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ASSESSMENT OF XYLANOLYTIC AND CELLULOLYTIC ACTIVITIES OF ANAEROBIC BACTERIAL COMMUNITY IN THE RUMEN OF CAMEL USING DIFFERENT LIGNOCELLULOSIC SUBSTRATES

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ABSTRACTS: ***The microbial community in the rumen of dromedary camel is predominated by lignocellulolytic anaerobic bacteria that make the greatest contribution in the digestion of poor-quality plant biomass. Consequently, camel rumen could be a promising source of lignocellulolytic enzymes with a wide range of applications, especially in bioenergy production. However, the majority of these bacteria were not cultivated and isolated, which represent a barrier towards the exploitation of this community in enzyme production. The goal of this study was to evaluate the endo-cellulase and endo-xylanase production ability of anaerobic bacterial community in the rumen of the camel. For that, rumen fluid from four camels fed Egyptian clover and wheat straw were inoculated into an anaerobic rumen bacterial media containing birchwood xylan, Filter paper, Wheat straw, Alfalfa hay as a carbon source. The maximum xylanase production was 1779.05 mU/ml at 7 days of incubation. Cellulose source impacted the cellulase yield and the highest production was 1389 mU/ml for the rumen samples incubated with Alfalfa hay for 48 hours. Our findings showed that anaerobic bacterial community in the rumen of the camel is an important source of fibrolytic enzymes.***

Key words: ***Anaerobic bacteria, Camel rumen Endo-cellulase, Endo-xylanase, In vitro.***

INTRODUCTION

Plant dry weight comprises 35–50% cellulose, 20–35% hemicellulose, and 5–30% lignin (Lynd *et al.,* 1999). Lignocellulolytic plant biomass is the most abundant renewable energy source (Whitaker, 1990; Kamble and Jadhav, 2012), and they represent the major component of agro-waste material, which is a rich and cheap source of cellulose and hemicellulose that could be converted to fermentable sugar using microbial enzymes (Kazeem *et al.,* 2017). This process could reduce the environmental problems associated with agro-wastes and contributes to the production of clean renewable Bioenergy (bioethanol and biogas), which might reduce the concerns about the depletion of non-renewable fossil fuels and its environmental effects (Rajoka *et al.,* 2012; Asem *et al.,* 2017).

Cellulose has a water-insoluble crystalline structure embedded in a lignin layer. Therefore, the hydrolysis of cellulose into available biosugar is difficult (Lai *et al.,* 2011). Lignocellulosic biomass needs to be first hydrolyzed into fermentable sugars by the synergetic work of different types of cellulases and xylanases enzymes (Fang *et al.,* 2008; Ahmed *et al.,* 2009; Seo *et al.,* 2013). The cellulases family consists of three major components, endoglucanase, exoglucanase and β-glucosidase (Coughlan, 1990). While, Xylanases family is more diverse than cellulase family and consist of at least ten

subfamilies, but the majority being endo-1,4-β-xylanases (Pollet *et al.,* 2010; Walia *et al.,* 2017). These enzymes work synergistically to break down the cellulose and xylane in the plant cell wall (Seo *et al.,* 2013; Asem *et al.,* 2017)..

Several microbial groups are involved in the production of cellulases and xylanases mainly include bacteria, fungi, yeast and protozoa (Béguin and Aubert, 1994; Chakdar *et al.,* 2016). However, the cost of the enzyme production is the major factor for their applications in the utilization of lignocellulosic biomass (Sukumaran *et al.,* 2005; Ibrahim *et al.,* 2013; Chakdar *et al.,* 2016). The cost could be reduced by utilization of cheap plant material in the enzyme production and the screening for new fibrolytic microorganisms and the innovation in the production process (Wang *et al.,* 2012).

