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Review article
Pregledni prispevek

BUTYRIVIBRIO STRAINS – IMPORTANT BUTYRATE PRODUCING BACTERIA FROM ANIMAL GASTRO-INTESTINAL TRACT AND CURRENT TAXONOMIC STATUS OF STRAINS IN THIS GENUS

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ABSTRACT

Bacteria belonging to or resembling the genus *Butyrivibrio* represent a significant proportion of bacterial isolates from different mammalian gastro-intestinal tracts from all over the world. The main characteristic of this genus is production of butyric acid, which has an important role in maintaining colonic health. The rumen strains – *B. fibrisolvens* are also known producers of conjugated linoleic acid (CLA), bacteriocins and several hydrolytic enzymes that contribute to effective forage digestion. Genus *Butyrivibrio* contains isolates that are very diverse in both genomic and phenotypic properties. Different researchers have proposed the grouping of isolates on the basis of some specific characteristics but up to now none was recognised as a new species. Therefore, a complex approach is needed to set basis for new species delineation and characterisations and to define a set of reasonable diagnostic methods for their discrimination.

Key words: rumen / microbiology / anaerobic bacteria / *Butyrivibrio* / diversity / taxonomy / species definition / butyrate / colonic health

BUTIRIVIBRIJI – POMEMBNI PRODUCENTI MASLENE KISLINE V PREBAVILIH ŽIVALI IN SEDANJE TAKSONOMSKO STANJE SEVOV V TEM RODU

IZVLEČEK

Butirivibriji so med najpogosteje izoliranimi bakterijami iz prebavil različnih sesalcev iz različnih delov sveta. Najpomembnejša lastnost predstavnikov tega rodu je tvorba maslene kisline, ki ima pomembno vlogo pri vzdrževanju zdravega stanja črevesa. Predstavniki vampne vrste tega rodu – *B. fibrisolvens* so tudi znani producenti konjugirane linolne kisline (CLA), bakteriocinov in več hidrolitičnih encimov, ki sodelujejo pri učinkoviti prebavi krme. Sevi s podobnimi lastnostmi so bili uvrščeni v rod *Butyrivibrio*, ki je zelo heterogen. To so potrdili tako z genomskimi kot tudi s fenotipskimi raziskavami. Raziskovalci so na osnovi določenih lastnosti predlagali razvrstitev izolatov v več skupin, vendar nihče ni osnoval nove vrste. Pri prerazporeditvi je treba upoštevati celosten pristop, ki bi utemeljil definiranje novih vrst in njihov opis kot tudi nabor smiselnih testov za njihovo razlikovanje.

Ključne besede: vamp / mikrobiologija / anaerobne bakterije / *Butyrivibrio* / raznolikost / taksonomija / definicija vrste / maslena kislina / zdravo stanje črevesa

INTRODUCTION

There has been extensive effort done in investigating the rumen ecosystem from different point of views in the last 50 years (Hobson, 1997). Special attention was paid to microbial

diversity among rumen bacteria, archea, fungi and protozoa. Several genera and species were defined and classified. Bryant and Small (1956) isolated anaerobic, nonsporeforming, monotrichous, curved rods that fermented glucose with the production of relatively large amounts of butyric acid. They stained Gram-negatively although later electron studies revealed typical Gram-positive ultrastructure of their cell wall that is too thin to retain the Gram stain complex during decoloration (Cheng and Costerton, 1977). This fact was confirmed also by studies of antibiotic sensitivities (Hespell *et al*., 1993) and electron microscopy (Cheng *et al*., 1989; Beveridge, 1990; Hespell *et al*., 1993). Those bacteria that corresponded to the above characteristics were placed in the new genus *Butyrivibrio*. Great diversity was observed among the isolates and the type strain ($D1^T$, ATTC 19171) was chosen to accommodate apparent common properties. No other correlation among other isolates could be determined at that time, so only one species was recognised and named *B. fibrisolvens*. This had been the only species for a long time. A new representative of the same genus was defined as *B. crossotus* (Moore *et al*., 1976). It was isolated from human feces and its characteristics resembled those of the rumen relative. These are the only recognised species of the genus *Butyrivibrio* at the moment (Euzéby, 1997 – the frequently updated list of accepted bacterial names with standing in the nomenclature).

