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Fibrolytic Bacterium Isolated From Buffalo Rumen Phylogenetically Closely Related to Butyrivibrios and Pseudobutyrivibrios

Somporn Poonko[1], Phoompong Boonsaen[1] and Suriya Sawanon[1,2,*]

ABSTRACT

Butyrivibrios and Pseudobutyrivibrios represent a significant proportion of ruminal bacteria and large culturable *Butyrivibrio* strains that contribute to fiber degradation and the utilization of various substrates. *Butyrivibrio* and *Pseudobutyrivibrio* are highly adaptable to ruminal conditions and play an important role in fiber fermentation. This study isolated the high potential fibrolytic and non-fibrolytic bacteria *Butyrivibrio* and *Pseudobutyrivibrio* from buffalo rumen and studied synergism between fibrolytic and non-fibrolytic bacteria in co-culture. Two mature, rumen-fistulated swamp buffaloes were fed roughage of either rice straw or rice straw plus concentrate. Roughage suspended in the rumen was used as the source of isolates. Six hours after feeding, roughage from the rumen was serially diluted and used for pure culture isolation by the roll tube technique under anaerobic conditions. Fiber degrading and cellobiose utilizing bacteria were collected and screened for further characterization, consisting of Gram-staining, 16S rDNA sequencing, utilization of different carbon sources, digestibility on fiber powders, intracellular fibrolytic enzyme activities, volatile fatty acid production and co-culture digestibility between fibrolytic *Butyrivibrio* and non-fibrolytic bacteria. A total of 165 Gram-negative fibrolytic bacteria were obtained from 1,125 colonies and then 21 fibrolytic and 20 non-fibrolytic strains were selected that could utilize cellobiose efficiently. Fibrolytic bacteria were identified as 10 strains of *Butyrivibrio* sp. and 4 strains of *Pseudobutyrivibrio* spp. and non-fibrolytic bacteria were identified as 4 strains of *Butyrivibrio* sp. and 3 strains of *Pseudobutyrivibrio* spp. Isolates of fibrolytic *Butyrivibrios* sp. and *Pseudobutyrivibrios* spp., especially *B. fibrisolvens* KU-F83, showed high digestibility of fiber powder and high intracellular fibrolytic enzyme activity. Co-culture of fibrolytic *B. fibrisolvens* KU-F83 and non-fibrolytic Butyrivibrios, Pseudobutyrivibrios and *S. ruminantium* S137 could ehance rice straw digestibility and the propionate portion while reducing cellulose digestibility.

Keywords: Butyrivibrios, Pseudobutyrivibrios, fibrolytic bacteria, non-fibrolytic bacteria, buffalo

INTRODUCTION

Roughage is one of the main ruminant animal feedstocks, which can be digested and fermented by rumen microbes (Krause *et al.,* 2003). The end products from microbial fermentation, such as volatile fatty acids and microbial cells, are used to produce meat or milk by ruminant animals and fibrolytic bacteria have an important role in fiber fermentation, especially *Fibrobacter*

1 Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.

2 Center for Advanced Studies for Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail: agrsusa@ku.ac.th

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succinogenes, Ruminococcus flavefaciens, and *R. albus* (Koike and Kobayashi, 2009). Butyrivibrios and Pseudobutyrivibrios contribute to fiber degradation and various substrate utilization in the rumen (Gylswyk *et al.,* 1996; Kopecny *et al.*, 2003; Grilli *et al.,* 2013). In particular, *Butyrivibrio fibrisolvens*, a hemicellulolytic butyrate producing bacterium, is commonly isolated from the rumen of domestic and wild animals (Hespell *et al.,* 1993). It is highly adaptable to rumen conditions and has a role in the biohydrogenation of unsaturated fatty acids (Boeckaert *et al*., 2008; Gudla *et al.,* 2012). The current study isolated the high potential fibrolytic bacteria, *Butyrivibrio* sp. and *Pseudobutyrivibrio* spp., from buffalo rumen and studied the synergism between fibrolytic bacteria and non-fibrolytic bacteria.

