colonized region, however, is the colon, with a total population of 10^{11}-10^{12} CFU/ml of contents (Cummings *et al.*, 1989). Consisting of higher levels of obligate anaerobes and lower levels of facultative aerobes (Rastall, 2004), the colonic microflora is very complex.

In terms of health, the most significant organisms are believed to be the bifidobacteria, the major component of the microbial barrier to infection (Gibson and Roberfroid, 1995). Bifidobacteria produce a range of antimicrobial agents that are active against Gram-positive and -negative organisms. Lactobacilli are also health positive and produce a range of antimicrobial agents, but they are present in much lower levels in the human colon. In addition to producing antimicrobial agents, a large population of beneficial bacteria competitively excludes pathogens by occupying receptor sites and competing for space, nutrients, etc (Rastall, 2004).

1.3.1 Utilization of prebiotics by probiotics

Throughout the gastrointestinal traet, pre- and probiotics work together to inhibit pathogenic bacteria, to alter the rate and extent of digestion and absorption of nutrients, to modify the barrier functions of the intestinal epithetlium, to enchance the immune system, to modulate ecology of gut microflora, and to improve overall health of colonic cells (Figure 1.2). The possible mechanisms of prebiotic action are illustrated in Figure 1.3.

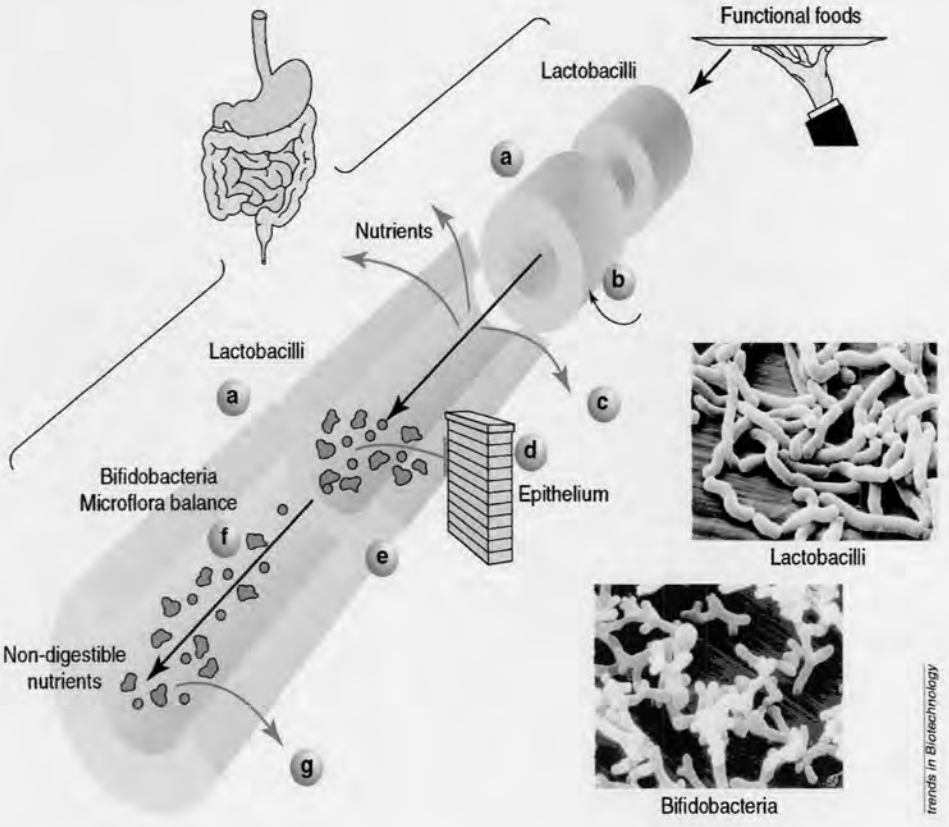


Figure 1.2 Targets throughout the gastrointestinal tract for functional-food ingredients. (**a**) Pre- and probiotics inhibit pathogenic bacteria at various sites, from Helicobacteria pylori in the gastric mucosa to Salmonella sp. and Clostridia sp. in the intestine. (**b**) Multiple ingredients alter the rate and extent of digestion of nutrients. (**c**) The absorption of nutrients and anti-nutritional factors throughout the stomach and intestine is affected by the presence, form and activity of functional-food components. (**d**) Pre- and probiotics modify the barrier functions of the intestinal epithelium. (**e**) Nutrients, from vitamins and minerals to probiotics, interact with and enhance the functions of gastrointestinal immune cells and, via systemic communication, the entire body's immune system. (**f**) Pre- and probiotics modulate the overall ecology of the gut microflora. (**g**) Fermentation products of fibers or non-digestible oligosaccharides and other components from the microflora not only nourish the intestine but also improve the differentiation, maturation and overall health of colonic cells. Adapted from F. Rochat, unpublished(German et al., 1999).

