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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Ability of the rumen bacterium *Pseudobutyrivibrio ruminis* strain k3 to utilize fructose, sucrose and fructose polymers

K. Stan-Głasek, A. Kasperowicz, J.P. Michalski[1], M. Taciak and T. Michałowski

The Kielanowski Institute of Animal Physiology of Nutrition, Polish Academy of Sciences, Instytucka 3, Jabłonna, Poland

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[1] Corresponding author:
e-mail: j.p.michalski@op.pl

ABSTRACT. The aim of the study was to evaluate in what manner rumen bacterium *Pseudobutyrivibrio ruminis* strain k3 uses and ferments timothy grass fructan, inulin, inulooligosaccharides, sucrose, fructose and glucose. The highest concentration of bacterial population was noted at 12 h of incubation for all cultures except from that on fructose which occurred at 24 h. The highest specific growth rates occurred on sucrose, timothy grass fructan and glucose, whereas the lowest on fructose. Protein productions on timothy grass fructan, glucose, sucrose and inulooligosaccharides were 66.4–73.5 mg/100 ml of culture, while on fructose and inulin – 59.9 and 34.5 mg/100 ml, respectively. Bacteria utilized more than 91% of initial dose of timothy grass fructan, sucrose, glucose, inulooligosaccharides and fructose, and less than 47% of inulin. Production of butyrate on timothy grass fructan, sucrose, inulooligosaccharides and glucose was higher than on fructose. In contrast lactate production on fructose and glucose was higher than on timothy grass fructan and inulooligosaccharides. The lowest production of both acids was on inulin and the highest on fructose, glucose and sucrose. It was stated that *Pseudobutyrivibrio ruminis* strain k3 was able to use sucrose and fructan of β-2,6-type originated from grasses but possessed limited capability of using inulin.

Introduction

Fructans are fructose polymers linked either by β-2,6- or β-2,1-glycosidic bonds, with glucose usually attached to the end of the chain. The first are called levans (or phleins) while the second – inulins. Levans are storage carbohydrates of cool-season grasses (*Gramineae*) with degree of polymerization up to 260 (in timothy grass) or 314 (in orchardgrass). Inulins occur in *Astereaceae*; their degree of polymerization in chicory is up to 35–45 (Nelson and Spollen, 1987; Van den Ende et al., 1996).

Grass fructans are natural components of ruminant diets. They may constitute approximately 25–30% of stem and leaves dry matter but usually they do not exceed 6% (Van Soest, 1982). When ruminants are fed grasses, fructans are completely digested and fermented in the rumen by rumen microbiota (Chesson and Forsberg, 1997). Lately, Hall and Weimer (2016) reported that capability of fermenting phleins is rather a property of saccharolytic not noncellulolytic rumen bacteria. On the other hand, Thomas (1960) indicated that bacterium *Streptococcus bovis* has the ability to use and ferment grass fructans. Investigations performed in our laboratory showed that bacteria *Treponema ziołeckii*, *Butyrivibrio fibrisolvens* 3071 (DSMZ) and *Pseudobutyrivibrio ruminis* strain 3 synthesized fructanolytic enzymes (Piknova et al., 2008; Kasperowicz et al., 2010, 2016). Apart from that, capability of fermenting range of fructans by *B. fibrisolvens* strain 3071 (DSMZ) was examined (Kasperowicz et al., 2016).

Strain k3 was isolated from the rumen of sheep on medium supplemented with timothy grass fructan as sole carbon source and identified as butyrivibrio-like bacteria (Ziolecki et al., 1992; Guczyńska, 1999). Verification of bacterium taxonomic affiliation by examination of 16S rRNA gene showed that strain k3 belongs to the species *Pseudobutyrivibrio ruminis* (Stan-Głasek, 2013). This species was emerged by Van Gylswyk et al. (1996) more than 20 years ago but its physiology has not been well documented yet (Kopečný et al., 2003; Koppová et al., 2006). The typical strain of the bacterium, i.e. strain A12-1^T, is able to ferment a range of carbohydrates (including glucose) to lactate, formate and butyrate. As far as we know, however, its capability to use and to ferment polymers of fructose has not been studied. This encourages us to undertake investigation to estimate possible contribution of *P. ruminis* strain k3 in fructan metabolism in the rumen. The fact that strain k3 was isolated on timothy grass fructan allowed us to suppose that this bacterium can use and ferment other fructose containing carbohydrates. Thus, the goal of this *in vitro* study was to investigate the ability of bacterium *P. ruminis* strain k3 to use and ferment fructose, sucrose and polymers of fructose with different glycosidic bonds and degree of polymerization.