MATERIALS AND METHODS

Rumen bacterial sampling and isolation

Two mature rumen-fistulated swamp buffaloes were used for rumen bacterial sampling. The buffaloes were individually penned and fed *ad libitum* on rice straw or rice straw plus 2 $kg.d^{-1}$ of 16% CP concentrate (41% cassava, 32% corn, 21% soybean meal, 1% urea, 1% NaCl and 1% premix for beef cattle). Roughage suspended (6 hr after feeding) or rumen contents were used as the source of rumen bacterial isolates; 0.5 g of rumen was add into a 4.5 mL dilution solution under anaerobic conditions then incubated on ice for 5 min and vortexed for 1 min to detach rumen bacterial cells from the fiber. Then, serial dilutions using the anaerobic dilution solution at 1×10^{-5} and 1×10^{-6} were inoculated into a basal medium containing 0.2% (weight per volume; w/v) glucose and cellobiose and 2.5% (w/v) (rumen fluid glucose and cellobiose agar) for pure culturing by the roll tube technique and then incubated at 38 ºC for 72 hr. A single colony was randomly picked up and introduced into a basal broth medium containing 0.2% (w/v) cellobiose (rumen fluid cellobiose medium; RC) incubated at 38 ºC for 24 hr and then the ruminal bacteria from the inoculated isolates were combined with the basal broth medium containing filter paper (Whatman® No.1) as a carbon source (rumen fluid filter paper medium) for selected fibrolytic bacteria. Non-fibrolytic associate fibrolytic bacteria were selected by their high growth rate in cellobiose as a carbon source in the basal medium. Fibrolytic bacteria (Butyrivibrios and Pseudobutyrivibrios) were selected then checked for fiber digestibility and intracellular fibrolytic enzyme activities (cellulase and xylanase). High potential fibrolytic and non-fibrolytic bacteria were collected and screened for identification with Gram staining, 16S rDNA sequencing and volatile fatty acid production. The selected pure cultures of fibrolytic and non-fibrolytic bacteria were stored in a reinforced clostridial agar medium at -80 ºC.

16S rDNA sequencing and phylogenetic analysis

Total DNA was extracted from bacterial cultures in the RC medium by boiling the cells in lysis buffer. Partial 16S ribosomal DNA was amplified using polymerase chain reaction (PCR; Multi Gene gradient thermal cycle; Labnet International Inc.; Edison, NJ, USA), with universal primers, 27F-Universal forward primer (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1492R-Universal reverse primer (5′ TACGGYTACCTTGTTACGACTT 3′). The PCR products were purified using a FavorPrep™ Gel/PCR Purification Mini Kit (Favogen Biotech Corp.; Taiwan.) and used for DNA sequencing (First Base Laboratories Sdn Bhd; Seri Kembangan, Selangor, Malaysia). The sequences obtained were run through BLAST in order to determine the closest identity of the bacteria in the GenBank database (BLAST N, http://www.ncbi.nlm.nih.gov/BLAST).

The generated sequences and reference sequences from the GenBank database were aligned using the multialigment program CLUSTAL X (version 1.83; Conway Institute; Dublin, Ireland).

The neighbor-joining method was used to perform phylogenetic analysis. The NJPLOT software was used to draw a phylogenetic tree (Perrière and Gouy, 1996). Bootstrap analysis was carried out with data resampling 1,000 times.

Carbon source utilization of selected non-fibrolytic bacteria

Glucose, cellobiose and xylose were used as the carbon source in a basal medium to investigate the utilization of different carbon sources of bacteria. Non-fibrolytic bacteria was used as the pre-culture in the RC medium for 24 hr followed by inoculation in medium containing 0.2% of glucose, cellobiose or xylose as a carbon sources, and then incubation at 38 °C. Growth of the bacteria was evaluated by measuring the turbidity (600 nm) of the cultures using a spectrophotometer (Optical density; OD_{600}; Amersham Biosciences Corp.; Piscataway, NJ, USA) described by Sawanon *et al.* (2003). Turbidity measurements were collected after incubation at 0, 2, 4, 6, 8, 10, 12 and 24 hr (Bertin *et al.,* 2013).

Fiber digestibility of pure culture fibrolytic bacteria and co-culture with non-fibrolytic bacteria

Fiber digestibility was tested using 1% w/v cellulose powder (Sigmacell; Sigma-Aldrich; St Louis, MO, USA) or rice straw as a carbon source in a basal medium. Fibrolytic *Butyrivibrio* strains were grown in basal medium containing cellulose powder as a carbon source (incubated at 38 °C for 24 hr) and non-fibrolytic *Butyrivibrio* sp. and *Pseudobutyrivibrio* spp. were grown in cellobiose as a carbon source (incubated at 38 °C for 12 hr) for pre-culture. *Selenomonas ruminantium* S137 as isolated by Sawanon *et al.* (2003) was used to study the enhancement of the digestibility of fibrolytic *Butyrivibrio*. A pre-culture (0.2 mL) was inoculated into cellulose powder or a rice straw basal broth medium and then incubated at 38 °C for 72 hr.

After incubation, the fiber cultures were cooled on ice for 30 min to detach the bacterial cells from the fiber particles (Minato and Suto, 1978) and centrifuged at 377×*g* for 10 min below 4 °C then the supernatant containing the bacterial cells was discarded. The fiber residue was washed with 10 mL of 0.5M potassium phosphate buffer and re-centrifuged (2,300×*g* at 4 °C for 10 min). The washed fiber residue was dried and weighed to calculate the dry matter (DM) fiber digestion of the isolated ruminal bacteria (Fukuma *et al*., 2012).

