IDENTIFICATION, MOLECULAR CHARACTERIZATION AND EVALUATION OF SOME RUMINAL BACTERIA THAT CATALYZE AND DEGRADE PLANT CELLULOSIC MATERIALS

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ABSTRACT

In the last few years there was a growing economic awareness about plant wastes and use it in compost production or in feeding of farm animals, some of these wastes are useless and cause a lot of problems in Egyptian environment. This study was designed to isolate, identify and evaluate some ruminal bacterial isolates to facilitate the *in vitro* degradation of certain local agro-industrial wastes (rice straw and corn stalks). Two bacterial isolates were isolated from rumen fluid of some Egyptian cattle and identified as *Fibrobacter succinogenes* and *Ruminococcus albus* depending on morphological, biochemical identification and molecular characterization using 16S rRNA genes. Two degenerate primers were used to detect about 1.6 kb PCR fragment, a partial sequences (709 and 401) bp were sequenced for *F. succinogenes* and *R. albus* respectively. The highest similarity was detected for both isolates which were 57% with *Streptomyces* sp. for *F. succinogenes* and 77% with *Enterobacter* sp. for *R. albus*. The two isolates were subjected *in-vitro* to catalyze and degrade rice straw and corn stalks. After four weeks of fermentation, degradation was measured depending on the bacterial growth and changes in chemical components of the degraded materials. The degradation of corn stalks was highly significant comparing to rice straw which inoculated by *F. succinogenes* or *R. albus*. Analysis of the chemical composition showed a decrease in Organic Matter (OM), Crude Fiber (CF), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), cellulose and hemicellulose content. The inoculum 7.5 mL/50 g of *Ruminococcus albus* is more efficient to degrade rice straw or corn stalks in four weeks fermentation period. Corn stalks are better than rice straw to enhance the cellulolytic bacteria to grow in all treatments.

Keywords: Rumen Bacteria, Cellulolytic Bacteria, *Fibrobacter Succinogenes*, *Ruminococcus Albus*, 16S rRNA

1. INTRODUCTION

The first chamber of ruminant's stomach (rumen) contains a large population of microbes. The rumen microbial ecosystem comprises at least 30 predominant bacterial species at a total concentration of 10^{10} to 10^{11}/mL of rumen fluid (Weimer, 2011; McSweeney and Mackie, 2012). Most of these microbes degrade cellulolytic materials. It is well known that cellulose is one of the most abundant biopolymers on earth and is an important structural component of the plant cell wall. Bacterial species of

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the rumen are considered important in determining the extent and rate of feed degradation and utilization for microbial protein production (Crossland *et al*., 2012). Since the establishment of effective methods to isolate and cultivate ruminal microbiota, a number of cellulolytic rumen bacteria, such as the genera *Fibrobacter*, *Ruminococcus*, *Clostridium*, *Lactobacillus* and *Prevotella*, have been isolated (Nikki *et al*., 2011). In addition to *F. succinogenes* and *R. flavefaciens*, *R. albus* is one of the main cellulolytic bacteria in the rumen (McSweeney and Mackie, 2012). *In vitro* studies have shown that *R. albus* became predominant over the other two fibrolytic species in co-cultures containing cellulose as well as in the rumen of cows (Wilson, 2011), but is outnumbered by the other rumen cellulolytic species, *R. flavefaciens* and *F. succinogenes*, in the rumen of sheep (McSweeney and Mackie, 2012). Ruminants have developed a unique microbial symbiosis in the rumen to utilize this plant tissue but non fermentable fiber can often be recovered from the faeces (Quiroz-Castaeda and Folch-Mallol, 2011; McSweeney and Mackie, 2012). Therefore, it can be hypothesized that the microbial population in the rumen is limiting and under normal feeding conditions, the fermentation of structural carbohydrates is a slow process (Messana *et al*., 2012). Therefore, efforts were done to enhance the microbial cellulosic digesting ecosystem. Thoetkiattikul *et al*. (2013) noticed that, there was an elevation of the fibrolytic bacterial numbers in the rumen by feeding a high-cellulose diet composed of purified wood cellulose. There was a 10-fold increase in the number of cellulolytic bacteria, but no significant increase in the digestion of alfalfa (lucerne) cellulose when it was placed in a nylon bag and suspended in the rumen for 24 h. Koike *et al*. (2000) reported a seasonal variation in cellulolytic bacterial species in the caecum of Hokkaido native horses: *F. succinogenes* was predominant in winter and *R. albus* and *R. flavefaciens* were predominant in summer. *F. succinogenes* was the most extensive lignocellulosic degradation *in vitro*; ferment cellobiose and glucose. If fiber digestibility is to be improved by microbial manipulation, a very significant, issue is whether introduced organisms can multiply and persist at levels in the rumen that are sufficient to improve fiber digestion (Fukuma *et al*., 2011).

