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COMPARISON OF THE GROWTH OF *LACTOBACILLUS ACIDOPHILUS* AND *BIFIDOBACTERIUM BIFIDUM* SPECIES IN MEDIA SUPPLEMENTED WITH SELECTED SACCHARIDES INCLUDING PREBIOTICS

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Abstract. The performed investigations evaluated the growth dynamics of *Lactobacillus acidophilus* DSM 20079, *Lactobacillus acidophilus* DSM 20242, *Bifidobacterium bifidum* DSM 20082, *Bifidobacterium bifidum* DSM 20215, *Bifidobacterium bifidum* DSM 20239, *Bifidobacterium bifidum* DSM 20456 in media supplemented with various saccharides, including prebiotic preparations. The addition of saccharides to the medium exerted a significant influence not only on the number of bacterial cells but also on their acid-creating capability. Glucose, lactose and saccharose turned out to be the easiest available saccharides for all the bacterial strains tested. In the media supplemented with these sugars the highest numbers of bacterial cells were determined. At the shortage of mono- and disaccharides, all strains of the bacteria tested were capable of utilising the prebiotic preparations as sources of carbon and energy in the media. The amount and isomeric forms of lactic acid produced by *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20239 were determined. Both strains meet the requirements adopted by the WHO and produce more than 70% lactic acid L(+) in the media with the addition of various saccharides. *Lactobacillus acidophilus* DSM 20079 was found to produce significantly higher amount of lactic acid in different media.

Key words: probiotics, prebiotic, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, lactic acid

INTRODUCTION

Prebiotics are food additives whose favourable effect on the human organism is associated with the stimulation of growth and activity of some strains of the native microflora or microflora introduced with the ingested food [De Vuyst 2002]. A prebiotic is defined as «a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in

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the colon» [Roberfroid 1998]. Prebiotics can be fermented through certain microorganisms e.g. lactic acid bacteria from *Bifidobacterium* and *Lactobacillus* belong to the most common species used as probiotics in the human diet [Fooks et al. 1999]. Principal end products of prebiotics bacterial fermentation in the colon are: CO_2, H_2, CH_4, short chain fatty acids (SCFA) and other organic acids [Roberfroid 2000, Cherbut 2002, Cummings and MacFarlane 2002, Mountzouris et al. 2002]. Reduction of gut pH through SCFA formation inhibits growth of pathogenic colon bacteria. In this way, the host's health condition is improved as well as his/her resistance to the penetration into the organism of potential allergens [Tannock 2002]. Different researchers consider fructans-fructo--oligosaccharides (FOS) in this inulin and oligofructose, lactulose, galacto-oligosaccharides (GOS), transgalacto-oligosaccharides (TOS), and iso-maltooligosaccharides (IMO) as prebiotics [Fooks et al. 1999, Blaut 2002, Rastall and Maitin 2002].

Bifidobacteria are known to have probiotic properties. The digestive system as well as mucous membranes of humans and animals constitute natural sites of *Bifidobacterium*, whereas *Lactobacillus* bacteria inhibit the digestive and urogenital systems [Klaenhammer 1995]. Bifidobacteria are among the first colonisers of the sterile gastrointestinal tract of newborns during breast feeding [Arunachalam 1999, Doleyres and Lacroix 2005]. Media for the culturing of *Bifidobacterium* require the presence of carbon source. Besides glucose all bifidobacteria are also capable of utilising galactose, lactose and usually fructose as carbon sources [Gomes and Malcata 1999], or products containing these carbohydrates, e.g. honey (fructose, galactose, oligosaccharide fraction in the structure) [Arunachalam 1999, Chick et al. 2001, Ustunal and Gandhi 2001, Shin and Ustunol 2005].

The genus of *Bifidobacterium* may, with the assistance of intracellular enzymes, break down polysaccharides which undergo conversion into glucose and fructose phosphates and, later on, can be metabolised in a way characteristic for bifidobacteria. Most strains of *Lactobacillus acidophilus* can ferment amygdalin, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, salicin, sucrose and trehalose [Gomes and Malcata 1999].

Predominant growth substrates for gut bacteria are of dietary origin and consist of foodstuffs that have not been absorbed in the upper gastrointestinal tract (resistant starch, dietary fibre, sugars, oligosaccharides, proteins, peptides and amino acids). There is also a quantitatively lower contribution from endogenous sources such a mucins [Fooks et al. 1999, Ouwehand et al. 2005].

The main development of functional food refers to food articles which contain probiotics and prebiotics are involved in the fortification of the bacterial microbiota in the intestines [Matilla-Sandholm et al. 2002, Mountzouris et al. 2002]. The list of prohealth ingredients includes: oligosaccharides, polyphenols, phospholipids, proteins and peptides, polyene fatty acids, minerals, vitamins, probiotics, phytocompounds and food fibre [Westrate et al. 2002]. Prebiotics may be very important in the nutrition of diabetics for whom the type and quantity of carbohydrates contained in the food products is very important [Biesalski 1999].

There is abundant literature dealing with the effect of prebiotics on the organism in *in vivo* experiments but few investigations were devoted to *in vitro* experiments on the impact of FOS on the growth of selected probiotic bacteria. The *in vitro* studies concern, primarily, milk products with the raffinose family oligosaccharides [Martínez-Villaluenga et al. 2006] or galactooligosascharides from dairy products and products from

lactose or whey (lactulose, lactitol, lactobionic acid) for selected bacteria [Gopal et al. 2001, Saarela et al. 2003]. Even if the *in vitro* growth of bacteria on FOS was tested, it did not involve bacteria tested in our study [Biedrzycka and Bielecka 2004, Maxwell et al. 2004, Pennacchia et al. 2006].