Material and methods

Bacterium and culture media

The bacterium *P. ruminis* strain k3 was isolated in our laboratory and so far is maintained in our culture collection on medium supplemented with the mixture of timothy grass fructan, inulin, inulooligosaccharides (IOS), sucrose, fructose and glucose at concentration 2 g/l. Its taxonomic affiliation was verified by 16S rRNA gene examination (Stan-Głasek, 2013). The medium for bacterium culture was prepared using anaerobic technique of Hungate (1969) and Holdeman and Moore (1973). The medium consisted of 700 ml of salt solution and 300 ml of clarified and pasteurized rumen fluid supplemented with 1 g casitone and 1 g yeast extract (both from Difco Laboratories, Detroit, MI, USA), 250 mg cysteine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA), 0.3 ml thioglycolic acid and 10 mg resazurine. The salt solution used to medium preparation was composed of (g per 700 ml): $(NH_4)_2SO_4$ 0.45, KH_2PO_4 0.45, K_2HPO_4 0.45, NaCl 0.9, $MgSO_4$ 0.09, $CaCl_2$ 0.09, $NaHCO_3$ 7.5 dissolved in distilled water. The medium was sterilized and carbohydrate mixture solution was prepared separately and added to the medium after sterilization. The media designated for fermentation experiments were supplemented with each carbohydrate listed above at concentration 5 g/l.

Determination of bacteria growth rate, microbial protein production, carbohydrate disappearance and the end products of fermentation

The medium (10 ml in volume) containing either glucose (as a reference), chicory inulin, sucrose and fructose (Sigma-Aldrich, St. Louis, MO, USA), IOS (Orafti, Oreye, Belgium) or timothy grass fructan was inoculated with 0.5 ml of culture grown overnight on the mixture of carbohydrates listed above and incubated at 40 °C. IOS consisted of 932 g/kg oligofructose with degree of polymerization of 2–8 and fructose, glucose and sucrose of 68 g/kg. Timothy grass fructan was obtained in the laboratory from timothy grass (*Phleum pratense*) grown on experimental field in Jabłonna (Poland) according to the method of Ziołecki et al. (1992). Sampling was performed just after inoculation and after 2, 4, 8, 12 and 24 h of incubation. The optical density of cultures (OD) was measured at 660 nm with the use of DU-64 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA). The bacteria specific growth rate was calculated from the increase of OD in exponential phase of growth. To determine microbial protein and carbohydrate contents, samples of bacterial cultures were centrifuged at 20 000 *g* and 4 °C for 10 min then the supernatants were saved for carbohydrate assay while the cell pellets were washed twice with NaCl solution at concentration 9 g/l and used for measurement of protein. The protein content was assayed with bicinchinonic acid (Sigma-Aldrich, St. Louis, MO, USA) reagent according to Smith et al. (1985) with bovine serum albumin as a standard. The carbohydrate content was determined by the anthrone method according to Southgate (1991) with calibration curve prepared separately for each carbohydrate. Disappearance of carbohydrate was expressed as percentage of its initial value. All not otherwise specified reagents were purchased from Avantor Performance Materials (Gliwice, Poland).

The short chain fatty acids content was measured by gas liquid chromatography according to Barszcz et al. (2011). The L(+) lactic acid was assayed enzymatically with D/L Lactic Acid Assay Kit (Megazyme, Wicklow, Ireland). The net production of the acids was expressed as the difference in content at the start and end of incubation.

Statistics

Calculations and graphs were performed using the Excel 2013 (Microsoft, Redmond, WA, USA). The statistical analysis was performed with use of the STATISTICA v. 13.1 (Dell Inc., Round Rock, TX, USA). The results of one-way ANOVA, followed by Fisher's least significant difference post-hoc test (LSD), were regarded statistically significant at $P \leq 0.05$. ANOVA was performed on the assumption of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). The results are means of the four values obtained in independent experiments expressed as mean ± standard deviation.

Results

Bacterial growth

The exponential phase of bacterial growth occurred between 8th and 12th h for all carbohydrates except that of inulin which was found to be between 4th and 8th h (Figure 1A). Maximal value of OD and calculated specific growth rate of timothy grass fructan and sucrose were higher than those of inulin, IOS or fructose (Figure 1A, Table 1; $P < 0.05$). Except medium with fructose and IOS, densities of bacterial cultures after 24 h of incubation were lower by 14–37% than at their highest values (Figure 1A, $P < 0.05$). Density of culture on IOS did not change in comparison with that at 12 h of incubation, while density of medium with fructose was higher by about 30% (Figure 1A, $P < 0.05$).