THE ROLE OF BUTYRATE-PRODUCING BACTERIA IN THE GASTRO-INTESTINAL TRACT

Communities of microorganisms in gastro-intestinal tract are believed to contribute to healthy gut function in a variety of ways, including protection against pathogens and production of nutrients for the colonic mucosa. Short-chain fatty acids (SCFA) formed by microbial fermentation have a beneficial effect on colonic epithelial cells (Barcenilla *et al*., 2000). SCFA stimulate both epithelial cell proliferation to exceed the rate of epithelial cell loss and might be an important physiologic stimulus to maintain the normal tissue mass. The effect is dose-dependent and varies among acids: n-butyric exerts the highest impact followed by propionic and acetic acid, respectively. SCFA have the stimulatory effect at neutral pH in protonated form. This effect is abolished by lowering the pH when accumulation of poorly absorbable acids (lactate and succinate) occurs. This shift of bacterial metabolism from SCFA to succinic and lactic acid can modify the epithelial cell kinetics considerably. Therefore it should be important to keep the lumen pH of large intestine above 6.0 to maintain normal cell number (Sakata and Inagaki, 2001). Butyrate in particular has an important role in the metabolism and normal development of colonic epithelial cells and has been implicated in protection against cancer (stimulates apoptosis of transformed cells) and ulcerative colitis (Barcenilla *et al*., 2000). In pigs, rats and humans n-butyrate and glutamine are major cell substrates. The intestinal flora can control the expression of target genes involved in the metabolism of epithelial colonic cells (mitochondrial 3-hydroxy 3-methyl glutaryl CoA synthase, controlling ketone body production from n-butyrate; and glutaminase responsible for the first step of glutamine utilisation in these cells) through n-butyrate production (Cherbury *et al*., 2001). Butyrate production is strongly influenced by feed composition (Barcenilla *et al*., 2000). There have been several studies investigating the diverse butyrate-producing microorganisms among which the most widely studied are bacteria from the genus *Butyrivibrio*. The representatives of this genus, especially strains currently constituting the species *B. fibrisolvens*, are of great importance because they have many hydrolytic enzymes important for forage digestion (Stewart *et al*., 1997) and produce bacteriocins (Kalmkoff *et al*., 1997) and conjugated linoleic acid (CLA) as well (Kepler *et al*., 1966). It is believed that *B. fibrisolvens* is one of the few, if not the only one, rumen bacterium capable to form CLA during the process of biohydrogenation of linoleic acid, but further work

will be needed to define the role of *B. fibrisolvens* and other ruminal bacteria in ruminant CLA production (Kim *et al*., 2000). CLA exhibits anticancerogenic effects, reduces the development of artherosclerosis and may regulate energy metabolism and nutrient partitioning resulting in reduction of body fat and enhancing lean body mass (Pariza *et al*., 1999; Dugan *et al*., 1999; Kritchevsky, 2000). Many bacteriocins have already found their application in extending shelf life as well as for controlling pathogenic bacteria (Kalmokoff and Teather, 1997) and so might butyrivibriocins (bacteriocins produced by strains of the genus *Butyrivibrio*) as well.

DIVERSITY OF BACTERIA BELONGING TO THE GENUS *BUTYRIVIBRIO*

Great diversity among different strains of *B. fibrisolvens* was already observed by Bryant and Small (1956), but nevertheless, only one species was defined. This variability in the characteristics was also observed later by many other independent studies. Species concept in microbiology cannot be easily defined and there is no official species definition today in microbiology, like there are no absolute boundaries for species delineation. The analyses of the 16S rRNA sequences (or genes coding for them) have been extensively applied for this purpose. But species definition can not and may not be based solely on one characteristic of the organism. It has been widely accepted among microbial taxonomists that species can be defined and delineated only after analyses of as many genomic and phenotypic characteristics as possible (Roselló-Mora and Amann, 2001). This is known as 'polyphasic taxonomy' (Vandamme *et al*., 1996). A very good review of species definition was made by Roselló-Mora and Amann (2001) who also proposed that species could be defined as 'a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics, and is diagnosable by a discriminative phenotypic property'.

Genomic diversity

Genomic diversity is being determined by methods that focus on DNA or RNA nucleotide sequences. This approach prevails in modern taxonomic studies because of immense development of these methods and also because of the present prevailing belief that classification should reflect genotypic relationships as it is written in the genome (Vandamme *et al*., 1996).

One of the classic methods is determination of DNA base ratio (G+C content) and is also part of the standard bacterial species description. In general representatives of well defined species should have G+C content within 5 mol% and representatives of the same genus within 10 mol% range (Roselló-Mora and Amann, 2001). G+C content of the DNA from *B. fibrisolvens* strains is between 39 and 48 mol% G+C, and majority falls in the range 39–42 mol% G+C (Mannarelli, 1988). Close relatives have following mol% G+C: *B. crossotus* 37% (Moore *et al*., 1976) and *Pseudobutyrivibrio ruminis* 40% (van Gylswyk *et al*., 1996).

Genome similarities can be determined also by different techniques of DNA-DNA hybridisations. Wayne *et al*. (1987) proposed that species generally would include strains with approximately 70% or greater DNA-DNA relatedness and with 5 °C or less ΔT_m (thermal denaturation midpoint). However Roselló-Mora and Amann (2001) suggest the wider range should be considered. Relative binding ratio (RBR) should be in range 50–70% and ΔT_m 5–7 °C. Several studies were made for *Butyrivibrio* strains. Mannarelli (1988) divided the strains in five groups according to the RBR, determined by two independent methods. Within the groups the RBR values were higher than 75%, whereas RBR with representatives of other groups were less than 50% (the majority less than 30%). The diversity was confirmed by the same method also by Hudman and Gregg (1989). Mannarelli *et al*. (1990) conducted comparative studies where analysis of extracellular polysaccharides (EPS) was included along with

hybridisation studies. The grouping based on hybridisation values and neutral sugar contents of EPS coincided.