The results of initiated studies in humans indicated that inulin, fructooligosaccharose or galactooligosaccharose may play important role for the absorption of Ca^{2+}, Mg^{2+} and Fe^{2+} [Roberfroid 2000, Tuohy et al. 2003]. Lowering of blood lipids has been associated with prebiotic consumption [Roberfroid 1998]. Inulin and oligofructose prebiotic properties were confirmed in numerous experiments carried out both on animals and people. Feeding of animals with diets enriched with preparations of non-digestible oligosaccharides resulted in some colonic microflora changes [Bielecka et al. 2002, Roberfroid 1998].

Another important question with regard to the effect of inulin or fructo-oligosaccharides on human fecal bifidobacterial counts is the dose-effect relationship. Within a range of 4-20 g or more, when initial bifidobacterial numbers are already high, it is difficult to further increase the size of the population by ingesting exogenous bifidobacterial cells. However, this does not exclude the possibility that, a dose-effect relationship might be observed if it were to be measured in the same group of volunteers with similar initial counts of bifidobacteria. But within the general population, in which fecal counts of bifidobacteria vary considerably, such a dose-effect relationship would be difficult to observe. It is generally regarded that at least 4 g/day but more preferably 8 g/day of FOS or GOS would be needed to significantly elevate bifidobacteria in the human gut [Alander et al. 2001, Losada and Olleros 2002, Matteuzzi et al. 2004, Scantlebury Manning and Gibson 2004].

If probiotics and a prebiotic occur together in one product, we refer to it as a synbiotic or eubiotic. The above-mentioned combination aims at increased survivability of the administered probiotic and facilitates its inoculation in the large intestine [Scantlebury Manning and Gibson 2004].

Our investigations fit into investigations on the evaluation of the efficiency of the application of prebiotics in combination with potentially probiotic bacteria. Therefore, it seems feasible to undertake *in vitro* research concerned with the growth dynamics of potentially probiotic bacteria in the media supplemented with different saccharides, including those recognised as prebiotics, and to study differences between bacterial species.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Lactobacillus acidophilus (DSM 20079 and DSM 20242) and *Bifidobacterium bifidum* (DSM 20082, DSM 20215, DSM 20239 and DSM 20456) were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). *Lactobacillus acidophilus* were grown in carbohydrate-free MRS medium [Saarela et al. 2003] and *Bifidobacterium bifidum* in carbohydrate-free Medium 58 (DSMZ) anaerobically at 37°C (pH = 6.5). The media were supplemented with sugar or fructooligosaccharids tested in amount 33.3% of dry mass of medium. The following saccharides were added:

glucose, fructose, lactose, saccharose (POCh, Poland), oligofructose (Raftilose® P95--Orafti, Belgium), inulin (Raftiline® HP-Orafti, Belgium).

Raftilose® P95 contains 95% oligofructose with the remaining 5% of glucose, fructose and saccharose. Raftiline® HP contains 100% inulin and is characterised by high purity. In comparison with standard inulin, Raftiline® HP is characterised by 50% reduction of its doses in products, while maintaining the same organoleptic properties and advantages associated with the substitution of fat [Roberfroid 2002].

The inoculum was prepared from strains stored at –70°C using appropriate media for 24 hours and it was propagated in the medium for the next 48 hours anaerobically at 37°C. The cells were centrifuged (15 min; 5000 rpm; at 4°C) and suspended in sodium chloride solutions (8.5 g × L^{-1}).

Bacteria prepared in this way provided the inoculum (2×10^7 cfu × mL^{-1}) which was used at the amount of 10% (v/v). The number of live (cfu × mL^{-1}) bacteria was determined by total plate count after 24 and 48 hours of cultivation. Medium without sugar and with glucose were used as controls.

Nephelometric method of measurement of bacterial growth

Growths of control cultures (glucose) and of test cultures (different sugars and prebiotics) were monitored after 24, 48, 72 h of incubation by measuring optical density at 600 nm using the spectrophotometer CADAS 3Os (Dr Lange, Germany). The references blanc were media with different sugars without bacteria [Gopal et al. 1996].

Supernatant pH determination after cultivation of bacteria

Bacterial cells were centrifuged after 48 hours (15 min; 5000 rpm; at 4°C) and pH of supernatants were measured.

Determination of the quantities of lactic acid [Ball 1990, Biorad 1994]

Lactic acid was determined on a Waters liquid chromatographer equipped in a refractometric detector (type Waters 410). The applied column was that of the Merck type POLYSPHER^R OAKC. A solution of sulphuric acid of 0.005 mol L^{-1} concentration was used as eluant at a flow of 0.5 mL min^{-1}. Assays were carried out at 32 RIU/F.S sensitivity of the detector. Samples were diluted depending on the predicted concentration of lactic acid. Measurements, file integration as well as the calculation of results were carried out with the assistance of the Millenium 32 computer program. Calculations and measurements were performed in relation to the previously prepared and investigated model solutions of the examined substance.

