DETERMINATION OF THE ACTIVITY OF ENZYMES PRODUCED BY INTESTINAL BACTERIA IN THE PRESENCE OF RESISTANT DEXTRIN

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Abstract — The aim of the study was to determine the activity of enzymes produced by intestinal bacteria in the presence of resistant dextrin. For this purpose, the concentration of β-glucosidase and β-glucuronidase was determined by spectrophotometric method in the post-culture fluid obtained as a result of joint cultivation of intestinal microorganisms of the genera: Lactobacillus, Enterococcus, Bifidobacterium, Clostridium, Escherichia, Prevotella and Bacteroides, which were isolated from the faeces of children. On the basis of the obtained results, it was found that the resistant dextrin caused a decrease in the activity of all tested enzymes. The concentration of enzymes was lower compared to the control samples, where the carbon source was glucose. The activity of the enzymes varied depending on the cultivation time and reached the highest values at 96 or 168 hours of cultivation.

Keywords — Faecal Enzymes, Intestinal Bacteria, Resistant Dextrin

I. INTRODUCTION

The group of microorganisms that inhabit the human body is 10^{14} (100 trillion) cells, belonging to approximately 500-1500 species. Their number is almost 10 times greater than that of the human body. The composition of the microbiota includes indigenous species, which constitute a protective barrier against the penetration of pathogens and have an impact on the formation of the immune system and allochthonous ones that enter from the external environment - they can be pathogenic. The system of microorganisms and their numbers are individual for each representative of the human species and are specific for particular places in the body [2].

The intestinal microbiota plays an important role in metabolic processes carried out in the human body. It produces enzymes that are not encoded by the human genome and are responsible, for example, for the breakdown of polysaccharides, polyphenols and for the synthesis of vitamins [1, 3].

The metabolic activity of the intestinal microflora, in addition to its positive effect on the human body, may also cause harmful effects, such as, for example, colorectal cancer. Intestinal bacteria have the ability to activate procarcinogenic compounds that enter the intestines with food and those that enter the colon with bile. In addition, they are able to enzymatically remove the detoxification group that is attached to the carcinogen in the liver. Another way of creating harmful metabolites by the gut bacteria is by converting endogenous substances via metabolic pathways into carcinogens or substances that induce tumor formation [3].

Fecal enzymes that have a significant impact on the formation of carcinogenic compounds are: β-glucosidase, α-glucosidase, β-galactosidase, α-galactosidase, β-glucuronidase, nitroreductase, azoreductase and 7-α-dehydroxylase. Compounds that arise as a result of their excessive activity include phenolic and indole compounds, flavonoid aglycones, azo dyes, estrogens, as well as secondary bile acids. All of the above-mentioned products may adversely affect human health [5].

Resistant dextrins are starch dextrinization products resistant to the action of hydrolytic enzymes in the human gastrointestinal tract. They contain short chains of linked glucose oligomers / polymers along with numerous types of digestible glycosidic linkages different from the typical glycosidic linkages present in starch. It is the formation of these bonds that makes the end products unavailable for human digestive enzymes, demonstrating the properties of resistant starch, which includes resistant dextrins. Resistant dextrins have been found to have many beneficial properties, resulting in their use in functional foods [4, 6].

Hence, the study investigated the activity of two faecal enzymes (β-glucosidase and β-glucuronidase) produced by deer bacteria and whether resistant dextrin will have a positive effect on the production of these enzymes.

II. MATERIAL AND METHODS

The scope of work included the determination of the concentration of fecal enzymes (β-glucosidase and β-glucuronidase) in post-culture fluid obtained as a result of co-culturing intestinal microorganisms of the genus: *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, *Clostridium*, Escherichia, *Prevotella* and *Bacteroides* which were isolated from the faeces of children. Bacterial cultures were incubated in a medium containing resistant dextin (appropriate samples) or

glucose (control samples) for 168 h. After 24 h, 48 h, 72 h, 96 h and 168 h of culture, post-culture fluid was collected in which the activity of fecal enzymes was determined.

The enzymes activity was measured by spectrophotometric method. Activity units were calculated as the amounts (μmol/L) of phenolphthalein (for β-glucuronidase) and nitrophenyl (for β-glucosidase) released per 1 hour, per 1 ml (U) per 1 mg of protein (U/mg).

III. RESULTS

In culture with resistant dextrin, the activity of β-glucosidase was variable and dependent on the time of the culture. At 24 hours of culture, the concentration of this enzyme ranged from 0.04 to 0.67 μM*U/mg (0.40 μM*U/mg on average). In the following hours of cultivation, an increase in the activity of this enzyme was found. The highest concentration of β-glucosidase was found at 96 hours of culture (from 0.34 to 0.77 μM*U/mg, average 0.58 μM*U/mg). At 168 hours of cultivation, the concentration of β-glucosidase fluctuated around similar values (Figure 1A).