Intracellular cellulase and xylanase activities analysis

The culture was grown in 20 mL of cellulose powder as a carbon source in a basal medium (incubated at 38 °C for 24 hr). The bacterial cells were harvested by centrifugation at 2,800×*g* for 10 min below 4 °C, washed twice using a 50 mM potassium phosphate buffer (pH 6.8), and re-suspended (2 mL) in the same buffer. Cells were disrupted using an ultrasonic disruptor (UD-201; Tomy Seiko Co.; Tokyo, Japan) on ice for 10 min and centrifuged at 2,800×*g* for 10 min below 4 °C to collect the cell-free extract as intracellular enzyme (Sawanon *et al.,* 2003; Kobayashi *et al.,* 1998). Xylanase and cellulase activities were determined by measuring the reducing sugar from xylan (1% xylan in sterilized 50 mM phosphate buffer; Sigma®) and carboxyl-methyl-cellulose (1% CMC in sterilized 50 mM phosphate buffer; Sigma®) by the dinitrosalicylic acid method (Miller, 1959), using D-xylose and D-glucose as standard substrates, respectively. One unit of xylanase or CMCase activity was defined as the amount of the enzyme that released one nanomole of xylose or glucose equivalent per minute from xylan or CMC, respectively. Cell protein concentrations were determined using Bradford protein assay (Bradford, 1976). Bovine serum albumin was used as a standard protein.

Volatile fatty acid analysis

Volatile fatty acid (VFA) samples of

pure culture were collected from the fibrolytic culture grown in the cellulose powder and from the non-fibrolytic culture grown in cellobiose as a carbon source in basal medium (24 hr for fibrolytic incubation and 12 hr for non-fibrolytic incubation, both at 38 °C). The VFA samples of co-culture digestibility between fibrolytic and non-fibrolytic strains were collected when incubation was completed for cellulose and rice straw digestibility analysis to correlate the VFA production with co-culture digestibility. The VFA samples were harvested by centrifugation at 2,800×*g* for 10 min below 4 °C then 1 mL of supernatant was collected for analysis using gas chromatography as described by Sawanon (2003).

RESULTS

Isolation and identification of isolated ruminal bacteria

The Butyrivibrios and Pseudobutyrivibrios samples in this study were isolated from buffalo rumen. In total, 1,125 colonies were picked up from roll tubes and transferred to RC medium for further analyses. The process found 165 Gram-negative fibrolytic bacteria and 21 isolates of high potential fibrolytic bacteria and 20 isolates of non-fibrolytic bacteria which could efficiently utilized cellobiose were chosen from the isolated strains and employed for 16S rDNA sequencing. The obtained 16S rDNA sequences were run through BLAST to determine the closest identity of the origin of the bacteria. Fourteen strains were identified as *Butyrivibrio* (Table 2), consisting of 10 fibrolytic strains and 4 non-fibrolytic strains. Seven strains were identified as *Pseudobutyrivibrio*—four fibrolytic bacteria strains and three non-fibrolytic strains. The phylogenetic tree of *Butyrivibrio* sp. and *Pseudobutyrivibrio* spp. with *Eubacterium ractale* as an out group is shown in Figure 1. All isolated strains were closely related to *Butyrivibrio* sp. and *Pseudobutyrivibrio* spp. in the GenBank data base and the phylogenetic tree of isolated bacteria demonstrated that the 16S rDNA of Pseudobutyrivibrios were closely linked with Butyrivibrios. The volatile fatty acid production of the isolated *Butyrivibrio* and *Pseudobutyrivibrio* strains are shown in Table 3; acetate and butyrate were the major products from the fiber fermentation of these species, which was similar to the report of Gylswyk *et al*. (1996).

Growth of non-fibrolytic bacteria isolation

The growth curves of non-fibrolytic *Butyrivibrio* and *Pseudobutyrivibrio* are shown in Figure 2. All strains of *Butyrivibrio* and *Pseudobutyrivibrio* could utilize glucose and cellobiose for efficient growth. In the glucose medium, *Butyrivibrios* sp. KU-NF6, KU-NF12 and KU-NF38 showed expotential growth at 6–8 hr and strain KU-NF24 at 6–10 hr. *Pseudobutyrivibrio* spp. KU-NF28 and KU-NF29 showed exponential growth at 6–10 hr and strain KU-NF7 at 8–12 hr. In the cellobiose medium, *Butyrivibrio* sp. KU-NF38 showed exponential growth at 6–8 hr, strains KU-NF12 and KU-NF24 at 6–10 hr and strain KU-NF6 at 6–10 hr. *Pseudobutyrivibrio* sp. KU-NF7 and KU-NF28 showed exponential growth at 6–10 hr and strain KU-NF29 at 8–10 hr. The growth efficiency of *Butyrivibrio* and *Pseudobutyrivibrio* isolates in xylose was less than in glucose and cellobiose in basal medium, with little change shown at OD_{600}. The exponential growth of *Butyrivibrio* sp. KU-NF6 and KU-NF24 was shown at 6–10 hr, and for strains KU-NF38 and KU-NF12 at 10–12 hr, while for *Pseudobutyrivibrio* sp. KU-NF7, KU-NF28 and KU-NF29 it was at 6–12 hr.

