

Processing of the lignocellulosic matters with cellulolytic bacteria isolated from the one hump camel foregut

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Abstract

Agricultural by-products are frequently used to feed livestock, but they often have low nutritional value. This study was conducted to improve nutritional value of wheat straw using cellulolytic bacteria isolated from the foregut of dromedary camel. Rumen fluid was collected from four fistulated camels. The cellulose-hydrolytic bacteria were isolated by using medium amended with carboxymethyl cellulose (CMC). The activity of carboxymethyl cellulase (CMCase) was determined by measuring the release of reducing sugars. The optimum pH and temperature for growth and CMCase activity of the isolates were determined. Three isolates (SH13, SH2, and SH22) showed maximum hydrolysis capacity and were selected for further investigation. The analysis of 16S rRNA gene sequences of these isolates showed that the isolates possessed 99, 94 and 98% similarity with *Escherichia coli*, and *Enterobacteriaceae*, respectively. Wheat straw was incubated with the isolated bacteria in liquid medium for 6 weeks. The results showed inoculation with SH22 isolates resulted in the highest disappearance of dry matter and crude protein content, and the lowest amount of neutral detergent fibre (NDF), iNDF and acid detergent fibre (ADF). Treatment with SH13 isolate had the highest digestibility of NDF and ADF, and treatment with SH2 had the highest truly degraded organic matter (TDOM) and partitioning factor (PF). Our data suggest that maybe bacteria isolated from camel foregut can increase digestibility and enhancing nutrition value of wheat straw.

Key words: Camel, Cellulolytic bacteria, Digestibility, Wheat straw

Introduction

Wheat straw is a major agro-residue that does not provide enough nutrients to ruminants, even for maintenance, due to high fibre and low nutritional value. Various processing methods have attempted to increase the accessibility to structural

carbohydrates and enhance degradability of wheat in the rumen, including physical (heat, steam, and pressure) and chemical treatments (acids, alkalis, NH₃ and ozone). Chemical methods are relatively cheap and easy. However, safety precautions are

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needed for these chemicals due to harmless and secondary pollution (Hendriks and Zeeman, 2009; Agbor et al, 2011). Previously, researchers have focused on biological alternatives, that are environmentally and widely acceptable (Sharma and Arora, 2013; Sánchez, 2009; Pérez et al, 2002). One of the biological methods uses microorganisms, such as fungi and bacteria, which have been found to degrade cellulose and other plant cell wall fibres. Although fungi have been widely studied in recent years, researchers have also been paying attention to various bacteria that produce cellulases because of high growth rate, expression of multi-enzyme complexes, and resistance to extreme environments (Martins et al, 2008; Marjamaa et al, 2013). Cellulolytic bacteria are present in a variety of sources, such as composting heaps, decaying agricultural wastes, soil, and gastrointestinal tract of herbivores (Gunavathy and Boominathan, 2015).

Camels belong to the family *Camelidae* of the sub-order *Tylopoda*. They are often referred to as pseudo-ruminants; their foregut consists of three compartments, instead of four in a true ruminant. Camels can utilize the abundance of low-quality shrubs and trees (Phillips et al. 2001), and can often browse on a broad spectrum of plants, like thorny bushes, halophytes, and aromatic types, which are mostly avoided by domestic ruminants (Iqbal and Khan, 2001). The high content of lignocellulose in the cell wall of these shrubs and trees, combined with their antinutritional factors, have prevented cattle and sheep from using these plants as a feed source (Samsudin et al, 2012). Whereas, camels are able to digest woody plant cell wall more efficiently than most ruminant animals (Samsudin et al, 2011; Turnbull et al, 2012). Thus, it is assumed that its foregut microbiota is very rich in a variety of enzymes that degrade lignocellulosic material. The aim of the present study was to improve the nutritional value of wheat

straw by cellulolytic bacteria isolated from the foregut of the dromedary camel.

Materials and Methods

Bacterial isolation from camel foregut and Determining of enzyme activity

The care and management of the camels and sampling procedures were conducted according to The Care and Use of Agricultural Animals in Research and Teaching guidelines (FASS, 2010). Foregut samples, for isolating cellulase-producing microorganisms, were obtained from four fistulated camels and placed in sealed plastic bags surrounded with ice. The samples were taken to the laboratory within 2 h, diluted with 100 mL physiological serum, and then one gram of the sample was homogenized in a constant-temperature oscillation water bath at 40 °C, for 30 min.

In order to isolate the cellulose-hydrolytic bacteria, one mL of sample was forwarded to the fresh 50 mL Bushnell Haas Medium (BHM) with carboxymethyl cellulose (CMC) as the sole carbon source in 100 mL sealed bottles under continuous CO₂ and incubated at 37°C for seven days.

In order to perform isolation, after enrichment in CMC-amended medium for more than five times, the inoculum (0.1 mL; successively diluted to 5-10 times) was streaked on BHM agar plates with CMC as a sole carbon source. The cellulose-hydrolytic bacteria were isolated using Bushnell Haas (BH) medium with carboxymethyl cellulose (CMC, Lo et al., 2009). In this step, for anaerobic growth conditions, incubation was performed in an anaerobic jar containing gas pack.

The carboxymethyl cellulase (CMCase) activity of the isolated cellulose-hydrolytic bacteria was specified by measuring the release of sugars reduced from CMC. The standard curves were prepared for glucose under assay conditions with 1% (w/v) CMC as the substrate (Miller, 1959). Then enzyme activity of isolates was performed as described in Harsini et al, (2019).