Over the last 25 years, techniques involving the analysis of rRNA or of the genes coding for rRNA (rDNA) have revolutionised prokaryotic taxonomy. There are several publicly accessible databases where 16S rRNA (or rDNA) sequences are deposited. Different algorithms and models of DNA analyses are used for sequence comparisons what usually results in construction of phylogenetic trees that show the most probable evolutionary pathway and relatedness among investigated microorganisms. But we have to be aware that those results do not necessarily reflect real historic development, because the results were obtained by comparing of only one trait. The better way is comparing as many characteristics as possible. It is expected that strains of the same species share more than 97% rRNA sequence similarities, but there are also some exceptions. Bacterial species definition can never be solely based on sequence similarity of rRNAs, but this can be quite a good orientation point. To date every new species description must include 16S rRNA sequence which is also deposited to public databases (Roselló-Mora and Amann, 2001). For the strains of the genus *Butyrivibrio* complete (Forster *et al*., 1996) or partial (Willems *et al*., 1996; Kopečný *et al*., 2001) 16S rRNA sequence comparisons were made and great diversity was determined. Minority of the strains had 16S rRNA sequence similarities higher than 97%. The majority of the strains had similarity among them less than 95%, in some cases even less than 90%. Forster *et al*. (1997) used oligonucleotide probes for studying those sequences. They confirm older findings of genetic heterogenity of this genus and propose grouping of the strains in at least three (Willems *et al*., 1996; Forster *et al*., 1996; Forster *et al*. 1997) or five (Kopečný *et al*., 2001) separated groups of cluster XIVa (Collins *et al*., 1994) of the *Clostridium* subphylum of the gram-positive bacteria. Kopečný *et al*. (2001) and Marinšek Logar *et al*. (2000) empowered that division with comparative analyses of cellular fatty acid analysis (FAMEs). They all agree that many characteristics of the type strain $D1^T$ are not very close to those of the majority of the strains that are confirmed to belong to the same genus. It is likely that the type strain is not closely related to other *Butyrivibrio* isolates (Manarelli *et al*., 1990; Hudman and Gregg, 1989; Forster *et al*., 1996).

Phenotypic diversity

Phenotype is the visible or otherwise measurable physical and biochemical characteristic of the organism, which is the result of the interaction of genotype and environment. It is necessary to standardise the treatment, inoculation and incubation of the organisms to compare the results from different studies credibly. The quantity or the ratios of substances or traits is considerably affected by the environmental conditions.

Classical phenotypic tests constitute the basis for the formal description of taxa and include description of morphological, physiological and biochemical traits. There are several commercial biochemical tests available, e.g. API and Biolog. However they have a limited scope of use depending on taxa that is to be investigated. The API tests showed to give no valuable information on taxonomic grouping of bacteria in the genus *Butyrivibrio* (Kopečny *et al*., 2001; Marinšek Logar *et al*., 2000). Already Bryant and Small (1956) found out that strains joined together to form one species *B. fibrisolvens* differed a lot in phenotypic characteristics. Similar conclusions were made after analysing cell wall structure (Hespell *et al*., 1993), fermentation of different substrates (Margherita *et al*., 1964; Shane *et al*., 1969; Kopečný *et al*., 2001; Marinšek Logar *et al*., 2000), fermentation products (Diez-Gonzales *et al*., 1999), antigen determinants (Hazlewood *et al*., 1986) and bacteriocin production and sensitivity towards them (Kalmokoff and Teather, 1997). Groupings of the isolates made by different methods and authors are difficult to compare because the authors usually analysed their own isolates and no study involves all the strains and all of the methods (Table 1). Nevertheless some strains were included in several

independent studies – grouping based on the respective results sometimes coincide, but this is not always the case. Therefore it would be necessary to examine which methods are the most appropriate for the species description and identification in the genus *Butyrivibrio*.