Non-fibrolytic bacteria were chosen on the study that could efficiently utilize cellobiose for growth from each species of Butyrivibrios and Pseudobutyrivibrios—*B. fibrisolvens* KU-NF6, KU-NF24 and *P. xylanivorans* KU-NF7—to study the co-culture digestibility with fibrolytic *Butyrivibrio* and *Pseudobutyrivibrio* to enhance fiber digestibility.

Table 1 Composition of experimental media.

Item	Basal medium (per 100 mL)	RGCA (per 100 mL)	RC (per 100 mL)	RF (per 100 mL)
Mineral solution I [a]	7.5 mL	7.5 mL	7.5 mL	7.5 mL
Mineral solution II [b]	7.5 mL	7.5 mL	7.5 mL	7.5 mL
Resazurin, 0.1% solution	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Rumen fluid [c]	30 mL	30 mL	30 mL	30 mL
Distilled water	50 mL	50 mL	50 mL	50 mL
Bactopeptone	0.2 g	0.2 g	0.2 g	0.2 g
Yeast extract	0.12 g	0.12 g	0.12 g	0.12 g
L-Cysteine-HCl.H_2O (Sigma®)	0.05 g	0.05 g	0.05 g	0.05 g
Glucose	-	0.1 g	-	-
Cellobiose	-	0.1 g	0.2 g	-
Filter paper	-	-	-	0.5×1.0 cm^2
Na_2CO_3, 8% solution	5.0 mL	5.0 mL	5.0 mL	5.0 mL
Agar	-	2.5 g	-	-

RGCA = Rumen fluid glucose and cellobiose agar, RC = Rumen fluid cellobiose medium, RF = Rumen fluid filter paper medium.

[a] = Contains 0.6 g of K_2HPO_4.per 100 mL.

[b] = Contains 0.6 g of KH_2PO_4, 1.2 g $(NH_4)_2SO_4$, 1.2 g NaCl, 0.25 g $MgSO_4$ and 0.12 g $CaCl_2 \cdot 2H_2O$ per 100 mL (Ogimoto and Imai, 1981).

[c] = Fresh ruminal fluid cheese-cloth filtered and autoclaved at 121 ˚C for 10 min and then centrifuged at 2,800×*g* for 15 min at 4˚C and stored at -20˚C.

Table 2 Identification of ruminal bacteria isolated from swamp buffalo rumen.

Isolate number	Nearest relative analysis		
	Accession number	Assignment	Identity
Fibrolytic bacteria			
KU-F133, KU-F135	EU714408	*Butyrivibrio* sp. K3	99%
KU-F110	AY699276	*Butyrivibrio fibrisolvens* L2	99%
KU-F14, KU-F83, KU-F132, KU-F134, KU-F136, KU-F140, KU-F143	U41167	*Butyrivibrio fibrisolvens* 49	99%
KU-F20, KU-F138, KU-F144	JQ316642	*Pseudobutyrivibrio* sp. 3C20C	99%
KU-F23	EU346757	*Pseudobutyrivibrio ruminis* CF1b	99%
Non-fibrolytic bacteria			
KU-NF12, KU-NF24	EU714408.1	*Butyrivibrio* sp. K3	99%
KU-NF38	U41167	*Butyrivibrio fibrisolvens* 49	99%
KU-NF6	EU684229.1	*Butyrivibrio fibrisolvens* WH-1	99%
KU-NF28	AY178843.1	*Pseudobutyrivibrio ruminis* Ce4	99%
KU-NF7, KU-NF29	JQ673415.1	*Pseudobutyrivibrio xylanivorans* 2	99%