The gastrointestinal of ruminant animal is inhabited by a diverse microbial community consist of bacteria, protozoa, fungi and archaea (Russell and Rychlik, 2001; Yeoman and White, 2014), which ferment indigestible lignocellulosic plant material that form the major component of animal diet into nutrients used for the growth of host animal (Creevey *et al.,* 2014). Anaerobic bacteria is the most predominant group in the microbial community in the rumen and they make the greatest contribution in the degradation of plant feedstuffs in the rumen (Henderson *et al.,* 2015; Gharechahi *et al.,* 2015).

The rumen microbiome is considered to be the most efficient microbial system at degrading lignocellulosic biomass (Flint *et al.,* 2008), and some cellulolytic and xylanolytic bacterial genera were isolated from the rumen, including *Rumminococcus* (Ekinci *et al.,* 2001), *Bacillus* (Seo *et al.,* 2013; Sadhu *et al.,* 2014), *Clostridium* (Khatab *et al.,* 2017) and *Prevotella* (Avguštin *et al.,* 1992). Some of these isolates were involved in the commercial production of fibrolytic enzymes (Seo *et al.,* 2013). Therefore, rumen has received a great interest for mining enzymes for biotechnological and industrial applications (Selinger *et al.,* 1996; Hess *et al.,* 2011; Wang *et al.,* 2013). However, The majority of rumen microorganisms are obligate anaerobic, which represents the major challenge to exploit and understand those microbial communities (Riberio *et al.,* 2016; Gharechahi and Salekdeh, 2018). Therfore, using Metagenomics and Metatranscriptomics technologies introduce a solution to examine and expand our understanding of the rumen microbial community (Riberio *et al.,* 2016; Wallace *et al.,* 2017). These techniques answer questions regarding the composition and relative abundance of microbial groups. However, these omics techniques do not address the questions regarding the metabolic activities of rumen microorganisms. Therefore, there is a need for more cultivation and physiological studies to verify predictions based on genome sequence data (Creevey *et al.,* 2014).

Camel, like other ruminant animals depends on microbial fermentation in the rumen to degrade the ingested feedstuff (Gharechahi *et al.,* 2015). Camels can utilize the low-quality shrubs that have a high content of lignocellulose and antinutritional factors, those plants are mostly avoided by other domestic ruminants (Iqbal *et al.,* 2001; Samsudin *et al.,* 2012). Consequently, camel rumen microbes must, therefore, have the capacity to degrade such poor-quality feeds (Gharechahi *et al.,* 2015). This speculation was supported by a metagenomics analysis in camel microbiome that revealed that camel microbiome contains a higher percentage of glycoside hydrolases compared with other gastrointestinal metagenomes from other herbivorous (Bhatt *et al.,* 2013;

Gharechahi and Salekdeh, 2018). Consequently, camel rumen microbiota can be a source of carbohydrate-active enzymes (CAZymes), that could be used in a wide range of biotechnological and industrial applications (Ameri *et al.,* 2018). However, Lignocellulolytic activities of camel rumen microbiome was not evaluated yet. Therefore, the objective of this study was to expand our knowledge regarding the metabolic capabilities of camel rumen microbiota by investigation the ability of anaerobic bacterial community in the rumen of the dromedary camel to produce xylanase and cellulase *in vitro.*

MATERIAL AND METHODS

Rumen sample collection

Rumen samples were collected from four adult male dromedary camels fed on Egyptian clover (*Trifolium alexandrinum*) and wheat straw. The samples were collected immediately after slaughtering in the Kom Hammada slaughtering house, Elbehra, Egypt. Rumen contents were strained immediately using two layers of cheese cloth to separate liquid and solid, and then liquid samples were cryopreserved using glycerol according to the protocol of Phillips and Gordon. (1988) for further processing. The project was approved and all samples were collected in accordance with the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Sadat City (Approval reference number: VUSC00008).