Assays of the L(+) and D(-) isomers of lactic acid

Assays of the L(+) and D(-) isomers of lactic acid were carried out by the chromatographic method Daicel Chemical Industries, Ltd. guidebook using the Waters liquid chromatographer with a UV detector (type Waters 2487). A Baker B.V. type Chiralpak MA (+) column was used to carry out assays. 2 mM concentration of $CuSO_4$ and 0.6 mL min^{-1} flow was used as an eluant. Measurements, file integration as well as the cal-

culation of results were carried out with the assistance of the Millenium 32 computer program.

Statistical assessment

All the media tested for each strain were prepared in five replications and the results are mean values from these repetitions. Statistical analysis of results using Excel 2000 software was preformed employing mean descriptive statistics and single-factorial analysis of variance ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of the addition of saccharides on changes in viable counts and active acidity of the medium

The addition of saccharides to the medium increased significantly both the total number of bacteria as well as their acid-creating capacity. In the case of cultivation of the *Lactobacillus acidophilus* in the medium without the addition of sugar, the total number of bacteria was found to decline. The total number of bacteria reached 5.13×10^5 cfu × mL^{-1} in the case of *Lactobacillus acidophilus* DSM 20079 and 3.2×10^3 cfu × mL^{-1} for *Lactobacillus acidophilus* DSM 20242 in the 48th hour of cultivation and was significantly lower than in the case when saccharides were added to the medium (Fig. 1 and 2). Glucose, lactose and saccharose were the most readily catabolised saccharides. Moreover, media supplemented with the above sugars gave the highest total number of bacteria for both *Lactobacillus* strains. In the 48th hour of cultivation of *Lactobacillus acidophilus* DSM 20079, the total number of bacteria in the medium with the addition of the above-mentioned sugars reached: 2.45×10^8 cfu × mL^{-1}; 1.46×10^7 cfu × mL^{-1} and 2.53×10^6 cfu × mL^{-1}, respectively. The above values were higher for *Lactobacillus acidophilus* DSM 20242 strain: 2.83×10^9 cfu × mL^{-1}; 4.06×10^8 cfu×mL^{-1} and 1.46×10^8 cfu × mL^{-1}, respectively. The *Lactobacillus* strains also utilised Raftilose and Raftiline in the medium as carbon sources. Although total number of bacteria developed during 48 hours of cultivation were not as high as in the case of easily-available carbon sources, nevertheless their numbers differed significantly in comparison with the medium without saccharide supplementation. In the case of the medium supplemented with Raftiline, the total number of bacteria in the 48th hour of cultivation was lower than in the case of Raftilose and amounted to 1.22×10^6 cfu × mL^{-1} and 6.28×10^6 cfu × mL^{-1} for *Lactobacillus acidophilus* DSM 20079 strain. In the case of *Lactobacillus acidophilus* DSM 20242 strain, these values for the medium with the above two sugars were higher and in the 48th hour reached: 1.18×10^7 cfu × mL^{-1} and 1.47×10^8 cfu × mL^{-1}, respectively. In the case of cultivation of all strains of *Bifidobacterium bifidum* in the medium without saccharide supplementation, significant increase in the total number of bacteria was recorded indicating that these bacteria utilised also other nutrients available in the «rich» medium. Nevertheless, the increase in the numbers of live cells was higher in the case of the addition of saccharides to the medium (Fig. 3-6). In the case of *Bifidobacterium bifidum* DSM 20082 and DSM 20239, the highest increase in the total number

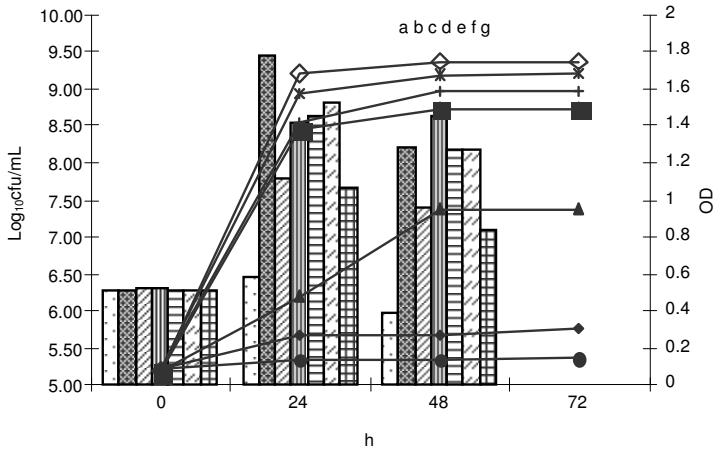

Fig. 1. Changes in the viable counts of *Lactobacillus acidophilus* DSM 20079 in the media supplemented with different saccharides and prebiotic preparations and OD changes. Means with different letters: a, b, c, d, e, f, g are significantly different ($P < 0.05$). Cfu: – without sugar, – in a medium containing glucose, – fructose, – lactose, – saccharose, – Raftilose, – Raftiline. Absorbance: ● – without sugar, ◇ – glucose, ▲ – fructose, + – lactose, ✱ – saccharose, ■ – Raftilose, ◆ – Raftiline