Table 1. *Butyrivibrio* strains most commonly analysed by different authors (abbreviations in the last 8 columns in the first row), their origins and grouping according to the individual study

Strain	Other designations	Origin & comment	Isolated by author/ref.	H '86	S '88	M '88	MS '90	HKC '93	WAC '96	DG '99	KMK '01
Butyrivibrio fibrisolvens											
D1	ATCC 19171, NCDO2221	cow, T	BS'56		I-A	*	8	*	r1/II	bk	Ts
A38	ATCC 27208, NCDO2222	cow	BS'56		I-A	*			r2/II	bk	
49	NCDO2223	cow	BS'56		IV-B	1	1	*	r8/III	tf	
H17c		steer	D'66		IV-B	1		*			GGAA
CE51		sheep	SGK'69		IV-C	1		*			Pr
CE52		sheep	SGK'69		IV-C	1					
12		sheep	GR'70		IV-C	1		*			
B 835	NCDO2398	heifer	H'73	*	I-B	3			r3/II	tf	
E9a		goat	D'77		I-B	3					
E21c		goat	D'77		I-B	3					
NOR-37	NCDO2249	cow	SR'72		I-B	3		*	r9/III	tf	
S-2		sheep	HD'79		I-B	*					
1L6-31	NCDO2400	cow	SR'72	*	I-C	3		*	r9		
ARD-22a		reindeer	D'75		II-A	*					
CF3		sheep	LD'85		III	2	1	*			
CF3c, CF3a, CF4c		sheep	LD'85		III	2					
X6C61		sheep	van Gylswyk (from S'88)		IV-A	4					
20-32		pig	Allison (from S'88)		V	*					
VV1		bison	Varel (from M'90)				6	*		tf	
UC12491, UC12254		steer	Kotarslo (from MS'90)				1				
PI-7		cow	H'86	*				*		tf	
JK 662, JK 670		sheep	Kopečny								Er
JK 669		sheep	Kopečny								GGAA
Bu43	Ce64	sheep	van Gylswyk (from WAC'96)						r7/II		GGAA
X2D62		sheep	van Gylswyk (from S'88)								GGAA
JK 609, JK 612, JK 614, JK 615		sheep	Kopečny								A
Mz3, Mz 9		cow	ZMA'97								A

continued overleaf

Strain	Other designations	Origin & comment	Isolated by author/ref.	H '86	S '88	M '88	MS '90	HKC '93	WAC '96	DG '99	KMK '01
OB 156		white-tailed deer, GM	F'97							tf	Pr
JK 10/1, JK 86, JK 170, JK 626		sheep	Kopečny								Pr
Mz7		cow	ZMA'97								Pr
AR11		sheep	HG'89								Pr
Butyrivibrio crossotus											
T9-40A	ATCC 29175, NCDO2416	human, T	MJH'76				6		r12/I		
Pseudobutyrivibrio ruminis											
A12-1	DSM 9787	cow, T	GHR'96								

Comments:
Isolated by author/ref.:

BS'56 = Bryant and Small, 1956
D'66 = Dehority, 1966
SGK'69 = Shane *et al*., 1969
GR'70 = van Gylswyk and Roche, 1970
SR'72 = Sharpe and Reiter, 1972
H'73 = Henderson, 1973
D'75 = Dehority, 1975
MJH'76 = Moore *et al*., 1976
D'77 = Dehority and Grubb, 1977
HD'79 = Hazlewood and Dawson, 1979
LD'85 = Lewis and Dehority, 1985
H'86 = Hazlewood *et al*., 1986
S'88 = Stack, 1988
M'88 = Mannarelli, 1988
HG'89 = Hudman and Gregg, 1989
MS'90 = Mannarelli *et al*., 1990
HKC'93 = Hespell *et al*., 1993
GHR'96 = van Gylswyk *et al*., 1996
WAC'96 = Willems *et al*., 1996
ZMA'97 = Zorec *et al*., 1997
F'97 = Forster *et al*., 1997
DG'99 = Diez-Gonzales *et al*., 1999
KMK'01 = Kopečny *et al*., 2001

Origin & comment.: T = type strain; GM = *B. fibrisolvens* that was successfully genetically modified by Gregg *et al*. (1994)
* = the strain was used in the study; other designations are original group designations as labelled in the original papers by the authors.

Moore *et al*. (1976) defined new species *Butyrivibrio crossotus*, with characteristics similar to *B. fibrisolvens*, on the basis of a morphological trait – lophotrichous flagella. It was isolated from human feces. Van Glyswyk *et al*. (1996) created new genus *Pseudobutyrivibrio* for those strains which phenotypically resembled strains placed in the genus *Butyrivibrio* but did not hydrolyse xylan or starch, neither were proteolytic. The only species is *P. ruminis*. The analyses of 16S rRNA (less than 95% of similarity to the closest related strains) confirmed this delineation.