Cultivation condition

The enrichment media that was used in this study was the modification of Medium 10 (M10) (Caldwell and Bryant, 1966). The xylanolytic and cellulolytic activities of anaerobic bacteria of camel rumen were evaluated in replicates in four media, one xylanolytic medium (X) enriched with birchwood xylan and media enriched with one of three fiber sources, Filter Paper (FP), Wheat Straw (WS), and Alfalfa Hay (AH) as shown in Table 1. The pH in all media was adjusted at 6.8. Enrichment medium (20 ml) Was prepared under anaerobic condition was dispensed in 50 ml-Serum bottles containing xylan or one of fiber sources, then the medium was sterilized by autoclaving at 121 ^{o}C for 15 min. The samples for enzyme quantification were picked at 4 times, 24 hours (hrs.), 48 hrs,72 hrs., and 7days for cellulase and 24 hrs, 48 hrs, and 7days for xylanase. Preserved rumen samples were thawed by rapid warming in tepid water and then 0.3 ml was inoculated to the culture medium, then the bottles were incubated at 39°C. The growth and the presence of bacteria was confirmed using the microscopic examination and the degradation of filter paper. Furthermore, yellow pigments were observed on the filter papers.

Cellulase and xylanase enzyme assay

Enzyme assays were performed in duplicate. Samples of growing cultures were collected after 24 hrs, 48 hrs,72 hrs, and 7days intervals for cellulase and 24 hrs, 48 hrs, and 7days for xylanase. The supernatant that served as the enzyme source was obtained by centrifugation of 1 ml of bacterial cultures at (3000 rpm, 15 min, 4°C. Cellulase and xylanase activities (mU/ ml) were measured using EnzChek Cellulase substrate that determines endo-1,4-β-glucanase and EnzChek Ultra Xylanase Assay Kit (Invitrogen, UK) that determines endo-1,4-β-xylanase using the reaction buffer as a negative control and according to the manufacturer recommendations. These quantification methods depend on the fluorescence substrates to evaluate the enzymes activities.

Table 1: The composition of anaerobic media (modification of Medium 10 (M10) (Caldwell and Bryant, 1966)) for the cultivation of cellulolytic and xylanolytic rumen bacteria.

Ingredient	Xylanolytic Medium(X)/L	Cellulytic Medium /L
Tryptone	2 g	2 g
Yeast Extract	0.5 g	0.5 g
Glucose	0.5 g	-
Maltose	0.25 g	-
Cellobiose	0.25 g	0.25 g
Lactic acid 85%	1.73 ml	-
Soluble Starch	0.5 g	-
Xylane	2 g	-
Mineral solution 1	37.5 ml	37.5 ml
Mineral solution 2	37.5 ml	37.5 ml
Resazurin 0.1%	1ml	1ml
VFA solution	4.5 ml	4.5 ml
Vitamins Solution	5ml	5ml
$FeSo_4$ solution	5ml	5ml
Hemin Solution 0.2%	5ml	5ml
Na_2Co3 8%	50 ml	50 ml
Cys. HCL 2.5%	20 ml	20 ml
water	630 ml	630 ml
Clarified rumen fluid	200 ml	200 ml
Cellulose Source for cellulytic Media		
Filter paper (FP)	-	2 disc / bottle
Wheat Straw (WS)	-	50-100 mg / bottle
Alfalfa Hay (AH)	-	50-100 mg / bottle

VFA:Volatile Fatty Acid; $FeSo_4$: Ferrous sulfate; Na_2Co_3: Sodium carbonate; Cys. HCL: L-Cysteine hydrochloride.

Statistical analysis

The statistical analyses were performed using the IBM SPSS version 20 software (SPSS, 1999). The difference in xylanase production at different incubation times was performed using Repeated Measures ANOVA and the difference was statistically different at $P < 0.05$. The differences in cellulase production using different cellulose sources and the production at different incubation time and the interaction between incubation time and cellulose sources were performed using Mixed ANOVA and the difference was statistically different at $P < 0.05$. A post hoc Tukey test was carried out to determine the significant differences.