Microbial protein production

The profiles of production showed that protein content increased until the end of the experiment in all culture media except that with inulin (Figure 1B). Maximal rate of microbial protein production occurred between 4th and 12th h for timothy grass fructan, sucrose and glucose (7.1–8.2 mg/h) and for fructose, inulin and IOS (3.6 – 5.2 mg/h) (Figure 1B, Table 1; $P < 0.05$). Final content of protein in cultures on IOS did not differ from those on glucose, timothy grass fructan, sucrose or fructose (Table 2). Net yield of protein on medium with timothy grass fructan (or glucose) was higher by about 20% than on fructose (Figure 1B, Table 2; $P < 0.05$). In culture on inulin microbial protein production was 1.7–2 fold lower than in those on other carbohydrates tested (Figure 1B, Table 2; $P < 0.05$).

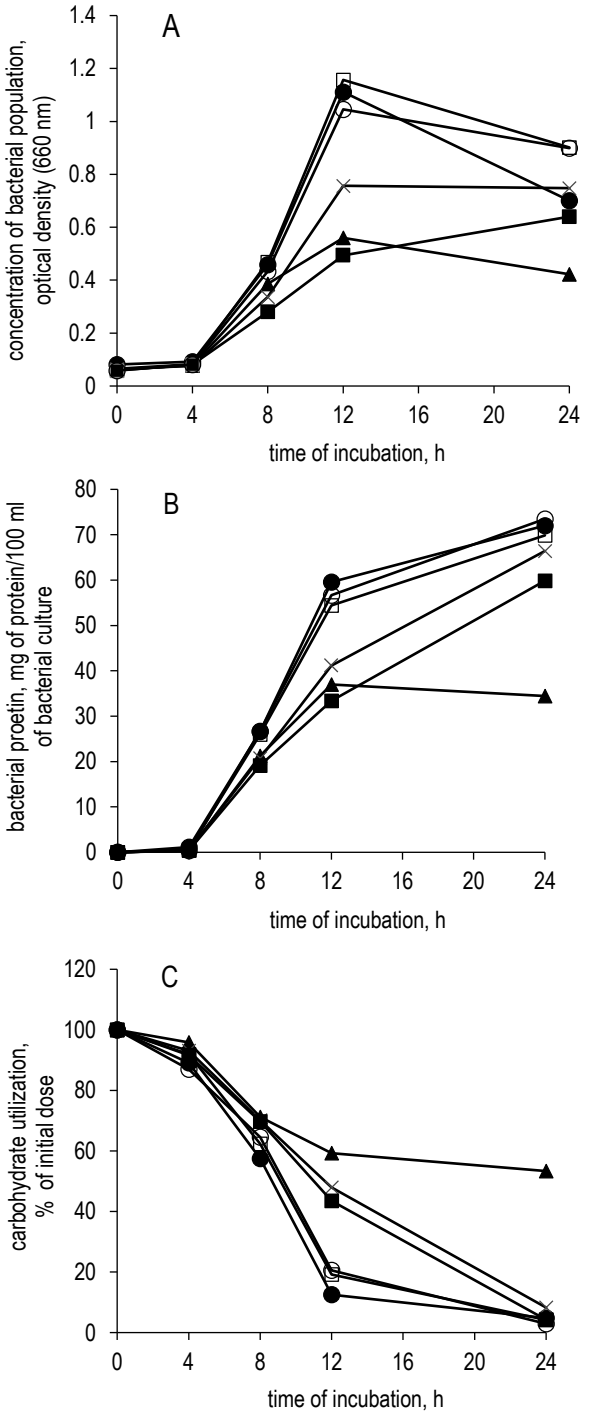

Figure 1. Concentration of bacterial population (A), bacterial protein (B) and carbohydrate content from growth medium (C) during 24 h incubation of bacterium *Pseudobutyrivibrio ruminis* strain k3 with: timothy grass fructan (●), sucrose (□), inulin (▲), inulooligosaccharides (x), fructose (■), glucose (○); mean values; n = 4

Carbohydrate disappearance

A slight drop in carbohydrate content in growth medium was detectable after 4 h of the experiment. Between 4th and 12th h of incubation the contents of timothy grass fructan, sucrose, and glucose were reduced to 10–20% of initial dose, those of fructose

Table 1. The rates of growth, disappearance of carbohydrates, microbial protein and organic acids production during exponential phase of growth of *Pseudobutyrivibrio* ruminis strain k3 on media supplemented with glucose and fructose containing carbohydrates