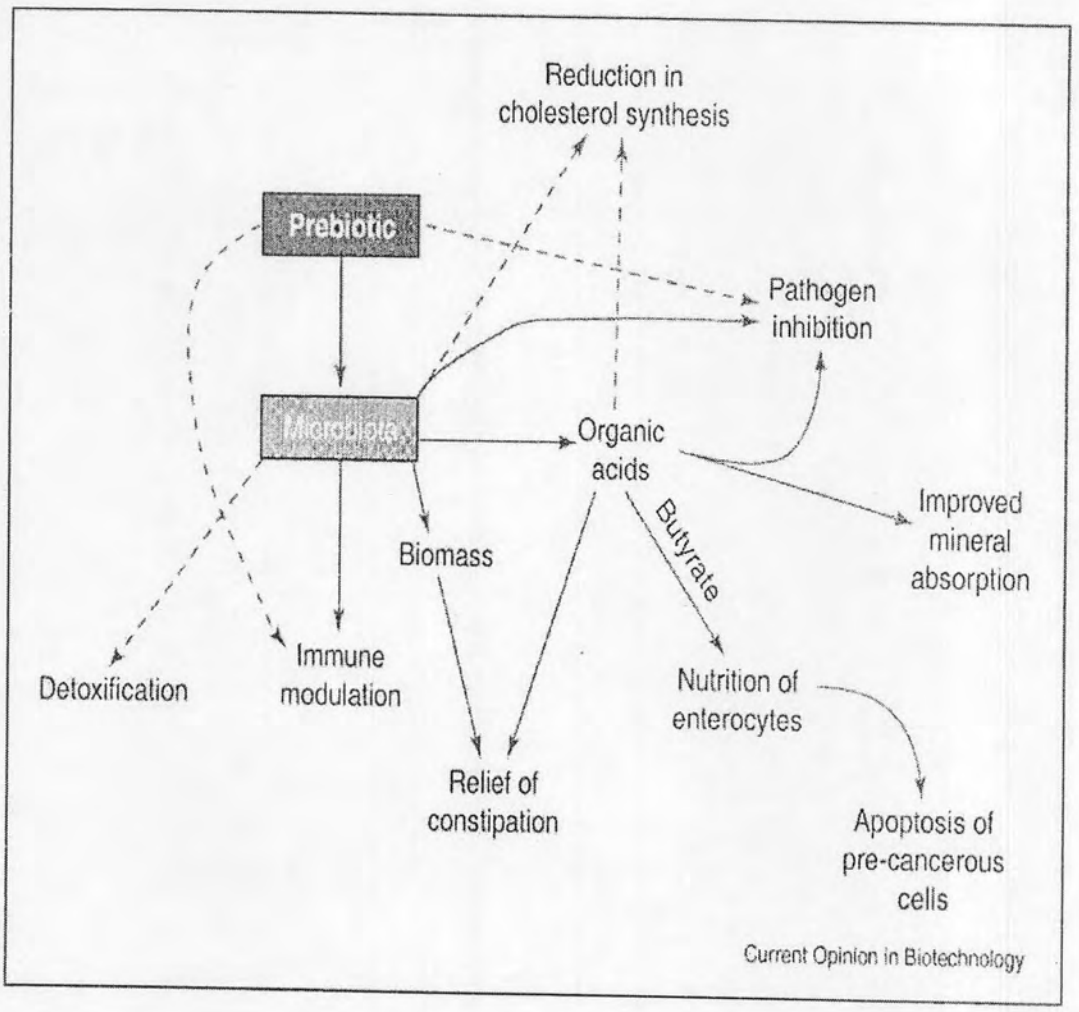


Figure 1.3 Schematic showing the possible mechanisms of prebiotic action. Solid lines indicate relatively well-established modes of action. Dotted lines indicate less well-established or speculative modes of prebiotic action. (Oywehand *et al.*, 2005)

1.4 Oligosaccharide production

1.4.1 Chemical production

Chemical methods for the synthesis of oligosaccharides are well developed (Paulsen, 1984; Schmidt, 1986). However, carbohydrates contain multiple hydroxyl groups of similar reactivity, so chemical methods for synthesis are complicated by the many protection steps that are necessary for regioselective synthesis (Figure 1.4).

The number of synthetic steps increases with the size of oligosaccharide, e.g. while synthesis of a disaccharide may require five to seven steps, a trisaccharide may require more than ten steps. Total yields are often low and large-scale synthesis is not practical. In addition, stereospecific reactions giving the correct anomer (α or β) of oligosaccharide products required are often difficult (Nilsson, 1988).

