SUGAR CATABOLISM IN BIFIDOBACTERIA

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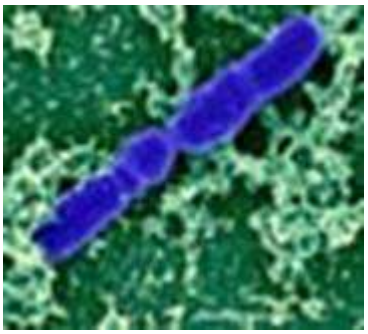


Introduction

The members of *Bifidobacterium* genus are gram positive, anerobic, non motile and no spore forming bacilli. Several species of bifidobacteria colonize the intestinal tract of a great number of animal species (1). Protection against enteric pathogens, immune system activation and vitamin production, are some of the the benefic properties, attributed to the members of the*Bifidobacterium* genus. Also, mechanisms involved with reduction of cholesterol levels and antitumoral activity, have been proposed (2).

Recently, administration of probiotic bifidobacteria and complex carbohydrates (prebiotics), in humans and farm animals, has been documented. Nevertheless, it is necessary to understand the catabolism of these bacteria, since this will allow the suitable administration of complex carbohydrates, to maintain a balanced gut microbiota, and guarantee the antagonistic activity against enteric pathogens. Synbiotics are defined as probiotic bacteria plus complex carbohydrates as prebiotics (3). Simultaneous administration of bifidobacteria and prebiotics, favors sugar catabolism and development of the microorganism. Also antagonic products production, like organic acids and bacteriocins, is stimulated. The aim of the present work is to make a bibliographical review about the bifidobacteria capacity to metabolize complex carbohydrate and their fermentation routes.

Physiology of Bifidobacteria

The members of the *Bifidobacterium* genus are considered anaerobes (4). However, the *B. longum* genome analysis does not show components that confirm their strict anaerobe condition (5). The degree of tolerance to oxygen depends on the species and the culture medium used (6) and it seems to be related to the source of the bacteria, since the human strains are more susceptible to air exposure, than those strains isolated from other animal sources (7). In our experience, a great number of bifidobacteria strains, isolated from the gastrointestinal tract of pigs, are aerotolerant (8).

Most of the *Bifidobacterium* species are not able to growth in a completely synthetic medium and require complex biological substances, as casein, bovine serum albumin digest, casein digest, hog gastric mucin or yeast extract (9; 10).

The culture media used for the isolation and purification of bifidobacteria, often are supplemented with substances with a low redox potential, such as cystine, cysteine, ascorbic acid or sodium sulfite (11). Different culture media have been proved for the specific isolation of bifidobacteria from fecal samples, and the obtained results have been quite satisfactory (12). However, it must be considered the characteristics of the desired strains, because some of them could have special carbohydrate requirements (13).

Complex Carbohydrates Catabolism

Most of the complex carbohydrates are not degraded in the stomach or the proximal small intestine, and these compounds can reach intact at the colon. On the contrary, simple sugars and disaccharides are absorbed in small intestine. Thus, the complex carbohydrates represent the main carbon and energy sources, for numerous bacterial species in the colon (14).

The information, about polysaccharides catabolism in bifidobacteria is fragmentary and it has been limited to the study of fructans or fructose polymers (15). However, it is interesting that more than 8.5% of the predicted proteins, from the genomic sequence of *B. longum*, would be involved in the transport and carbohydrate metabolism. This is equivalent to more of 30%, than the observed in other organisms (16).

Bifidobacteria are able to use galacto, manno and fructo-oligosaccharides, at different levels and with different intensities. These differences can be due to the individual nature of each strain, since strains belonging to the same species, and originated from different culture source, have different patterns of carbohydrate fermentation (17). In the particular case of fructo-oligosaccharides, the discrepancies observed, *in vitro*, can be due to differences in the fructan hydrolyzing enzyme expression (18). In addition, some *B. longum* strains are capable to ferment several plant gums, two of which (arabic and ghatti gums), are not fermented by many other bacterial species (19).

