NOTE

Institute of Bioscience¹ and Department of Biochemistry and Microbiology, Faculty of Science and Environmental Studies², Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Mitsuokella jalaludinii sp. nov., from the rumens of cattle in Malaysia

G. Q. Lan,¹ Y. W. Ho¹ and N. Abdullah²

Author for correspondence: Y. W. Ho. Tel: +60 3 8946 8067. Fax: +60 3 8942 3087.
e-mail: ywho@ibs.upm.edu.my

Five strains of phytase-producing, Gram-negative, non-spore-forming, non-motile, small, stout, rod-shaped, strictly anaerobic, fermentative bacteria were isolated from the rumens of cattle in Malaysia. All five strains had morphological, physiological and biochemical features in common. Although these strains had many physiological and biochemical characteristics that were identical to those of the *Mitsuokella multacida* type strain (ATCC 27723^T), they could be distinguished from this species by means of the following characteristics: a smaller cell size (1.2–2.4 µm long and 0.6–0.8 µm wide); a lower final pH value (3.8–4.0) in peptone/yeast extract/glucose broth; inhibition by 0.001% brilliant green; insensitivity to kanamycin (100 µg ml⁻¹) and penicillin (10 µg ml⁻¹); a higher optimum growth temperature (approx. 42 °C); the ability to grow at 45 and 47 °C; the ability to ferment glycerol, sorbitol and amidon; and the inability to ferment mannitol, rhamnose, D-tagatose and melezitose. The G+C content of the type strain (M 9^T) of these five strains was 56.9 mol%. Analysis of the 16S rRNA gene sequence of type strain M 9^T indicated that the strain falls within the genus *Mitsuokella*. The sequence similarity between type strain M 9^T and *Mitsuokella multacida* was 98.7%. The DNA–DNA relatedness between type strain M 9^T and *Mitsuokella multacida* type strain DSM 20544^T (= ATCC 27723^T) was 63.8%, indicating that, in spite of a high level of similarity for the 16S rRNA gene sequence, type strain M 9^T is independent of *Mitsuokella multacida* at the species level. On the basis of these results, a new species, *Mitsuokella jalaludinii* sp. nov., is proposed for these strains. The type strain is M 9^T (= DSM 13811^T = ATCC BAA-307^T).

Keywords: *Mitsuokella jalaludinii*, rumen bacteria, phytase-producing bacteria

Mitsuoka *et al.* (1974) isolated a large number of bacterial strains from the faeces of humans, pigs, dogs and chickens; these strains appeared to be identical to, or closely related to, *Megamonas hypermegas* (basonym: *Bacteroides hypermegas*). These Gram-negative, non-spore-forming, non-motile, strictly anaerobic, stout rods could ferment a wide range of carbohydrates and were named *Bacteroides multiacidus* by Mitsuoka *et al.* (1974). Later, Shah & Collins (1982) assigned *B. multiacidus* to a new genus, *Mitsuokella*, and named it *Mitsuokella multiacidus*, which was recently corrected to *Mitsuokella multacida* by Euzéby (1998). In the process of screening for phytase-producing bacteria from the rumens of cattle in

Malaysia, five isolates were found to be related to *Mitsuokella multacida*. To verify the systematic position of these phytase-producing bacterial isolates, a study of their morphological and physiological characteristics, 16S rRNA gene sequence, G+C contents and DNA–DNA hybridization was undertaken.

For the isolation of phytase-producing rumen bacteria, rumen fluid was sampled from fistulated cattle (*Bos indicus*) fed 60% commercial concentrate and 40% oil palm frond for 15 d. The samples were taken 4 h after the onset of morning feeding. The anaerobic techniques described by Hungate (1950, 1969) for rumen bacteria, with the modifications of Bryant & Burkey (1953) and Bryant (1972), were used for isolation and maintenance of the bacterial cultures. The rumen fluid was serially diluted to 10⁻⁹; 0.1 ml was inoculated into roll-tubes containing modified phytase-screening

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain M 9^T is AF479674.