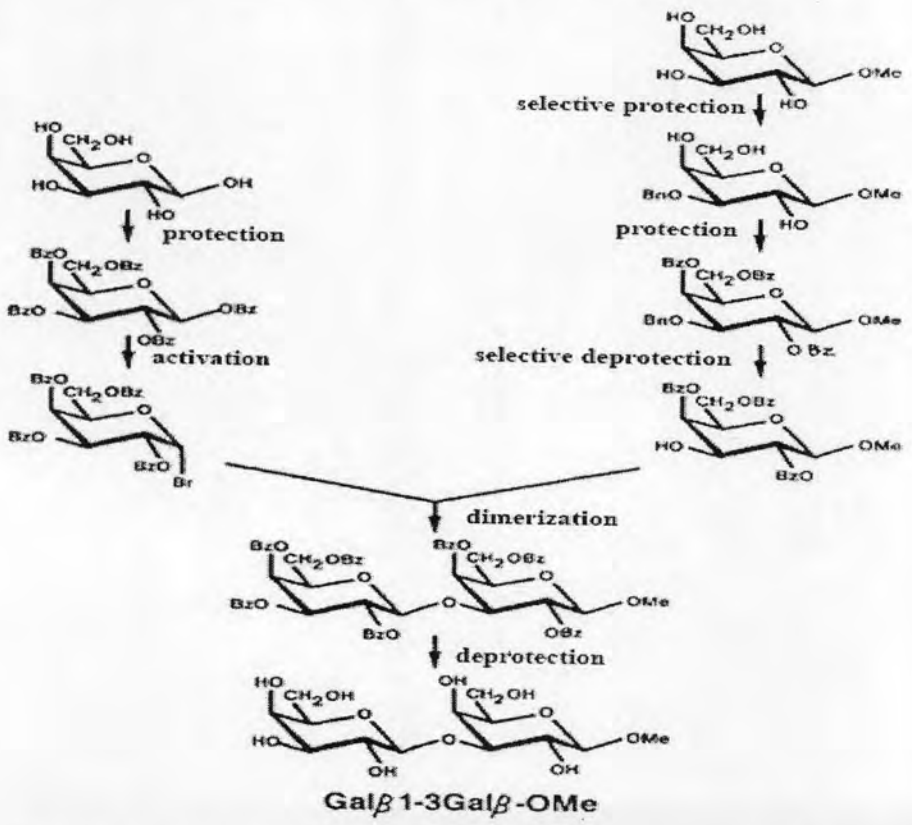


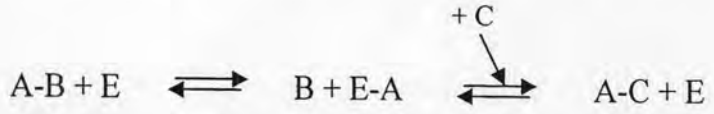
Figure 1.4 Typical chemical synthesis of a disaccharide (Nilsson, 1988)

1.4.2 Enzymatic production

The use of enzymes in the synthesis of complex carbohydrates offers several advantages over the chemical methods. Through enzyme catalysis, regiospecific and regioselective reactions can be efficiently proceeded without protection of the hydroxyl groups. These reactions are taken place under mild conditions, often at room temperature and close to neutral pH, and organic solvents and hazardous chemicals or catalysts can be avoided. Production of the enzyme catalyst by fermentation facilitates large scale synthesis while immobilization of the enzyme allows reuse. Two types of enzyme have been used for synthesis of complex oligosaccharides, the glycosyltransferase (EC 2.4) and the glycosidase (EC 3.2) (Nilsson, 1988).

1.4.2.1 Glycosyltransferase

Glycosyltransferase (EC 2.4) catalyzes the stereo- and regiospecific transfer of a monosaccharide from a donor substrate (glycosyl nucleotide) to an acceptor substrate. This method of oligosaccharide synthesis has the advantage of high efficiency and selectivity. The major drawbacks are the requirement for a complex glycosyl donor and the relative inaccessibility of the glycosyltransferases; moreover, the nucleotide sugar required is costly. Long chain oligosaccharides are usually produced by this reaction (Nilsson, 1988; Crout and Vic, 1998). The energy necessary for the synthesis reaction is provided by the original osidic bond and stored as a covalent glycosyl-enzyme intermediate E-A (Monsan and Paul, 1995).



A-B, donor carbohydrate or glycoside; E, glycosyltransferase; C, acceptor

Some glycosyltransferases such as cyclodextrin glucosyltransferase (EC 2.4.1.19), fructosyltransferase or levansucrase (EC 2.4.1.10) do not require nucleotide sugar for activitation of the donor substrate (Maitin *et al.*, 2004). Their uses for industrial oligosaccharide synthesis become real after these enzyme have been commercially produced.