Strains can be grouped also on the basis of chemotaxonomic methods – retrieving and analysing occurrence and quantity (ratios) of different chemical compounds present in selected organism. Chemical composition of the cell is genetically controlled and a highly stable trait that directly reflects diversity coded in the genome. Therefore the results of standardized methods can be used to establish taxonomic relationships among studied organisms. The most frequently studied are cell wall composition, lipid and polyamines profiles (Roselló-Mora and Amann, 2001). Hespell *et al*. (1993) analysed cell wall composition of some *Butyrivibrio* strains and Stack (1988) and Mannarelli *et al*. (1990) extracellular polysaccharides. The most extensive studies were done analysing cellular fatty acids (Miyagawa, 1982; Moore *et al*., 1994; Kopečný *et al*., 2001; Marinšek Logar *et al*., 2001) as fatty acid content of all lipids is determined by particular type of biosynthetic pathway of a given species (Welch, 1991). The major cellular constituents (especially cellular fatty acids) of species in a genus are relatively uniform and they can be useful in indicating the genus to which organisms with characteristics unlike those of any described species might be assigned or in excluding unknown strains from particular genera

(Moore *et al*., 1994). Results of all the studies point out that strains currently forming one species differ significantly in these characteristics. This fact reflects the diversity in the genus *Butyrivibrio* and supports the need for new species formation.

Table 2. Methods used by different authors for classifying the strains of the genus *Butyrivibrio*

METHOD	MHH '64	SGK '69	H '86	S '88	M '88	TG '85	HG '89	MS '90	HKC '93	F'96 &97	WAC '96	DG '99	MZK '01	KMK '01
16S rRNA sequences analyses										+	+			+
DNA-DNA hybridisation					+		+	+						
EPS analyses				+				+						
Cell wall									+					
mAb	+		+											
Fermentation of different sugars and enzyme activities	+	+	+				+					+		+
Fermentation products		+				+	+							+
FAMEs													+	+
Number of strains included	5	19	68	37	39	26	8	19	13	6 and 127	40	8	45	55
Method accordance	div.	div.	div.	-	-	-	yes	yes	-	-	-	-	-	yes
Number of suggested groups	no	2	no	5	5	3	4	9	no	3	3g/12r	2	6	min 5

Comments:
Authors:

MHH'64 = Margherita *et al*., 1964
SGK'69 = Shane *et al*., 1969
TG'85 = van der Toorn and van Gylswyk, 1985
H'86 = Hazlewood *et al*., 1986
S'88 = Stack, 1988
M'88 = Mannarelli, 1988
HG'89 = Hudman and Gregg, 1989
MS'90 = Mannarelli *et al*., 1990
HKC'93 = Hespell *et al*., 1993
WAC'96 = Willems *et al*., 1996
F'96&97 = Forester *et al*., 1996 and 1997
DG'99 = Diez-Gonzales *et al*., 1999
KMK'01 = Kopečny *et al*., 2001
MZK'01 = Marinšek Logar *et al*., 2001

+ = the method was applied by the author
Method accordance:
div. = just diversity was confirmed
- = only one method was used
yes = methods were in accordance
Number of suggested groups:
no = no grouping was made
3 g/12r = 12 ribotypes in 3 different phylogenetic lineages.

CONCLUSION

Great phenotypic and genotypic diversity of *Butyrivibrio* strains was observed and confirmed by different authors since the genus was established by Bryant and Small in 1956. Therefore the need for defining new species within this genus or even placing some of the strains in closely related genera *Pseudobutyrivibrio* and *Clostridium* is evident. In order to reclassify those strains, not rarely just one trait of *Butyrivibrio* strains was analysed (especially 16S rRNA) and also represented the basis for the putative reclassification. This approach is not appropriate and can not represent the only argument for such an act. It can have just an orientation value and can direct further analyses of other characteristics. On the other hand there are also studies where

more characteristics were analysed and grouping of the strains was based on combination of the results (Table 2). For revealing new relations between strains and for defining and describing new species the polyphasic approach has to be applied (Rosellló-Mora and Amann, 2001), hence the later approach is more appropriate. As many as possible phenotypic and genomic characteristics of as many as possible strains have to be analysed and described. It would be ideal to use joined forces of many laboratories that would examine the same strains by methods they are most skilful in and have good analytical techniques. Results given would represent strong argument for the reclassification. There is also a need to define a set of relatively simple but reliable identification tests in order to identify and classify new isolates in previously defined and established species and genera. Publishing the article in the International Journal of Systematic and Evolutionary Microbiology (former Int J Syst Bacteriol) (IJSEM, 2002; Euzéby, 1997) would grant new species naming and standing in taxonomy. Such an approach already gave results in two new species definition (Kopečný *et al*., 2002), but there are many strains that have to be analysed jet and new species will likely be defined in the near future.