Indices	Timothy grass fructan	Inulin	Inulooligosaccharides	Sucrose	Fructose	Glucose	*P*-value
Specific growth rate of bacteria per h	$0.163^{d} \pm 0.017$	$0.077^{b} \pm 0.006$	$0.105^{c} \pm 0.006$	$0.172^{d} \pm 0.021$	$0.054^{a} \pm 0.005$	$0.153^{d} \pm 0.021$	0.001
Carbohydrate disappearance, mg/h	$11.24^{b} \pm 0.87$	$6.14^{a} \pm 1.31$	$5.54^{a} \pm 1.10$	$10.79^{b} \pm 1.45$	$6.56^{a} \pm 1.34$	$10.99^{b} \pm 1.32$	0.001
Microbial protein production, mg/h	$8.24^{b} \pm 0.66$	$5.19^{a} \pm 0.66$	$5.11^{a} \pm 0.85$	$7.10^{b} \pm 1.42$	$3.56^{a} \pm 1.22$	$7.51^{b} \pm 1.54$	0.001
Butyrate production, mg/h	$0.019^{c} \pm 0.005$	$0.004^{a} \pm 0.002$	$0.011^{b} \pm 0.005$	$0.018^{c} \pm 0.002$	$0.008^{ab} \pm 0.002$	$0.010^{ab} \pm 0.006$	0.001
Lactate production, mg/h	$0.378^{c} \pm 0.043$	$0.142^{a} \pm 0.015$	$0.222^{b} \pm 0.038$	$0.398^{c} \pm 0.040$	$0.240^{b} \pm 0.028$	$0.362^{c} \pm 0.023$	0.001

$^{a–d}$ – mean values within a row marked with different superscript letters are significantly different at $P < 0.05$; mean values ± SD

and IOS to 43–48%, and that of inulin to 60% (Figure 1C; $P < 0.05$). Till the end of the experiment contents of the first five carbohydrates decreased of 3–8% whereas inulin remained on the similar level as 12 h earlier (Figure 1C, Table 2).

Fermentation products

It was found that in relation to carbohydrate the concentration of acetic acid diminished during incubation from approximately 1.15–1.2 mM/100 ml at the start to 0.85–1.1 mM/100 ml at the end of the experiment (Figure 2A). The content of propionate was about 0.22 mM/100 ml and did not change during the incubation time (Figure 2B). The highest rate of lactate production for medium with fructan, sucrose, glucose and inulin occurred between 8th and 12th h of the experiment and those with fructose or IOS between 4th and 24th h (Figure 2C, Table 1; $P < 0.05$). The rate was by 33–44% higher for fructan, sucrose and glucose in comparison to IOS and fructose and by 60–64% higher than for inulin (Table 1; $P < 0.05$). Finally, lactic acid level in post culture media with fructose and glucose was by 16–31% higher than in those with timothy grass fructan and IOS, and on medium with inulin was about 2.5–3 fold lower than on other media (Table 2; $P < 0.05$).

Butyric acid content increased during the incubation from 0.2 mM/100 ml to about 0.3–0.45 mM/100 ml with the highest rate of production in media with fructan and sucrose compared to other carbohydrates (Figure 2D; $P < 0.05$). Net yields of butyrate in media with fructan, IOS, sucrose and glucose were by 60% higher than in medium with fructose and about 2-fold higher than in medium supplemented with inulin (Table 2; $P < 0.05$).

The sum of butyric and lactic acids in medium with timothy grass fructan was lower by 14–20% than on those with sucrose, glucose or fructose but similar to that on IOS and over twice times higher than in medium with inulin (Table 2; $P < 0.05$).

Table 2. Carbohydrate utilization, microbial protein and organic acids production after 24 h of incubation of *Pseudobutyrivibrio ruminis* strain k3 on media supplemented with glucose and fructose containing carbohydrates

Indices	Timothy grass fructan	Inulin	Inulooligosaccharides	Sucrose	Fructose	Glucose	*P*-value
Carbohydrate utilization, % of initial dose	$95.21^{bc} \pm 1.25$	$46.65^{a} \pm 4.57$	$91.74^{b} \pm 2.91$	$95.82^{c} \pm 1.16$	$95.77^{bc} \pm 2.62$	$97.09^{c} \pm 2.24$	0.001
Microbial protein, mg/100 ml	$72.01^{c} \pm 1.64$	$34.47^{a} \pm 6.51$	$66.42^{bc} \pm 9.19$	$69.86^{bc} \pm 6.84$	$59.86^{b} \pm 6.05$	$73.48^{c} \pm 11.92$	0.001
Butyrate production, mM/100 ml	$0.255^{c} \pm 0.020$	$0.116^{a} \pm 0.023$	$0.231^{c} \pm 0.024$	$0.252^{c} \pm 0.038$	$0.160^{b} \pm 0.014$	$0.221^{c} \pm 0.018$	0.001
Lactate production, mM/100 ml	$2.640^{b} \pm 0.174$	$1.094^{a} \pm 0.364$	$2.898^{bc} \pm 0.099$	$3.100^{cd} \pm 0.171$	$3.473^{e} \pm 0.146$	$3.369^{de} \pm 0.215$	0.001
Butyrate + lactate production, mM/100 ml	$2.895^{b} \pm 0.180$	$1.210^{a} \pm 0.388$	$3.129^{bc} \pm 0.092$	$3.352^{cd} \pm 0.203$	$3.633^{d} \pm 0.159$	$3.590^{d} \pm 0.215$	0.001