Marked developments have occurred in bacterial identification and classification since the application of molecular technologies to this task. The impetus for major change has resulted from the observation that 16S ribosomal DNA (rDNA) sequences can be used as evolutionary chronometers (Shakira *et al*., 2012). Some regions of the 16S rDNA molecule are conserved throughout all bacterial species and can be used to align sequences obtained from different isolates (Yang *et al*., 2010a). Analysis of rDNA give a basis for describing microbial communities without the limitations imposed by culture based methods and biochemical identification (Petri *et al*., 2012). Similarities in nucleotide sequences serve to relate microorganisms and can be used to identify uncultured microbes in environmental studies. Recent studies using comparative sequencing of bacterial 16S rDNA clearly indicated that there is a high degree of genetic divergence among rumen isolates previously thought to represent strains of a single species (Yang *et al*., 2010b; Chaudhary *et al*., 2012; Petri *et al*., 2012).

This study is interested in isolation and identification of some cattle ruminal bacteria depending on its 16S rDNA genes and can *in vitro* degrade rice straw and corn stalks.

2. MATERIALS AND METHODS

2.1. Sampling

Representative samples (at least 500 g) of rumen content were collected from cattle, the sample were brought immediately to the laboratory, containing proportions of solid and liquid materials which approximated those in the original was continually gassed with CO_2. The samples were then strained through four-layer sterilized cheesecloth. The obtained rumen fluid was then used for isolating of fiber-digesting bacteria. Most probable number calculations were then used to estimate the numbers of total bacterial count and cellulolytic bacterial count in rumen fluid on nutrient broth and cellulose broth medium, respectively (Nikki *et al*., 2011).

2.2. Isolation and Identification of Fiber-Digesting Rumen Bacteria

Sterilized test tubes containing cellulose broth medium supplemented with strips of filter paper were firstly inoculated with a loop of previously prepared

rumen fluid for culturing of cellulolytic bacteria. The tubes were incubated in an anaerobic incubator (Hirayama Manufacturing Corp., Tokyo, Japan) at 39°C for 21 days and scored positive if there was any visual breakdown of the cellulose strips. In order to isolate the cellulolytic bacteria, tubes that were scored positive vortexed for 30 sec and 1 mL of bacterial culture was serially diluted ten-fold up to 10^{-7}. Three dilutions (10^{-5}, 10^{-6} and 10^{-7}) were used to inoculate petri dishes containing cellulose agar medium. After incubation for 96 h at 39°C, single colonies showing zones of clearance on cellulose agar were picked and inoculated anaerobically at 39°C into a broth of cellulose medium with (0.2%) glucose. After another 48 h of incubation, the cultures were examined microscopically to check the purity, morphology and gram staining characteristics. The colonies were picked up, purified then maintained on nutrient agar at 4°C (Nikki *et al.*, 2011). The biochemical identification of the isolates were carried out and reconfirmed in the Micro Analytical Centre, Cairo University, Giza, Egypt.

2.3. DNA Extraction and PCR

DNA was extracted from the isolated bacteria by using AxyPrep Bacterial Genomic DNA Miniprep Kit (*Axygen Biosciences* USA). The isolated DNA was applied for PCR test. Two PCR Primers [Forward (AGA GTT TSA TCC TGG CTC AG)] and [Reverse (ACG GMT ACC TTG TTA CGA CTT)] were used in this study to amplify a partial segment of bacterial ribosomal DNA. PCR amplification was performed by using a 50 µL (total volume) mixture containing 1.25 U of *Taq* DNA polymerase (*Amplitaq Gold*, *Perkin-Elmer*), 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.5 mM MgCl$_2$, each deoxynucleoside triphosphate at a concentration of 200 µM, 25 pmol of each primer and 10-50 ng of the extracted DNA. The thermal cycle involved 5-min pre-heating at 94°C before 30 cycles consisting of 1 min at 94°C, 1 min at 50 and 2 min at 72°C. Finally, a 10-min extension at 72°C was performed. Amplification of PCR products of the proper size was confirmed by electrophoresis through 1.5% (w/v) agarose gel (Sigma) in TBE buffer, stained with ethidium bromide (Shakira *et al.*, 2012). The PCR product of each sample was subjected to electrophoresis on 1.2g % agarose gel. A 1.6 kb PCR

fragment was observed and eluted from the gel using QIAquick Gel Extraction Kit (250) (cat. no. 28706 QIAgen). The forward primer was used in sequencing of the eluted PCR products; the sequencing process was done in Sigma (Germany) by the automated sequencer.