1.4.2.2 Glycosidase

Glycosidase (glycosylhydrolase) (EC 3.2), a hydrolase, is responsible for the hydrolytic cleavage of glycosidic bonds in nature. The glycosidase, in general, is divided into two groups: exoglycosidases cleave glycosidic bonds at the nonreducing end of the oligosaccharides, and endoglycosidases cleave internal glycosidic bonds. The advantage of utilizing glycosidase is that this group of enzyme required relatively simple glycosyl donors and generally more available and less expensive. Short chain oligosaccharides are generally produced by this enzyme (Nilsson, 1988; Ichikawa *et al.*, 1992; Crout and Vic, 1998).

The hydrolytic activity of glycosidase can also be used for preparation of oligosaccharides from larger carbohydrate structure. Oligosaccharide synthesis is carried out either as an equilibrium-controlled or as a kinetically-controlled process.

1.4.2.2.1 Equilibrium-controlled synthesis

Equilibrium-controlled synthesis is reversion of the hydrolytic reaction (reverse hydrolysis reaction). In reverse hydrolysis (sometimes called the thermodynamic approach) a high concentration of reactant, high temperature, low water activity and long incubation time are used to drive the reaction towards

equilibrium (Nilsson, 1988; Monsan and Paul, 1995; Crout and Vic, 1998). This approach constitutes a very cost-effective and simple procedure, these is very attractive for industrial scale-up (Crout and Vic, 1998).

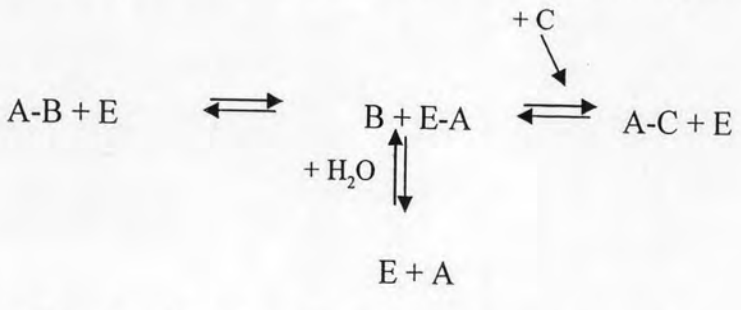
$$A\text{-}H + B\text{-}OH \rightleftharpoons A\text{-}B + H_2O$$

where A and B are glycosyl moieties

Glycosidases are widely available and some are highly thermostable. This allows operating at high temperature. The main limits of the reverse hydrolysis approach are the low activity of glycosidase in concentrated carbohydrate solutions, the low yield of carbohydrate conversion and the production of isomer mixture (Monsan and Paul, 1995).

1.4.2.2.2 Kinetically-controlled synthesis

Kinetically-controlled synthesis (transglycosylation) uses glycosidase enzyme to catalyze the transfer of a glycosyl moiety from a glycoside derivative onto an acceptor molecule, which generally contains a hydroxyl group:



A-B, glycoside donor, E, Glycosidase; C, acceptor.

In this approach, the reaction efficiency is kinetically controlled, as the product, A-C, is a potential substrate of the glycosidase enzyme (Monsan and Paul, 1995). However, the donor glycoside is consumed during the reaction and its reuse is not possible. Hydrolysis reaction competes and the maximum yield depends on the rate of product formation relative to the rate of hydrolysis (Nilsson, 1988).

Thus, the enzymatic synthesis can result in a wide variety of oligosaccharideshas. Transfer reactions usually take place from a specific donor to different acceptors forming structurally different acceptor products without mediation of nucleotide-activated sugars and/or cofactors. Typical transfer reactions in carbohydrate bioconversion have usually been found in dextransucrase (EC 2.4.1.5), fructosyltransferase (E.C.2.4.1.10) and galactosyltransferase (E.C.3.2.1.23) (Kim *et al.*, 2001).

1.4.3 Current commercial oligosaccharide production process

Industrial production processes of NDPs have been established, either by extracting the NDOs from natural sources, by hydrolyzing polysaccharides, are by enzymatic or chemical synthesis from disaccharide substrates.With the exception of soybean oligosaccharides and raffinose (which are produced by direct extraction) and lactulose (which is produced by isomerization reaction), the NDOs are manufactured through enzymatic processes. They are either "built up" from simple sugars, such as sucrose or lactose, by enzymatic transglycosylation reactions, or formed by controlled enzymatic hydrolysis of polysaccharides, such as starch or xylan (Figure1.5) (Sako *et*

al., 1999). Typically, cheap oligosaccharides. such as sucrose, lactose and starch-derived oligomers, are utilized as donors. These processes usually produce a range of oligosaccharides differing in degree of polymerization and sometimes in the position of the glycosidic linkages. Unreacted substrates and monosaccharides are also present in the reaction mixtures together with oligosaccharide products. Such contaminating sugars are often removed by membrane or chromatographic procedures to yield higher-grade products that contain purer oligosaccharides (Crittenden and Playne, 1996).

