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Biohydrogen production by bacteria isolated from manures of three different bovines using synthetic starch wastewater as substrate

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Aims: This study aimed at investigating the various hydrogen-producing bacteria isolated from three different bovine manure samples (dairy cow, feedlot cow and free grazing cow manures).
Methodology and results: Nutrient broth (NB) and Reinforced *Clostridium* medium (RCM) broth were used for the isolation of facultative and strict anaerobic bacteria. The isolates were subjected to batch fermentation to determine their capability to produce hydrogen (H_2) using synthetic starch wastewater as substrate in 60 mL serum bottles. Biogases of interest (hydrogen, carbon dioxide, methane) produced were analyzed using a Residual Gas Analyser (RGA), which is a mass spectrometry-based analytical system. A total of 52 isolates were obtained of which 13 of the isolates showed the ability to produce H_2, with the NF6 isolate having the highest production of 6.85 × 10-4 % partial pressure [% (pp)].
Conclusion, significance and impact study: From this study, culturable hydrogen-producing bacteria were successfully isolated from the three bovine manures. The results give an insight on the types of H_2 producing bacteria present in the manure samples. These isolates will be useful for the further studies of H_2 production using waste sources as substrates. The production of H_2 by NF6 isolate will be optimized by varying parameters during the batch fermentation.

Keywords: Biohydrogen, bacteria, bovine manures, fermentation, Residual Gas Analyser (RGA)

INTRODUCTION

Over the years fossil fuel has been the main source of energy, as its discovery has brought about rapid development, industrialization and urbanization on a global scale. The production and utilization of these fossil fuels and its by-products has resulted in various environmental damages; aquatic pollution (mostly in the form of oil spillage) and the emission of greenhouse gases, such as carbon dioxide, nitrogen dioxide, methane and other chemicals. These gases are in turn responsible for the current global warming problems and climate change (Yokoi *et al.,* 2001; Mohan *et al.*, 2008). The estimated increase of energy consumption to over 54% between the year 2001 to 2025 (Carere *et al.*, 2008), the subsequent depletion of non-renewable fossil fuel resources, and the environmental problems caused has resulted in the rapid development of renewable and sustainable energy.

Hydrogen (H_2) has emerged as the cleanest of all renewable alternative energy sources, having the highest gravimetric energy content of 122 kJ/g (Carere *et al.*, 2008; Levin *et al.*, 2004). It is a carbon-neutral energy carrier, and it has the possibility of replacing gasoline, diesel and ethanol due to its high energy conversion rate of about (50-70%) via fuel cells (Zhang *et al.*, 2007). Production of H_2 is mainly from electrolysis, photolysis, and thermolysis of water, steam reformation and thermochemical methods of fossil fuel, thermal cracking of natural gas and coal gasification (Das and Veziroglu, 2001). H_2 can also be as a result of the pyrolysis biomass to produce various gas mixtures.

Biological production of H_2 is claimed to be an ideal, clean energy resource for the future and has attracted increasing interest due to its flexibility, low energy demand and environmental-friendliness (Kim *et al.*, 2011). Biological method of production also facilitates waste treatment and recycling process, as various waste-containing materials can be used (Das and Veziroglu, 2001).

Substrates which have been employed for H_2 production, include agricultural produce containing starch or lignocellulosic content, such as corn, wheat, oilseeds, rice and corn starch (Carere *et al.,* 2008); agro-industrial waste; sweet potato starch residue (Yokoi *et al.,* 2001), waste from cheese industry (Carere *et al.,* 2008), and pineapple waste (Wang *et al.,* 2006; Lens *et al.*, 2005); municipal waste and waste from paper mill; palm oil mill

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effluent (POME) (Ismail *et al.*, 2010); sago starch in wastewater (Rafiani *et al.*, 2011), wastewater from food and brewery industries (Kapdan and Kargi, 2006); animal fats and manure and dairy waste (Chaucheyras-Durand *et al.*, 2010), due to their low cost and abundance. Theses wastes are rich in carbohydrates which can be easily broken down through different pathway by various microorganisms (Lateef *et al.*, 2012). They are renewable, readily available and are most times constantly produced in bulk (Prakasham *et al.*, 2009). Thus, the production of H_2 from these wastes can go a long way in minimizing waste accumulation and help maintain a sustainable ecosystem (Lakshmidevi and Muthukumar, 2010).