2.4. Nucleotide Sequence Analysis

Database searches with determined sequences were conducted by using the BLASTN programs in the GenBank on web site http://blast.ncbi.nlm.nih.gov/Blast.cgi. The sequences were aligned online by using BLASTN, version 2.2.21+ (on the web site http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) and the alignments were refined by visual inspection. The nucleotide sequences reported in this study have been registered in the EMBL database nucleotide under accession nos. (GQ505949) for *F. succinogenes* and (GQ505950) for *R. albus*.

2.5. Preparation of Pre-Bacterial Cultures

Three litters of Luria Broth (LB) medium were divided equally into 3 conical flasks (2L), sterilized, then inoculated separately with one of each of bacterial strains and rumen fluid, incubated anaerobically at 39°C for 48h to obtain high density growth (1 mL contained $2\text{-}3\times10^{7}$ viable cells) (Nikki *et al.*, 2011).

2.6. Cellulosic Materials Bio-Treatment

Rice straw and corn stalks were collected from farms of rice and corn in El-Beheira governorate (Delta of the Nile north of Cairo, Egypt lat. 30.921076375384878, long. 30.2728271484375). The chopped Rice straw (3-5cm) and the crushed corn stalks were weighted (50g), packed and autoclaved at 121°C for 30 min, then stored at room temperature until use. The Molasses Urea Medium (MUM) containing (5%) crude sugar cane molasses (about 50% w/w total sugars) (Sugar Refinery Factory at El-Hawamdia, Giza, Egypt) and (3%) urea (46% N w/w) (Abo Qir Fertilizers Company, Alexandria, Egypt) was prepared for all fermentation treatments. 50 ml of MUM was mixed with (zero, 2.5, 5, 7.5 and 10 mL) of *F. succinogenes* or *R. albus* or rumen fluid (positive

control) cultures and the final volume was adjusted to 80 mL by sterilized water, then added to 50 g of the rice straw or corn stalks in sterilized polyethylene bags. The negative control was bags supplied with 80 mL of sterilized water. All bags were tightly closed and incubated at 39°C for 6 weeks. Representative samples were taken after zero, 2, 4 and 6 weeks of inoculation. Bacteria were counted on standard cellulose agar plates (Anand *et al.*, 2010). The contents of all bags were oven dried at 65°C for 24 h.

2.7. Analytical Methods

Dried samples were ground through a Wiley Mill fitted with a 1.5 mm screen. The chemical composition of the cellulosic materials were carried out using the standard laboratory analytical procedures for biomass analysis provided by the National Renewable Energy Laboratory (NREL, USA) and methods developed by the Association of Official Analytical Chemistry for biomass analysis. Dry matter analyzing methods (DM, method 930.15), crude protein (CP, method 954.01), crude fiber (CF, method 962.09), ether extract (EE, method 920.39) and ash (method 942.05) (AOAC, 1995). Neutral Detergent Fiber (NDF) was assayed with a heat stable amylase and expressed inclusive of residual ash, Acid Detergent Fiber (ADF) expressed inclusive of residual ash and Acid Detergent Lignin (ADL) were determined according to Van Soest *et al.* (1991). Cellulose and hemicellulose values were calculated according to the results of analysis as: Cellulose = ADF-ADL and Hemicellulose = NDF-ADF. The volatile or organic matter portion of the dried samples was determined by burning at 550°C in a muffle furnace (Blue M Electric Company, Blue Island, USA) for 4 h. Organic Matter (OM) was calculated as: 100-ash %, while carbon content (%) was determined by dividing the volatile fraction by 1.83. Carbon content (%) = (100-ash %)/1.83 (Barrington *et al.*, 2002). The obtained data were statistically analyzed using the general linear model procedure described in SAS statistical software User's Guide as the following model:

$$Y_{ijk} = \mu + M_i + L_j + P_k + E_{ijk}$$

Where:
Y_{ijk} = Observed trait

μ = The overall mean
M_i = The effect of ith inculants
L_j = The effect of jth level of inoculants
P_{ij} = the effect of kth period of incubation and E_{ijk} = Random error.

Mean differences between experimental groups were tested by Duncan's multiple range test (Duncan, 1955).

3. RESULTS

3.1. Fiber-Degrading Bacteria

The estimated total number of viable bacteria in ruminal fluid was 5.0×10^9 cfu ML^{-1} while the number of cellulolytic bacteria was 3.2×10^7 cfu mL^{-1}. Two bacterial isolates showed the highest cellulose clearance zones on cellulose agar medium. These isolates were typically Gram-negative short rods or Gram-positive cocci. The first isolate was identified as *Fibrobacter succinogenes*. It was Gram-negative none motile rods but can become coccoid or oval on culture. The other one was Gram-positive cocci and identified as *Ruminococcus albus*, produced a yellow pigment when grown on cellulose.