Rys. 1. Zmiana liczby żywych bakterii *Lactobacillus acidophilus* DSM 20079 w podłożu z dodatkiem różnych sacharydów i preparatów prebiotycznych oraz zmiana absorbancji podczas hodowli. Średnie oznaczone różnymi literami: a, b, c, d, e, f, g są statystycznie różne ($P < 0,05$). Cfu: – bez cukru, – w pożywce zawierającej glukozę, – fruktozę, – laktozę, – sacharozę, – Raftilose, – Raftiline. Absorbancja: ● – bez cukru, ◇ – glukoza, ▲ – fruktoza, + – laktoza, ✱ – sacharoza, ■ – Raftilose, ◆ – Raftiline

of bacteria was recorded in the medium with the addition of glucose and total number of bacteria reached: 2.16×10^9 cfu × mL^{-1} and 6.21×10^7 cfu × mL^{-1} in the 48th hour, respectively (Fig. 3 and 5). In the case of *B. bifidum* DSM 20215 and *B. bifidum* DSM 20456 in the medium supplemented with lactose, the total number of bacteria in the 48th hour of cultivation amounted to: 4.21×10^8 cfu × mL^{-1} and 4.14×10^8 cfu × mL^{-1}, respectively (Fig. 4 and 6). All strains of *Bifidobacterium bifidum* grew better in the medium with the addition of Raftilose than Raftiline. In the 48th hour of cultivation, the total number of bacteria in the medium with Raftilose for the individual strains reached the following values: DSM 20082 – 1.18×10^9 cfu × mL^{-1}; DSM 20215 – 2.25×10^9 cfu × mL^{-1}; DSM 20239 – 1.1×10^7 cfu × mL^{-1} and DSM 20456 – 3.87×10^8 cfu × mL^{-1}. Total number of bacteria of all strains were significantly higher in the medium supplemented with Raftilose than in the medium with the addition of Raftiline ($P < 0.05$). In the medium supplemented with Raftiline, the determined values were: DSM 20082 – 3.41×10^8 cfu × mL^{-1}, DSM 20215 – 5.17×10^8 cfu × mL^{-1}; DSM 20239 – 6.32×10^6 cfu × mL^{-1} and DSM 20456 – 5.02×10^7 cfu × mL^{-1} and were by one order of magnitude lower than in the case of the medium supplemented with Raftilose. In the case of all strains, optical density value of the medium reached higher values for cultures in the

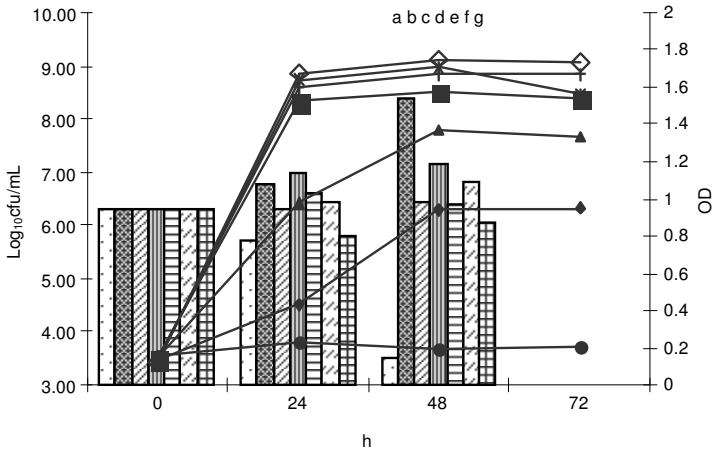

Fig. 2. Changes in the viable counts of *Lactobacillus acidophilus* DSM 20242 in the media supplemented with different saccharides and prebiotic preparations and OD changes. Explanations see Figure 1

Rys. 2. Zmiana liczby żywych bakterii *Lactobacillus acidophilus* DSM 20242 w podłożu z dodatkiem różnych sacharydów i preparatów prebiotycznych oraz zmiana absorbancji podczas hodowli. Objaśnienia patrz rysunek 1

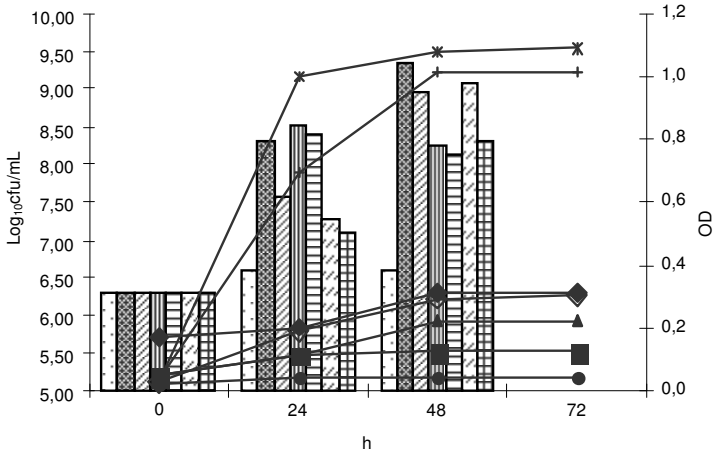

Fig. 3. Changes in the viable counts of *Bifidobacterium bifidum* DSM 20082 in the media supplemented with different saccharides and prebiotic preparations and OD changes. Explanations see Figure 1

Rys. 3. Zmiana liczby żywych bakterii *Bifidobacterium bifidum* DSM 20082 w podłożu z dodatkiem różnych sacharydów i preparatów prebiotycznych oraz zmiana absorbancji podczas hodowli. Objaśnienia patrz rysunek 1