$^{a–d}$ mean values within a row marked with different superscript letters are significantly different at $P < 0.05$; mean values ± SD

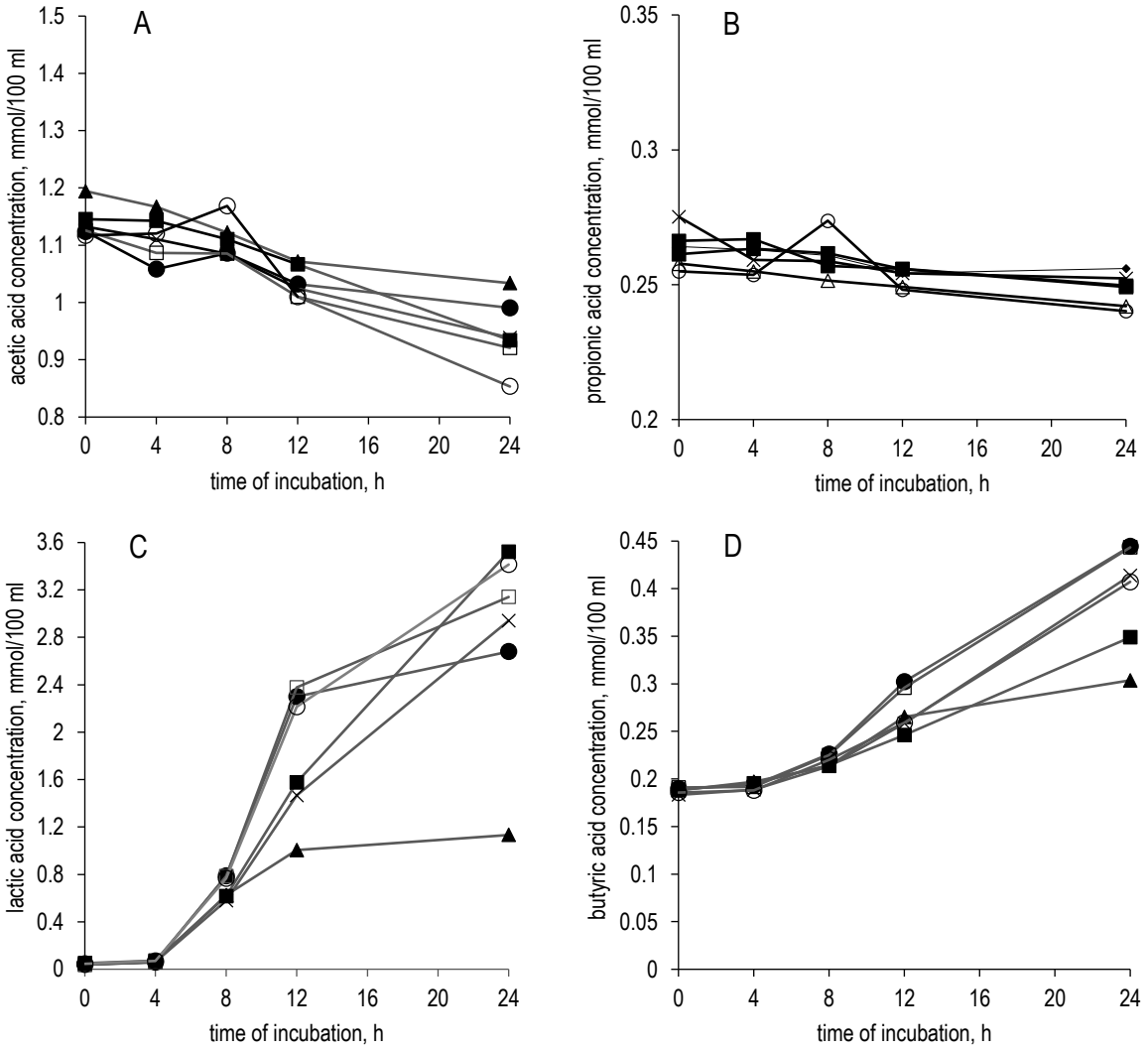

Figure 2. Changes in concentration of acetic acid (A), propionic acid (B), lactic acid (C) and butyric acid (D) during incubation of *Pseudobutyrivibrio ruminis* strain k3 with particular carbohydrates: fructan (●), sucrose (□), inulin (▲), inulooligosaccharides (x), fructose (■), glucose (○), mean values; n = 4