The rumen compartment of herbivorous animals provides an anaerobic environment for numerous microfloras. The pH (5.5-5.8) at the rumen is maintained as a result of the rumination process making the environment favorable for fermentation (Russell and Rychlik, 2001; Gonzalez *et al.*, 2012). Tonnes of cow manures are produced daily, and are disposed into landfills or applied on lands as a form of organic fertilizer which enhances the soil fertility (Swain and Ray, 2006). Anaerobic digestion of these manures is considered as a means of energy generation (Abubakar and Ismail, 2012). Till recent there are no concise explanation in the nature of the microbial population found in the rumen of cattle, which would affect the type of fermentation pathway and the end products obtained from the fermentation. Possible explanations are; the digestive capability of the rumen microbes and the type of feed given to the cattle (Wanapat *et al.,* 2000; Khejomsart *et al.,* 2011), fiber content and the ruminal degradability of the bacteria (Reynolds, 2006; Zebeli *et al.,* 2010).

Fermentation pathway mostly leading to the production of acetate/butyrate favors the production of H_2 as compared to that which produces propionate/lactic acid volatile fatty acids (VFA) (Antonopoulou *et al.*, 2008). Metabolic pathways involved in these two types of bacteria are thus different. Fibre-digesting bacteria yield acetate and butyrate as its main metabolic volatile fatty acid (VFA) metabolites while the amylolytic bacteria mostly produce propionate. In the production of H_2, pathways which lead to the production of acetate and butyrate as its main VFA are known to favor the production of H2 more than those which lead to the production of propionate (Matsumoto and Nishimura, 2007).

Bacterial species isolated from cow manures include: *Selenomonas ruminantium*, *Megasphaera elsdenii* (Wolin, 1975); ruminal bacteria *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens*, *Streptococcus bovis*, *Lactobacillus fermentens*, *Fibrobacter succinogenes* (Russell and Rychlik, 2001), *Paenibacillus polymixa* (Kanso *et al.*, 2011) and *Bifidobacterium* (Swain *et al.*, 2006). *Bacillus* species such as; *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus licheniformis* (Swain *et al.,* 2006). Bacteria which have been involved in H_2 production are mostly strict anaerobes belonging to the genera *Clostridia* (Chen *et al.*, 2008). Among the species include: *Clostridium pasteurianum* (Liu and Shen, 2004), *C. thermolacticum* from lactose (Collet *et al.*, 2004; Carere *et al.*, 2008), *C. bifermentants* (Wang *et al.*, 2006), *C. paraputrificum* (Evvyernie *et al.*, 2001) and *C. buytricum* (Yokoi *et al.*, 2001).

Species from the genus *Enterobactericeae* also have the ability to produce H_2 by metabolising glucose through the mixed acid or the 2,3-butanediol fermentation yielding CO_2 and H_2 from formic acid (Kumar and Das, 2001; Kapdan and Kargi, 2006). *Enterobacter aerogenes* (Fabiano and Perego, 2002) and *E. cloacae* IIT-BT 08 (Dutta *et al.*, 2009) are the most widely studied species in the genus. Kumar and Das, (2000) investigated the production of H_2 from glucose substrate yielding 2.2 mol of H_2/mol of glucose by *E. cloacae* IIT-BT 08. Trace amount of H_2 production have been observed from *Escherichia coli* (Redwood *et al.*, 2008). The genera *Bacillus* has also been reported to play a significant role in the fermentation process effectively producing H_2 (Kotay and Das, 2007).