Although it seems that exist a better efficiency in the transport systems of dimeric or oligomeric carbon sources, in these organisms (20), the enzymes necessary for the breakage of di and oligosaccharides could be induced, according to changes in growth and metabolite requirements (21, 22). For example, it has been observed that in *B. infantis* the presence of fructose in the culture media, induces the synthesis of fructofuranosidase (23). This enzyme is necessary for the breakdown of fructo-oligosaccharides.

Metabolic adaptation, to catabolize di and oligosaccharides, may display an advantage for bifidobacteria, in their competition with other microorganisms, which are in the normal microbiota of the intestinal tract, where oligo and polysaccharides are the main sugars (24). Therefore, *B. longum* posses 8 transporters with high affinity, for the uptake of oligosaccharides with less than 8 ramifications, the highest number for any prokaryote genome published (25).

Carbohydrate Fermentation

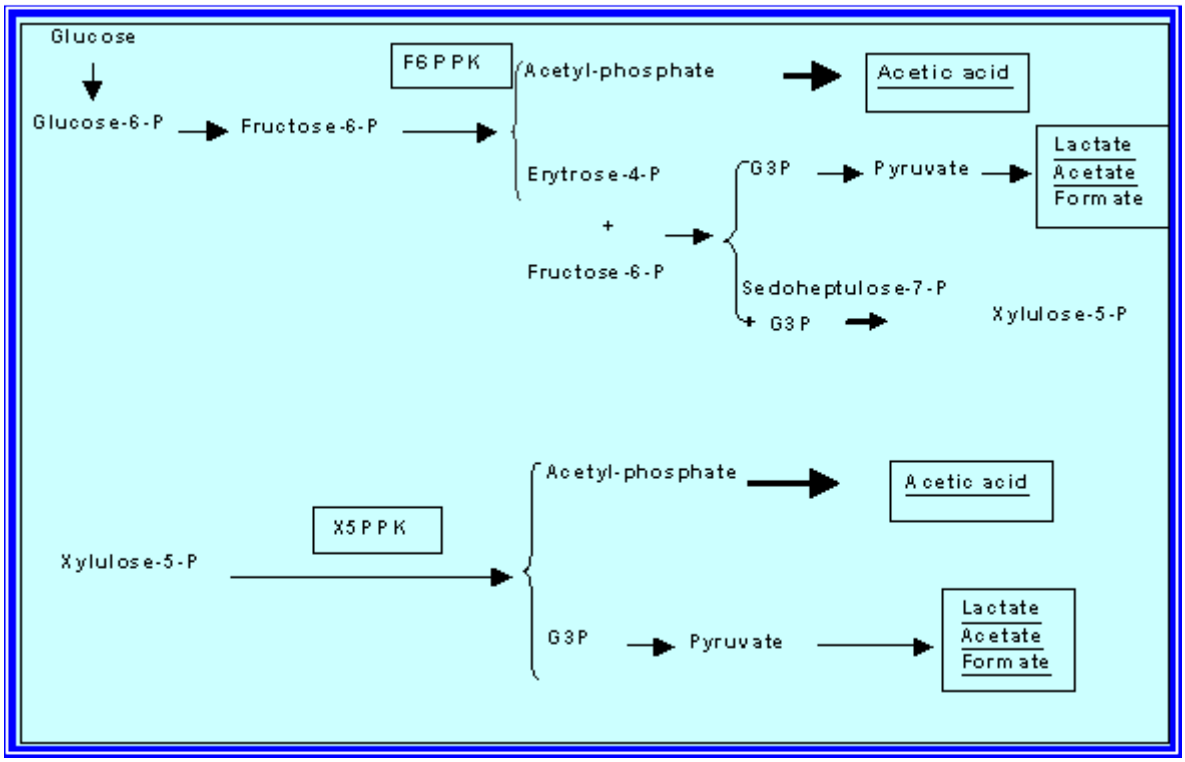
In the genus *Bifidobacterium*, hexoses are degraded only and exclusively by the fructose 6-phosphate pathway, as shown in figure 1 (26, 27). The “bifid shunt”, or glucose catabolism through the fructose 6-phosphate phosphoketolase (EC 4.1.2.22), generates acetyl phosphate and eriytrose 4-phosphate. The final products of the fermentation route are formed by the sequential action of the enzymes: transaldolase (EC 2.2.1.2), transketolase (EC 2.2.1.1.) and xylulose 5-phosphate phosphoketolase (EC 4.1.2.9) which generates glyceraldehyde 3-phosphate that enters to the Embden-Meyerhoff-Parnas pathway (28).