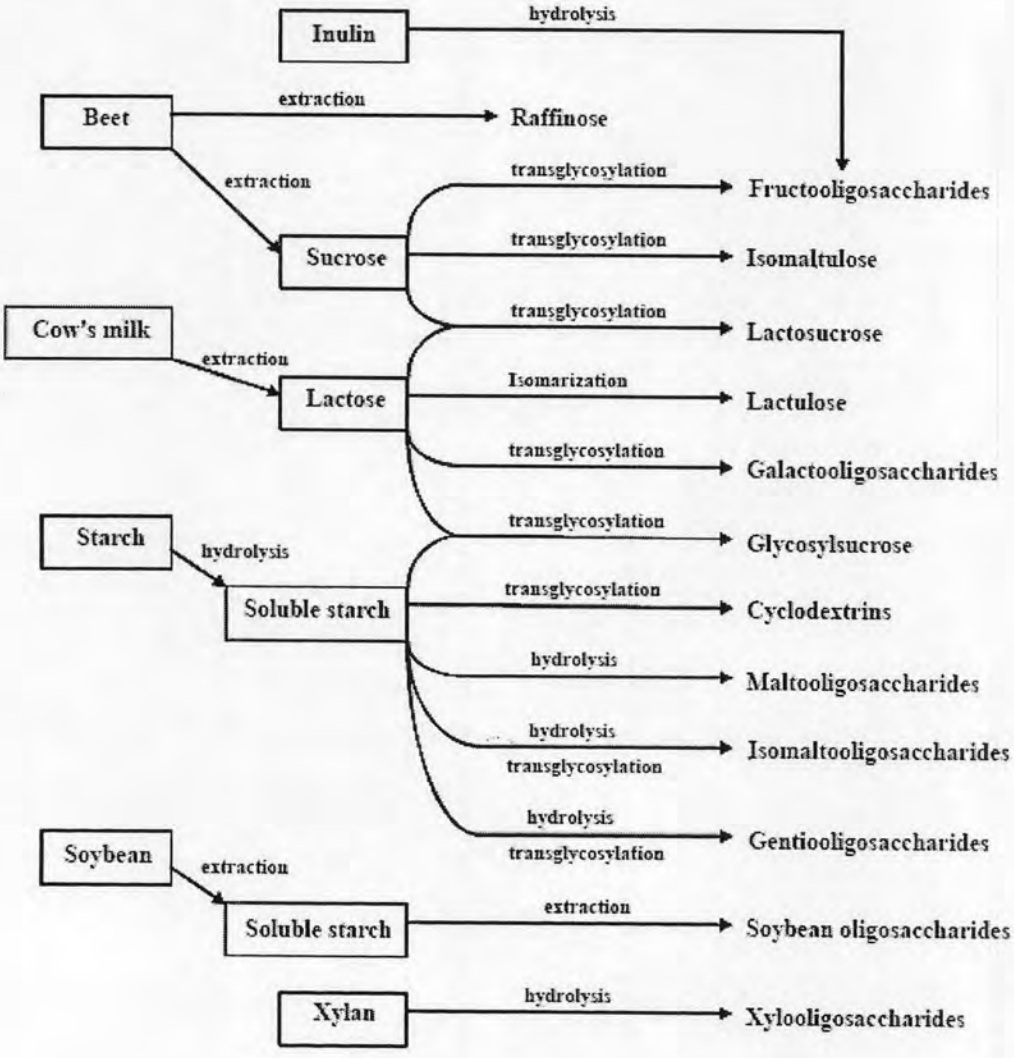


Figure 1.5 Schematic representation of production processes of non-digestible oligosaccharides (Sako *et al.*, 1999)

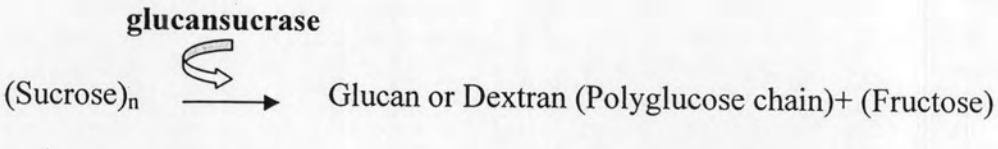
1.5 Glucansucrase (EC 2.4.1)