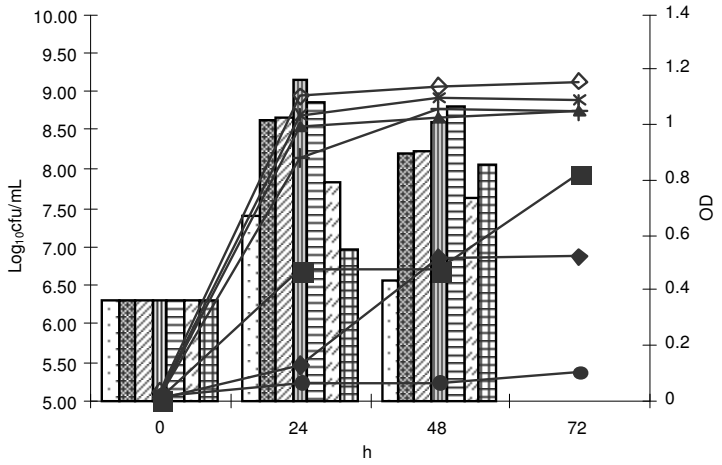

Fig. 4. Changes in the viable counts of *Bifidobacterium bifidum* DSM 20215 in the media supplemented with different saccharides and prebiotic preparations and OD changes. Explanations see Figure 1

Rys. 4. Zmiana liczby żywych bakterii *Bifidobacterium bifidum* DSM 20215 w podłożu z dodatkiem różnych sacharydów i preparatów prebiotycznych oraz zmiana absorbancji podczas hodowli. Objaśnienia patrz rysunek 1

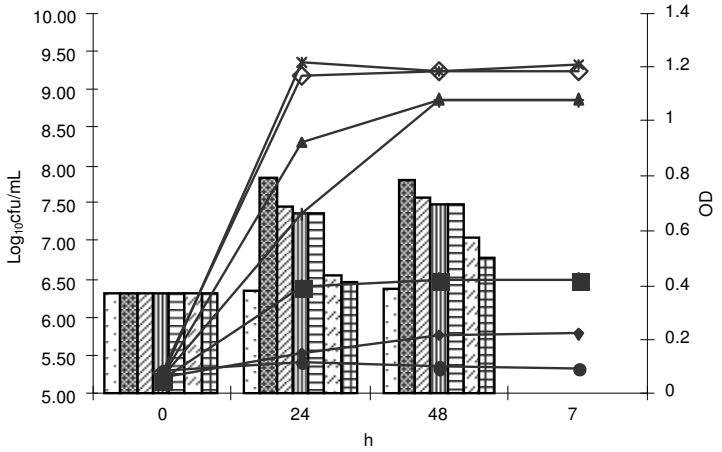

Fig. 5. Changes in the viable counts of *Bifidobacterium bifidum* DSM 20239 in the media supplemented with different saccharides and prebiotic preparations and OD changes. Explanations see Figure 1

Rys. 5. Zmiana liczby żywych bakterii *Bifidobacterium bifidum* DSM 20239 w podłożu z dodatkiem różnych sacharydów i preparatów prebiotycznych oraz zmiana absorbancji podczas hodowli. Objaśnienia patrz rysunek 1

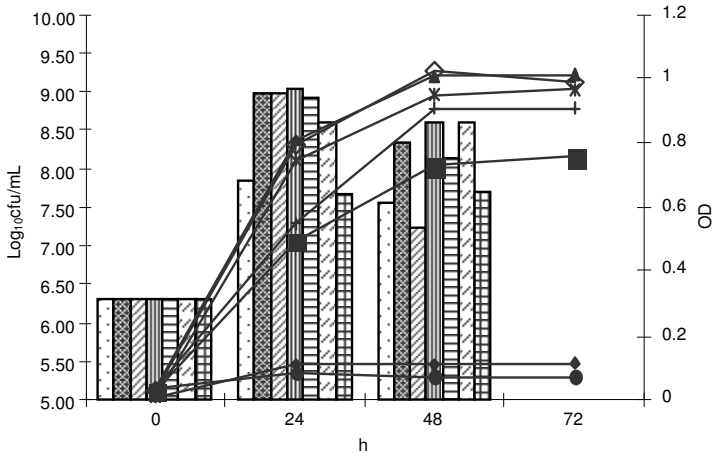

Fig. 6. Changes in the viable counts of *Bifidobacterium bifidum* DSM 20456 in the media supplemented with different saccharides and prebiotic preparations and OD changes. Explanations see Figure 1

Rys. 6. Zmiana liczby żywych bakterii *Bifidobacterium bifidum* DSM 20456 w podłożu z dodatkiem różnych sacharydów i preparatów prebiotycznych oraz zmiana absorbancji podczas hodowli. Objaśnienia patrz rysunek 1

medium with Raftilose than with Raftiline – we obtained higher biomass yield of bacterial cells. The tested strains of *Bifidobacterium bifidum* utilized saccharose or lactose much better than prebiotics.

Therefore, the experiments confirmed that glucose is the easiest available saccharide for the tested strains. Some strains of *Bifidobacterium bifidum* utilise lactose as readily as saccharose. At the shortage of saccharides in the medium, all strains of the tested bacteria are capable to utilise the prebiotic substances. Higher total number of bacteria was recorded in the media with Raftilose and Raftiline in comparison with cultures without saccharide supplementation.

