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FERMENTATION OF OLIGOSACCHARIDES BY HUMAN INTESTINAL BACTERIA AND POSSIBLE EFFECTS ON PROTEIN FERMENTATION

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SUMMARY

Oligosaccharides are used in foods to increase the number of beneficial intestinal bacteria, especially bifidobacteria and lactobacilli. This selective stimulation may be too limited to obtain a healthy intestinal flora. It is suggested that to obtain a healthy intestinal flora the fermentation of proteins and amino acids by the intestinal flora should be reduced, while stimulating its saccharolytic activity. This may be achieved using oligosaccharides that are fermented by a large number of bacterial species in the large intestine.

INTRODUCTION

During the last few decades much interest developed in modifying the intestinal flora, to obtain a more healthy flora. Although there is still much controversy on the actual definition of a "healthy intestinal flora", it is generally accepted that a healthy flora should contain high numbers of saccharolytic bacteria (bifidobacteria and lactobacilli) and as few as possible of proteolytic bacteria (e.g. enterobacteria and clostridia) and/or proteolytic activity . Both bifidobacteria and lactobacilli are considered to have positive effects on general health. These effects include boosting the immune system of the host, protection against possible pathogens (notably *E. coli* and *Salmonella* spp), production of vitamins, limitation of putrefactive products in the faeces, production of short-chain fatty acids, decomposition of possible carcinogens and a lowering effect on serum cholesterol levels. Bacterial strains capable of inducing some of the effects mentioned above are designated as probiotics (Fuller, 1992; Hoover, 1993; Sanders, 1993; Gibson and Roberfroid, 1995)

Not all of the above mentioned claims have been proven (or disproven) sufficiently as yet in *in vivo* experiments with healthy human volunteers. Some effects have only been observed *in vitro* (e.g. production of vitamins and bacteriocins), and it is questionable whether these effects occur *in vivo* as well (Sanders, 1993).

Though the effects on healthy humans may be marginal, people with gastro-intestinal disorders clearly benefit from bifidobacteria and other probiotics. This merits the claim that, in order to improve or stabilise the intestinal flora, a large number of bifidobacteria is advisable (Isolauri et al, 1991; Isolauri et al, 1994; Malin et al., 1996).

There are several ways to obtain a large number of bifidobacteria in the large intestine and faeces. The easiest way to do this is to consume a product containing a large number of living probiotic cells. However, there are a few drawbacks to this approach. First, the cells have to be alive in the product. Secondly, the cells need to survive the stomach-bile barrier in sufficient numbers. Thirdly bifidobacteria and lactobacilli produce lactic and acetic acid, which will influence the taste of the product. This has restricted the product range mainly to dairy products. Finally the strains generally do not or poorly grow in the intestine and they hardly ever colonize (Fuller, 1992; Sanders, 1993; Tamime et al., 1995).

Another approach to increase the number of bifidobacteria in the large intestine is to ingest a selective substrate for bifidobacteria, a so-called bifidogenic factor. In this approach the host's own flora is stimulated. No living cells have to be consumed. As bifidobacteria are dominantly saccharolytic, the research focused mainly on non-digestible carbohydrates (Yazawa et al, 1978; Yazawa and Tamura, 1982; O'Sullivan, 1996). Unfortunately it is very difficult to observe changes in the intestinal flora. The problem is mainly due to the immense complexity of the microbial ecosystem in the large intestine. The intestinal flora is composed of around 10^{12} bacteria, divided among maybe as much as 400 different species. Each of these species has its own niche and often depends on other species for survival (Hill, 1986; Cumming and Macfarlane, 1991).
A major drawback to the use of oligosaccharides as bifidogenic factors is that most oligosaccharides are not fermented selectively by bifidobacteria, but by a large number of bacterial species. This non-specific effect has lead to the idea that it may be more beneficial to increase the general saccharolytic activity, rather than increasing bifidobacteria specifically. This paper deals firstly with the fermentation of oligosaccharides and secondly with the possible effects of oligosaccharides in limiting the end products of protein metabolism.