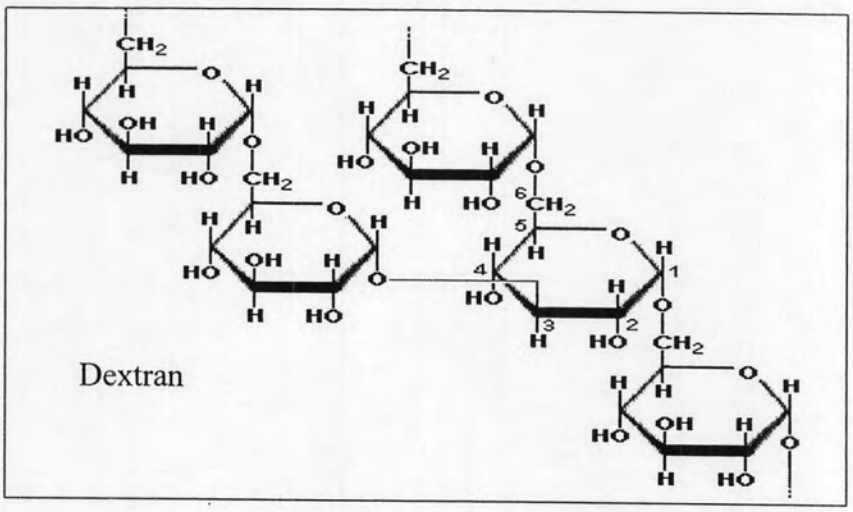
Glucansucrase is a family of the enzyme glucosyltransferase, consisting of subfamilies dextransucrase, mutansucrase, alternansucrase and reuteranesucrase. The enzyme in this family catalyzes three kinds of reaction; (1) transglucosylation reaction reading to synthesis of polyglucosylfructosides from sucrose (2) hydrolysis of sucrose and (3) acceptor reaction (intermolecular transglucosylation) corresponds to the transfer of glucosyl units from the sucrose donor onto various acceptor molecules (if a mono or disaccharide is used as acceptor, low molecular weight oligosaccharides (Hijum *et al.*, 2006).

Within the glucansucrase family, the member enzymes show different linkage specificity of glucosyltransferase. Dextransucrase (EC 2.4.1.5) is a glucosyltransferase that produces a glucan called dextran, which consists of 95% of α 1-6 glycosidic linkages and 5% of α 1-3 branched linkages. Whereas mutansucrase (EC 2.4.1.5) produced a polymer with only α 1-3 linkages in the main chain called mutan. Another enzyme of particular interest is the alternansucráse (EC 2.4.1.140), this enzyme synthesizes from sucrose an alternating α 1-6 and α 1-3 linked D-glucan, called alternan. For reuteransucrase, this enzyme synthesizes α 1-4 linked D-glucan and α 1-6 branched linkages, called reuteran (Hijum *et al.*, 2006).

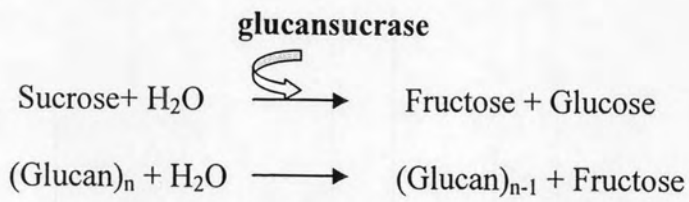
The glucansucrase enzyme catalyzes the following reactions :