Also Śliżewska and Libudzisz [2001] reported differences in the growth of intestinal bacteria from *Lactobacillus* in the presence of fructo-oligosaccharides. The lowest increase of biomass was recorded in the media containing inulin and inulin preparations (Raftiline® HP and ST) and it was found to depend on the strain. The lowest biomass increase was also confirmed by the optical density of the medium with the Raftiline® HP supplementation. We recorded higher optical density values in the medium with the addition of Raftiline® HP than Raftilose® P95 only for *Bifidobacterium bifidum* DSM 20082. The above-mentioned researchers also obtained a slightly higher biomass yield in the media containing oligofructose (Raftilose® P95 and L60) in comparison with the increase in the presence of inulin. Nearly all of *Lactobacillus* and *Bifidobacterium* strains tested in our experiments in the media supplemented with the Raftilose® P95 certified the above investigations but only for *Bifidobacterium* optical density values were lower than cultures in the media containing mono- and disaccharides. *Lactobacillus* strains tested in our investigations achieved higher optical density values in the medium supplemented with the Raftilose® P95 than in the medium with the addition of

fructose. Moreover, Śliżewska and Libudzisz [2001] showed that glucose and saccharose are the best sources of carbon for *Lactobacillus acidophilus*. Our experiments also proved that, from among the examined carbon sources, both tested *Lactobacillus acidophilus* utilised glucose and saccharose best. It was in the media with these very additives that we recorded the highest total number of bacteria as well as the highest optical density values.

Raftiline and Raftilose were also utilized by all the *Bifidobacterium* strains in experiments conducted by Mayer et al. [2003]. In their studies, Rada et al. [2002] emphasised different growth, in the medium supplemented with various saccharides, dependent on the species or even strain of *Bifidobacterium*. They also indicated that there is *Bifidobacterium pseudolongum* which does not ferment glucose. In the case of *Bifidobacterium bifidum* examined in this study, this did not occur and all strains achieved a distinctly higher growth in the medium supplemented with glucose than with prebiotic preparations.

Possibilities of synbiotic production were also reported by Kneifel et al. [2000] who observed growth of *Lactobacillus* and *Bifidobacterium* in media with the addition of prebiotics. In addition, numerous researchers emphasize a better growth of bacteria derived from intestines in the medium supplemented with inulin with DP > 10. Other authors reported that galacto-oligosaccharides and fructo-oligosaccharides with lower DP are best in supporting the growth of bifidobacteria and carbohydrates with high DP are poor substrates for bifidobacteria [Bruno et al. 2002].

Table 1 presents acidity of media after 48 hours of cultivation of different strains in the media supplemented with saccharides. The ready-to-use media for all strains before inoculation were characterised by pH = 6.5. It changed only slightly after 48 hours, when no saccharides were added to the medium. The lowest pH value of 5.97 was recorded for the *Bifidobacterium bifidum* DSM 20239. However, for the culture of this strain, the authors recorded the highest pH changes after 48 hours in the media with all types of saccharides. The pH values of the media in the cultures of all bacterial strains in the medium with the addition of glucose differed statistically significantly in comparison with those with the addition of Raftilose and Raftiline ($P < 0.05$). Also cultures supplemented with prebiotic preparations differed between one another statistically significantly ($P < 0.05$). The medium pH supplemented with Raftilose dropped, for *Lactobacillus acidophilus* DSM 20079 and *Lactobacillus acidophilus* DSM 20242 to the values of 3.81 and 3.68, respectively. These values were lower in comparison with the addition to the medium of Raftiline and amounted to: 5.9 and 5.79, respectively. *Bifidobacterium bifidum* reduce less the pH of the Raftilose-containing medium in comparison with *Lactobacillus acidophilus*. The respective values amounted to: 4.27 – for the *Bifidobacterium bifidum* DSM 20082; 4.23 – for *Bifidobacterium bifidum* DSM 20215; 4.02 – for *Bifidobacterium bifidum* DSM 20239 and 4.56 – for *Bifidobacterium bifidum* DSM 20456. These values are significantly different for the cultures of these strains in the media supplemented with fructose, lactose and saccharose. In addition, cultures of *Bifidobacterium bifidum* supplemented with Raftiline reduced the pH of the medium more than those with *Lactobacillus acidophilus*. These values amounted to: 5.12 – *Bifidobacterium bifidum* DSM 20082; 5.03 – for *Bifidobacterium bifidum* DSM 20215; 4.82 – for *Bifidobacterium bifidum* DSM 20239 and 5.51 – for *Bifidobacterium bifidum* DSM 20456. We recorded significant differences for cultures with the addition of other saccharides. In the case of the culture with *Lactobacillus acidophilus* DSM

Table 1. Medium pH value after 48 hours of cultivation six probiotics strains with the addition of different saccharides and prebiotic preparations

Tabela 1. Zmiana pH podłoża po 46 h hodowli sześciu probiotycznych szczepów w podłożu z dodatkiem różnych sacharydów i preparatów prebiotycznych