Results

The current study is a preliminary study to assess the ability of anaerobic bacteria in rumen of dromedary camel to produce bacterial cellulase and xylanase (*in vitro*) using rumen samples from dromedary camel fed Egyptian clover and wheat straw. The rumen samples were inoculated to anaerobic bacterial media enriched with xylane and different sources of cellulose to examine the effect of cellulose source on cellulase production. Moreover, the effect of incubation time on xylanase and cellulase production was also tested.

Xylanase production

Optimizing xylanase production at different incubation type is important, the bacterial xylanase (endo-1,4-β- xylanase) production was evaluated by incubating camel rumen samples in anaerobic bacterial medium containing birch wood xylane at a range of incubation time 24 hrs, 48 hrs and 7days at 38^{o}C and pH=6.8 (Figure 1). The analysis of xylanase production showed that the xylanase was raised gradually and reached maximum activity at 7 days. The overall mean production was 124.5 ± 37.6 mU/ml (mean ± sd) at 24 hrs, 315.3 ± 113.4 at 48 hrs, and 1779.05 ± 102.9 at 7 days. The difference in xylanase production at different incubation times was significant (P < 0.01) (Figure 1).

Cellulase production at different incubation times and using different cellulose sources

Bacterial cellulase (endo-1,4-β-glucanase) production was evaluated by incubating camel rumen samples in anaerobic bacterial media containing one of three different sources of cellulose, Filter Paper (FP), Wheat Straw (WS) and Alfalfa Hay (AH) at different incubation times 24 hrs, 48 hrs,72 hrs and 7 days at 38^{o}C and pH=6.8 to optimize the enzyme production. The results indicated that the production of cellulase was changed with increasing the incubation time and was dependent on the cellulose type. The production in FP media was raised slowly by increasing incubation time and the highest production was observed at 7 days (Table 2). A similar trend was observed in WS media; however, the production declined at 72 h then increased and the highest production was observed at 7 days. The highest cellulase production in AH media was registered at 48 hrs then decreased (Table 2). The Tuckey test showed that the difference in cellulase production between cellulose sources was significant (P < 0.01), the difference was significant between AH to WS and FP. Furthermore, the difference in cellulase production at different incubation time (P < 0.01).

A comparison of cellulase production of the anaerobic bacterial community using different cellulose sources at 48 hrs and 7 dayas revealed that the cellulase yield was increased by approximately 37-fold from 48 hrs to 7days in FP media. While the yield increased by 42.95 % from 48 hrs to 7 days in WS media. In contrast, the cellulase yield was decreased by 43.5 % from 48 hrs to 7 days in AH media (Figure 2). These results showed that the highest cellulase production in the current study was observed with anaerobic bacterial media inoculated with Alfalfa hay at 48 hrs. In addition, the wheat straw media could be effective in cellulase production at 48 hrs. The interaction between time and substrate was significant (P < 0.01).