Table 1. Media used for physiological tests of bacterial strains

Characteristic	Medium*
Methyl red reaction (MR test)	Glucose phosphate medium
Acetylmethylcarbinol production (Voges-Proskauer test)	Glucose phosphate medium
Nitrate reduction	Nitrate broth containing 1 % glucose
Catalase production	PYG medium
Indole production	CMC medium
H ₂ S production	Christensen's medium
Growth in 4.5 % NaCl	Nutrition medium containing 1 % glucose
Gelatin liquefaction	Gelatin nutrition medium containing 1 % glucose
Tween 80 hydrolysis	Tween 80 medium
Growth at 20, 45 and 47 °C	PYG medium
Decarboxylase production	Møller's medium
Urease production	PY-urea broth

* All media were bubbled with oxygen-free CO₂ gas before autoclaving, and the pH was adjusted before use if necessary.

(MPS) agar medium. The MPS medium was modified from the phytase-screening medium of Howson & Davis (1983) by the addition of 1 g peptone (Difco) and 37.5 ml vitamin-free casein l⁻¹ medium. The inoculated tubes were incubated at 39 °C for 6 d. After the incubation period, bacterial colonies with surrounding clear zones were transferred to modified M 10 (MM 10) medium and incubated at 39 °C for 24 h. The MM 10 medium was modified from Medium 10 (Caldwell & Bryant, 1966) by replacing K₂HPO₄ and KH₂PO₄ with sodium phytate. Pure cultures were obtained by repeated reinoculation and reisolation in roll-tubes containing MPS agar medium. Pure cultures were stored at -70 °C in MM 10 medium and subcultures were made every 4 months. The type strain of *Mitsuokella multacida*, ATCC 27723^T (= NCTC 10934^T = DSM 20544^T) obtained from the American Type Culture Collection, was used as the reference strain in the morphological and physiological studies.

For the morphological study, pure bacterial strains were streaked on peptone/yeast extract/glucose (PYG) agar medium (Holdeman *et al.*, 1977) under anaerobic conditions and incubated at 39 °C in an atmosphere of 100 % CO₂ for 2 d. The cellular appearance was determined from Gram-stained smears examined with a light microscope (Leitz Aristoplan). Colonies were examined for size, colour and elevation. Morphological characteristics and size were further studied with a scanning electron microscope (JSM 6400; JEOL). The bacterial strains were grown on PYG agar medium at 39 °C for 48 h, after which the bacterial cells were harvested and prepared for scanning electron microscopy using the methods described by Sieo *et al.* (1999).

The medium used for preparation of inocula and for most of the physiological studies was PYG medium. Acid production from carbohydrates was determined

by using the API kit system (bioMérieux). The formation of gas in the fermentation of glucose (1 %) was detected in PYG broth by the appearance of bubbles in a Durham tube. The final pH values were measured electrometrically in PYG broth by incubating the bacterial strains for 48 h. The volatile and non-volatile fatty acids produced from the fermentation of PYG broth containing 1 % glucose were determined by GC (GC-14A; Shimadzu) using the method described by Kageyama *et al.* (1973). Other physiological characteristics studied included acetylmethylcarbinol production (Voges-Proskauer test), nitrate reduction, catalase production, indole production, H₂S production, urease production, Tween 80 hydrolysis, gelatin liquefaction, arginine acid decarboxylase production, growth in 4.5 % NaCl, and growth at 20, 45 and 47 °C. The media used for the determination of these physiological characteristics are shown in Table 1. Growth stimulation by bile or glucose, determined by adding 2 % bile or 1 % glucose to peptone/yeast (PY) medium (Holdeman *et al.*, 1977), was also studied. The growth was monitored by measuring the optical density (OD₆₀₀) using a spectrophotometer (DU-65; Beckman). The stimulation effects were determined by comparing the optical density (OD₆₀₀) values of the experimental and control tubes. Tolerance to inhibitory substances was determined in PYG broth containing one of the following substances: 0.001 % brilliant green, 0.005 % crystal violet, neomycin (25 µg ml⁻¹), kanamycin (100 µg ml⁻¹), penicillin (10 µg ml⁻¹), erythromycin (50 µg ml⁻¹) and bacitracin (3 U ml⁻¹). All the tests in the physiological studies were repeated three times, each with duplicates.