OLIGOSACCHARIDES

Oligosaccharides are naturally present in many foods, mainly from plant origin, but can also be synthesized enzymatically. Daily consumption of natural oligosaccharides is about four grams in a Western style diet (Roberfroid et al, 1993).
Natural oligosaccharides can be synthesized as such by the plant or animal, but they may also be breakdown products of plant cell walls or storage polymers. Animal oligosaccharides are generally produced in the small intestine as products from the turnover of the mucous tissues in the GI tract. These mucins have a complicated structure and lack a homogeneous backbone. Plant oligosaccharides are mainly derived from inulin, resistant starch and cell wall polysaccharides. The latter group consists of a large number of carbohydrates that are generally composed of a homogenous backbone with side chains of different length and composition. The production and characterization of plant cell wall oligosaccharides will be discussed in the following paper.
Synthetic oligosaccharides can again be divided in several groups. The first group consists of oligosaccharides which are made enzymatically using transglycosylation techniques. The oligosaccharides in this group are sometimes identical to natural oligosaccharides. The second group consists of chemically modified natural oligosaccharides. This modification can be performed either by a physical (heat, e.g. lactulose) or chemical process (hydrogenation, e.g. lactitol) (Röper and Koch, 1988).

FERMENTATION

Most oligosaccharides cannot be absorbed or fermented in the upper gastro-intestinal tract. These oligosaccharides reach the colon unaltered and are designated as non-digestible oligosaccharides. In the large intestine these oligosaccharides may be fermented by the intestinal flora. Fermentation of carbohydrates in general results in the production of short-chain fatty acids, lactate and gases. The short-chain fatty acids and lactate are absorbed by the colon mucosa and either assimilated in the mucosa or transported to the liver (Hentges, 1983).

Fermentation of an oligosaccharide is dependent on its structure, size and solubility. Therefore different oligosaccharides will be fermented by different specific sub-groups of intestinal

bacteria (Table 1). When an oligosaccharide is fermented by bacteria that are considered beneficial to human health, it can be designated as a prebiotic (Gibson and Roberfroid, 1995).

Table 1 : Fermentation of some commercial oligosaccharides by some intestinal bacteria

species	TOS*	LAS	LOS	GLL	FOS	NEP	NEP	KES	NYS	OF	INU
Bacteroides distasonis	+	+	+/-	+	+	-	+	+	+	+	+
B. fragilis	++	+	+/-	+	+	-	+	+	+	++	+
B. ovatus	+			-	+	-	+	+	+	+	+
B. thetaiotaomicron		+			+		+	+	+	+	+
B. vulgatus	+	+		+	-	-	+	+	+	+	+
Bifidobacterium adolescentis	++	+	+++	+	+++	+	+	+	+	++	++
Bi. bifidum	++	-	++	+	-	-	-	-	-	-	-
Bi. breve	++	+	+++	+	+++	-	+	+	-	++	-
Bi. infantis	++	+	+++	+	+++	+	+	+	+	++	++
Bi. longum	++	+	+	+	+++	+	+	+	+	++	-
Clostridium bifermentans	-	-	-	-	-						
Cl. butyricum	-	+	++	-	+++	+	+	+	+	++	-
Cl. clostridiiforme			+	-	+	-	+	+	-	-	-
Cl. innocuum		-		-	+	-				+	+
Cl. paraputrificium	-	-/+	+/-		-	-	-	-	-	+/-	-
Cl. perfringens	-	+	+	-	+	-	-	+/-	-	-	-
Cl. ramosum		+	+	-	++	+	+	+	-	+	+
Escherichia coli	++	-	-	-	-	-	-	+/-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-	+	-	+	+
Ec. faecium	+/-			-	++	-	-	+	-	+	+
Eu. aerofaciens	-	-		-	+	-	+	+	-	-	-
Fusobacterium necrophorum	-			-	-	-				-	-
Klebsiella pneumoniae	++				+/-	-	+	+	+	+	-
Lactobacillus acidophilus	-	-/+	-	+	+	-	+	+	-	++	++
Lb. casei	++	-	-	-	++	-	-	-	-	+	+
Lb. fermentum	+				-		-	-	-		
Lb. gasseri		-		-	+++	-					
Lb. plantarum	++						+	+	-		
Lb. salivarius	++	-		-	++	-	+	+	-		
Megamonas hypermegas		-		+		-	+	+	+	++	+
Megasphaera elsdenii		-		-	-	-	-	-	-		
Mitsuokella multiacidus		-		-		-	+/-	+	-	++	+
Peptostreptococcus anaerobius				-	-	-					
Prevotella melaninogenica					+/-	-	+	+	+		
Ruminococcus productus				-		-					
Streptococcus mutans					+	+					
St. mitis	++				+						
St. salivarius	-/++				+						