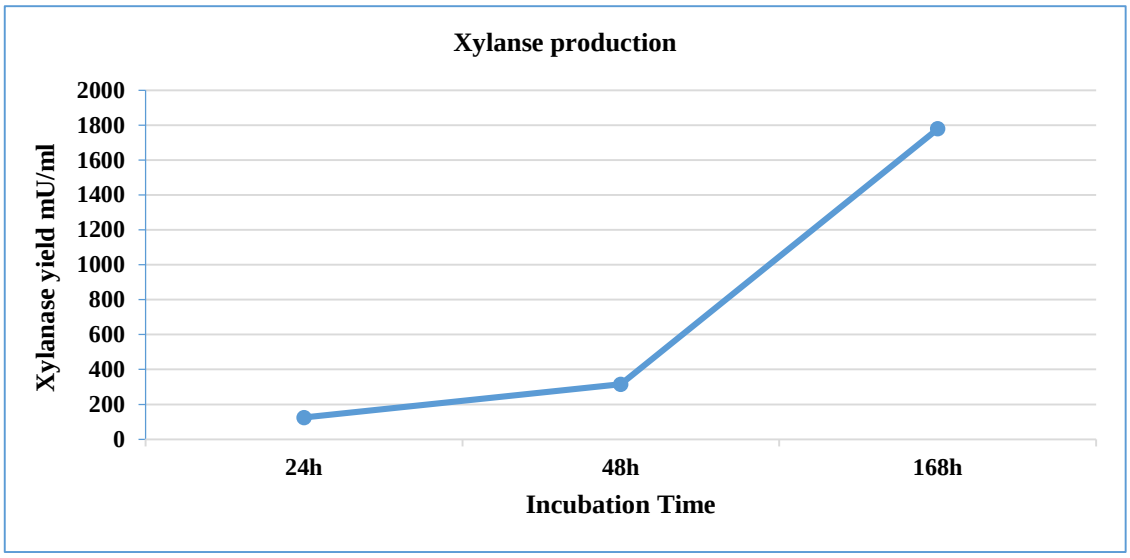

Figure 1: Effect of incubation time on endo-xylanase activity (mU / ml) of anaerobic bacterial community in the rumen of dromedary camel.

Table 2: Effect of different cellulose source and incubation times on endo-cellulase activity (mU / ml) (mean ± SD) of bacterial community in the rumen of dromedary camel.

Cellulose Source	Incubation Times			
	24 hours	48 hours	72 hours	7 days
Filter Paper (FP)	20.8 ± 3.2	21.1 ± 2.8	49.4 ± 19.1	799.2 ± 452.9
Wheat Straw (WS)	48.7 ± 33	422.8 ± 87.9	193.5 ± 35.3	604.4 ± 122.23
Alfalfa Hay (AH)	47.85 ± 3.8	1389 ± 350.7	959.9 ± 470	784.7 ± 418.1

