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

Ahmed A. Mansour, T.S. El-Tayeb and N.E. El-Bordeny

Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt and Department of Medical Biotechnology, College of Applied Medical Sciences, University of Taif, (branch Truba), KSA

Department of Agricultural Microbiology, Department of Animal Production, Faculty of Agriculture, Ain Shams, University, Shoubra El-Kheima, Cairo, Egypt

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...
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 cellulosytic 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 cellulosytic 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 cellulosytic 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.

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 cellulosolytic 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 (AmpliTag Gold, Perkin-Elmer), 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.5 mM MgCl₂, 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 2-3×10⁷ 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
- \( \mu \) = The overall mean
- \( M_i \) = The effect of \( i^{th} \) inculants
- \( L_j \) = The effect of \( j^{th} \) level of inculants
- \( P_k \) = the effect of \( k^{th} \) 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 \( 5.0 \times 10^9 \text{cfu ML}^{-1} \) while the number of cellullolytic bacteria was \( 3.2 \times 10^7 \text{cfu ML}^{-1} \). 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 $6.5 \times 10^6$ cfu/g (1.1 fold) and $6.1 \times 10^6$ cfu/g (1.05 fold) respectively and gave a growth on corn stalks with rates of $7.9 \times 10^6$ cfu/g (1.5 fold) and $6.7 \times 10^6$ 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](image-url)
Fig. 2. Comparison of in vitro degradation of Rice Straw (RS) or Corn Stalks (CS) at different incubation time by rumen fluid \( F. \) succinogenes \( \square \) or \( R. \) albus \( \triangle \) (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 × 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

| Time after inoculation (weeks) | Volume of each treatment added to 50 g rice straw | Volume of each treatment added to 50g corn stalks |
|-------------------------------|-----------------------------------------------|-----------------------------------------------|
| Treatments                    | 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 | 2.0000 | 4.0000 | 5.0000 | 3.4125^c |
| 2                             | 1.7000 | 3.2000 | 4.200  | 5.200  | 5.0000 | 3.2000 | 4.3000 | 5.200  | 6.0000 | 3.2000 |
| 4                             | 2.2000 | 4.3000 | 5.800  | 6.0000 | 6.0000 | 3.1000 | 4.1000 | 2.1000 | 2.1000 | 2.1000 |
| 6                             | 2.1000 | 4.1000 | 5.300  | 5.0000 | 5.0000 | 3.1000 | 4.1000 | 2.1000 | 2.1000 | 2.1000 |
| Fibrobacter succinogenes       | 0      | 1.0000 | 2.1000 | 3.500  | 4.3000 | 0.9000 | 2.4000 | 3.8000 | 4.5000 | 3.4125^c |
| 2                             | 2.3000 | 3.4000 | 4.600  | 5.5000 | 3.2000 | 3.6000 | 4.7000 | 5.200  | 5.6000 | 3.4125^c |
| 4                             | 2.7000 | 4.5000 | 6.100  | 5.8000 | 4.0458 | 3.5000 | 4.8000 | 6.7000 | 6.7000 | 4.4687^b |
| 6                             | 2.6000 | 4.5000 | 6.300  | 5.2000 | 3.1000 | 4.4000 | 2.1000 | 2.1000 | 2.1000 | 2.1000 |
| Ruminococcus Albus             | 0      | 1.1000 | 2.5000 | 4.100  | 5.0000 | 1.2000 | 2.6000 | 4.0000 | 4.8000 | 4.6000 |
| 2                             | 3.1000 | 4.5000 | 5.600  | 6.5000 | 3.2000 | 3.8000 | 5.3000 | 5.6000 | 4.4687^b |
| 4                             | 4.7000 | 5.5000 | 6.500  | 6.3000 | 4.8234 | 3.7000 | 5.2000 | 7.9000 | 7.7000 | 5.0532^c |
| 6                             | 4.3000 | 5.2000 | 6.500  | 6.5000 | 2.7333 | 5.5000 | 7.5000 | 7.8000 | 7.8000 | 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 Control | Rumen fluid | F. succinogenes | R. albus | Corn stalks Control | Rumen fluid | F. succinogenes | R. albus |
|--------------------|---------------------|-------------|-----------------|----------|---------------------|-------------|-----------------|----------|
| DM                 | 93.26               | 94.78       | 93.26           | 93.48    | 94.84               | 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               | 56.21       | 58.90           | 56.09    | 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 SS5 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 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 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, Thermosospora 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 CO2 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 cellulolytic materials was increasing CP and ash contents and decreasing OM, CF, NDF, ADF, cellulose and hemicellulose.

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