3.2. Bacterial Identification and Characterization at Molecular Level

Total DNA was extracted from the two bacterial isolates (*F. succinogenes* and *R. albus*) and subjected to PCR conditions with degenerated primers of 16S rDNA. About 1.6 Kb PCR products was performed and sequenced by Sigma Company (Germany) using the forward primer and the sequenced parts were 709 and 401 bp for *F. succinogenes* and *R. albus* respectively. Each sequence was aligned with different bacterial DNA sequences stored in NCBI GenBank database. The highest Query coverage of *F. succinogenes* sequence was 57% with *Streptomyces* sp., while it was 77% with *Enterobacter* sp. in the case of *R. albus*.

3.3. Cellulolytic Bacterial Growth on Rice Straw and Corn Stalks

The isolated bacterial strains (*F. succinogenes* and *R. albus*) and the cattle rumen fluid were evaluated in the polyethylene plastic bags for their degradation

efficiencies on either rice straw or corn stalks. The degradation effect was assessed by monitoring the bacterial growth and changes in chemical composition of rice straw and corn stalks. Data in **Table 1 and Fig. 1** showed significant increases of bacterial counts on fermented materials due to almost all inoculation treatments. However, the counts were dropped or remained constant in the last two weeks of fermentation. The inoculation with rumen fluid (positive control) gave less bacterial counts on either rice straw or corn stalks comparing with single inoculations of each of *F. succinogenes* or *R. albus* (**Table 1 and Fig. 1**). The inoculations with (7.5 mL/50g) of either rice straw or corn stalks (in all fermentation periods) achieved significantly higher or sometimes close counts of cellulolytic bacteria comparing with those in case of inoculation with (10 mL/50g). After 4 weeks of fermentation, the 7.5 mL/50g inoculation exhibited the highest growth of *R. albus* or *F. succinogenes* on rice straw with rates of 6.5×10^6 cfu/g (1.1 fold) and 6.1×10^6 cfu/g (1.05 fold)

respectively and gave a growth on corn stalks with rates of 7.9×10^6 cfu/g (1.5 fold) and 6.7×10^6 cfu/g (1.3 fold) respectively, comparing with the positive control.

3.4. Degradation Activity of Cellulolytic Bacteria on Rice Straw and Corn Stalks

Cellulolytic bacteria (*F. succinogenes* and *R. albus*) showed higher (p<0.05) degradation activity expressed in cell wall constitutes (NDF, ADF, cellulose and hemicelluloses) compared with rumen fluid at different incubation periods (**Table 2**) and (**Fig. 2**) and different inoculation levels (**Fig. 3**) for both roughage materials (rice straw and corn stalks). The data in **Table 2** showed that, no difference between *F. succinogenes*, *R. albus* or rumen fluid which decreased the OM, CF, NFE, NDF, ADF, cellulose and hemicellulose content compared to the untreated materials. Gradual increase in degradation activity were recorded for *R. albus*, *F. succinogenes* and rumen fluid parallel to the increase in incubation time up to 4 weeks then tend to constant (**Fig. 2**).
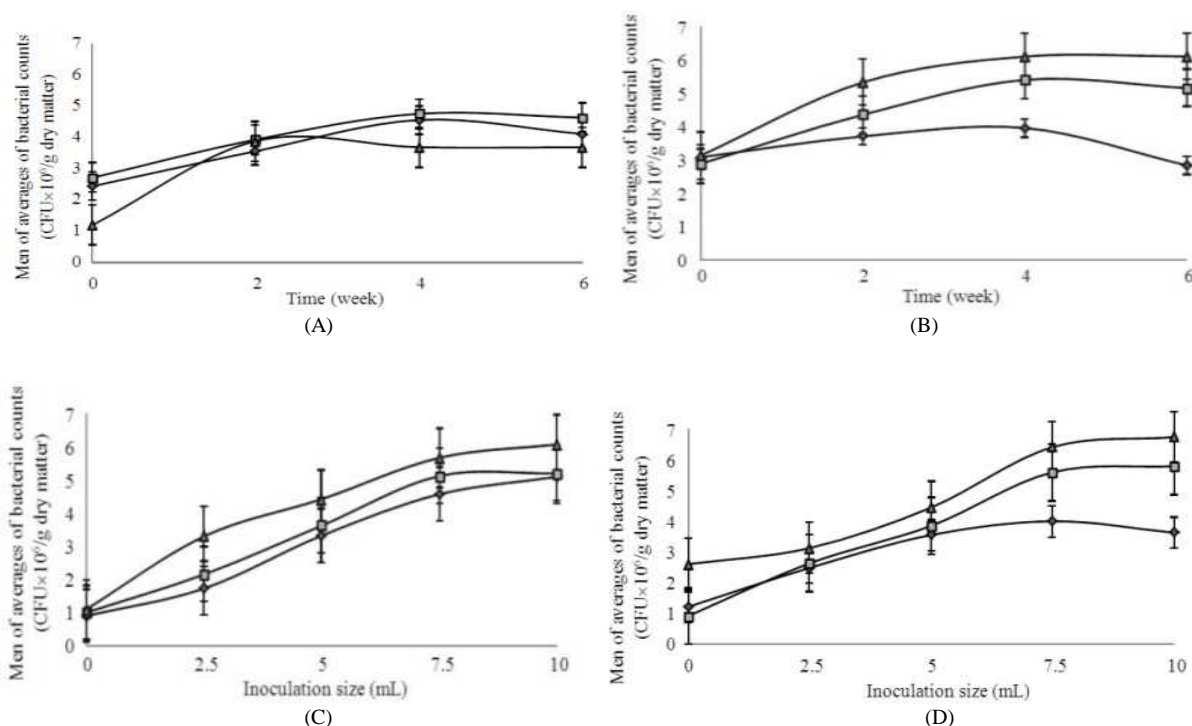