Thermophilic bacteria are also another group of organisms which has been studied for the production of H_2. *Thermoanaerobacterium saccharolyticum*, *T. thermosulfurigenes* and thermophilic bacilli, isolated from hot spring has been used in biohydrogen production from wastewater containing starch (Rafiani *et al.*, 2011). H_2 production has also been reported by *T. thermosaccharolyticum* and *Desulfotomaculum geothermicum* (Shin *et al.*, 2004), *Rhodobacter capsulatus* and *Thermohydrogenium kirishis* (Teplyakov *et al.*, 2002). The ability to produce H_2 has also been reported using some groups of aerobic bacteria, which include *Aeromonas* spp., *Vibrio* spp. and *Pseudomonas* spp. (Kapdan and Kargi, 2006).

This paper describes an investigation on the various hydrogen-producing bacteria isolated from three different bovine manure samples (dairy cow, feedlot cow and free grazing cow manures) using synthetic starch wastewater as substrate. Single colonies were successfully isolated and the results obtained give an insight on the types of bacteria found in the different manures. The isolates would be useful for further studies of H_2 production using different waste sources as substrates.

MATERIALS AND METHODS

Sample collection

Samples of the cow manure were collected in a sterile 50 mL centrifuge tubes, from three different farm houses; dairy cow and free grazing cow farm houses located in Ulu Tiram and feedlot cow farm house located in Tebrau, Johor Bahru, Malaysia. Fresh manures (when possible) were used in order to minimize contamination by soil microorganisms. The samples were stored at –20 °C and kept till further use.

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Isolation of hydrogen-producing bacteria

Isolation of facultative anaerobes

The manure sample (1 g) was serially diluted using distilled water to make up to 10^{-10} dilutions. Inoculation were then made on Nutrient Agar (NA) plates and incubated at 37 °C for 24 h. Colonies which appeared on the plate were sub-cultured onto NA plates to obtain single colonies which were then further stored at –80 °C in 80% glycerol stock solution resulting to approximately 20% (v/v). Bacterial colonies originating from this technique of isolation are named DC (dairy cow manure), FDC (feedlot cow manure) and FGC (free grazing cow manure).

Isolation of strict anaerobes

Hungate technique was employed for the isolation of strict anaerobes (Hungate, 1969). Anaerobic environment were created in the media by purging with nitrogen gas in order to remove the oxygen present. Oxygen removal is detected by color change from green to pink as a result of the indicator dye (Resazurin) initially added to the media. The Reinforced *Clostridium* Media (RCM) and Nutrient Broth (NB) were cooled to 50 °C. Each sample (1 g) were aseptically transferred into 70 mL serum bottles of both RCM broth and NB and incubated at 37 °C for 48 h. Prior to the inoculation into RCM broth, 1.5% (v/v) of glucose solution and 5% (v/v) of vitamin solution were added to the media using a needle and syringe. Vitamin solution used had the following composition in g/100 mL; 1 g of cysteine, 2.0 mg of *ρ*-aminobenzenoic acid and 1.6 mg of biotin.

Inoculated samples were then serially diluted in NB and RCM broth followed by inoculation into their respective agar. The rolling-tube method was employed for the inoculation into the RCM agar. In this method, samples were inoculated into the respective agar, swirled briefly and rolled on ice to facilitate the solidifying of the agar onto the sides of the serum bottles. Rolling-tube method of inoculation is mostly used in situations where streak plate method on solid agar media cannot be employed. Media used for isolation is heated and oxygen is driven off by displacing it with nitrogen. Several dilutions are made on the inoculum suspension and mixed with the media in order to obtain cultures with appropriately-spaced colonies (Hungate and Macy, 1973).

Bacterial colonies originating from this technique of isolation are named and differentiated based on the media used: ND (dairy cow manure), NG (feedlot cow manure) and NF (free grazing cow manure) from isolation using NB; RD (dairy cow manure), RG (feedlot cow manure) and RF (free grazing cow manure) from isolation using RCM.