POVZETEK

Vamp je kompleksen ekosistem, ki ga sestavljajo predstavniki velikega števila vrst mikroorganizmov. Ti igrajo različne vloge pri razgradnji hrane in funkciji vampa. Vampne mikroorganizme so raziskovalci intenzivno proučevali in jih razvrstili v več rodov in vrst na osnovi določenih lastnosti. Tako so paličaste anaerobne bakterije, ki so se barvale po gramu negativno, niso sporulirale in so glukozo fermentirale do znatnih količin maslene kisline, uvrstili v rod *Butyrivibro* z edino vampno vrsto *B. fibrisolvens*. Predstavniki te vrste imajo kar nekaj zanimivih lastnosti za potencialno biotehnološko uporabo. Že tvorba maslene kisline je pomembna za delovanje in vzdrževanje zdravega stanja črevesa. Poleg tega so butirivibriji eni izmed redkih vampnih producentov konjugirane linolne kisline (CLA), s kopico blagodejnih učinkov (antikancerogeno delovanje, zmanjševanje nastanka arterioskleroze in vsebnosti maščob v tkivu). Tudi njihovi bakteriocini (butirivibriocini) bi lahko našli podobna polja uporabe kot že uveljavljeni bakteriocini mlečnokislinskih bakterij. Poleg tega imajo na voljo obsežen hidrolitični encimski sistem, ki sodeluje pri učinkoviti prebavi krme. Že utemeljitelja tega rodu pa sta opazila veliko raznolikost sevov, ki jih takrat še nista uvrstila v več vrst. Tudi raziskovalci, ki so proučevali tako genomske kot fenotipske lastnosti butirivibrijev, so to ugotovitev potrdili. Nekateri so seve na podlagi rezultatov svojih analiz razporedili v več skupin. Te razdelitve je težko primerjati med seboj, saj nobena študija ne obsega vseh pomembnejših sevov kot tudi ne vseh metod. Za definiranje novih vrst, oziroma mogoče prerazporeditve v nov rod, bi bilo potrebno proučiti kar se da največ sevov z različnimi metodami. Tak polifazni pristop bi lahko utemeljil definiranje novih vrst sevov iz današnjega rodu *Butyrivibrio*. V najnovejši objavi (Kopečný *et al*., 2002) so s takim pristopom avtorji uspeli definirati dve novi vrsti. Vseeno pa bo potrebno proučiti še mnogo sevov in zelo verjetno je, da bo kmalu opredeljeno še več novih vrst v tem rodu.

REFERENCES

Barcenilla, A./ Pryde, S.E./ Martin, J.C./ Duncan, S.H./ Stewart, C.S./ Henderson, C./ Flint, H.J. Phylogenetic relationships of butyrate-producing bacteria from the human gut. Appl. Environ. Microbiol., 66(2000), 1654–1661.

Beveridge, T.J. Mechanism of Gram variability in select bacteria. J. Bacteriol., 172(1990)3, 1609–1620.

Bryant, M.P./ Small, N. The anaerobic monotrichous butyric acid-producing curved rod-shaped bacteria of the rumen. J. Bacteriol., 72(1956), 16–21.

Chebury, C./ Andrieux, C./ Duee, P.H./ Darcy-Vrillon, B. Action of n-butyrate at the level of gene expression in the colonic mucosa: lessons from pig and rat expreiments. In: Gut environment of pigs (Eds.: Piva, A./ Bach Knudsen, K.E./ Lindberg, J.E.). Nothingam, Nothingam University press, 2001, 95–104.

Cheng, K.J./ Costerton, J.W. Ultrastructure of *Butyrivibrio fibrisolvens*: a gram-positive bacterium? J. Bacteriol., 129(1977)3, 1506–1512.

Cheng, K.J./ Phillippe, R.C./ McLean, R.J.C./ Costerton, J.W. The characterization and ultrastructure of two new strains of *Butyrivibrio*. Can. J. Microbiol., 35(1989), 274–282.

Collins, M.D./ Lawson, P.A./ Willems, A./ Cordoba, J.J./ Fernandez-Garayzabal, J./ Garcia, P./ Cai, J./ Hippe, H./ Farrow, J.A.E. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int. J. Syst. Bacteriol., 44(1994)4, 812–826.

Dehority, B.A. Characteristics of several bovine rumen bacteria isolated with xylan medium. J. Bacteriol., 91(1966), 1724–1729.

Dehority, B.A. Characterization of the predominant bacteria occurring in the rumen of goats (*Capra hircus*). Appl. Environ. Microbiol., 33(1977), 1030–1036.

Dehority, B.A. Characterization studies on rumen bacteria isolated from Alaskan reindeer (*Rangifer tarandus* L.). In: Proceedings of the First International Reindeer and Caribou Symposium, Fairbanks, 1972-08-9/11. Anchorage, University of Alaska, 1975, 228–240.

Diez-Gonzales, F./ Bond, D.R./ Jennings, E./ Russell, J.B. Alternative schemes of butyrate production in *Butyrivibrio fibrisolvens* and their relationship to acetate utilization, lactate production and phylogeny. Arch. Microbiol., 171(1999), 324–330.

Dugan, M.E.R./ Aalhus, J.L./ Jeremiah, L.E./ Kramer, J.K.G./ Schaefer, A.L. The effects of feeding conjugated linoleic acid on subsequent pork quality. Can. J. Anim. Sci., 79(1999)1, 45–51.

Euzéby, J.P. List of bacterial names with standing in nomenclature. Int. J. Syst. Bacteriol., 47(1997), 590–592. (09. feb. 2002) http://www.bacterio.cict.fr/b/butyrivibrio.html (11. feb. 2002).