Fig. 1. Growth of total rumen bacteria ◇, *F. succinogenes* ▢ and *R. albus* △ on rice straw (A) and corn stalks (B) at different incubation time, while (C) and (D) on rice straw and corn stalks at different inoculation sizes respectively
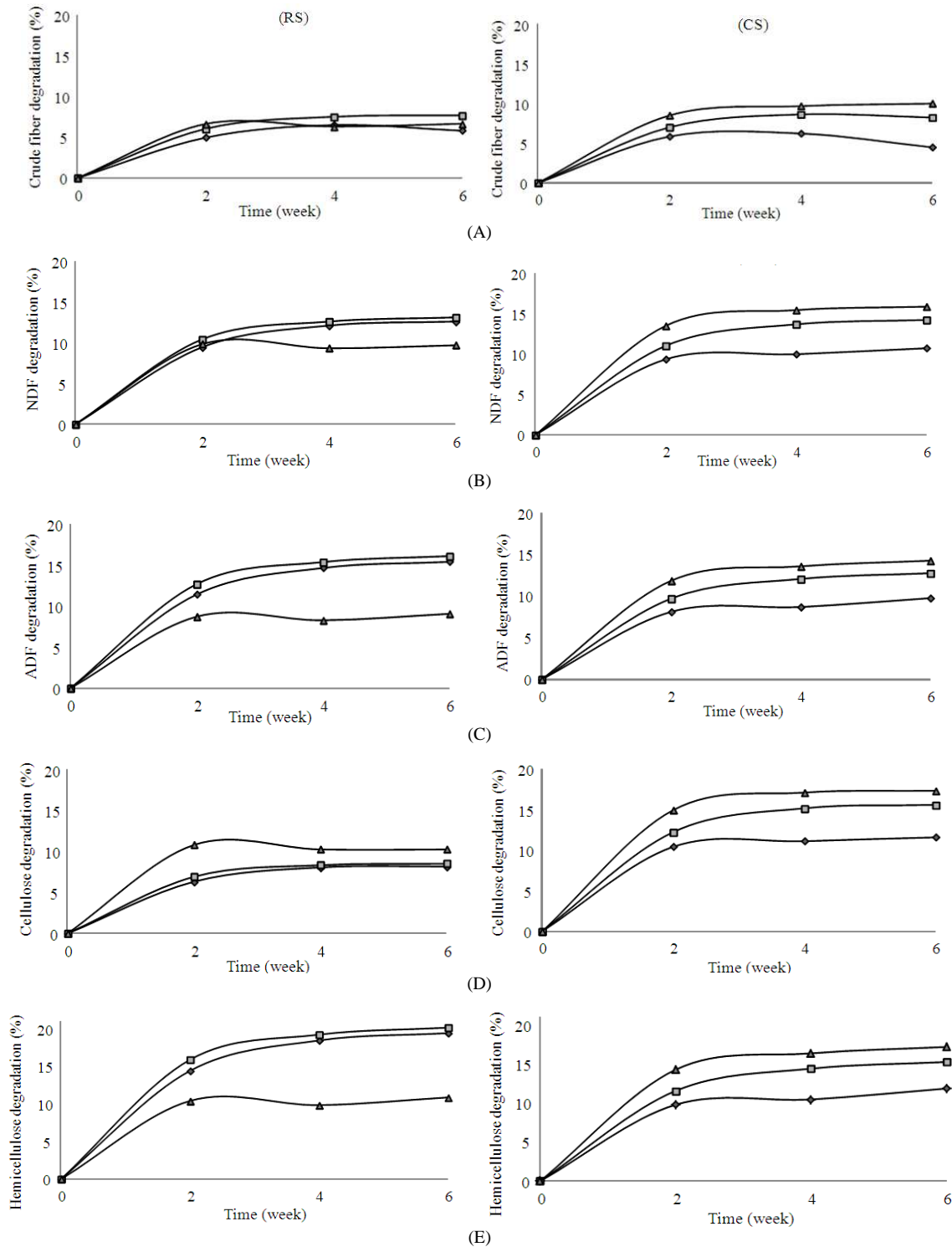
Fig. 2. Comparison of *in vitro* degradation of Rice Straw (RS) or Corn Stalks (CS) at different incubation time by rumen fluid ◇ *F. succinogenes* ☐ or *R. albus* △ (A) = crude fiber (B) = NDF (C) = ADF (D) = cellulose and (E) = Hemicellulose degradation
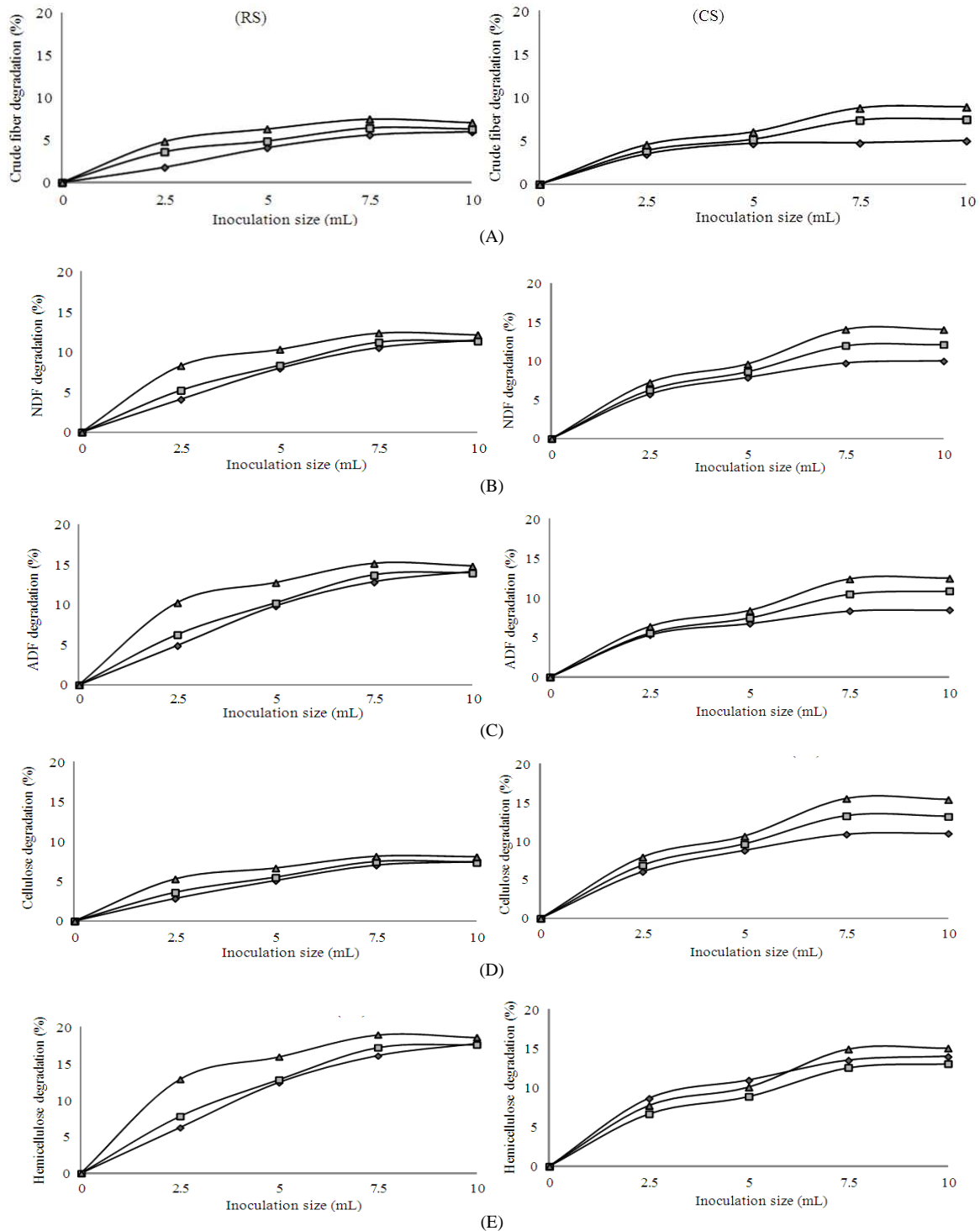
Fig. 3. Comparison of *in vitro* degradation of Rice Straw (RS) or Corn Stalks (CS) at different inoculation volumes by rumen fluid ◆ *F. succinogenes* ▢ or *R. albus* ▲ (A) = crude fiber, (B) = NDF, (C) = ADF, (D) cellulose and (E) = Hemicellulose degradation

