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ORIGINAL PAPER

β-glucuronidase and β-glucosidase Activity of *Lactobacillus* and *Enterococcus* Isolated from Human Feces

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Abstract

The domination of microorganisms characterized by excessive activity of the so-called fecal enzymes may be one of the reasons of the large intestine cancers. These enzymes are mainly those that belong to the hydrolase and reductase classes and their excessive activity may lead to disorders in the functioning of the digestive tract. The aim of tise research was to determine the activity of β-glucuronidase and β-glucosidase of *Lactobacillus* and *Enterococcus* strains isolated from the feces of healthy children, aged 1 and 8, and adults, aged 30 and 80. The analysis included 10 strains isolated from the feces of individuals in each of the age groups. β-glucuronidase activity in the case of the isolates from children, depending on the strain, equaled from about 0.15 mM/h/mg of protein to 0.26 mM/h/mg of protein and was lower, respectively, by 52.35% and 57.81%, than the β-glucosidase activity. Simultaneously, the activity of the *Lactobacillus* enzymes from children was 2.4 times higher, and in case of the isolates obtained from adults they were 4.6 and 2.7 times higher than the activity of the *Enterococcus* enzymes. The highest β-glucuronidase activity was observed in *Lactobacillus* isolates coming from an 80-year-old subject. The differences between the activity of *Enterococcus* β-glucuronidase isolated from the feces of 1 and 8 year old children were statistically insignificant. On the other hand, in the case of the subjects aged 30 and 8 the isolates were characterized by activity lower by, respectively, 48% and 37% than the isolates coming from children. The highest β-glucosidase activity was discovered in the case of *Lactobacillus* and *Enterococcus* coming from children, which was higher by 32% than the activity of the isolates from adult persons. Therefore, it was determined that the activity of β-glucuronidase of *Lactobacillus* strains isolated from feces from people aged 80 was the highest, and the isolates of the examined microorganisms coming from children were characterized by the highest β-glucosidase activity.

Key words: β-glucuronidase, β-glucosidase, intestinal bacteria

Introduction

The large intestine is a complex ecosystem of microorganisms that performs a very important immunological function since it takes part in inhibiting the growth of harmful bacteria and also in processing food components supplied to the organism. The collection of microorganisms of the large intestine is dominated by absolute anaerobes that belong to *Bacteroides*, *Clostridium*, *Ruminococcus*, *Butyrovibrio*, *Fusobacterium*, *Eubacterium*, *Peptostreptococcus* and *Bifidobacterium* (Ouwehand *et al.*, 2002). It also includes bacteria species that may induce disorders in the functioning of the digestive tract, especially when they become the dominant ones (McGarr *et al.*, 2005). Excessive activity of the so-called fecal enzymes, *i.e.* the enzymes of bacteria present in the large intestine, generates many genotoxic, mutagenic and carcinogenic products, or it transforms pro-carcinogenic substances into carcinogenic ones. The enzymatic activity of intestinal microorganisms may therefore induce the formation of digestive tract cancer, including mainly the cancer of the large intestine (Burns *et al.*, 2000). The presence of bacterial strains characterized by high activity of β-glucuronidase (EC 3.2.1.31) or β-glucosidase (EC 3.2.1.21) in the large intestine may be a risk factor leading to the formation of a tumor (De Preter *et al.*, 2008).

β-glucuronidase (Glucuronohydrolase of β-D-glucuronides) hydrolyzes β-D-glucuronides to glucuronic acid and aglycone that may have the form of an alcohol, rest of organic acid, amine, imine or a thiol compound. The formation of glucuronides is catalyzed by UDP-glucuronylotransferase. From the liver, where their synthesis takes place, they are partially removed with bile to the large intestine. There, under