Tests of Within-Subjects Effects time*substrate p=0.0001

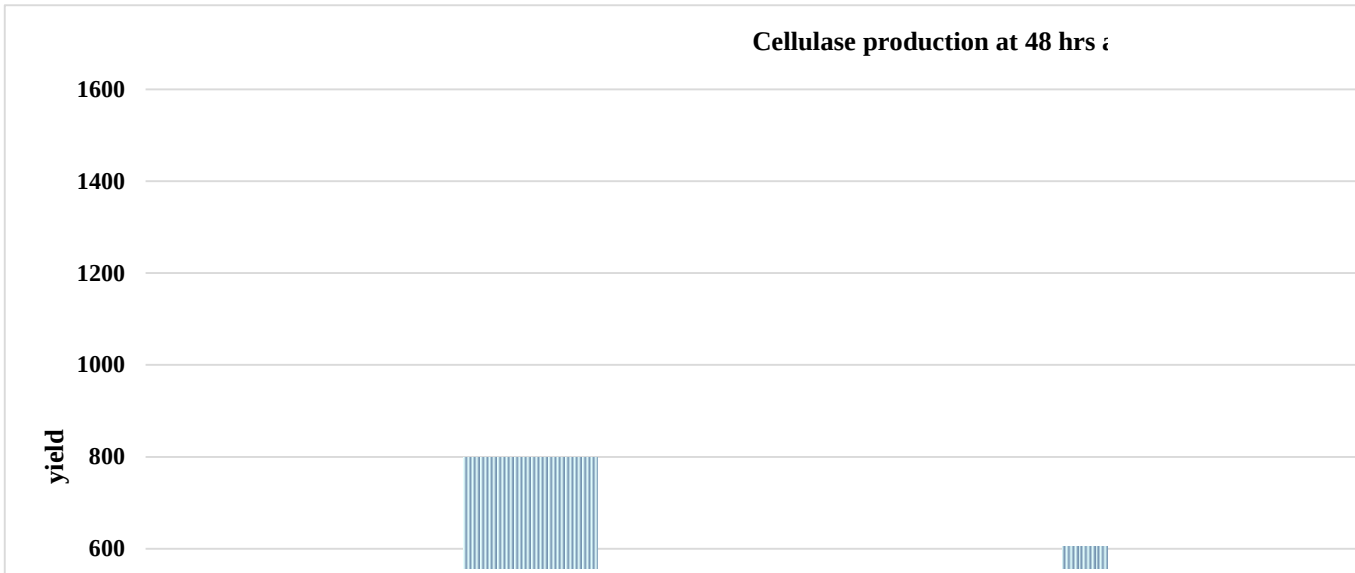

Figure 2: A comparison of endo-cellulase activity (mU / ml) of anaerobic bacterial community in the rumen of dromedary camel using different carbon substrates at 48 hrs and 7 days.

Discussion

Camel rumen is a rich source of lignocellulolytic enzymes and different microorganisms producing them, thus it represents a good source of enzymes and productive bacteria for different biotechnological purposes (Zorec *et al.,* 2014; Ameri *et al.,* 2018; Gharechahi and Salekdeh, 2018). Metagenomic analysis of camel rumen bacteria suggested that the camel rumen metagenome is enriched for genes involved in cellulose and xylan degradation more than other ruminant animals; in addition, genes encoding endoglucanases and endoxylanases were over-represented in the camel rumen's metagenome (Gharechahi and Salekdeh, 2018). However, only a small proportion of rumen bacteria were isolated, which represent a barrier towards the exploitation of this community in enzymes production (Creevey *et al.,* 2014; Nyonyo *et al.,* 2014). This work demonstrates the possibility of production of cellulase and xylanase using camel rumen contents inoculated to anaerobic bacterial media enriched with xylane and different fiber sources, including filter paper, wheat straw and alfalfa hay.

Xylanolytic activities

Xylanase production in the current study increased continuously by increasing the incubation time and reached the maximum at 7 days (Figure 1), this finding had a similar trend to results on different xylanolytic gut bacteria (Asem *et al.,* 2017). However, the xylanase production in the present study was lower than the production of *Bacillus* isolated from the rumen of the Korean goat (Seo *et al.,* 2013). On the other hand, the anaerobic bacterial community in this study produced more xylanase than the aerobic fungi (Salmon *et al.,* 2014) and anaerobic rumen fungi of camel gut (Rabee *et al.,* 2018). Xylanase in this study was quantified as endo-1,4-β-xylanase attacks 1,4-linkages, which represents the major component of xylanases family. Moreover, it has a greater catalytic versatility and can catalyze the hydrolysis of even cellulose and cellobios (Pollet *et al.,* 2010).

Cellulolytic activities

In this study, the cellulase activity was examined at different times, the result revealed that cellulase production varied by increasing the incubation time and the production reached the optimum at 7 days in media containing FP or WS (Table 2). Unlikely, the cellulase production in AH media reached the maximum at 48 hrs and decreased sharply. This results in the same line with the results of cellulolytic bacteria isolated from goat and swine (Seo *et al.,* 2013; Yang *et al.,* 2014; Asem *et al.,* 2017), and cow manure (Sadhu *et al.,* 2014).

Effect of carbone source on cellulase yield

The activities of fibrolytic enzymes are strongly influenced by the growth substrate (William and Withers, 1982; Ekinci *et al.,* 2001). In the present study, different sources of cellulose were examined to explore their impact on cellulase yield. The result revealed that maximum production was obtained with media containing alfalfa hay (AH) at 48 hrs followed by media containing filter paper (FP) at 7d (Table 2). Therefore, the alfalfa hay could be used to produce cellulase from anaerobic rumen bacteria. Cellulase production in the current study was higher than cellulase production of *Bacillus* isolated from cow dung (Sadhu *et al.,* 2014) and other cellulolytic bacteria isolated from goat and swine (Asem *et al.,* 2017). Furthermore, it was higher than aerobic fungi (Salmon *et al.,* 2014) and

anaerobic rumen fungi in camel rumen that were incubated in alfalfa hay (Rabee *et al.,* 2018).