Table 1. Average cellulolytic bacteria counts (cfu $\times 10^6$/1 gm) incubated with rice straw or corn stalks after zero, 2, 4 and 6 weeks of inoculation at 39°C with different enhancing fiber-degrading treatments

Treatments	Time after inoculation (weeks)	Volume of each treatment added to 50 g rice straw					Volume of each treatment added to 50g corn stalks				
		2.5 mL	5.0 mL	7.5 mL	10 mL	Mean	2.5 mL	5.0 mL	7.5 mL	10 mL	Mean
Rumen fluid	0	0.9000	1.7000	3.000	4.2000	3.7375[c]	1.2000	2.2000	4.0000	5.0000	3.4125[c]
	2	1.7000	3.2000	4.200	5.2000		2.4000	3.6000	4.7000	4.2000	
	4	2.2000	4.3000	5.800	6.0000		3.2000	4.3000	5.2000	3.2000	
	6	2.1000	4.1000	5.300	5.0000		3.1000	4.1000	2.1000	2.1000	
Fibrobacter	0	1.0000	2.1000	3.500	4.3000		0.9000	2.4000	3.8000	4.5000	
succinogenes	2	2.3000	3.4000	4.600	5.5000		2.8000	3.8000	5.3000	5.6000	
	4	2.7000	4.5000	6.100	5.8000	4.0458[b]	3.5000	4.8000	6.7000	6.7000	4.4687[b]
	6	2.6000	4.5000	6.300	5.2000		3.3000	4.4000	6.6000	6.4000	
Ruminococcus	0	1.1000	2.5000	4.100	5.0000		1.2000	2.6000	4.0000	4.8000	
Albus	2	3.1000	4.5000	5.600	6.5000		3.9000	4.5000	6.3000	6.7000	
	4	4.7000	5.5000	6.500	6.3000	4.8234[a]	3.7000	5.2000	7.9000	7.7000	5.0532[a]
	6	4.3000	5.2000	6.500	6.5000		3.7000	5.5000	7.5000	7.8000	
Mean		2.4944[c]	3.7917[b]	5.125[a]	5.4143[a]		2.7333[c]	3.9500[b]	5.2583[a]	5.3114[a]	

The values are mean of three replicates. Standard deviation was within 10%. Values followed by the same (a, b, or c) letter do not significantly differ from each other, according to Duncan's at 5% level

Table 2. Chemical analysis (%) and cell wall composition (%) of treated rice straw and corn stalks as affected by ruminal bacteria and rumen fluid inoculations at the level of 7.5 mL after 4 weeks of incubation period at 39°C

Chemical analysis	Rice straw				Corn stalks			
	Control	Rumen fluid	*F. succinogenes*	*R. albus*	Control	Rumen fluid	*F. succinogenes*	*R. albus*
DM	93.26	94.78	93.26	93.48	92.84	94.47	92.73	93.10
OM	85.78	81.47	81.36	80.54	89.11	86.65	86.22	85.93
CP	4.08	8.62	8.69	8.62	4.03	9.65	9.80	9.93
CF	38.88	34.36	35.13	34.12	37.01	34.21	34.02	32.46
EE	1.21	1.29	1.29	1.30	1.31	1.33	1.40	1.46
Ash	14.22	18.53	18.64	19.46	10.89	13.35	13.78	14.07
NDF	80.40	65.11	66.12	63.58	81.36	67.15	68.11	65.07
ADF	48.52	36.21	38.90	36.09	37.66	32.25	31.53	31.00
ADL	10.30	10.01	9.82	9.65	7.20	6.22	6.02	5.90
Cellulose	38.22	26.20	29.08	26.44	30.46	26.03	25.51	25.10
Hemicellulose	31.88	28.90	27.22	27.49	43.70	34.90	36.58	34.07
% C	46.87	44.52	44.46	44.01	48.69	47.35	47.11	46.96
% N	0.65	1.38	1.39	1.38	0.64	1.54	1.57	1.59
C:N ratio	71.81	32.28	31.98	31.91	75.52	30.67	30.05	29.55

The values are mean of three replicates. Standard deviation was within 10%. Control treatment = Raw materials without bacterial inoculation. DM = Dry matter, OM = Organic matter, CP = Crude protein, CF = Crude fiber, EE = Ether extract, NDF = Neutral detergent fiber, ADF = Acid detergent fiber and ADL = Acid detergent lignin

Also, degradation activity increased gradually by both cellulolytic bacteria and rumen fluid paralleled to increasing inoculation size up to 7.5 mL then tend to be constant (**Fig. 3**).