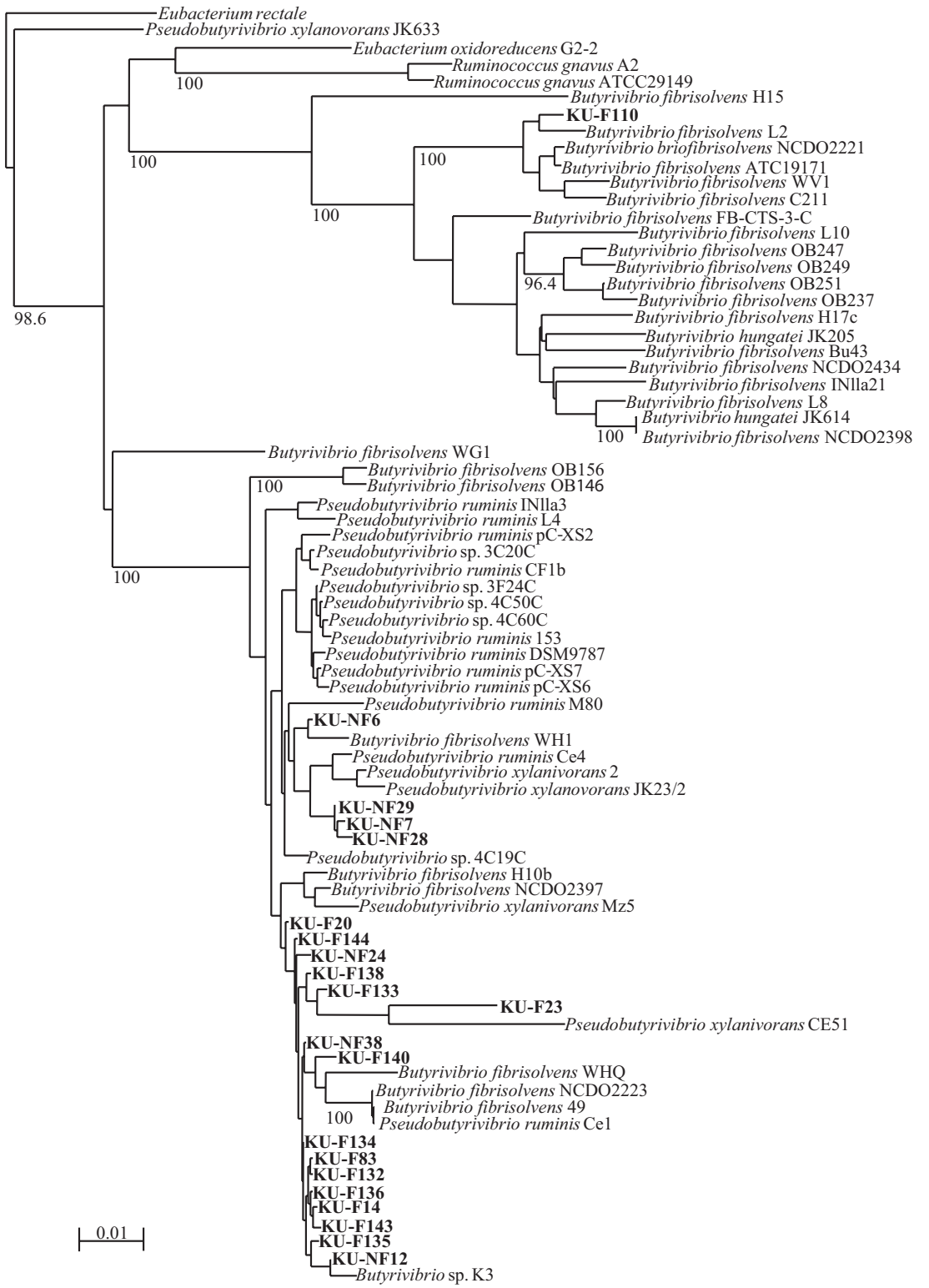

Figure 1 Phylogenetic tree of *Butyrivibrio* sp. and *Pseudobutyrivibrios* spp. based on 16S rDNA sequences.