Characterizations of the isolates and biochemical analysis

The facultative anaerobe isolates were subjected to sugar fermentation test using Triple Sugar Iron Agar (TSIA) medium, to determine the ability of the isolates to ferment sugars and produce gas. TSIA, a differential medium which is mostly used to differentiate enteric bacteria based on their ability to ferment sugars and reduce sulfur. The media contains three different types of sugars (lactose, sucrose and a small amount of glucose); upon fermentation of any of these sugars a color change is observed in the medium (red-yellow) as a result of the phenol red pH indicator. The medium can also be used to determine the ability of an organism to produce gas during the fermentation process, this is observed as cracks in the medium or the entire slant or agar would be raised above the bottom of the test tube. Observation made in the media was recorded accordingly.

Biochemical tests are usually done to determine the genus of an unknown bacterial species. The facultative isolates screened for the production of gas subjected to; citrate utilization test, urease test, indole production test, nitrate reduction test and catalase test, according to standard procedure. Gram staining was performed on both facultative and strict anaerobic isolates. Results from the analysis were used to find the closest match and to assign the bacterial signature with a known bacterial genus according to Bergey's Manual of Determinative Bacteriology (Breed *et al.*, 1957).

H_2 production assay

The ability of an isolate to produce H_2 was determined by subjecting the colonies to batch fermentation using synthetic starch wastewater as the substrate. The wastewater solution was prepared in the following compositions (in mg/L, unless stated): starch 70; peptone 90; yeast extract 12; ammonium chloride 96; $MgSO_4 \cdot 7H_2O$ 24; $MnSO_4 \cdot 5H_2O$ 2.16; $FeSO_4 \cdot 7H_2O$ 10; $CaCl_2 \cdot 2H_2O$ 2.4; $ZnSO_4 \cdot 7H_2O$ 0.106; $CoCl_2 \cdot 6H_2O$ 52.6 µg; $CuSO_4 \cdot 5H_2O$ 4.5 µg; 0.5 M potassium phosphate buffer, pH 7.0±0.2 (Klatt and LaPara, 2002; Chen, 2000). The solution was flushed with nitrogen gas, and then 40 mL was dispensed into 60 mL serum bottle, capped with rubber stopper and sealed with aluminium cover. Bacterial cells were grown overnight in NB and RCM broth and 10% of the culture was used as inoculum to inoculate the fermentation medium (Khamaiseh *et al.*, 2012). A Buchner flask (150 mL) was connected to the serum bottles during the fermentation process to collect the gases produced. The flask was initially vacuumed using a vacuum pump at 0.7 bar for 3 min before connecting to the serum bottle. Both the serum bottle and flask was incubated at 37ºC for 24 h. *Clostridium* sp. was used as positive control for H_2 production.

Gases produced were then analyses using the Residual Gas Analyser Model Cirrus 2 (MKS Instruments, MA, USA), which is equipped with a pump to vacuum the gases produced during Buchner flask into the machine.

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H_2, CO_2 and CH_4 content were recorded as partial pressure (Torr) of the percentage of each of the gas presents.

RESULTS AND DISCUSSION

A total of 31 facultative anaerobe colonies were isolated. From the isolates obtained, dairy and feedlot cow manures gave 12 and 11 colonies respectively, while the free grazing cow manure yielded 8 colonies, as shown in Table 1. A total of 21 strict anaerobe colonies were isolated with 7 colonies each from the manure samples. Eight colonies were isolated from NB and 13 colonies from RCM Broth, as shown in Table 2.

Table 1: Facultative and strict anaerobic bacteria isolates obtained from the three manure samples.

Types of isolates	Types of media	Types of manure samples			Total
		Dairy cow	Feedlot cow	Free grazing cow	
Facultative	NB	12	11	8	31
Strict	NB	3	2	3	8
	RCM	4	5	4	13

Table 2: Sugar fermentation test for the facultative anaerobes.