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the influence of bacterial β-glucuronidase, they are subject to hydrolysis to aglycones (Beaud *et al.*, 2005; De Moreno de leBlanc *et al.*, 2005). In patients with diagnosed tumors of the large intestine high activity of β-glucuronidase was observed, which suggests that this enzyme plays an important role in promoting large intestine tumors (Kim *et al.*, 2001). Among the intestinal microflora the highest activity of β-glucuronidase is shown by *Escherichia coli*, *Clostridium paraputrificum*, *Clostridium clostridioforme*, *Clostridium perfringens*, *Bacteroides fragillis*, *Bacteroides vulgatus*, *Bacteroides uniformie*, *Ruminococcus gnavus* and species that belong to the genera *Peptostreptococcus*, *Staphylococcus* and *Eubacterium* (De Moreno de leBlanc *et al.*, 2005; Nakamura *et al.*, 2002).

β-glucosidase (β-D-glucoside glucohydrolase, amygdalase) hydrolyzes glycosides to sugar groups, *i.e.* glycones, and to non-sugar groups, *i.e.* aglycones (Rafter *et al.*, 2002). A diet composed of large amounts of glycosides of plant origin (*e.g.* flavonoids) may be the reason for the formation of harmful substances in the large intestine. Glycosides that have not been digested are transported to the large intestine where they are hydrolyzed under the influence of bacterial β-glucosidase. The aglycones that are formed in such a way often manifest toxic and carcinogenic properties, *e.g.* querecin that is one of the promoters of the large intestine tumors and is formed in the process of rutin transformation involving β-glucosidase (De Preter *et al.*, 2008). This enzyme is present in the cells of some of the microorganisms located in the large intestine and the highest activity is shown by *Bacteroides uniformis*, *Clostridium paraputrificum*, *Clostrdium clostridioforme* and *Enterococcus faecalis* (De Preter *et al.*, 2008; Nakamura *et al.*, 2002). The qualitative and quantitative composition of the intestinal microflora in healthy human beings contains a dominant number of microorganisms favorable to their health and it is relatively balanced. The dominance of some of the intestinal bacteria species and an increase in the activity of the so-called fecal enzymes may lead to increased production of carcinogenic compounds.

The aim of the research was to determine the activity of β-glucuronidase and β-glucosidase of *Lactobacillus* and *Enterococcus* bacteria. *Enterococcus*, although usually are not the dominant microorganisms in the large intestine, consist a constant and typical element of this ecosystem. On the other hand, *Lactobacillus* that belongs to the intestinal endogenic microorganisms is considered to have a favorable influence on the activity of the digestive tract (Russel *et al.*, 2001). The present article describes the changes in the activity of the large intestine bacteria enzymes isolated from healthy persons, including children aged 1 and 8 and adults aged 30 and 80.

Experimental

Material and Methods

Bacterial *Lactobacillus* and *Enterococcus* strains used for the purpose of the research were isolated from the feces of 5 healthy children (aged 1 and 8) and 5 adults (aged 30 and 80). The above-mentioned genera were isolated on selective media: *Lactobacillus* on Rogosa medium, and *Enterococcus* on BE medium with bile and esculin. The material for the determination of the enzymatic activity was a 24-hour culture of the tested bacteria. The enzymatic activity of β-glucuronidase and β-glucosidase was determined spectrophotometrically using the wavelengths 540 nm and 450 nm. The method was based on color reaction between a substrate and the examined enzyme. The activity of β-glucuronidase and β-glucosidase was determined with the method described by Freeman (1986), using as a substrate phenolphthalein-β-D-glucuronide (Sigma) for β-β-glucuronidase and p-nitrophenyl-β-D-glucopiranoside (Sigma) for β-glucosidase. The adopted activity unit equaled such amount of phenolphthalein (for β-glucuronidase) and p-nitrophenyl (for β-glucosidase) expressed in mM that was released during the reaction in 1 hour, calculated per 1 mg of protein. The total concentration of protein in bacterial cells was determined with the use of Lowry method as described (Lipińska *et al.*, 1999). The results were elaborated based on statistical analysis.

