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 this 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, Butyryrovia, 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-glucuronoyltransferase. From the liver, where their synthesis takes place, they are partially removed with bile to the large intestine. There, under...
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 fragilis, Bacteroides vulgatus, Bacteroides uniformis, Ruminococcus gravis 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. quercetin 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, Clostridium 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-glucopyranoside (Sigma) for β-glucosidase. The adopted activity unit equaled such amount of phenolphthalein (for β-glucuronidase) and p-nitrophenol (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
The activity of β-glucuronidase and β-glucosidase of bacteria isolated from feces of person in different age

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Age (years)</th>
<th>Activity*</th>
<th>Activity*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>β-glucuronidase</td>
<td>β-glucosidase</td>
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<tr>
<td>Lactobacillus</td>
<td>1</td>
<td>0.135–0.275</td>
<td>0.021–0.048</td>
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<td></td>
<td>8</td>
<td>0.164–0.237</td>
<td>0.019–0.042</td>
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<tr>
<td></td>
<td>30</td>
<td>0.159–0.217</td>
<td>0.018–0.029</td>
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<tr>
<td></td>
<td>80</td>
<td>0.257–0.313</td>
<td>0.020–0.032</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>1</td>
<td>0.073–0.103</td>
<td>0.013–0.015</td>
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<td></td>
<td>8</td>
<td>0.070–0.114</td>
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<td>30</td>
<td>0.032–0.053</td>
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<td></td>
<td>80</td>
<td>0.044–0.064</td>
<td>0.006–0.009</td>
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* activity of β-glucuronidase (mM phenolphtalein /h/mg of protein), β-glucosidase (mM p-nitrophenyl /h/mg of protein)

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 equalled only 18%. A similarly varied
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 1). 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 adults. 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 (Pawlowska 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-glucuronidase 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.

**Literature**