Our findings highlight the anaerobic bacterial community in camel rumen as a promising source for cellulase and xylanase to meet the global demand for these enzymes, as most of the commercial lignocellulolytic enzymes are being produced by fungi, which have a slower growth rate and longer fermentation period than bacteria. Consequently, the cost of production is high (Westers *et al.,* 2004; Yang *et al.,* 2014; Maki *et al.,* 2011; Ladeira *et al.,* 2015; Gaur *et al.,* 2015). On the other hand, the anaerobic bacterial community in the current study has been cultivated with cheap and available sources of cellulose (wheat straw and Alfalfa hay). Wheat straw exhibited a good cellulase production at 48 hrs (422.8 mU / ml) comparing with the production at 7 days (604.4 mU / ml), this indicated that wheat straw also could be recommended for the production at 48 hrs, and that might save the time and the cost of production compared with FP media (Figure 2). Using cheap substrates for cellulase production is recommended to save the cost of production (Wang et al., 2012).

Higher cellulase yield in AH medium could be attributed to the lower lignin content in Alfalfa compared to wheat straw (Martin and Mertens, 2005; Shrivastava *et al.,* 2014). Lignin restricts the microbial degradation of plant cell wall carbohydrates (Shrivastava *et al.,* 2014). Furthermore, the fiber in Alfalfa has a higher digestion rate than grass fiber (Martin and Mertens, 2005), which might increase the bacterial count (Chung and Hungate, 1976). Hespell and Argyle. (1987) investigated the digestion of alfalfa hay by anaerobic rumen bacteria *in vitro*, and they noticed that glucose started to disappear after 12 hrs and was completely gone by 48 hrs, hemicellulose and cellulose digestion began after 12 hrs, these findings could illustrate the results of our study. The decrease in production in AH after 48 hrs could be explained as a result of the excessive consumption of nutritional ingredients in the medium (Yang *et al.,* 2014). Endo-Cellulase hydrolyzes cellulose by cutting the internal amorphous sites and soluble derivatives of the cellulose molecule, which produce oligosaccharides of different lengths (Kuhad *et al.,* 2011; Fariq, 2016).

Lignocellulolytic bacteria in camel rumen

Previous studies on microbial community in camel rumen revealed that the bacterial community in the rumen of the camel is largely dominated by cellulolytic and xylanolytic bacterial genera that include *Fibrobacter, Butyrivibrio, Clostridium, Ruminococcus, Treponema, Bacilli,* and *Prevotella* (Samsudin *et al.,* 2011; Samsudin *et al.,* 2012; Gharechahi *et al.,* 2015). These genera are the major contributors to lignocellulose degradation in the rumen (Gharechahi and Salekdeh, 2018). Camel has the ability to retain ingested material in the rumen for a longer time than other ruminant and the pH in camel rumen is closer to neutral, which support colonization of cellulolytic bacteria and the efficient degradation of fibrous diets (Stevens and Hume, 1998; Jouany, 2000; Samsudin *et al.,* 2011).

The high proportion of cellulolytic and hemicellulolytic bacteria in the rumen of camel reflects the ability of camel to utilize the abundance of low-quality shrubs and poor quality forages, which are mostly avoided by domestic ruminants (Iqbal and Khan *et al.,* 2001; Gharechahi *et al.,* 2015; Gharechahi and Salekdeh, 2018). Samsudin *et al.* (2012) used rumen content from dromedary camels to inoculate three different enrichment media contain different fiber