Results

The aim of the conducted research was to determine the activity of β-glucuronidase and β-glucosidase of *Lactobacillus* and *Enterococcus* isolated from the feces of healthy children, aged 1 and 8, and adults aged 30 and 80. The analysis included 10 strains isolated from human feces in each of the age groups.

The activity of β-glucuronidase of *Lactobacillus* coming from children, depending on the strain, in interval equaled from 0.135 to 0.275 mM/h/mg of protein. In the case of the adults, the activity of this enzyme ranged from 0.159 mM/h/mg of protein to 0.313 mM/h/mg of protein. *Enterococcus* strains coming from children were characterized by β-glucuronidase activity within the range 0.07–0.114 mM/h/mg of protein, and in the case of the subjects aged 30 and 80, the activity of the enzyme equaled from 0.032 to 0.064 mM/h/mg of protein. The activity of β-glucosidase of *Lactobacillus* strains obtained from children equaled from 0.021 to 0.048 mM/h/mg of protein, while in case of adults it equaled from 0.018 to 0.032 mM/h/mg of protein. These differences were statistically significant. The isolates of *Enterococcus*

Table I
The activity of β-glucuronidase and β-glucosidase of bacteria isolated from feces of person in different age

Bacterium	Age (years)	Activity*	
		β-glucuronidase	β-glucosidase
Lactobacillus	1	0.135–0.275	0.021–0.048
	8	0.164–0.237	0.019–0.042
	30	0.159–0.217	0.018–0.029
	80	0.257–0.313	0.020–0.032
Enterococcus	1	0.073–0.103	0.013–0.015
	8	0.070–0.114	0.015–0.017
	30	0.032–0.053	0.007–0.010
	80	0.044–0.064	0.006–0.009

* activity of β-glucuronidase (mM phenolphtalein /h/mg of protein), β-glucosidase (mM p-nitrophenyl /h/mg of protein)

from children were characterized by β-glucosidase activity at the level from 0.013 to 0.017 mM/h/mg of protein, and in adults the activity of this enzyme was very low and equaled from 0.006 to 0.01 mM/h/mg of protein (Table I).

It was discovered that the average activity of both β-glucuronidase and β-glucosidase of *Lactobacillus* isolates coming from children was 2.3 and 2.4 times higher, respectively, and in the case of the isolates coming from adults it was 4.6 and 2.7 times higher than the activity of these enzymes from *Enterococcus* (Figure 1 and 2). The highest *Lactobacillus* β-glucuronidase activity was observed in isolates from an 80-year-old subject (0.313 mM/h/mg of protein) (Table I). The differences between the activity of β-glucuronidase of *Enterococcus* isolated from the feces of 1 and 8-year-old children were statistically insignificant. In the case of children the activity of the *Enterococcus* isolates was higher by 48% in comparison to 30-year-old subjects and by 37% in comparison to the oldest group. *Lactobacillus* bacteria, depending on the strain, were characterized by varied β-glucuronidase activity. The differences for the activity of this enzyme in isolates coming from children was 2-fold, while in the case of the 80-year-olds this difference equaled only 18%. A similarly varied