Discussion

Comparing the ability of bacterium *P. ruminis* strain k3 to use two types of fructose polymers as well as the relative carbohydrates it can be stated that bacterium was able to grow on all tested carbohydrates. However higher values of maximal density and higher specific growth rate of bacterial cultures on timothy grass fructan, sucrose or glucose than on IOS, inulin and fructose suggest the differences among carbohydrates seem to indicate timothy grass fructan as more effective substrate for bacterium than inulins. When considering glucose as the reference source of carbon and fructose, as another simple sugar and integral building block of each polymer, it can be stated that the growth patterns of bacteria on sucrose and timothy grass fructan fit to that represented by glucose while those on inulin and IOS are closer to that on fructose. Earlier examination of the relative of *P. ruminis*, bacterium *B. fibrisolvens* 3071 (DSMZ), showed that the specific growth rate was higher when bacteria were cultured on medium with sucrose or timothy grass fructan than on fructose (Kasperowicz et al., 2016). On the other hand, Hall and Weimer (2016) studying the pure strains of rumen bacteria found that specific growth rates on fructan from orchardgrass were 19–68% slower than on fructose and 22–75% than on glucose.

Similar assignment to that described above could be applied when the rate of production of microbial protein is analyzed, certifying the statement that the inulin type fructans and fructose were inferior source of carbon for bacterium to the timothy grass fructan or sucrose and glucose. On the other hand final yield of protein on IOS similar to that

on timothy grass fructan and higher than on inulin suggests the importance of the β-2,1 fructan chain length for ability of bacterium to convert it to microbial protein. There is no data in the literature enabling comparison of microbial protein production by pure strains of rumen bacteria growing on fructose polymers. Studies of Hall and Weimer (2016) with mixed rumen microbiota *in vitro* showed higher rate of microbial N accumulation on medium with phlein than with inulin. However, the authors reported that final accumulation of protein on phlein was similar to that on inulin. On the other hand Biggs and Hancock (1998) showed no differences in degradation paths of bacterial levan and inulin by rumen contents *in vitro*. The question is if the capabilities of single bacterial species to use substrate should be compared with those of mixed rumen microorganisms.

Carbohydrate disappearance profiles seemingly followed the profiles of bacterial growth and microbial protein production showing that bacterium was able to intake the timothy grass fructan, sucrose, glucose more rapidly than IOS and fructose and indicating that inulin was used only partially. It seems that various patterns of carbohydrate depletion from the medium can arise from capabilities of bacterium to produce enzymes necessary for digestion of polymers with the β-2,6 bonds of timothy grass fructan and β-2,1 of inulins and sucrose, and/or costs of delivery of carbohydrates to the cell. It was previously shown that bacterium *P. ruminis* strain k3 synthesizes specific endolevanase enabling the digestion of timothy grass fructan to oligosaccharides as intermediate products (Stan-Głasek, 2013). The lack of similar enzyme for digestion of inulin type fructans could make long chain inulins inaccessible for bacterium. However, another fructanolytic enzyme found in strain k3 was unspecific β-fructofuranosidase capable to digest oligosaccharides of both β-2,6 and β-2,1 types. So this could explain why the use of heterogenous inulin composed of polymers with various degrees of polymerization was limited. On the other hand, apart from endolevanase and β-fructofuranosidase, strain k3 was found by Kasperowicz et al. (2010) to produce sucrose phosphorylase enabling bacterium the cleavage of sucrose to fructose and energy-rich glucose-1-phosphate and potentially to save the energy for cellular processes (Reid and Abratt, 2005). Furthermore, transport of carbohydrates to bacterial cell can take place in several different ways, for example as simple sugar or as oligosaccharides. The former is sometimes considered as less effective than the latter (Martin, 1994; Hopkins et al., 1998). Thus differences in transport efficiency could be visible in the profiles of bacterial growth, microbial protein production and carbohydrate disappearance from the medium.