Isolates	Reactions in the TSIA medium		
	Butt*	Slope*	H_2S production§
DC10(2)1	Y	Y	-
DC10(2)2	Y	YG	-
DC10(2)3	Y	Y	-
DC10(2)4	Y	Y	-
DC10(3)1	Y	Y	-
DC10(3)2	Y	YG	-
DC10(3)3	Y	YG	-
DC10(3)4	R	R	-
DC10(4)1	Y	YG	-
DC10(4)2	Y	Y	-
DC10(4)3	Y	YG	-
DC10(5)1	Y	Y	-
FDC10(2)1	Y	Y	-
FDC10(3)1	Y	Y	-
FDC10(3)2	Y	Y	-
FDC10(4)1	Y	[1]R	-
FDC10(4)2	Y	Y	-
FDC10(4)3	Y	Y	-
FDC10(4)4	Y	Y	-
FDC10(4)5	Y	Y	-
FDC10(5)1	Y	Y	-
FDC10(5)2	Y	Y	-
FDC10(6)1	Y	Y	-
FGC10(2)1	Y	YG	-
FGC10(3)2	Y	YG	-
FGC10(3)1	Y	Y	-
FGC10(3)3	Y	YG	-
FGC10(4)1	Y	YG	-
FGC10(4)2	Y	Y	-
FGC10(4)3	Y	Y	-
FGC10(4)4	Y	YG	-

DC, dairy cow manure sample; FDC, feedlot cow manure sample; FGC, free grazing cow manure sample; *Y, medium changed color to yellow (signifies acid production); *YG, medium changed color to yellow, gas is produced; 1R, no color change in the medium; §+, blackening of medium.

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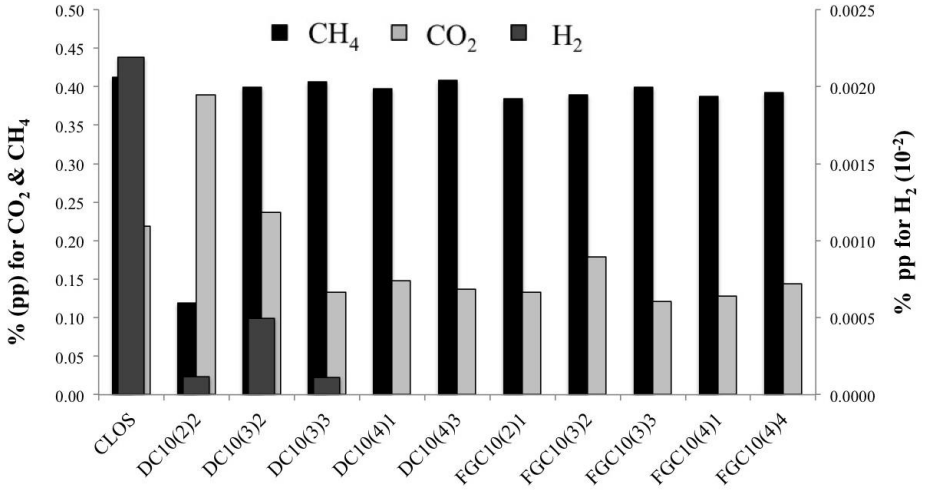

Figure 1: Gas production (H_2, CO_2 and CH_4) by facultative anaerobes. CLOS, positive control; DC, dairy cow manure sample; FGC, free grazing cow manure sample.

The 31 facultative anaerobe colonies were first subjected to the sugar fermentation test to screen for gas production using TSIA as medium. It was found that all (except two) colonies have the ability to ferment the carbohydrates (glucose, sucrose and lactose) present in the TSIA medium. Ten of the colonies, five from dairy cow manure and five colonies from free grazing cow manure have the ability to produce gas (Table 2). None of the colonies from feedlot cow manure showed the ability of gas production. Gas produced in the TSIA media was detected through the presences of bubbles, cracks, pushing up of agar from the bottom or from the side of the agar.