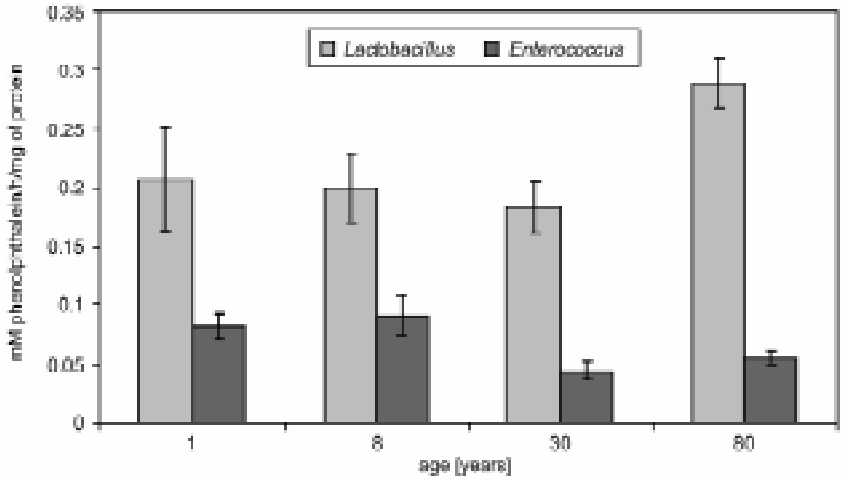

Fig. 1. Average activity of β-glucuronidase of *Lactobacillus* and *Enterococcus* isolates

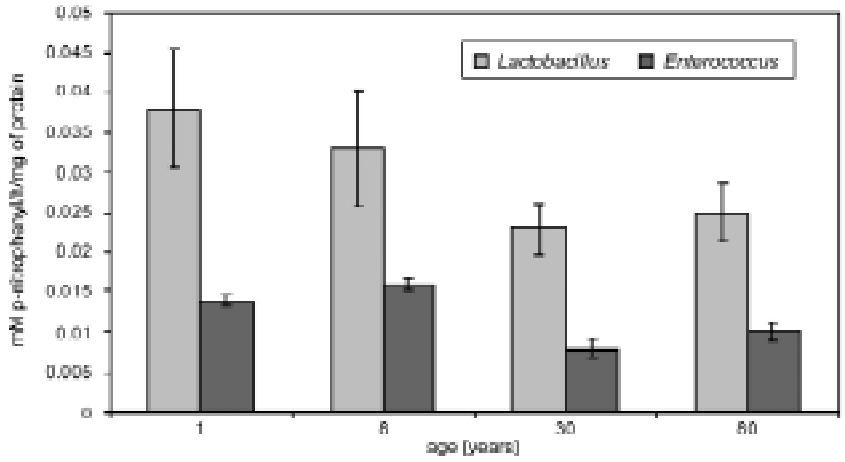

Fig. 2. Average activity of β-glucosidase of *Lactobacillus* and *Enterococcus* isolates.

activity of β-glucosidase was observed in the isolates of *Enterococcus* coming from children (2.2-fold), whereas in the adult groups this difference equaled 37.5% (Table I). The highest enzymatic activity of β-glucosidase both regarding *Lactobacillus* and *Enterococcus* was discovered in the isolates obtained from the feces of children, *i.e.* 0.048 mM/h/mg and 0.017 mM/h/mg respectively. It was determined that the activity of β-glucosidase of the *Lactobacillus* isolates coming from children was by 32% higher than the activity of the isolates from adults. It was also observed that the differences between the activity of β-glucosidase of the *Lactobacillus* and *Enterococcus* isolates coming from a one-year-old and an eight-year-old were statistically insignificant (Fig. 2). Moreover, it was shown that the activity of β-glucuronidase of *Lactobacillus* strains isolated from the feces of the 80-year-olds was the highest, and the isolates of the examined microorganisms coming from children were characterized by the highest activity of β-glucosidase.