The nucleotide sequence of the 16S rRNA gene of one strain i.e. type strain M 9^T was determined. The methods used for genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and

purification of the PCR products were similar to those described previously by Rainey *et al.* (1996). Purified PCR products were sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) as directed by the manufacturer's protocol. Sequence reaction products were electrophoresed using the Applied Biosystems 373A DNA Sequencer. The resulting sequence data were manually aligned using the Alignment Editor ae2 (Maidak *et al.*, 1996) and compared with representative 16S rRNA gene sequences of organisms belonging to the Gram-positive bacteria (Maidak *et al.*, 1996). For comparison, 16S rRNA gene sequences were obtained from the EMBL database or the database of the Ribosomal Database Project (Maidak *et al.*, 1996). The 16S rRNA gene-similarity values were calculated by pairwise comparison of the sequences within the alignment. For construction of the phylogenetic dendrogram, the Phylogeny Inference Package (PHYLIP) (Felsenstein, 1993) was used. Pairwise evolutionary distances were computed from percentage similarities by the correction of Jukes & Cantor (1969), and, on the basis of the evolutionary distance values, the phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). The root of the tree was determined by including the 16S rRNA gene sequence of *Bacillus subtilis* in the analysis.

For studies on the G + C content (mol %) of the DNA of type strain M 9^T, cells were disrupted by passage through a French pressure cell (Aminco) and the DNA was then purified on hydroxyapatite according to the procedures of Cashion *et al.* (1977) and Visuvanathan *et al.* (1989). The DNA was hydrolysed with P₁ nuclease and the nucleotides were dephosphorylated with bovine alkaline phosphatase (Mesbah *et al.*, 1989). The resulting deoxyribonucleosides were analysed by using HPLC apparatus fitted with an LKB 2150 high-pressure pump and an LKB 2151 UV detector (270 nm) connected to a Shimadzu CR-3A integrator. The Nucleosil 100-5C18 analytical column (250 × 4 mm) was equipped with a Nucleosil 100-5C18 pre-column (20 × 4 mm; MELZ VDS). The chromatography running temperature was 26 °C, the solvent used was 0.6 M NH₄H₂PO₄/acetonitril (80:6, v/v; pH 4.4), the flow rate was 0.7 ml min⁻¹, and the pressure was approximately 140 bars (Tamaoka & Komagata, 1984). A non-methylated Lambda-DNA with a G + C content of 49.858 mol % (Sigma) was used for calibration. The G + C content was calculated from the ratio of deoxyguanosine (dG) and deoxyadenosine (dA) according to the method of Mesbah *et al.* (1989).

DNA–DNA hybridization was also determined. DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970), with the modifications described by Huss *et al.* (1983) and Escara & Hutton (1980), using a Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermoprogammer and

Fig. 1. Scanning electron micrographs showing the rod forms of (top) *Mitsuokella multacida* ATCC 27723^T and (bottom) *Mitsuokella jalaludinii* sp. nov. Bar, 10 µm.

plotter. Renaturation rates were computed with the TRANSFER.BAS program of Jahnke (1992).

From a total of 125 bacterial strains isolated from the rumens of cattle, five strains which showed strong abilities to hydrolyse sodium phytate were studied and identified. Observations using light microscopy and scanning electron microscopy showed that all five strains had features in common. They were Gram-negative, non-spore-forming, non-motile, small, short rods (1.2–2.4 µm long and 0.6–0.8 µm wide) with rounded ends and occurred singly, in pairs, in short chains or in irregular groups (Fig. 1). Colonies on PYG agar were circular with regular margins, greyish-white, convex with smooth surfaces and 1–4 mm in diameter after 48 h incubation at 39 °C.