Analysis of H_2 production using Residual Gas Analyser (RGA) by measuring the pressure (Torr) of gases present in the Buchner collecting flasks showed that 3 facultative anaerobe isolates produced H_2 at a detectable level (Figure 1). They are colonies DC10(2)2, DC10(3)2 and DC10(3)3. Results obtained are in percentage of partial pressure [%(pp)] of the individual gases (H_2, CO_2 and CH_4) produced.

All of the facultative anaerobe colonies appear circular in form, having entire margins with sizes ranging from 0.2-0.7 cm in diameter. The colonies had flat, convex, raised and punctiform elevation, opaque characteristics, cream in color, with smooth surfaces. In order to identify the identities of the facultative anaerobe colonies that show H_2 production, biochemical tests were performed for partial identification. Table 3 summarizes the results obtained. The biochemical tests showed that the strains are able to reduce nitrate, produce indole and show positive catalase test, but were unable to utilize citrate as a growth substrate nor produce urease. DC10(2)2 and DC10(3)3 are Gram negative bacilli while DC10(3)2 is a Gram negative coccus. From the biochemical tests results obtained (Table 3), it can be suggested that the facultative anaerobes might belong to the genus *Enterobacteriaceae*, *Aeromonas*, *Veillonella* and *Acinetobacter*.

Analysis of H_2 production by the strict anaerobe colonies shows that 2 strict anaerobes isolated using RCM (RG4 and RF4) (Figure 2) are able to produce H_2 via starch fermentation, and all strict anaerobe colonies isolated using NB are able to produce H_2 (Figure 3).

For partial identification, Table 4 shows the result of biochemical tests (Gram stain and catalase test) performed on strict anaerobes. Catalase tests are negative for all the strict anaerobes, isolates from RCM medium are both Gram positive cocci while those from NB are either Gram positive bacilli (ND5, ND6, ND7, NG5, NF6, NF7), Gram negative bacillus (NG6) or Gram positive coccus (NG7).

Table 3: Biochemical tests for the facultative anaerobe colonies. DC: dairy cow manure.

Isolates	Biochemical tests					
	Gram staining*	Citrate utilisation	Urease	Indole	Catalase	Nitrate reduction
DC10(2)2	(-) bacillus	–	–	+	+	+
DC10(3)2	(-) coccus	–	–	+	+	+
DC10(3)3	(-) bacillus	–	–	+	+	+

*Gram staining, (Gram positive/negative) (cell shape).

+, Positive test results; –, Negative test results.

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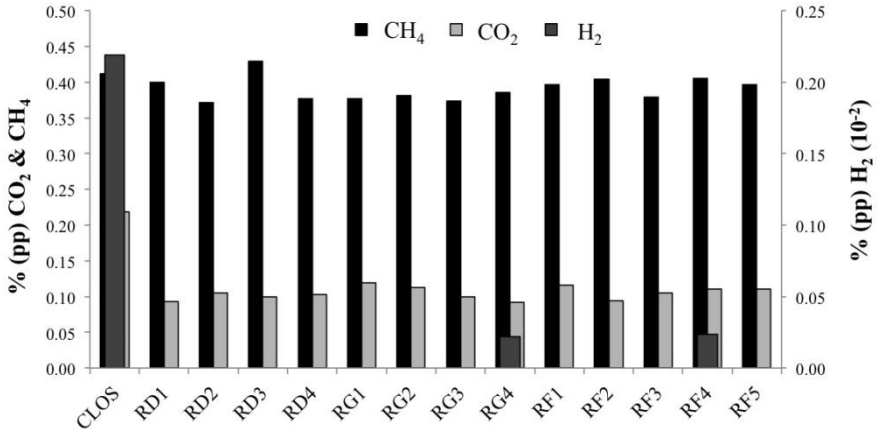

Figure 2: Gas production (H_2, CO_2 and CH_4) by strict anaerobe colonies isolated using RCM medium. CLOS, positive control; RD, dairy cow manure sample; RG, feedlot cow manure sample; RF, free grazing cow manure sample.