* TOS= trans-galactosyl oligosaccharides, LAS = lactosucrose, LOS = lactitol oligosaccharides, GLL = 4'-galactosyllactose, NEP = Neosugar P, KES = kestose, NYS = nystose, OF = oligofructose, INU = inuline. Signs are taken directly form original tables. Plus-signs denote fermentation.
Data are taken from : Fujita et al., 1991; Tanaka et al., 1983; Yanahira et al., 1995; Hayakawa et al., 1990; Mitsuoka et al., 1987; Wada, 1992

Most of the data from Table 1 are obtained using different procedures, which may result in different results (e.g. the results obtained with NEP in Table 1). To determine the ability of a certain strain to ferment a carbohydrate two methods are generally used. The first method only measures the pH shift in the medium and compares this to a carbohydrate free medium and

medium with glucose as a negative and positive control respectively. This easy way of measuring the fermentability, however, has several drawbacks.
First the fermentation is dependent on the medium used. Several species possess both proteolytic as well as saccharolytic activity. Proteolytic activity, especially in a rich medium after 48h incubation, may have increased the pH, due to formation of ammonia and amines, resulting in false-negative results. Reducing the protein content will emphasise saccharolytic activity and show more positive results (Hartemink et al, 1997).
Secondly, the purity of a product is not always known. Traces of glucose, or another easily fermentable carbohydrate, may already give rise to a change in pH, especially in a non-buffered medium. This would result in false-positive results. Addition of a buffer may compensate for this unwanted acid formation.
Finally, the enzymes necessary for the fermentation are sometimes inducible. This indicates that results may be influenced by pre-culturing conditions.
The second method uses optical density as a measure of growth, again compared with glucose and a carbohydrate-free blank. However, this method has similar drawbacks as the pH-method. The medium will also interfere with the results similar as described above, and presence of fermentable contaminants will result in some growth. The induction effect is again similar. A specific problem with optical density measurements may be that growth on different sugars may affect the density of a solution. An insoluble polymer may be formed and, on the other hand, nutritionally stressed cells may be smaller, both effects will influence the optical density. Counting viable cells by plating at different times may be a more reliable, but also more time-consuming method, which overcomes most of these problems.
In our opinion the best way to determine whether a carbohydrate is degraded and fermented, is to analyse the carbohydrate-composition of the medium before and after the incubation. This is relatively simple using HPLC, but much more time-consuming and expensive than the classical methods. The next paper will show the differences between traditional methods and HPLC determinations.

PROTEOLYSIS AND OLIGOSACCHARIDES

The second major source of energy for intestinal bacteria is protein, originating either from the ingested food, or from endogenous secretions. The proteins are first degraded to individual amino acids which may be fermented. Fermentation of amino acids results in the formation of several products, such as ammonia, indoles, skatoles and biogenic amines. Many of these products are considered potential harmful to the large intestinal mucosa. Several of these products have also been implicated with mutagenesis and colon cancer. For these reasons protein fermentation is regarded as an undesirable process for human health (Hill, 1995).
Many different species of intestinal bacteria are capable of degrading and fermenting protein. Only a limited number of these bacteria is solely dependent on the fermentation of proteins, most possess both a saccharolytic as well as a proteolytic metabolism (e.g. most clostridia, *E. coli*). Other bacteria, like bifidobacteria and lactobacilli, do not show high proteolytic activity (Hill, 1995).
As fermentation of amino acids may yield products harmful to the host, it may be important to reduce this fermentation to a minimum.
There are several hypothetical ways to achieve reduction of amino acid fermentation; reduce the amount of available protein, suppress the proteolytic bacteria and suppress the amino acid fermentation directly.