Medium Pożywka	*Lactobacillus acidophilus*		*Bifidobacterium bifidum*			
	DSM 20079*	DSM 20242*	DSM 20082*	DSM 20215*	DSM 20239*	DSM 20456*
Without sugar Bez cukru	6.40 ±0.05 g	6.34 ±0.04 e	6.13 ±0.05 e	6.26 ±0.04 e	5.97 ±0.06 g	6.16 ±0.06 e
Glucose Glukoza	3.72 ±0.03 b	3.67 ±0.03 b	3.71 ±0.02 a	3.68 ±0.03 a	3.52 ±0.04 a	3.93 ±0.04 a
Fructose Fruktoza	4.30 ±0.03 e	4.24 ±0.04 c	3.78 ±0.03 b	3.65 ±0.04 a	3.66 ±0.03 c	4.04 ±0.05 b
Lactose Laktoza	3.90 ±0.03 d	3.70 ±0.05 b	3.83 ±0.02 b	3.76 ±0.03 b	3.73 ±0.03 d	4.00 ±0.03 a,b
Saccharose Sacharoza	3.60 ±0.04 a	3.61 ±0.03 a	3.71 ±0.03 a	3.63 ±0.02 a	3.57 ±0.02 b	3.95 ±0.04 a,b
Raftilose	3.81 ±0.03 c	3.68 ±0.05 b	4.27 ±0.04 c	4.23 ±0.04 c	4.02 ±0.05 e	4.56 ±0.05 c
Raftiline	5.90 ±0.05 f	5.79 ±0.05 d	5.12 ±0.05 d	5.03 ±0.05 d	4.82 ±0.04 f	5.51 ±0.05 d

*Mean and standard deviation.
Means in the columns with different letters: a, b, c, d, e, f, g are significantly different ($P < 0.05$).
*Średnio i odchylenie standardowe.
Wartości w kolumnach oznaczone różnymi literami: a, b, c, d, e, f, g są statystycznie różne ($P < 0,05$).

20079, significant differences were found between the pH of cultures supplemented with glucose (3.72 ±0.03) and Raftilose (3.81 ±0.03), whereas for *Lactobacillus acidophilus* DSM 20242 – there were no significant differences between culture with the addition of glucose (3.67 ±0.03), lactose (3.70 ±0.05) and Raftilose (3.68 ±0.05; $P > 0.05$). We observed significant differences between the pH value of the media with glucose (3.72 ±0.03) and saccharose (3.60 ±0.04) for *Lactobacillus acidophilus* DSM 20079. In the case of cultures with *Bifidobacterium bifidum* DSM 20082, no significant differences were observed between the pH of the media containing glucose (3.71 ±0.02) and saccharose (3.71 ±0.03) as well as between fructose (3.78 ±0.03) and lactose (3.83 ±0.02). There were also no significant differences for culture of *Bifidobacterium bifidum* DSM 20215 with glucose (3.68 ±0.03), saccharose (3.63 ±0.02) and fructose (3.65 ±0.04). The same can be said about cultures of *Bifidobacterium bifidum* DSM 20456 supplemented with glucose (3.93 ±0.04), saccharose (3.95 ±0.04) and lactose (4.00 ±0.03) as well as fructose (4.04 ±0.05), lactose (4.00 ±0.03) and saccharose (3.95 ±0.04; $P > 0.05$). The pH of the media containing fructose (3.66 ±0.03) and saccharose (3.57 ±0.02) for *Bifidobacterium bifidum* DSM 20239 failed to show significant differences. Therefore, the above discussed research results confirm data about the significance of differences of bacterial cultures in media supplemented with prebiotics and other easily available saccharides.

Analysing the correlation of pH changes of the medium with the total number of bacteria during the cultivation in media containing different saccharides it can be no-

ticed that in the case of all strains, the pH reduction was accompanied by the increase in the total number of bacteria. In the medium without saccharide supplementation, the authors recorded the lowest total number of all tested strains in comparison with the saccharide-containing cultures. Higher pH value was determined and, at the same time, lower amount of bacteria was determined for *Lactobacillus acidophilus* cultivated in the medium supplemented with Raftiline in comparison with cultures grown in media supplemented with other saccharides.

The same regularity also referred to the bacteria from *B. bifidum* DSM 20239. In the case of the remaining bacterial strains, in the 48th hour, only slight differences in the pH changes and the determined viable counts were recorded.

Passing intact to the large intestine and not digested in the initial sections of the gastrointestinal tract of the host, prebiotics become the only source of carbon for the bacteria inhabiting its final section. The above-discussed investigations demonstrated that all the tested bacteria strains are capable of utilising substances of fructo-oligosaccharide nature in metabolic processes as a carbon source. This property is a very advantageous trait because it can contribute to the development of prebiotic bacteria in the gastrointestinal tract.

Impact of the addition of saccharides on the quantity and form of the lactic acid

The results of the research presented in Table 2 showed that the carbon source effects on the production of lactic acid and only marginally influences proportions of its isomers. *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20239 fulfilled the WHO requirements and produced over 50% L(+) lactic acid. In addition, *Lactobacillus acidophilus* DSM 20079 produced statistically significantly more lactic acid in the media supplemented with different saccharides than *Bifidobacterium bifidum* DSM 20239 ($P > 0.05$).