sources, including cotton thread, filter paper, and neutral detergent fiber from lucerne hay, the results showed that the fiber type influenced bacterial species that grow in the fiber-enriched medium. Moreover, members related to the phylum Firmicutes were dominant and some of the bacteria involved in fiber digestion were assigned to *Fibrobacters*. In another study by Gharechahi and Salekdeh. (2018), the metagenomics analysis of camel rumen microbiota revealed that species related to phylum Firmicutes and Fibrobacteres were rich in cellulases and hemicellulases lineages that have a possible role in the degradation of lignocellulose. These findings explain that the variation in cellulase yield among the substrates in the current study could be attributed to the variation in the composition of bacterial community associated with the carbon sources. In the current study, the partially degraded filter papers showed a yellow pigment, which indicates to the presence of *Ruminococcus* that produce cellulase and xylanase (Ekinci *et al.,* 2001).

All the previous speculations confirm that camel rumen is a promising source of cellulolytic and xylanolytic enzymes and bacteria that could be used in a wide range of applications in many fields. However, more studies are recommended to isolate cellulolytic and xylanolytic bacteria from camel rumen and to make more production optimizations under different cellulolytic and xylanolytic substrates, pH and temperatures. Xylanases and cellulases have numerous applications worldwide; for example, it is used for biobleaching of pulps in the paper industry, brewing, laundry detergents and in backing industry. Furthermore, it can be included in animal feed additives, probiotics, and biofuel production; also, it can be involved in the recycling of waste paper (Kuhad *et al*., 2011; Fariq, 2016; Chakdar *et al*., 2016). In this study, we quantified the cellulase and xylanase using Fluorescence substrates that enable the highly sensitive detection of xylanolytic and cellulolytic activities and allow to the differentiation between exo and endo activities (Helbert *et al.* 2003; Khatri *et al.* 2016).

Conclusion

It can be concluded that the rumen fluid of the dromedary camel is a promising source of lignocellulolytic enzymes that could be used in a wide range of applications. The incubation time has an impact in cellulase and xylanase yield. In addition, the cellulose source influenced the cellulase production, where Alflalfa hay supported the highest cellulase production, which offer the possibility to reduce the cost of cellulase production using cheap cellulos sources.

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تقييم الانشطة الهاضمة للسليولوز والزيلان للبكتريا اللاهوائية فى كرش الابل باستخدام مصادر لجنوسليولوز مختلفة

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الملخص العربى

المجتمع الميكروبى بكرش الابل العربى يسوده البكتريا اللاهوائية الهاضمة للمواد اللجنوسليولوزية والتى تساهم بالنصيب الاكبر فى هضم الاعلاف الفقيرة وبالتالى فان كرش الابل قد يكون مصدر واعد للانزيمات الهاضمة للجنوسليولوز والتى لها العديد من التطبيقات فى الصناعة . على الرغم من ذلك فأن اغلب أنواع هذه البكتريا لم يتم عزلها واستزراعها مما يمثل عائق لاستغلال هذه البكتريا فى انتاج الانزيمات. هذه الدراسة تهدف الى تقييم قدرة البكتريا اللاهوائية فى كرش الابل على انتاج انزيمى السليوليز والزيلانيز . تم جمع اربع عينات سائل كرش من ابل تغذت عل البرسيم وتبن القمح ثم تم تلقيح هذه العينات فى بيئة بكتريا لاهوائية تحتوى على الزيلان، ورق الترشيح، تبن القمح، او دريس البرسيم الحجازى كمصادر للمواد اللجنوسليولوزية. لوحظ ان انتاج الزيلان سجل اعلى مستوى له بعد سبعة ايام وان مصدر السليولوز اثر على انتاج السليوليز وكان اعلى انتاج فى حالة البيئة التى تحوى دريس البرسيم الحجازى عند ٤٨ ساعة .

نتائج هذه الدراسة تشير الى ان البكتريا اللاهوائية بكرش الابل مصدر هام لانتاج الانزيمات الهاضمة للسليولوز.

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