Discussion

The age of a person may have a very significant influence on the number and activity of intestinal microorganisms. Intestinal microflora in children is better known than that in the case of the elderly, which is indicated by the fact that the number of described bacteria species coming from the large intestine of children equals 70%, and from the elderly only 8%. The intestinal microflora of an infant is dominated by *Escherichia coli* and enterococci (Rada *et al.*, 2006). The settlement of bacteria in the intestine is influenced by the mode of nutrition. It can be recognized whether a child is breastfed or whether it receives artificial baby milk on the basis of its intestinal microflora. When a child is breastfed by its mother, its intestinal microflora is richer in bacteria producing lactic acid (*Lactobacillus* and *Bifidobacterium*). After about 7–10 days after the delivery the microflora becomes more varied and absolute anaerobes can also be found (*Clostridium* and *Bacteroides*) (Strus *et al.*, 2002). The formation of an adult intestinal ecosystem takes place when children are 7–10 years old. In the case of the elderly, we observe an increase in the number of *Clostridium*, and a decrease in the number of *Bifidobacterium*, as well as an increase of the pH of the intestinal content up to 7–7.5 (Kurokawa *et al.*, 2007). These changes may result in a different amount of enzymes released to the intestinal environment, including the elevated activity of enzymes that are harmful to human health. Therefore, in this work research was undertaken with the intention of determining the activity of β-glucuronidase and β-glucosidase of *Lactobacillus* and *Enterococcus* isolates. The highest activity was discovered in the case of the *Lactobacillus* isolates coming from the 80-year-olds, and the *Enterococcus* isolates were most active in the case of children. It was proven that the activity of both β-glucuronidase and β-glucosidase of the *Lactobacillus* isolates was several times higher than the enzymatic activity of the *Enterococcus* isolates in each age group. This result emphasizes the fact that some endogenic (intestinal) bacteria – *Lactobacillus* or *Bifidobacterium* – to which only positive properties are attributed, may manifest enzymatic activity harmful to human health (*Bifidobacterium longum*) (Russel *et al.*, 2001). The activity of β-glucuronidase of *Lactobacillus* bacteria isolated from the feces of 80-year-olds was the highest among the examined age groups. The reason for this may be a diet rich in fats and poor in probiotic products coming from bacterial species showing properties favorable for the functioning of the digestive tract. A decrease in the activity of the examined enzymes may be achieved by means of the application of probiotic preparations containing controlled strains that can replace the host's own lactic acid bacteria strains manifesting too high activity of the so-called fecal enzymes. The change in the β-glucuronidase and β-glucosidase activity was observed during the application of fermented diary drinks containing *Lactobacillus casei* strain Shirota in the case of healthy adult subjects. The β-glucuronidase activity of the microorganisms present in their large intestine decreased by 30%, and the activity of β-glucosidase by 29% in comparison to the control groups (Edited by Yakult Central Institute for Microbiological Research, 1999). Similarly, a diet supplement in the form of a probiotic strain *Lactobacillus casei* DN-11401 administered to children after liver transplant led to a decrease in the activity of β-glucuronidase by 41% in comparison to children that did not receive such a diet (Pawłowska *et al.*, 2007). High level β-glucuronidase activity of in the case of lactic acid bacteria isolates taken from the group of old people may be related to typical changes in the species composition regarding this group of bacteria, which is also connected with the risk of tumors. The lack of statistically significant differences between the activity of β-glucosidase in the isolates of *Lactobacillus* and *Enterococcus* coming from the two groups of children and the two groups of adults makes it possible to determine the changes in the activity of this enzyme occurring between children and adults. The research results present a decrease in the activity of β-glucosidase in the case of both *Lactobacillus* and *Enterococcus* in adults compared to children. The increased level of the activity of this enzyme in children may be related to the kind of nutrition characteristic for this age group. A diet rich in milk influences shaping of the intestinal ecosystem in a child. A diet containing *Lactobacillus rhamnosus* LC705

and *Propionibacterium freudenreichii* ssp. *shermanii* JS resulted in a decrease of the activity of β-glucosidase in healthy persons by 10% in comparison to the control group (Hatakka *et al.*, 2008). The changes in the number of the intestinal microorganisms that accompany aging may induce an increase or a decrease of the activity level of the so-called fecal enzymes, and thus they can influence the presence of toxic and often carcinogenic substances in an organism.

The conducted research showed that there is a variety as for the activity of β-glucuronidase and β-glucosidase concerning the strains of *Lactobacillus* isolated from subjects of different age and proved that there is an unfavorable increase in the activity of β-glucuronidase accompanying aging. The activity of β-glucosidase of *Lactobacillus* and *Enterococcus* strains was decreased in case of adults in comparison to children, which suggests that the diet of adults ought to be enriched with probiotic products containing bacteria beneficial for human beings.

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