Fermentation profile of carbohydrates was examined in relation to their ability to produce acetic, propionic, butyric and lactic acids. The capacities of bacterium to produce acetic and propionic acids were negligible or even none, since concentration of the first diminished in relation to that at start of the experiment while the content of the second remained at the same level throughout the incubation. It cannot be excluded that bacterium was able to produce acetate and then to use it. Latham and Legakis (1976) documented that strains of *B. fibrisolvens* were able to produce acetate which was then taken up and this was dependent on glucose concentration, growth rate of bacteria and initial level of acetic acid in growth medium. On the other hand fermentation products of glucose by *P. ruminis* strain A 12-1^{T}, i.e. the type strain of the species, were similar to those found in our study with the exception that, besides formate, acetate was produced in amounts below 1 mM (Van Gylswyk et al., 1996). Other authors (Gill and King, 1958; Diez-Gonzalez et al., 1999) showed that such fermentation of carbohydrates can be an alternative for some bacteria from butyrivibrio group.

The lactic and butyric acids were the main products of fermentation. The content of lactate in post-culture media was approximately ten times higher than that of butyrate suggesting that most of energy originated from fermentation of carbohydrates is used by lactic acid. However, omitting the medium with glucose the production of both acids in exponential phase of growth appeared to run according to the patterns described for bacterial growth, protein production and carbohydrate decrease from culture medium showing the close relation among their synthesis and occurred microbial processes. What is more, higher final concentration of lactic acid in cultures on fructose or glucose than on timothy grass fructan or IOS could suggest that simple sugars generated more lactate originating energy then polysaccharides. Nevertheless, higher level of butyrate in post-culture with timothy grass fructan and IOS than in that with fructose could indicate polymers as promoting higher synthesis of this acid in comparison to the building monomer. In general, the sum of acids generated during fermentation of carbohydrates was higher in cultures with fructose, glucose and sucrose, than in that with timothy grass fructan

apparently showing the higher energy efficiency of first carbohydrates in comparison with the remaining. This was, however, accompanied by lower or comparable with that on timothy grass fructan microbial protein content suggesting either higher cost of its synthesis or other energy-spending pathways, not examined in this study.

Conclusions

Results of our *in vitro* study lead to conclusion that bacterium *Pseudobutyrivibrio ruminis* strain k3 possesses capability of using and fermenting fructose, sucrose and polymers of fructose. Ability of strain k3 to use fructans seems to be depended on fructans type of bonds and their degree of polymerization. Further *in vitro* experiments on mixed cultures are needed to verify the obtained results taking into account microbial diversity in the rumen.

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References

Barszcz M., Taciak M., Skomiał J., 2011. A dose-response effects of tannic acid and protein on growth performance, caecal fermentation, colon morphology and β-glucuronidase activity in rats. J. Anim. Feed Sci. 20, 613–625, https://doi.org/10.22358/jafs/66219/2011

Biggs D.R., Hancock K.R., 1998. In vitro digestion of bacterial and plant fructans and effects on ammonia accumulation in cow and sheep rumen fluids. J. Gen. Appl. Microbiol. 44, 167–171, https://doi.org/10.2323/jgam.44.167

Chesson A., Forsberg C.W., 1997. Polysaccharide degradation by rumen microorganisms. In: P.N. Hobson, C.S. Stewart (Editors). The Rumen Microbial Ecosystem. Blackie Academic & Professional. London (UK), pp. 329–381, https://doi.org/10.1007/978-94-009-1453-7_8

Diez-Gonzalez F., Bond D.R., Jennings E., Russell J.B., 1999. Alternative schemes of butyrate production in *Butyrivibrio fibrisolvens* and their relationship to acetate utilization, lactate production, and phylogeny. Arch. Microbiol. 171, 324–330, https://doi.org/10.1007/s002030050717

Gill J.W., King K.W., 1958. Nutritional characteristics of a *Butyrivibrio*. J. Bacteriol. 75, 666–673

Guczyńska W., 1999. Substrate preferences of some of rumen bacteria in metabolism of sugars. PhD Thesis (in Polish). The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences. Jabłonna (Poland)

Hall M.B., Weimer P.J., 2016. Divergent utilization patterns of grass fructan, inulin, and other nonfiber carbohydrates by ruminal microbes. J. Dairy Sci. 99, 245–257, https://doi.org/10.3168/jds.2015-10417

Holdeman L.V., Moore W.E.C., 1973. Anaerobe Laboratory Manual. 2nd Edition. The Virginia Polytechnic Institute and State University Anaerobe Laboratory. Blacksburg, VA (USA)

Hopkins M.J., Cummings J.H., Macfarlane G.T., 1998 Inter-species differences in maximum specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and other simple carbohydrate sources. J. Appl. Microbiol. 85, 381–386, https://doi.org/10.1046/j.1365-2672.1998.00524.x

Hungate R.E., 1969. A roll tube method for cultivation of strict anaerobes (IV) In: J.R. Norris, D.W. Ribbons (Editors). Methods in Microbiology. Vol. 3 Part B. Academic Press. Cambridge, MA (USA), pp. 117–132, https://doi.org/10.1016/S0580-9517(08)70503-8