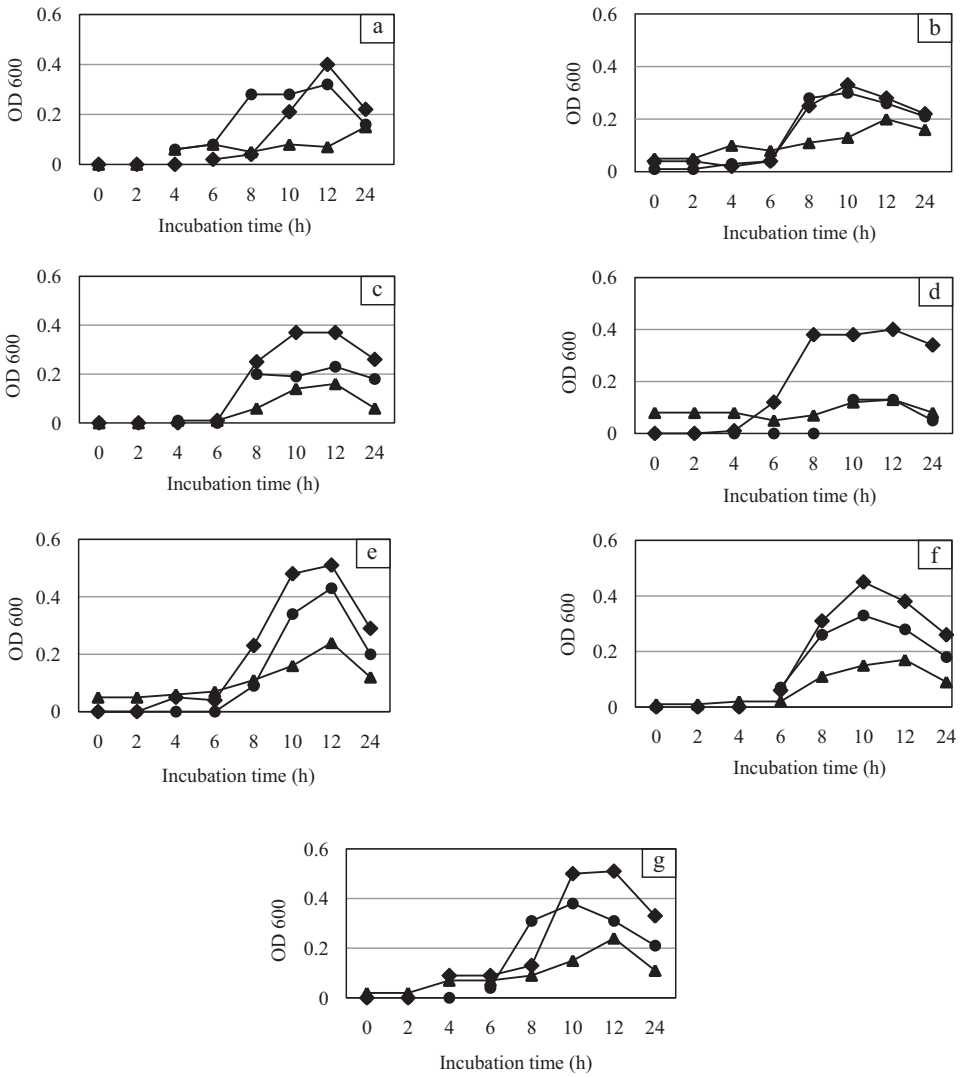

Figure 2 Growth curves of isolated non-fibrolytic strains closely related to *Butyrivibrio* sp.: (a) KU-NF6, (b) KU-NF12, (c) KU-NF24 and (d) KU-NF38 and *Pseudobutyrivibrio* spp.: (e) KU-NF7, (f) KU-NF28 and (g) KU-NF29 in basal medium containing glucose (–●–), cellobiose (–◆–) and xylose (–▲–) as a carbon source.

Fibrolytic activity of isolated strains

The fibrolytic activity of the *Butyrivibrio* and *Pseudobutyrivibrio* strains is shown in Table 4. The digestibility values of the *Butyrivibrio* strains were 5.32–20.59% DM and 9.88–20.87% DM and for the *Pseudobutyrivibrio* strains were 7.84–22.23% DM and 13.73–15.11% DM on the cellulose powder and rice straw, respectively. The total cellulase and xylanase activities of the *Butyrivibrio* strains were 5.09–10.09 and 3.99–11.59 $nMol.min^{-1}.mL^{-1}$ of culture, respectively. The total cellulase and xylanase activities of the *Pseudobutyrivibrio* strains were 5.22–7.86 and 3.86–7.81 $nMol.min^{-1}.mL^{-1}$ of culture, respectively. *B. fibrisolvens* KU-F83 showed high fiber digestibility and high fibrolytic enzyme activity and was chosen for the study of the co-culture digestibility with isolated non-fibrolytic bacteria.

Table 3 Volatile fatty acids (VFAs) of fibrolytic and non fibrolytic bacteria isolated from buffalo rumen.