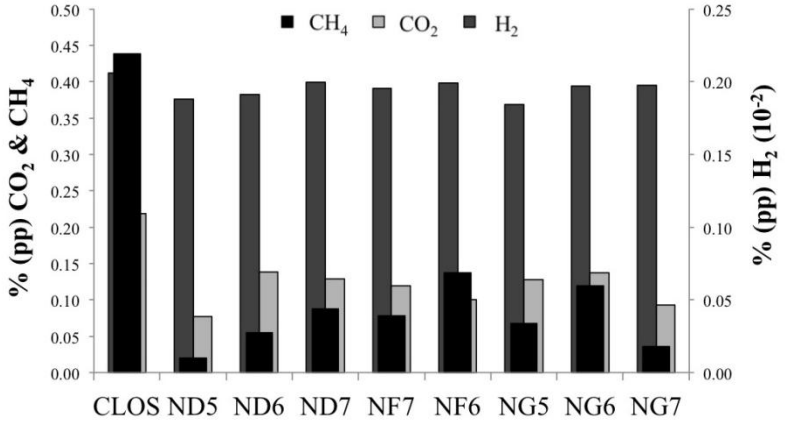

Figure 3: Gas production (H_2, CO_2 and CH_4) by strict anaerobe colonies isolated using NB medium. CLOS, positive control; ND, dairy cow manure sample; NG, feedlot cow manure sample; NF, free grazing cow manure sample.

Characterization and identification of anaerobes to genus level using biochemical tests are usually challenging to be carried out and are limited to certain test. The results obtained are also not as reliable as compared to the results obtained for facultative anaerobes. This could be due to the biochemical tests that are usually done in the presence of oxygen. Classification of strict anaerobes might require additional biochemical tests such as determination of metabolic end product [usually analyzed by gas liquid chromatography (GLC)], spore formation for some anaerobes, and in some cases determination of antibiotic resistance or susceptibility.

Figure 4 shows the H_2 production ability by colonies from both facultative anaerobes and strict anaerobes, for comparison purposes. It can be seen that NF6, isolated from the feedlot cow manure, showed the highest H_2 production of 0.0685 x 10-2 % (pp) among all the isolates subjected to fermentation.

H_2 production analysis showed that H_2 is detected in most of the bacteria isolated from dairy cow and free grazing cow manure samples. This could be attributed to the type of microbes found in the rumen of the different types of cattle and the fermentation pathway employed by them. Among the factors known to contribute to this is the types of cattle feed, which determine the type of bacteria.

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Table 4: Biochemical tests for the strict anaerobes.

Isolates	Biochemical tests	
	Gram staining*	Catalase
RG4	(+) coccus	–
RF4	(+) coccus	–
ND5	(+) bacillus	–
ND6	(+) bacillus	–
ND7	(+) bacillus	–
NG5	(+) bacillus	–
NG6	(-) bacillus	–
NG7	(+) coccus	–
NF6	(+) bacillus	–
NF7	(+) bacillus	–

RG, feedlot cow manure sample (RCM isolation); RF, free grazing cow manure sample (RCM isolation); ND, dairy cow manure sample (NB isolation); NG, feedlot cow manure sample (NB isolation); NF, free grazing cow manure sample (NB isolation).
*Gram staining, (Gram positive/negative) (cell shape); +, Positive test results; -, Negative test results.
Note: Other tests are not possible to be performed on the strict anaerobes due to the nature of the tests that usually requires aerobic environment.