Forster, R.J./ Gong, J./ Teather, R.M. Group-specific 16S rRNA hybridization probes for determinative and community structure studies of *Butyrivibrio fibrisolvens* in the rumen. Appl. Environ. Microbiol., 63(1997)4, 1256–1260.

Forster, R.J./ Teather, R.M./ Gong, J./ Deng, S.J. 16S rDNA analysis of *Butyrivibrio fibrisolvens*: phylogenetic position and relation to butyrate-producing anaerobic bacteria from the rumen of white-tailed deer. Lett. Appl. Microbiol., 23(1996), 218–222.

Gregg, K./ Cooper, C.L./ Schafer, D.J./ Sharpe, H./ Beard, C.E./ Allen, G./ Xu, J.W. Detoxification of the plant toxin fluoroacetate by genetically modified rumen bacterium. Bio\Technology, 12(1994), 1361–1365.

Hazlewood, G.P./ Dawson, R.M.C. Characteristics of a lipolytic and fatty acid-requiring *Butyrivibrio* sp. isolated from the ovine rumen. J. Gen. Microbiol., 112(1979), 15–27.

Hazlewood, G.P./ Theodorou, M.K./ Hutchings, A./ Jordan, D.J./ Galfre, G. Preparation and characterisation of monoclonal antibodies to a *Butyrivibrio* sp. and their potential use in the identification of rumen Butyrivibrios, using an enzyme-linked immunosorbent assay. J. Gen. Microbiol., 132(1986), 43–52.

Henderson, C. The effects of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. Camb., 81(1973), 107–112.

Hespell, R.B./ Kato, K./ Costerton, J.W. Characterization of the cell wall of *Butyrivibrio fibrisolvens*. Can. J. Microbiol., 39(1993), 912–921.

Hobson, P.N. Introduction. In: The rumen microbial ecosystem (Eds.: Hobson P.N./ Stewart, C.S.). London, Thomson Science, 1997, 1–9.

Hudman J.F/ Gregg K. Genetic diversity among strains of bacteria from the rumen. Current Microbiol., 19(1989), 313–318.

IJSEM, International journal of systematic and evolutionary microbiology (till dec. 1999 Int J Syst Bacteriol). Society for general microbiology. http://ijs.sgmjournals.org (11. feb. 2002).

Kalmokoff, M.L./ Teather, R.M. Isolation and characterization of a bacteriocin (butyrivibriocin AR10) from the ruminal anaerobe *Butyrivibrio fibrisolvens* AR10: evidence in support of the widespread occurrence of bacteriocin-like activity among ruminal isolates of *B. fibrisolvens*. Appl. Environ. Microbiol., 63(1997), 394–402.

Kepler, C.R./ Hirons, K.P./ McNeill, J.J./ Tove, S.B. Intermediates and products of biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. J. Biol. Chem., 241(1966), 1350–1354.

Kim, Y.J./ Liu, R.H./ Bond, D./ Russell, J.B. Effect of linoleic acid concentration on conjugated linoleic acid production by *Butyrivibrio fibrisolvens* A38. Appl. Environ. Microbiol., 66(2000)12, 5226–5230.

Kopečný, J./ Marinšek Logar, R./ Kobayashi, Y. Phenotypic and genetic data supporting reclassification of *Butyrivibrio fibrisolvens* isolates. Folia Microbiol., 46(2001)1, 45–48.

Kopečný, J./ Marinšek Logar, R./ Zorec, M./ Mrázek, J./ Kobayashi, Y. *Butyrivibrio hungatei* sp. nov., and *Pseudobutyrivibrio xylanivorans* sp. nov., butyrate-producing bacteria from the rumen. Int. J. Syst. Evolut. Microbiol., (2002), in press.

Kritchevsky, D. Antimutagenic and some other effects of conjugated linoleic acid. Brit. J. Nutr., 83(2000), 459–465.

Lewis, S.M./ Dehority B.A. Microbiology and ration digestibility in the hindgut of the ovine. Appl. Environ. Microbiol., 50(1985), 356–363.

Mannarelli, B.M. Deoxyribonucleic acid relatedness among strains of the species *Butyrivibrio fibrisolvens*. Int. J. Syst. Bacteriol., 38(1988)4, 340–347.

Mannarelli, B.M./ Stack, R.J./ Lee, D./ Ericsson, L. Taxonomic relatedness of *Butyrivibrio*, *Lashnospira*, *Roseburia*, and *Eubacterium* species as determined by DNA hybridization and extracellular-polysaccharide analysis. Int. J. Syst. Bacteriol., 40(1990)4, 370–378.

Margherita, S.S./ Hungate, R.E./ Storz, H. Variation in rumen *Butyrivibrio* strains. J. Bacteriol., 87(1964), 1304–1308.