Kasperowicz A., Stan-Głasek K., Guczynska W., Piknová M., Pristaš P., Nigutová K., Javorský P., Michałowski T., 2010. Fructanolytic and saccharolytic enzymes of the rumen bacterium *Pseudobutyrivibrio ruminis* strain 3 – preliminary study. Folia Microbiol. 55, 329–331, https://doi.org/10.1007/s12223-010-0051-4

Kasperowicz A., Stan-Głasek K., Taciak M., Michałowski T., 2016. The fructanolytic abilities of the rumen bacterium *Butyrivibrio fibrisolvens* strain 3071. J. Appl. Microbiol. 120, 29–40, https://doi.org/10.1111/jam.12976

Kopečný J., Zorec M., Mrázek J., Kobayashi Y., Marinšek-Logar R. 2003. *Butyrivibrio hungatei* sp. nov. and *Pseudobutyrivibrio xylanivorans* sp. nov., butyrate-producing bacteria from the rumen. Int. J. Syst. Evol. Microbiol. 53, 201–209, https://doi.org/10.1099/ijs.0.02345-0

Koppová I., Lukáš F., Kopečný J., 2006. Effect of fatty acids on growth of conjugated-linoleic-acids-producing bacteria in rumen. Folia Microbiol. 51, 291–293, https://doi.org/10.1007/BF02931816

Latham M.J., Legakis N.J., 1976. Cultural factors influencing the utilization or production of acetate by *Butyrivibrio fibrisolvens*. J. Gen. Microbiol. 94, 380–388, https://doi.org/10.1099/00221287-94-2-380

Martin S.A., 1994. Nutrient transport by ruminal bacteria: a review. J. Anim. Sci. 72, 3019–3031, https://doi.org/10.2527/1994.72113019x

Nelson C.J., Spollen W.G., 1987. Fructans. Physiol Plant. 71, 512–516, https://doi.org/10.1111/j.1399-3054.1987.tb02892.x

Piknova M., Guczynska W., Miltko R., Javorsky P., Kasperowicz A., Michalowski T., Pristas P., 2008. *Treponema zioleckii* sp. nov., a novel fructan-utilizing species of rumen treponemes. FEMS Microbiol. Lett. 289, 166–172, https://doi.org/10.1111/j.1574-6968.2008.01383.x

Reid S.J., Abratt V.R., 2005. Sucrose utilisation in bacteria: genetic organization and regulation. Appl. Microbiol. Biotechnol. 67, 312–321, https://doi.org/10.1007/s00253-004-1885-y

Smith P.K., Krohn R.J., Hermanson G.T., Mallia A.K., Gartner F.H., Provenzano M.D., Fujimoto E.K., Goeke N.M., Olson B.J., Klenk D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85, https://doi.org/10.1016/0003-2697(85)90442-7

Southgate D.A.T., 1991. Determination of Food Carbohydrates. 2nd Edition. Elsevier Applied Science. London (UK)

Stan-Głasek K., 2013. The ability of the rumen bacteria *Pseudobutyrivibrio ruminis* strain k3 to utilize sucrose and the polymers of fructose. PhD Thesis (in Polish). The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences. Jabłonna (Poland)

Thomas G.J., 1960. Metabolism of the soluble carbohydrates of grasses in the rumen of the sheep. J. Agric. Sci. 54, 360–372, https://doi.org/10.1017/S0021859600021304

Van den Ende W., Mintiens A., Speleers H., Onuoha A.A., van Laere A., 1996. The metabolism of fructans in roots of *Cichorium intybus* during growth, storage and forcing. New Phytol. 132, 555–563, https://doi.org/10.1111/j.1469-8137.1996.tb01874.x

Van Gylswyk N.O., Hippe H., Rainey F.A., 1996. *Pseudobutyrivibrio ruminis* gen. nov., sp. nov., a butyrate-producing bacterium from the rumen that closely resembles *Butyrivibrio fibrisolvens* in phenotype. Int. J. Syst. Evol. Microbiol. 46, 559–563, https://doi.org/10.1099/00207713-46-2-559

Van Soest P.J., 1982. Carbohydrates. In: Nutritional Ecology of the Ruminant. O & B Books, Inc. Corvallis, OR (USA), pp. 95–117

Ziołecki A., Guczyńska W., Wojciechowicz M., 1992. Some rumen bacteria degrading fructan. Lett. Appl. Microbiol. 15, 244–247, https://doi.org/10.1111/j.1472-765X.1992.tb00774.x