In bifidobacteria, pyruvate can follow two metabolic pathways: (1) reduction to lactate by lactate derhydrogenase (EC 1.1.1.27) or (2) the splitting by phosphoroclastic enzyme (not yet detected) to form formic acid and acetyl phosphate (29). Nevertheless, the organic acid production could differ between the exponential and the stationary phase, and between different members of the genus. For example in *B. animalis*, acetic acid production stays in both phases, but lactic acid production is remarkably high in the early stages of the fermentation and later it decreases. In contrast, formic acid production is low during the first hours and later it is increased. This can indicate that a high intracellular sugar concentration stimulates the lactic acid production, whereas a less fermentable carbohydrate and a low intracellular sugar concentration could stimulate the formic acid production to produce ATP, necessary for the growth in the presence of sugars that are slowly metabolized (30).

In *B. pseudolongum*, the acetate/lactate proportion observed is 2.4:1 in media containing glucose, whereas in the presence of pectin, the fermentation pattern alters in favour of acetate and succinate, at expense of formate, lactate and ethanol (31). In addition, the presence of some individual carbohydrate, can direct the metabolism towards the production of biomass (glucose) or organic acids (fructose) in *B. infantis* (32).

Respect to the complex carbohydrate fermentation in bifidobacteria, Crociani and colleagues (33) found that most of 290 strains, belonging to 29 species of *Bifidobacterium* genus, are able to ferment $_{D}$-galactosamine, $_{D}$-glucosamine, amylose and amylopectin. In this study, the authors detected that many of species isolated from animal habitats, showed a reduced fermentation activity. On the other hand, *B. bifidum* and *B. infantis* were the only species able to ferment $_{D}$-glucuronic acid, whereas *B. longum* strains were able to ferment arabinogalactans, arabic and ghatti gums. Finally, *B. breve, B. infantis* and *B. pseudocatenulatum*, were able to ferment a-$_{L}$-fucose. This gives us an idea, of the diversity in the capacity of carbohydrate catabolism, between the different species of the *Bifidobacterium*genus, which could be related to the type of ecological niche that each species colonize.

Figure 1: The “bifid shunt” (bifidobacteria fermentation route).
F6PPK: Fructose 6-phosphate phosphoketolase; X5PPK: Xylulose 5-phosphate phosphoketolase



Fructose 6-Phosphate Phosphoketolase, A Key Enzyme In Bifidobacteria

Phosphoketolases catalyse an irreversible thiamine diphosphate dependent phosphorolytic reaction, in which D-xylulose 5-phosphate (xylulose 5-phosphate phosphoketolase or X5PPK, EC 4.1.2.9) or D-fructose 6-phosphate (fructose 6-phosphate phosphoketolase or F6PPK, EC 4.1.2.22) is split, in acetyl-phosphate and glyceraldehyde-3-phosphate or erytrose-4-phosphate, respectively. In both cases, the reaction requires the presence of inorganic phosphorous (34).

The heterogeneity observed between different phosphoketolase enzymes (F6PPK), within the *Bifidobacterium*genus, according to distinct electrophoretic migration patterns and molecular masses, has been discussed previously (35). These differences were confirmed in studies that showed differences at the molecular mass level and, an aspect of great importance: specificity of substrate. It is neccessary to emphasize that the strains isolated from human sources, present two enzymes with phsophoketolase activity: F6PPK and X5PPK, which have been identified as two separated bands. On the contrary, in strains of other animal sources, the presence of a single enzyme with activity on both substrates is characteristic (36). However, the analysis of nucleotide sequences for the phosphoketolase gen in several species of bifidobacteria, does not allow explain the differences observed phenotipically (37).