Abundant growth occurred under strict anaerobic conditions. Although some growth occurred under aerobic conditions, it was not sustained after about four or five subcultures. The optimum temperature for growth was 42 °C and growth was still good at 47 °C. Growth was markedly enhanced by the presence of glucose in the medium, but not by bile.

Table 2. Characteristics of *Mitsuokella jalaludinii* sp. nov. that are distinctly different from those of the *Mitsuokella multacida* type strain (ATCC 27723^T)

+, Positive reaction; –, negative reaction. The following characteristics apply to both *M. jalaludinii* sp. nov. and *M. multacida* type strain ATCC 27723^T: (1) an inability to grow at 20 °C; (2) the end-products from glucose fermentation are lactic acid, succinic acid and acetic acid; (3) no copious gas is produced from glucose; (4) tests for gelatin liquefaction, indole production, catalase production, urease production, hydrogen sulfide production, Tween 80 hydrolysis, arginine acid decarboxylase, and growth in 4.5% sodium chloride are negative; (5) the test for nitrate reduction, the methyl red test and the Voges–Proskauer test are positive; (6) L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, inositol, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, D-raffinose, D-turanose, D-arabitol and 5-keto-gluconate are fermented; (7) erythritol, D-arabinose, L-xylose, adonitol, methyl β -xyloside, L-sorbose, dulcitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, inulin, glycogen, xylitol, β -gentiobiose, D-lyxose, D-fucose, L-fucose, L-arabitol, gluconate and 2-keto-gluconate are not fermented; (8) resistance to neomycin, bacitracin and erythromycin; (9) intolerance of 0.005% crystal violet; (10) stimulation of growth by glucose but not by bile.

Characteristic	<i>M. jalaludinii</i> sp. nov.	<i>M. multacida</i> ATCC 27723 ^T
Cell size (μ m)	1.2–2.4 $\times$ 0.6–0.8	2.0–10.0 $\times$ 0.6–0.8
Final pH in glucose broth	3.8–4.0	4.1–4.3
Optimum growth temp. (°C)	42	37
Growth at:		
45 °C	+	–
47 °C	+	–
Growth in the presence of:		
Brilliant green (0.001%)	–	+
Kanamycin (100 μ g ml ^{–1})	+	–
Penicillin (10 μ g ml ^{–1})	+	–
Acid from:		
Glycerol	+	–
Sorbitol	+	–
Amidon	+	–
Rhamnose	–	+
Mannitol	–	+
D-Tagatose	–	+
Melezitose	–	+
Source of strains	Cattle	Humans

The other physiological and biochemical characteristics of the strains are summarized in Table 2. Many of the characteristics were similar to those of the *Mitsuokella multacida* type strain (ATCC 27723^T), particularly the fermentation products of glucose. However, there were a number of characteristics that were distinctly different and could be used to distinguish these strains from the *Mitsuokella multacida* type strain (ATCC 27723^T), as follows: a smaller cell size (1.2–2.4 μ m in length and 0.6–0.8 μ m in width); a lower final pH value (3.8–4.0) in PYG broth; inhibition of growth by 0.001% brilliant green; insensitivity to kanamycin (100 μ g ml^{–1}) and penicillin (10 μ g ml^{–1}); a higher optimum growth temperature (approx. 42 °C); the ability to grow at 45 and 47 °C; the ability to ferment glycerol, sorbitol and amidon; and the inability to ferment mannitol, rhamnose, D-tagatose and melezitose (Table 2).