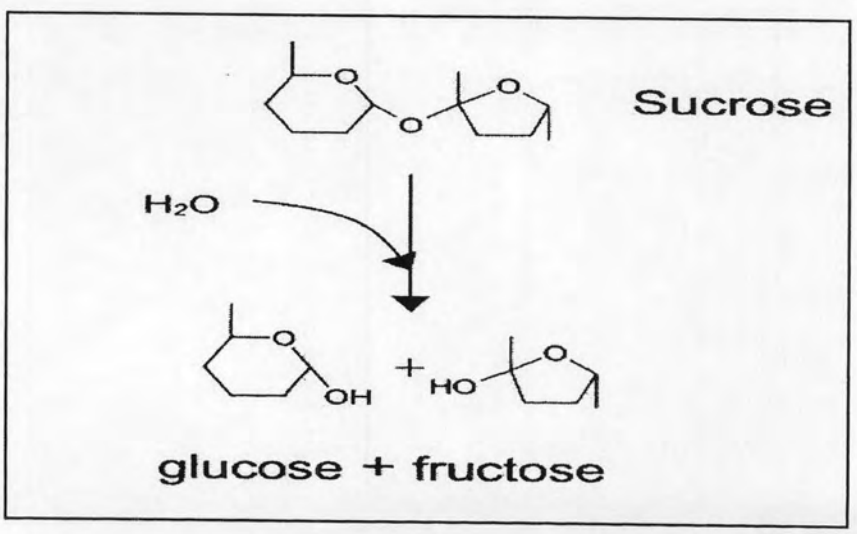
1.5.1 Polymerization reaction



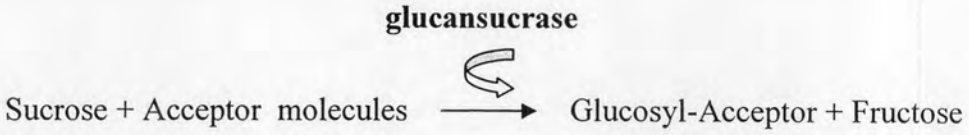


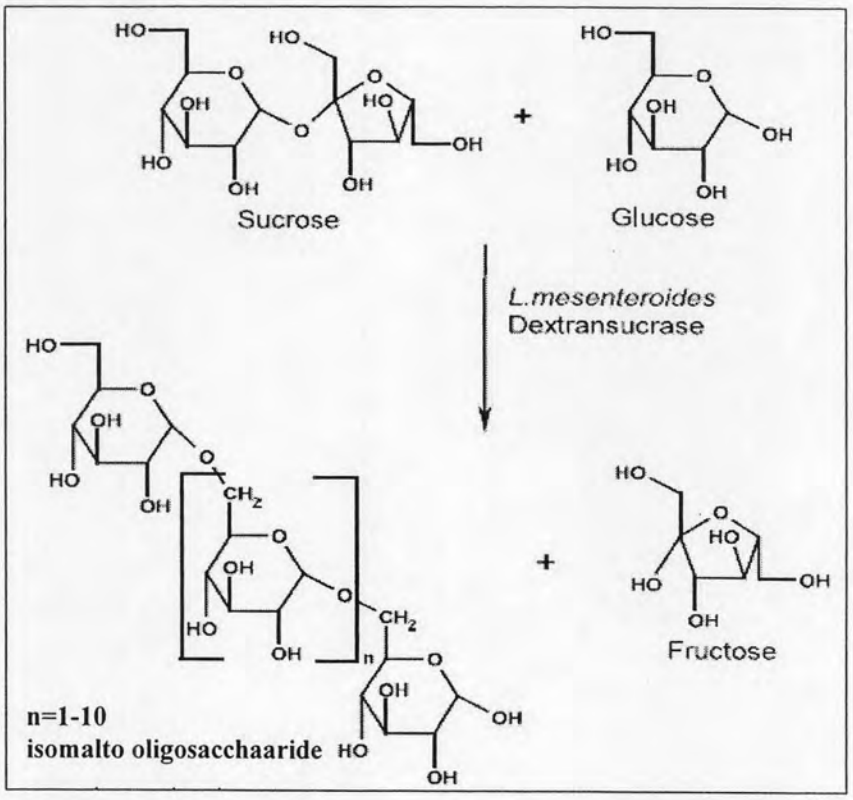
1.5.2 Hydrolysis reaction





1.5.3 Acceptor reaction





1.6 Transglucosylation activity for synthesis of prebiotic oligosaccharides by glucansucrases

Gluco-oligosaccharide (GOS) preparation has been enzymatically synthesized by the action of glucosyltransferase to transfer glucose molecules from sucrose donor to an acceptor such as isomaltose, the product name is isomalto-oligosaccharides (IMO), the structure are composed of glucose monomers linked by α1-6 glucosidic

linkages (Gibson *et al.*, 2004). They are extraordinary important as functional food ingredients owing to their prebiotic properties (Ghazi *et al.*, 2006).

The safety and health benefits of GOS have been reviewed. GOS has low sweetness, it is scarcely hydrolyzed by the digestive enzymes in the intestine, thus not utilized as an energy source in the body. They are non-cariogenic, encourage the growth of beneficial bifidobacteria, and decrease the levels of serum cholesterol, phospholipids, and triglycerides (Manning, 2004).

GOS are marketed in Japan as dietary supplements and functional food. They are being developed in the United States for similar uses. Glucansucrase is commercially attractive for the production of Gluco-oligosaccharides through its transfer reactions of glucosyl from sucrose donor and various types of acceptors. resulting in the formation of oligosaccharides (Bertrand *et al.*, 2006).