Reduction of the amount of available protein is difficult, as a large part of the protein is host-derived (mucus, enzymes). Reduction of the protein content of foods will thus have a limited effect.
Suppressing the proteolytic species might be achieved by increasing the number of saccharolytic bacteria. Due to increased numbers, acid production and competition for other essential nutrients, the saccharolytic bacteria will suppress the proteolytic bacteria.
Suppressing the amino acid fermentation by making available fermantable carbohydrate may be the most promising option. This way the species capable of both amino acid fermentation and saccharolysis will change their metabolic activity towards saccharolysis. This will result in increased saccharolysis and decreased amino acid fermentation.
The last two mechanisms may be achieved using oligosaccharides. The concept of prebiotics was to increase the number of beneficial organisms by selective fermentation. There have been several publications showing an increased number of bifidobacteria after ingestion of oligosaccharides (Wang and Gibson, 1995; Bouhnik et al, 1996; Kashimura et al., 1989; Höffmann and Bircher, 1969; Yoneyama et al., 1992; Ogata et al., 1993; Benno et al., 1987). Unfortunately, there does not seem to be a decrease in the number of most other species, not even after prolonged ingestion of oligosaccharides. The presence of large numbers of other bacteria indicates that the exclusion mechanism is not completely effective in healthy humans. On the other hand several other publications have shown that activity of enzymes related to proteolysis as well as the concentrations of end-products of amino acid fermentation were reduced after ingestion of oligosaccharides (Goldin and Gorbach, 1984; Rowland et al, 1986; Terada et al., 1992; Rowland and Tanaka, 1993; Bouhnik et al., 1996). This fact indicates that, even though the bacterial numbers did not change, the metabolic activity has changed towards a more healthy situation.

CONCLUSIONS AND SUGGESTIONS

As a conclusion of the above arguments it can be suggested that it is not necessary to increase the number of bifidobacteria or any other specific bacterial group in the intestinal contents, but to increase general saccharolytic activity and decrease the general amino acid fermentation.
In our opinion the best way to reduce amino acid fermentation is to increase the saccharolytic activity of the intestinal flora, not just to increase the number of bifidobacteria. A reduction of amino acid fermentation has been observed *in vivo* after ingestion of non-digestible oligosaccharides.
At present oligosaccharides used as prebiotics are selected on the basis of selective fermentation by bifidobacteria. In our hypothesis that would be too restricted. We would propose that to be effective in reducing the amino acid fermentation, an oligosaccharide should be fermented by a large number of bacterial species. In addition to this, we would propose that an oligosaccharide should preferably be fermented by those bacteria that possess both saccharolytic activity as well as amino acid fermentation activity.
Although there are many indications that oligosaccharides are capable in reducing the amino acid fermentation, this has to be studied in more detail.
In addition to this, there are no well-defined markers (either chemical or enzymatical) for amino acid fermentation. The markers used at present, e.g. indoles, skatole, cresol, azoreductase and nitroreducatse activity, are limited to a specific group of organisms (Hill, 1986, 1995). For example indole is always produced from tryptophan, but not all species capable of amino acid fermentation produce indole. A reduction of indole may thus be an indication of reduced amino acid fermentation, but reduced amino acid fermentation does not always result in a reduced indole concentration. The same holds for the other markers used at

present. An ideal marker has not yet been developed. At present many different markers, like end-products and enzyme activities, have to be analysed to determine whether amino acid fermentation has been reduced.

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