Approximately 95% of the 16S rRNA gene sequence of type strain M 9^T was determined and was found to

comprise 1543 nucleotide bases. Comparison of the 16S rRNA gene sequence of type strain M 9^T with those of some related taxa demonstrated that the strain was closely related to *Mitsuokella multacida*, having a sequence similarity of 98.7%. The phylogenetic dendrogram based on the evolutionary distance value shows that strain M 9^T belongs to the *Clostridium* subphylum of the Gram-positive bacteria (Fig. 2). Within this subphylum, strain M 9^T is a member of the *Sporomusa* sub-branch with the highest 16S rRNA gene sequence similarity with *Mitsuokella multacida*. Thus, the phylogenetic dendrogram clearly shows that type strain M 9^T falls within the genus *Mitsuokella* and that the sequence similarity between M 9^T and *Mitsuokella multacida* is higher than 97%, suggesting that they are closely related and that DNA–DNA hybridization studies should be carried out to verify the taxonomic relationship between these two strains.

The G + C content of the DNA of strain M 9^T was determined as 56.9 mol%, which was similar to that of

Fig. 2. Phylogenetic dendrogram of type strain M 9^T, based on 16S rRNA gene sequence data. The bar represents the scale of estimated evolutionary distance (i.e. a mean of 10 substitutions at any nucleotide position, per 100 nucleotide positions).

Mitsuokella multacida type strain A 405-1^T (= ATCC 27723^T) (57.3 mol %; Mitsuoka *et al.*, 1974).

The genomic DNA–DNA relatedness between strain M 9^T and *Mitsuokella multacida* type strain DSM 20544^T (= ATCC 27723^T) was determined. The DNA–DNA hybridization value between strain M 9^T and *Mitsuokella multacida* type strain DSM 20544^T (= ATCC 27723^T) was 63.8 %. This low hybridization value indicates that strain M 9^T is independent of *Mitsuokella multacida* at species level.

On the basis of the results of this study, it can be seen that a number of biochemical and physiological characteristics, as well as the smaller size and the DNA–DNA hybridization value, clearly distinguish strain M 9^T from *Mitsuokella multacida*, which is the sole species in the genus. Therefore, it is evident that strain M 9^T is a previously undescribed *Mitsuokella* species, for which we propose the name *Mitsuokella jalaludinii*.

Description of *Mitsuokella jalaludinii* sp. nov.

Mitsuokella jalaludinii (jal.al.u.di'ni.i. N.L. gen. n. *jalaludinii* of Jalaludin, in honour of S. Jalaludin, an animal nutritionist and Vice-Chancellor of Universiti Putra Malaysia, who has contributed significantly to rumen microbiology).

Gram-negative, non-spore-forming, non-motile, small, stout rods (1.2–2.4 µm in length and 0.6–0.8 µm in width). Cells occur singly, in pairs, in short chains or in irregular groups. Colonies on PYG agar after 2 d incubation are greyish-white, roughly circular and convex with smooth surfaces and regular edges. Strictly anaerobic. The final pH in PYG broth is 3.8–4.0. There is no copious gas formation from glucose. The major end-products from glucose fermentation are lactic acid, succinic acid and some acetic acid. Growth is stimulated by fermentable carbohydrates but not by bile. The optimum growth temperature is 42 °C. Growth occurs at 45 and 47 °C. Gelatin liquefaction, indole production, catalase production, urease production, hydrogen sulfide produc-

tion, Tween 80 hydrolysis, arginine acid decarboxylase and growth in 4.5 % sodium chloride are negative. The nitrate-reduction test, the methyl red test and the Voges–Proskauer reaction are positive. Glycerol, L-arabinose, ribose, D-xylose, galactose, glucose, fructose, D-mannose, inositol, sorbitol, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, D-raffinose, amidon, D-turanose, D-arabitol and 5-keto-gluconate are fermented. Erythritol, D-arabinose, L-xylose, adonitol, methyl β-xyloside, L-sorbose, rhamnose, dulcitol, mannitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, inulin, melezitose, glycogen, xylitol, β-gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, gluconate and 2-keto-gluconate are not fermented. Resistant to kanamycin, neomycin, penicillin, bacitracin and erythromycin. Intolerant to 0.005 % crystal violet and 0.001 % brilliant green. Full 16S rRNA gene sequence similarity and DNA–DNA relatedness to *Mitsuokella multacida* are 98.7 and 63.8 %, respectively. The G + C content of the DNA is 56.8 mol %. Isolated from the rumens of cattle. The type strain is M 9^T (= DSM 13811^T = ATCC BAA-307^T).