Several works on transglucosylation of interesting compounds to make useful prebiotic oligosaccharides by the action of glucosyltransferase or glucansucrase have been conducted. The transglucosylation activity for the production of prebiotic oligasaccharides has been extensively reviewed by many authors (Sangeetha *et al.*, 2004). Examples are as follows:

- In 1997, Lee and his colleagues reported the production of gluco-oligosaccharides (GOS) by an acceptor reaction using two types of glucansucrase from *Streptococcus sobrinus*, GTF-S and GTF-I. Acceptor reaction of GTF-S with maltose acceptor, gave a great number of GOS ranging from DP (degree of polymerization) 2 – DP 15. However, the

amount of dextran and DP of oligosaccharides was shown to be dependent on the sucrose to maltose ratio for both GTF-S and GTF-I. A maximum GOS yield of 69% was achived at the acceptor reaction with GTF-I and when the molar ratio of sucrose/maltose is 2:1,in which GOS of DP6-DP9 were major oligosaccharides which amounted for 17% dextran. The polymeric size of GOS could be controlled by varying the ratio of sucrose to the acceptor.

- In 2001, Arguello and his colleagues reported the synthesis of novel oligosaccharides from sucrose donor and cellobiose acceptor by alternansucrase. Interestingly, alternansucrase produced a series of oligosaccharides from cellobiose. Their structures were determined by MS and NMR . Two trisaccharides are first produced: α-D-glucopyranosyl-(1-2)-[β-D-glucopyranosyl- (1- 4) -D-glucopyranose] (compound A) and α-D-glucopyranosyl-(1- 6)- [β-D-glucopyranosyl-(1-4) -D-glucopyranose] (compound B). Then, compound B can in turn be glucosylated leading to the synthesis of a tetrasaccharide with an additional α -(1-6) linkage at the non-reducing end (compound D). The presence of the α -(1-3) linkage occurred only in the pentasaccharides (compounds C1 and C2) formed from tetrasaccharide D. Compounds B, C1, C2 and D were never described before. They were produced efficiently only by alternansucrase. Their presence emphasizes the difference existing in the acceptor reaction selectivity of the various glucansucrases.

- In 2007, Hee Nam and his colleagues reported the synthesis of Thermo- and Acid- Stable novel Oligosaccharides (TASO) from sucrose by using

dextransucrase from *Leuconostoc mesenteroides* B-512 FMCM with high concentration of sucrose. The degree of polymerization (DP) of oligosaccharides synthesized was from 2 to 11. TASO resisted hydrolysis of its glycosidic linkages at 140 °C and pH 6.0 for 1 h. It was stable at pHs ranging from 2 to 4 at 120 °C. These oligosaccharides effectively inhibited the formation of insoluble glucan, the growth and acid production of *Streptococcus sobrinus.* However, it stimulated the growth of probiotic organisms such as *Bifidobaterium* sp. TASO potentially can be used as sweeteners for the food and beverages where thermo- and acid-stable properties are required and as potential inhibitors of dental caries.

- In 2009, Cote reported the production of novel oligosaccharides by alternansucrase with gentiobiose acceptor for food and feed and elimination of bitterness. The initial product is a single trisaccharide, α-D-Glu-(1-6)-β-D-Glu-(1-6)-D-Glu.Two tetrasaccharides were formed in approximately equal quantities: α-D- Glu-(1-3)-α-D-G1u (1-6)-β-D-Glu-(1-6)-D-Glu and α-D-Glu-(1-6)-α-D-G1u (1-6)-β-D-Glu-(1-6)-D-Glu. Just one pentasaccharides was isolated from the reaction mixture, α-D- Glu-(1-6)-α-D-G1u (1-3)-α-D- Glu-(1-6)-β-D-Glu-(1-6)-D-Glu. The hypothesis that the enzyme is incapable of forming two consecutive α- (1-3) linkaged, and does not form products with more than two consecutive α- (1-6) linkaged, apparentyl applies to other acceptors as well as to maltose. The glucosylation of gentiobiose reduces or eliminates its bitter teste.

From previous studies , *Bacillus licheniformis* TH 4-2 was isolated from soil in Thailand and screened for thermotolerant bacteria and thermoactive levansucrase enzyme (Ammar *et al.*, 2002). The focus of this research is on the search for Glucansucrase activity of *Bacillus licheniformis* TH4-2 and the use in transglucosylation from sucrose donor to suitable acceptor for production of prebiotic oligosaccharides with new structure or new property.

The Objectives of this research

1. Optimization of cultivation condition for high glucansucrase production
2. Preparation of purified glucansucrase from *Bacillus licheniformis* TH 4-2 and characterization of glucansucrase
3. Synthesis and detection of prebiotic oligosaccharide products
4. Determination of transglucosylation efficiency
5. Optimization of transglucosylation reaction
6. Larger scale preparation, isolation, and charaterization of prebiotic oligosaccharide product
7. Determination of biological properties of prebiotic oligosaccharide product