The study of the X5P/F6PPK in *B. lactis*, a strain recovered from a dairy product, reveals that this enzyme is a multimeric protein with a molecular mass of 550 kDa. The enzyme is an homohexamer, and the hybridization analysis indicated, that one single copy of the gene, is present on the *B.* lactis chromosome. Finally, the transcriptional analysis revealed, that this gene is not cotranscribed with other adjacent genes (38). *Bifidobacterium longum*, from the human gastrointestinal microbiota, showed a F6PPK with a molecular mass estimated in 300 $\pm$ 5 kDa. Nevertheless unlike *B. lactis*, in this species, the enzyme is formed by two different types of subunits: α and β, with molecular masses of 93 $\pm$ 1 and 59 $\pm$ 0.5 kDa, respectively. The functional enzyme is formed by a tetramer $\alpha_2\beta_2$or a dimer αβ (39).

It is important to set attention in the fact that, the enzyme that is conformed by a single type of subunit (*B. lactis*) has activity on both substrates, whereas the enzyme that is formed by two different types of subunits (*B. longum*) only acts on F6P. This point would support the hypothesis that the species of human source could come from a different ancestor, than those recovered of animal sources (40). Another possibility is, the strains that colonize

the intestinal tract of animals, like human, whose diet includes glucose or fructose polymers, have a major activity of F6PPK. By the contrary the bifidobacteria that colonize the intestinal tract of other animals, like cows or rats, whose diet includes hexoses and pentoses polymers would be capable of metabolize the monomers by an enzyme with dual substrate affinity.

Conclusions

The catabolism of simple and complex carbohydrates does not seem to have a specific pattern within the*Bifidobacterium* genus. That is, great differences with respect to the complex carbohydrates use exist, between the different species belonging to this genus. Nevertheless, all of them have a common feature: requires the phosphoketolase activity, to be capable to carry out the catabolism of monosaccharides: pentoses or hexoses. For that reason the study of probiotics and prebiotics, must consider the precise requirements of those strains that will be used, depending on the commercial interest, that is for human or farm animals.

Abstract

In recent years, an increasing interest in the study of the bacteria with potential probiotic activity exists, especially in those genera that constitute the intestinal microbiota, as the members of the *Bifidobacterium* genus. Nevertheless, to guarantee a successful administration of probiotic bacteria, it is necessary to consider the nutritional and environmental requirements of these microorganisms, to exert their probiotic effect. In bifidobacteria, as in other genera of the intestinal microbiota, catabolism and capacity to generate antagonic products, depends on metabolic activity, as complex carbohydrates catabolism, phosphoketolase activity, and monosaccharide fermentative routes. This is a bibliographical review about the bifidobacteria catabolism of complex carbohydrates, and their fermentative routes in the gut.

Key words: bifidobacteria, probiotic, catabolism, carbohydrate, phosphoketolase.

Resumen

En la actualidad, hay un gran interés en el estudio de las bacterias con actividad probiótica, especialmente en aquellos géneros que constituyen la flora interstinal, como los son los miembros del género *Bifidobacterium.* Sin embargo, para garantizar una administración acertada de bacterias probióticas, es necesario considerar los requisitos nutricionales y ambientales de estos microorganismos, para ejercer su efecto probiótico. En bifidobacterias como en otros géneros de la flora intestinal, el catabolismo y la capacidad para generar productos antagónicos, depende de la actividad metabólica, como del catabolismo complejo de los carbohidratos y la actividad de la fosfoketolosa, y de las rutas de fermentación de los monosacáridos. Esta es una revisión bibliográfica acerca del catabolismo de los carbohidratos complejos en bifidobacterias y sus rutas de fermentación en el intestino

Palabras claves: bifidobacteria, probiotico, catabolismo, carbohidratos, fosfoketolasa

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