Isolated bacteria strain	VFA (mMol.L^{-1} culture)				
	Acetate	Propionate	Butyrate	iso-Butyrate	Valarate
Fibrolytic species					
Butyrivibrio sp. KU-F133	20.91	1.34	3.61	0.43	0.06
Butyrivibrio sp. KU-F135	3.59	0.40	2.53	0.45	0.05
B. fibrisolvens KU-F110	1.72	0.64	1.55	0.35	0.11
B. fibrisolvens KU-F14	16.56	1.64	0.13	0.41	0.37
B. fibrisolvens KU-F83	27.04	1.60	4.90	0.39	0.10
B. fibrisolvens KU-F132	2.61	0.97	3.42	0.35	0.12
B. fibrisolvens KU-F134	20.24	1.83	4.41	0.37	0.08
B. fibrisolvens KU-F136	6.68	1.15	3.13	0.38	0.09
B. fibrisolvens KU-F140	6.33	1.40	2.63	0.39	0.08
B. fibrisolvens KU-F143	29.64	1.65	4.67	0.61	0.05
Pseudobutyrivibrio sp. KU-F20	4.95	0.26	2.28	-0.10	0.09
Pseudobutyrivibrio sp. KU-F138	3.40	1.04	3.33	0.36	0.09
Pseudobutyrivibrio sp. KU-F144	13.28	1.39	3.69	0.45	0.09
Pseudobutyrivibrio ruminis KU- F23	3.15	0.73	2.99	0.31	0.11
Non-fibrolytic species					
Butyrivibrio sp. KU-NF12	-8.34	-0.55	2.08	0.37	-0.34
Butyrivibrio sp. KU-NF24	-12.64	-0.75	2.03	0.45	-0.38
B. fibrisolvens KU-NF38	8.95	0.46	1.90	0.38	-0.36
B. fibrisolvens KU-NF6	-3.58	1.12	2.99	2.07	3.66
P. ruminis KU-NF28	-12.60	-0.58	2.26	0.38	-0.33
P. xylanivorans KU-NF7	1.37	0.12	2.23	0.50	-0.14
P. xylanivorans KU-NF29	-13.12	-0.73	2.11	0.39	-0.42

Fiber digestibility and VFA production of co-culture isolated strains

The fibrolytic bacterium, *B. fibrisolvens* KU-F83 was co-cultured with non-fibrolytic *B. fibrisolvens* KU-NF6, *Butyrivibrio* sp. KU-NF24, *P. xylanivorans* KU-NF7 and *S. ruminantium* S137. The results in Tables 5 and 6 show that co-culturing could enhance rice straw digestibility (2.81–4.85% DM) but reduced cellulose digestibility (3.52–6.15% DM). However, these results showed that co-culture with *S. ruminantium* S137 could enhance the rice straw digestibility and propionate production.

DISCUSSION

Butyrivibrios are the major fibrolytic bacteria isolated from buffalo rumen in this study (47.61% of total isolated bacteria). The homology analysis of the 16S rDNA sequences of islolated fibrolytic and non-fibrolytic bacteria revealed a closely relationship between *Butyrivibrio* sp. and *Pseudobutyrivibrio* spp. They ferment many sugars such as glucose, cellobiose, sucrose, galactose xylose or mannose and their major fermention products are acetate, butyrate and formate (Gylswyk *et al.,* 1996).

Table 4 Mean values (±SD) of fiber digestibility and intracellular fibrolytic enzyme activity of fibrolytic *Butyrivibrios* and *Pseudobutyrivibrios* isolated from buffalo rumen.

Isolated bacterial strain	Digestibility (%)		Total enzyme activity (nMol.min^{-1}.mL^{-1} culture)		Protein concentration (mg.mL^{-1})	Specific enzyme activity (nMol.min^{-1}.mg^{-1} protein)	
	CP	RS	Cellulase	Xylanase		Cellulase	Xylanase
B. fibrisolvens KU-F14	9.10±0.39	16.55±1.48	6.32	5.19	0.0734	86.05	70.63
B. fibrisolvens KU-F110	6.81±2.33	11.52±0.22	5.84	4.02	0.0577	101.29	69.65
B. fibrisolvens KU-F132	5.32±0.30	9.88±0.56	6.50	3.99	0.0929	70.02	42.94
B. fibrisolvens KU-F134	12.97±0.35	17.44±0.62	10.41	9.23	0.1911	54.48	48.28
B. fibrisolvens KU-F136	12.31±0.54	15.51±1.22	5.09	4.92	0.0836	60.83	58.84
B. fibrisolvens KU-F140	7.16±0.51	14.34±0.99	5.34	4.65	0.0502	106.35	92.49
B. fibrisolvens KU-F143	20.59±0.58	17.49±0.18	6.18	4.85	0.1670	37.00	29.06
B. fibrisolvens KU-F83	20.29±0.61	20.87±0.53	9.72	11.59	0.1577	61.63	73.46
Butyrivibrio sp. KU-F133	9.98±0.54	16.74±2.42	5.90	3.99	0.1623	36.34	24.56
Butyrivibrio sp. KU-F135	7.99±1.97	13.17±0.88	7.24	4.72	0.1559	46.43	30.28
Pseudobutyrivibrio ruminis KU-F23	9.95±1.36	15.43±0.68	6.36	4.44	0.0916	69.38	48.42
Pseudobutyrivibrio sp. KU-F138	13.42±1.35	15.11±1.20	5.22	5.12	0.1753	29.80	29.20
Pseudobutyrivibrio sp. KU-F144	22.23±0.40	19.58±0.59	7.34	7.82	0.0993	73.85	78.63
Pseudobutyrivibrio sp. KU-F20	7.84±1.17	13.73±0.79	7.86	3.86	0.0941	83.51	41.02

CP = Cellulose powder

RS = Rice straw

Table 5 Cellulose digestibility (mean±SD) and volatile fatty acid production of co-culture between isolated *B. fibrisolvens* KU-F83 and non-fibrolytic bacteria.