References

- Bryant, M. P. (1972). Commentary on the Hungate technique for culture of anaerobic bacteria. *Am J Clin Nutr* **25**, 1324–1328.
- Bryant, M. P. & Burkey, L. A. (1953). Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. *J Dairy Sci* **36**, 205–217.
- Caldwell, D. R. & Bryant, M. P. (1966). Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Appl Microbiol* **14**, 794–801.
- Cashion, P., Hodler-Flanklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethylsulphoxide solution: acceleration of renaturation rate. *Biopolymers* **19**, 1315–1327.

- Euzéby, J. P. (1998).** Taxonomic note: necessary correction of specific and subspecific epithets according to Rules 12c and 13b of the International Code of Nomenclature of Bacteria (1990 Revision). *Int J Syst Bacteriol* **48**, 1073–1075.
- Felsenstein, J. (1993).** PHYLIP (Phylogeny Inference Package), version 3.5.1. Seattle: Department of Genetics, University of Washington.
- Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (1977).** *Anaerobe Laboratory Manual*, 4th edn. Blacksburg: Virginia Polytechnolgy Institute and State University.
- Howson, S. J. & Davis, R. P. (1983).** Production of phytate-hydrolysing enzyme by some fungi. *Enzyme Microb Technol* **5**, 377–382.
- Hungate, R. E. (1950).** The anaerobic mesophilic cellulolytic bacteria. *Bacteriol Rev* **14**, 1–49.
- Hungate, R. E. (1969).** A roll tube method for cultivation of strict anaerobes. *Methods Microbiol* **3B**, 118–132.
- Huss, V. A. R., Festl, H. & Schleifer, K. H. (1983).** Studies on the spectrometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jahnke, K.-D. (1992).** Basic computer program for evaluation of spectroscopic DNA renaturation data from GILFORD System 2600 spectrometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.
- Jukes, T. H. & Cantor, C. R. (1969).** Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–123. Edited by H. N. Munro. New York: Academic Press.
- Kageyama, K., Hori, H. & Sato, K. (1973).** Simultaneous determination of volatile fatty acids and lactic acid in silage by gas chromatography. *Jpn J Zootech Sci* **44**, 465–469.
- Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1996).** The ribosomal database project (RDP). *Nucleic Acids Res* **24**, 82–85.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Mitsuoka, T., Terada, A., Watanabe, K. & Uchida, K. (1974).** *Bacteroides multiacidus*, a new species from the faeces of humans and pigs. *Int J Syst Bacteriol* **24**, 35–41.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996).** The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage; proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Saitou, N. & Nei, M. (1987).** The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shah, H. N. & Collins, M. D. (1982).** Reclassification of *Bacteroides multiacidus* (Mitsuoka, Terada, Watanabe and Uchida) in a new genus *Mitsuokella*, as *Mitsuokella multiacidus* comb. nov. *Zentbl Bakteriell Parasitenkd Infektionskr Hyg 1 Abt Orig C* **3**, 491–494.
- Sieo, C. C., Abdullah, N., Jalaludin, S. & Ho, Y. W. (1999).** Cellular structure involved in the attachment of *Ruminococcus albus* strain D3 to microcrystalline cellulose avicel. *Asia Pacific J Mol Biol Biotechnol* **7**, 143–149.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Visuvanathan, S., Moss, M. T., Stanford, J. L., Hermon-Taylor, J. & McFadden, J. J. (1989).** Simple enzymic method for isolation of DNA from diverse bacteria. *J Microbiol Methods* **10**, 59–64.