Śliżewska and Libudzisz [2001] tested 15 strains of intestinal bacteria from *Lactobacillus* in MRS media which contained fructo-oligosaccharides and obtained species- and strain-dependent average production of lactic acid ranging from 1.06 g × L^{-1} to 6.25 g × L^{-1}. It was considerably lower than in the media containing mono- and disaccharides (from 8.42 g × L^{-1} to 10.09 g × L^{-1}). Results obtained for *Lactobacillus acidophilus* DSM 20079 in our investigations are similar to those reported by Śliżewska and Libudzisz. However, *Lactobacillus acidophilus* DSM 20079 produced in the medium supplemented with Raftilose 8.0 g × L^{-1} lactic acid and this value is higher than that reported by the above-mentioned authors. However, in the medium containing fructose and lactose, the production of lactic acid by this strain was lower than in the data reported by other researchers and amounted to: 5.87 g × L^{-1} and 7.48 g × L^{-1}, respectively. In the case of the media supplemented with glucose and saccharose, the tested strain produced quantities of lactic acid comparable with other strains of *Lactobacillus*. Literature data also mention the proportion of the L(+) lactic acid in the presence of fructo-oligosaccharides which ranged, depending on the species, from 50.6% to 90.3%, while in the presence of simple sugars, depending on the species and the type of sugar, from 52.2% to 88.1% [Śliżewska and Libudzisz 2001]. Data obtained in our experiments, both for *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20239 are in agreement with literature reports.

Table 2. Content of lactic acid and its forms after 48 hours cultivation of bacteria in media supplemented with different saccharides

Tabela 2. Zawartość kwasu mlekowego i jego form po 48 h hodowli bakterii w podłożach z dodatkiem różnych sacharydów

Medium Pożywka	*Lactobacillus acidophilus* DSM 20079			*Bifidobacterium bifidum* DSM 20239		
	Lactic acid* g 100 g^{-1}	L(+) %	D(-) %	Lactic acid* g 100 g^{-1}	L(+) %	D(-) %
Without sugar Bez cukru	0.200 ±0.02	90.46	9.54	0.129 ±0.01	70.28	29.72
Glucose Glukoza	0.860 ±0.05	68.50	31.50	0.619 ±0.05	69.87	30.13
Fructose Fruktoza	0.587 ±0.05	61.53	38.47	0.531 ±0.03	70.85	29.15
Lactose Laktoza	0.748 ±0.03	68.19	31.81	0.399 ±0.03	78.88	21.12
Saccharose Sacharoza	0.968 ±0.05	70.97	29.03	0.580 ±0.02	70.48	29.52
Raftilose	0.800 ±0.04	71.03	28.97	0.227 ±0.01	72.94	27.06
Raftiline	0.264 ±0.02	79.20	20.80	0.145 ±0.01	70.03	29.97

*Mean and stadard deviation.
*Średnio i odchylenie standardowe.

The L(+) form is readily absorbed by the human organism and is completely metabolised providing an energetic substrate, whereas the D(-) form of lactic acid is absorbed much slower. That is the reason why the D(-) form of lactic acid is the main pH-reducing element found in the gastrointestinal tract and its quantities ingested with food should be as low as possible [Grzybowski et al. 1997].

It is also noticed that the quantity of lactic acid produced in different media was correlated with pH changes of cultures in these media (Table 1 and 2). Despite the fact, that in certain cases, the authors failed to record significant differences between the pH values of the media, the performed statistical analysis of the produced lactic acid in the media supplemented with different saccharides revealed significant differences between individual cultures ($P < 0.05$).

CONCLUSION

Investigations presented in this paper show that the tested strains of bacteria could become components of synbiotic preparations which could be used in future. The examined strains are capable of utilising Raftilose and Raftiline preparations as sources of energy and meet the requirements adopted by the WHO producing over 70% of L(+) lactic acid in media containing different saccharides. The performed experiments confirmed differences between *Lactobacillus* and *Bifidobacterium* also with regard to their utilisation of saccharides.

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PORÓWNANIE WZROSTU BAKTERII Z GATUNKU *LACTOBACILLUS ACIDOPHILUS* I *BIFIDOBACTERIUM BIFIDUM* NA PODŁOŻACH Z DODATKIEM WYBRANYCH SACHARYDÓW W TYM PREBIOTYKÓW

Streszczenie. W pracy podjęto badania nad dynamiką wzrostu bakterii *Lactobacillus acidophilus* DSM 20079, *Lactobacillus acidophilus* DSM 20242, *Bifidobacterium bifidum* DSM 20082, *Bifidobacterium bifidum* DSM 20215, *Bifidobacterium bifidum* DSM 20239, *Bifidobacterium bifidum* DSM 20456 w podłożach z dodatkiem różnych sacharydów, w tym preparatów prebiotycznych. Dodatek sacharydów do podłoża znacząco wpłynął zarówno na liczbę komórek bakterii, jak i ich zdolność kwasotwórczą. Najłatwiej dostępnymi sacharydami dla wszystkich testowanych szczepów okazały się: glukoza, laktoza i sacharoza i to w podłożu z dodatkiem tych właśnie cukrów oznaczano najwyższą liczbę komórek bakterii. Przy deficycie mono- i disacharydów wszystkie szczepy testowanych bakterii były zdolne do wykorzystania preparatów prebiotycznych jako źródeł węgla i energii w pożywkach. Oznaczono ilość i formy izomeryczne produkowanego kwasu mlekowego przez *Lactobacillus acidophilus* DSM 20079 i *Bifidobacterium bifidum* DSM 20239. Obydwa szczepy spełniają wymagania stawiane przez WHO, produkując powyżej 70% kwasu mlekowego L(+) w podłożach z dodatkiem różnych sacharydów. Istotnie wyższe ilości kwasu mlekowego na różnych podłożach produkuje szczep *Lactobacillus acidophilus* DSM 20079.

Słowa kluczowe: probiotyki, prebiotyki, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, kwas mlekowy

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