Mono-culture	Cellulose Digestibility (%)	Volatile fatty acid (mMol.L^{-1} culture)					
		Acetate	Propionate	iso-Butyrate	Butyrate	iso-Valerate	Valerate
B. fibrisolvens KU-F83	16.09±0.53	-0.05	0.17	0.13	1.73	0.14	0.20
B. fibrisolvens KU-F83 + *B. fibrisolvens* KU-NF6	9.94±0.83	-0.21	0.09	0.14	1.32	0.14	0.15
B. fibrisolvens KU-F83 + *P. xylanivorans* KU-NF7	11.33±2.59	-0.24	0.05	0.01	1.51	0.01	0.02
B. fibrisolvens KU-F83 + *Butyrivibrio* sp. KU-NF24	10.24±0.79	-0.18	0.06	0.01	1.52	0.01	0.02
B. fibrisolvens KU-F83 + *S. ruminantium* S137	12.57±0.15	-0.15	0.43	0.01	1.44	0.01	0.01

Table 6 Rice straw digestibility (mean±SD) and volatile fatty acid production of co-culture between isolated *B. fibrisolvens* KU-F83 and non-fibrolytic bacteria.

Culture	Rice straw Digestibility (%)	Volatile fatty acid (mMol.L^{-1} culture)					
		Acetate	Propionate	iso-Butyrate	Butyrate	iso-Valerate	Valerate
B. fibrisolvens KU-F83	16.97±1.06	0.37	0.18	0.02	1.75	0.06	0.07
B. fibrisolvens KU-F83 + *B. fibrisolvens* KU-NF6	21.82±1.30	-0.09	0.08	0.00	1.52	0.04	0.02
B. fibrisolvens KU-F83 + *P. xylanivorans* KU-NF7	21.06±1.74	0.18	0.14	0.00	1.83	0.03	0.02
B. fibrisolvens KU-F83 + *Butyrivibrio* sp. KU-NF24	19.78±1.11	-0.47	-0.03	-0.01	1.10	0.02	0.00
B. fibrisolvens KU-F83 + *S. ruminantium* S137	21.63±1.62	-0.32	0.19	-0.01	1.12	0.02	0.00

Butyrivibrio sp. and *Pseudobutyrivibrio* spp. isolation showed differences in fiber digestibility and fermentation products. Some strains of isolated Butyrivibrios and Pseudobutyrivibrios showed ability to digest filter paper, cellulose and rice straw but some could not utilize filter paper as reported by Sawanon and Kobayashi (2007) where the *B. fibrisolvens* strain S-28 could digest 4.0 ± 1.61 and 12.7% DM of rice straw and alfalfa, respectively. The digestibility of isolated fibrolytic *Butyrivibrios* is related to intracellular fibrolytic enzyme activity; high digestibility strains showed high fibrolytic enzyme activity. Since *B. fibrisolvens* is known to utilize cello- (Russell, 1985), xylo- (Cotta, 1993) and malto-oligosaccharides (Cotta, 1992), a similar explanation based on cross feeding can be adopted.

Utilization of oligosaccharides and lower molecules such as cellobiose is crucial in further fiber digestion (Russell, 1985). Koike and Kobayashi (2009) indicated that *in vitro* fiber digestibility can be improved by co-cultures between fibrolytic and non-fibrolytic species such as a combination of the fibrolytic species *F. succinogenes*, *R. flavefaciens* or *R. albus* with non-fibrolytic *Treponema* or *Butyrivibrio* species. The isolated fibrolytic *B. fibrisolvens* and non-fibrolytic *S. ruminantium* synergize to improve rice straw digestion and increase the propionate portion because the fibrolytic and non-fibrolytic bacteria are associated in hydrogen transfer and/or the removal and exchange of metabolites between species (Sawanon *et. al*., 2011).

CONCLUSION

Isolates of fibrolytic Butyrivibrios and Pseudobutyrivibrios, specifically *B. fibrisolvens* KU-F83, showed high digestibility of fiber powder and high intracellular fibrolytic enzyme activity. A co-culture of fibrolytic *B. fibrisolvens* KU-F83 with non-fibrolytic Butyrivibrios, Pseudobutyrivibrios and *S. ruminantium* S137 could ehance rice straw digestibility but reduced cellulose digestibility and the total VFA production from celulose and rice straw fermentation. *S. ruminantium* S137 could enhance the propionate portion when co-cultured with *B. fibrisolvens* KU-F83. Therefore, other fermentation products of co-culture between fibrolytic and non-fibrolytic *Butyrivibrios* need to be studied in addition to co-culture digestibility with other non-fibrolytic stains.

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