Marinšek Logar, R./ Kopečny, J./ Zorec, M. Variability of *Butyrivibrio fibrisolvens* estimated by cellular fatty acid profiles, DNA restriction analysis and fermentation patterns. Reprod. Nutr. Dev. 40(2000)2, 182–183.

Marinšek Logar, R./ Zorec, M./ Kopečný, J. Reliable identification of *Prevotella* and *Butyrivibrio* spp. from rumen by fatty acid methyl ester profiles. Folia Microbiol. 46(2001)1, 57–59.

Miyagawa, E. Cellular fatty acid and fatty aldehyde composition of rumen bacteria. J. Gen. Appl. Microbiol., 28(1982), 389–408.

Moore, L.V.H./ Bourne, D.M/ Moore, W.E.C. Comparative distribution and taxonomic value of cellular fatty acids in thirty-three genera of anaerobic gram-negative bacilli. Int. J. Syst. Bacteriol., 44(1994)2, 338–347.

Moore, W.E.C./ Johnson, J.L./ Holdeman, L.V. Emendation of *Bacteriodes* and *Butyrivibrio* and descriptions of *Desulfomonas* gen. nov. and ten new species in the genera *Desulfomonas, Butyrivibrio, Eubacterium, Clostridium,* and *Ruminococcus*. Int. J. Syst. Bacteriol., 26(1976), 238–252.

Pariza, M.W./ Park, Y./ Cook, M.E. Conjugated linoleic acid and the control of cancer and obesity. Toxicol. Sci., 52(1999), 107–110.

Rosselló-Mora, R./ Amann, R. The species concept for prokaryotes. FEMS Microbiol. Rev., 25(2001), 39–67.

Sakata, T./ Inagaki A. Organic acid production in the large intestine: implication for epithelial cell proliferation and cell death. In: Gut environment of pigs (Eds.: Piva, A./ Bach Knudsen, K.E./ Lindberg, J.E.). Nothingam, Nothingam University press, 2001, 85–94.

Shane, B.S./ Gouws, L./ Kistner, A. Cellulolytic bacteria occurring in the rumen of sheep conditioned to low-protein teff hay. J. Gen. Microbiol., 55(1969), 445–457.

Sharpe, M.E./ Reiter, B. Common antigenic determinant in a rumen organism and in *Salmonellae* containing the antigen O4. Appl. Microbiol., 24(1972), 615–617.

Stack, R. Neutral sugar composition of extracellular polysaccharides produced by strains of *Butyrivibrio fibrisolvens*. Appl. Environ. Microbiol., 54(1988), 878–883.

Stewart, C.S./ Flint, H.J./ Bryant, M.P. The rumen bacteria. In: The rumen microbial ecosystem (Eds.: Hobson P.N./ Stewart, C.S.). London, Thomson Science, 1997, 10–72.

van der Toorn, J.J.T.K./ van Gylswyk, N.O. Xylan-digesting bacteria from the rumen of sheep fed maize straw diets. J. Gen. Microbiol., 131(1985), 2601–2607.

van Gylswyk, N.O./ Hippe, H./ Rainey, F.A. *Pseudobutyrivibrio ruminis* gen. nov., sp. nov., a butyrate-producing bacterium from the rumen that closely resembles *Butyrivibrio fibrisolvens* in phenotype. Int. J. Syst. Bacteriol., 46(1996)2, 559–563.

van Gylswyk, N.O./ Roche, C.E.G. Characteristics of *Ruminococcus* and cellulolytic *Butyrivibrio* species from the rumens of sheep fed differently supplemented teff (*Eragrostic tef*) hey diets. J. Gen. Microbiol., 64(1970), 11–17.

Vandamme, P./ Pot, B./ Gillis, M./ De Vos, P./ Kersters, K./ Swings, J. Polyphasic taxonomy, a consensus approach to bacterial systematisc. Microbiol. Rev., 60(1996)2, 407–438.

Wayane, G.L./ Brenner, D.J./ Colwell, R.R./ Grimont, P.A.D/ Kandler, O./ Krichevsky, M.I./ Moore, L.H./ Moore, W.E.C./ Murray, R.G.E./ Stackebrandt, E./ Starr, M.P./ Trüper, H.G. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int. J. Syst. Bacteriol., 37(1987)4, 463–464.

Welch, D.F. Applications of cellular fatty acid analysis. Clin. Microbiol. Rev., 4(1991)4, 422–438.

Willems A./ Amat–Marco M./ Collins M.D. Phylogenetic analysis of *Butyrivibrio* strains reveals three distinct groups of species within the *Clostridium* subphylum of the gram-positive bacteria. Int. J. Syst. Bacteriol., 46(1996)1, 195–199.

Zorec, M./ Marinšek Logar, R./ Avguštin, G. Isolation of butyric acid producing xylanolytic bacteria from the rumen. Zb. Bioteh. Fak. Univ. Ljubl., Kmet. Zooteh., 70(1997), 21–29.