4. DISCUSSION

By comparing between our isolates and bacteria which were identified at molecular level and registered in EMBL database, similarity alignment did not exceed than 77%, for that, we used the traditional microbial identification methods (morphological and biochemical tests) to identify our isolates and we registered the sequenced 16S rDNA sequences as a novel sequences which can be used in the future to identify the *F. succinogenes* and *R. albus* at molecular level.

The natural rumen fluid contains a balanced number of microorganisms including bacteria that may catalyze nutrients in a harmony of cooperation while the number of bacterial cells is elevated when exists alone (*in-vitro*) in a suitable media without any competition with other bacterial species. In a related study, Matulova *et al.* (2005) grew *F. succinogenes* for up to 4 days with 100 mg of wheat straw by solid state fermentation. About 50 mg of straw was degraded with a relative increase in microbial growth. In another comparative study,

Matulova *et al*. (2008) found *R. albus* 20 appeared to be less efficient than *F. succinogenes* S85 for the degradation of wheat straw in 4 days solid-state fermentation experiment. Microbial growth reached a maximum between 24 and 48 h and then remained fairly constant. By comparing elevation of the cellulose and hemicelluloses degradation possesses occurred by inoculation with *F. succinogenes*, *R. albus* or the rumen fluid, each strain was highly degraded the plant materials than the rumen fluid and this may be attributed to: (1) *F. succinogenes* or *R. albus* were supportive to fiber degradation, while the rumen fluid contained many species not involved in fiber degradation associated with fiber degrading species. The rumen ecosystem comprises a diverse population of no cellulolytic activity obligatory anaerobic bacteria, fungi and protozoa (Otajevwo, 2011). (2) *F. succinogenes* or *R. albus* secreted cell wall degrading enzymes (e.g., cellulases, xylanases, â-glucanases and pectinases) (Hyun-Sik *et al*., 2007; Abd El-Galil and Abou-Elenin, 2011). Abd El-Galil and Abou-Elenin (2011) found that rice straw treated with *R. albus* and *Clostridium cellulovorans* recorded lower CF, NDF, ADF, cellulose and hemicellulose than untreated rice straw. Also the inoculation of rice straw, bagasse and corn stalks with *Cellulomonas cellulase*, *Acetobacter xylinum*, *Thermonospora fusca*, *Ruminococcus albus* and *Bacillus* sp. for two months led to degrade the fiber contents and cell wall constitutes and attributed these decrease in CF, NDF, ADF, cellulose and hemicelluloses for treated material to cellulase enzymes secreted by cellulolytic bacteria.

Bacteria in rumen fluid may have a common system of cellulolytic enzymes and utilize the available carbohydrates (soluble carbohydrate, crude fiber and its fractions) as a source of carbon to produce CO_2 and energy. It uses the generated energy and nitrogen sources to grow and consequently decreased the OM content and increased ash content in treated materials. Considering CP content, it is clear that treated materials had higher CP content compared to untreated. The higher CP content recorded for treated materials could partly due to: (1) the reduction of organic matter contents as carbohydrates. (2) Trapping of excess ammonia in the biological medium and its subsequent conversion to microbial protein (Gupta *et al*., 1988). (3) Nitrogen content of added urea, about 3% of the medium (Abd El-Galil and Abou-Elenin, 2011). Also the treatment with fungus of roughage materials resulted in an increase in CP content in these materials. In our future research, we will try to isolate some bacteria from camel's rumen fluid and evaluate its ability to degrade different wastes of Egyptian crops.

5. CONCLUSION

Using of PCR technique to detect 16S rDNA and sequencing of the resulted products can be used as specific markers for the identified bacteria. Two bacteria (*Fibrobacter succinogenes* and *Ruminococcus albus*) were isolated from rumen fluid of some Egyptian cattle and molecularly characterized depending in 16S rRNA genes. 1.6 kb PCR fragment was produced for each isolate and a partial sequences (709 and 401) bp were sequenced for *F. succinogenes* and *R. albus* respectively.

The degradation of corn stalks was highly significant comparing to rice straw which inoculated by *F. succinogenes* or *R. albus*. The Organic Matter (OM), Crude Fiber (CF), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), cellulose and hemicellulose content were decreased in both of rice straw and corn stalks after *in-vitro* treatments for four weeks with each isolates. The inoculum 7.5 mL/50 g of *Ruminococcus albus* is more efficient to degrade rice straw or corn stalks in four weeks fermentation period. Corn stalks are better than rice straw to enhance the cellulolytic bacteria to grow in all treatments.

In general the main effect of cellulolytic bacterial growth on celluloytic materials was increasing CP and ash contents and decreasing OM, CF, NDF, ADF, cellulose and hemicellulose.

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