In culture with glucose, the β-glucosidase activity was also variable and time-dependent, and also higher than when resistant dextrin was used as the carbon source. For all samples, the highest concentration was recorded at the 96 hour of culture (from 0.72 to 1.04 μM*U/mg, average 0.94 μM*U/mg) and it was on average

0.36 μM*U/mg higher than in a resistant dextrin culture (Figure 1B).

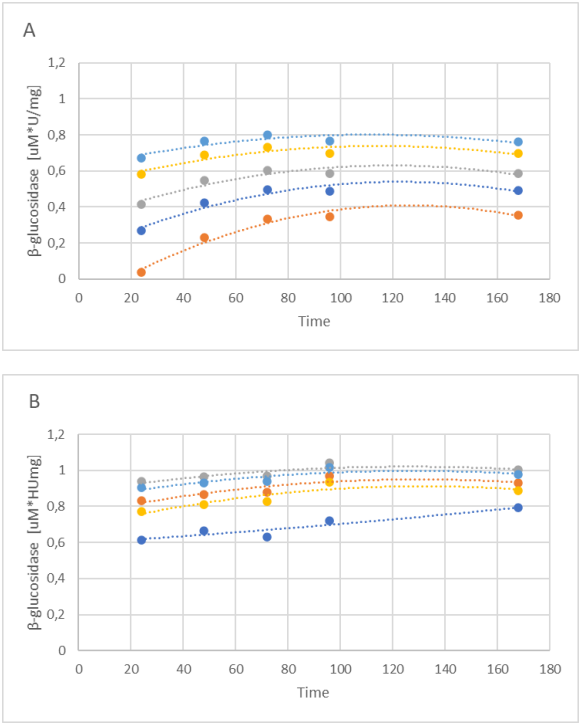


Figure 1. β-glucosidase activity in medium with resistant dextrin (A) and glucose (B).

The β-glucuronidase activity was also varied and depended on the cultivation time. The lowest activity of this enzyme in the samples with resistant dextrin was recorded at 24 hours of cultivation (from 0.43 to 2.53 μM*U/mg, average 1.49 μM*U/mg). With the passage of time of cultivation, the activity of this enzyme increased and its highest value was recorded at 168 hours of cultivation (from 1.23 to 2.91 μM*U/mg, average 2.15 μM*U/mg) (Figure 2A).

β-glucuronidase was more active in glucose culture than in the dextrin resistant trials. At 24 hours of culture, the concentration of this enzyme ranged from 2.93 to 4.08 μM*U/mg (mean 3.63 μM*U/mg) and was, on average, higher by 2.14 μM*U/mg than in the resistant dextrin culture. Observing the dynamics of the activity of this enzyme, it was noted that it was the highest at 168 hours of cultivation (from 4.59 to 5.08 μM*U/mg, on average 4.89 μM*U/mg) and it was on average higher by 2.74 μM * H / mg than trials with resistant dextrin (Figure 2B).

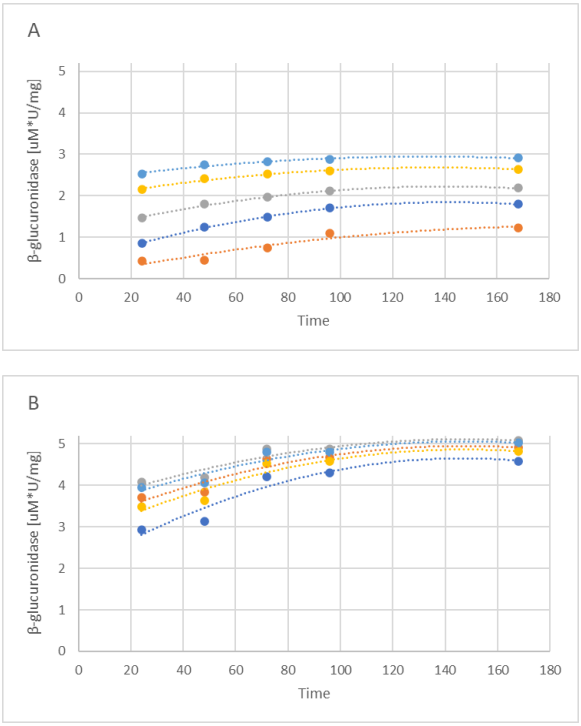


Figure 2. β-glucuronidase activity in medium with resistant dexrite (A) and glucose (B)

IV. CONCLUSION

The activity of faecal enzymes (β-glucosidase and β-glucuronidase) varied and depended on the time of culture. The tested fecal enzymes obtained the highest activity at 96 or 168 hours of cultivation. The resistant dextrin decreased the concentration of all tested faecal enzymes. The concentration of enzymes was lower compared to the control samples (with glucose).

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