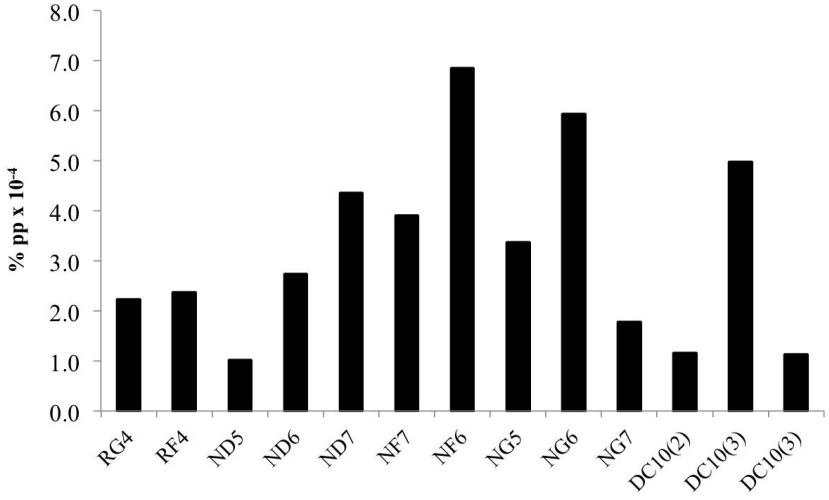

Figure 4: Comparison of H_2 production by the different isolates. RG, feedlot cow manure sample (strict anaerobe, RCM isolation); RF, free grazing cow manure sample (strict anaerobe, RCM isolation); ND, dairy cow manure sample (strict anaerobe, NB isolation); NF, free grazing cow manure sample (strict anaerobe, NB isolation); NG, feedlot cow manure sample (strict anaerobe, NB isolation); DC, dairy cow manure sample (facultative anaerobe).

that colonize the rumen, as reported by others (Khejornsart *et al.*, 2011). Rumen microorganisms are mainly consisting of cellulolytic bacteria, other than anaerobic fungi and protozoa. We observed that during sampling, the different farms (dairy, feedlot and free-grazing) fed different feed to the cows. Diet for dairy cows mainly consists of watermelon and its waste, while for feedlot cows it is mainly pineapple waste. Free-grazing cows feed on grass. Although the actual fiber content of these feeds is not known, it is possible that they affect the microbial population in the rumen, particularly the cellulolytic bacterial population.

The different types of feed substrate can then in turn affect the pH of the rumen (Calsamiglia *et al.*, 2008). Previous studies have shown that maximum H_2 yield is obtained at an acidic or slightly acidic pH (Kumar and Das, 2000; Khanna *et al.*, 2011). Average rumen fermentation phase has been shown to be at pH 6.0 to 6.2 (Krause and Oetzel, 2006). Khanna and co-workers (2011) observed that the optimum operational pH for *Enterobacter cloacae* strain IIT-BT08 to yield the highest H_2 production from glucose *in vitro* is at pH 6.5, while *Citrobacter freundii* strain CWB1952 has been shown to produce H_2 at pH 5.9 (Hamilton *et al.*, 2010), using batch

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method with glucose as substrate. These demonstrate that for optimum H_2 production by bacteria, a slightly acidic pH is required. As different types of substrate may contribute to different pH conditions in the rumen, this explains the differing populations of H_2-producing bacteria in the three cow types investigated.

CONCLUSION

Several bacterial colonies have successfully been isolated from the 3 different cow manures, with dairy cow manure samples giving the most hydrogen-producing bacteria. From the results of the biochemical test and morphological characteristics obtained, partial identification suggests that the facultative anaerobe isolates might belong to the genus *Enterobacteriaceae, Aeromonas*, *Acinetobacter* and *Veillonella* while the strict anaerobes require further analysis for identification. It was found that the manures of dairy and free grazing cows produce more hydrogen-producing bacteria, compared to the feedlot cow, probably due to the different feed composition that affects the microbial ecosystem in the rumen of the animals.

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