Optimization of temperature and pH for bacteria growth and enzymes production

To select the appropriate temperature and pH for the growth of bacterial isolates, the growth curves of these bacteria were drawn at two temperatures (25 and 39 °C) and three pH (5.2, 5.7 and 6.2) in a broth culture medium in anaerobic condition. Therefore, 18 250 ml flasks (2 temperatures with 3 pH and 3 replicates) for each isolate were prepared and 150 ml of BHM, containing carboxymethyl cellulose. The pH values were adjusted using 1N NaOH and 1N HCL. The flasks were sterilized at 121 °C, 15 psi, and after cooling, a bacterial suspension of 0.5 McFarland standard (0.05 mL of 1.175% BaCl₂.2H₂O, with 9.95 ml of 1% H₂SO₄) was added at a rate 1% of the culture medium per flask. These bacterial cultures were placed in an incubator with the desired temperatures. For the growth of bacterial isolates within 24 hours, every two hours the growth rate of each bacterial strain was measured by spectrophotometer at 600 nm, and then the bacterial growth curve was plotted.

For enzyme production of bacterial isolates, samples were taken at 24, 48 and 72 hours from the culture media, and then the enzymatic activity of the samples was measured by spectrophotometer (Biochrom Libra S22, Cambridge, United Kingdom, Ziemer et al, 2002).

Genomic DNA extraction and amplification of 16S rRNA gene for bacteria identification

Boiling method was used to extract the genomic DNA of the selected isolates (Ausubel et al. 1995). The DNA quality was assessed by OD260/OD280 ratios (>1.8) and samples were visualized on 0.8% agarose gel.

The 16S rRNA gene was amplified with universal primers in a PCR amplification, using a Bio-Rad i-cycler, following the protocol of Weisburg et al., (1991). PCR products were visualized on 1% agarose gel (Fig. 1). The PCR products was sequenced by NGS method. The quality of sequences was high and thus edited using Bioedit. The

GenBank data (National Center for Biotechnology Information, NCBI) were used to compare PCR sequences using BLAST algorithm.

Processing of wheat straw with isolated bacteria and in vitro digestibility

The processing of wheat straw with isolated bacteria was done according to Harsini et al, (2019). For this purpose, 20 1 Erlenmeyer flasks (4 treatments [3 bacterial isolates + controls] in 5 replicates) were prepared, each filled with 200 ml of BHM and 5% substrate (10 g per flask) under continuous CO₂. After sterilizing, each flask was inoculated with 2 ml of bacteria cultured in broth medium with 1 McFarland concentration. Thereafter, all flasks were cultured in the shaking incubator for 6 weeks at 39 °C. After incubation, the flask contents were filtered and the residues were collected and dried. *In vitro* gas production was done according to Menke and Steingass (1988). Rumen fluid was collected from four fistulated sheep, before morning feeding, maintained on 60% hay and 40% concentrate diet for two weeks. Rumen fluid was strained using four layers' cheesecloth and maintained at 39 °C. Next, vials containing 200 mg samples were inoculated with 30 ml buffered rumen fluid under continuous CO₂, and were incubated at 39 °C for 2, 4, 6, 8, 24, 48, 72 and 96 h in triplicate. The cumulative gas production was calculated by non-linear regression through using an exponential model as follows:

$Y = b(1 - e^{-ct})$; Where Y represents gas volume at time t (mL), b shows the gas production potential (mL), t is time (h) and c stands for fractional rate of gas production (mLh⁻¹). The fermentation parameters such as partitioning factor and microbial biomass were estimated according to Makkar and Becker's (1998).

The *in vitro* digestibility of dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) of samples (7

replicates per treatment) were determined based on the method of Tilley and Terry (1963). Rumen fluid was collected from four fistulated sheep, before morning feeding, and was strained using four layers' cheesecloth and maintained at 39 °C. Next, tubes containing 500 mg samples (Samples processed with bacteria and wheat straw as control) were inoculated with 50 ml buffered rumen fluid under continuous CO₂. This method consists of two consecutive digestion phases. During the first digestion phase, plant materials were incubated with rumen micro-organisms and under anaerobic conditions for 48 h at 39° C followed by a 48-h acid-pepsin digestion phase under anaerobic conditions at 39° C. After the 96-h period of incubation, residual materials were collected and oven dried (105° C for 12 h).

Processed samples with isolated bacteria were dried using an oven at 55 °C. Following the AOAC (1990) procedure numbers 930.15, 924.05 and 984.13, the DM, ash, and N content were respectively analyzed. Neutral detergent fibre (NDF) was determined without sodium sulfite and expressed exclusive of residual ash (Van Soest et al., 1991). ADF was determined and expressed exclusive of residual ash (AOAC, 1990; # 973.18).

General Linear Model (GLM) (SAS Institute, 2001) was used for data analysis according to the statistical model: $Y_{ij} = \mu + T_i + e_{ij}$; where Y_{ij} stands for the observation, μ represents the mean, T_i shows the effect of isolated bacteria on substrates and e_{ij} illustrates the standard error of term, as well. Duncan multiple comparison test was employed to compare the means. Significance level was considered to be ≤ 0.05 , if there are no other signs.

Results

Isolated bacteria

Twelve cellulose-degrading bacterial strains were isolated from the foregut of dromedary camels. Six of these isolates showed hydrolyzing zones on agar plates containing CMC-Na after Congo-red staining. Three isolates (SH2, SH13, and SH22) showed maximum hydrolysis capacity and were selected for further tests. The clear zone diameters and hydrolysis capacity are given in Table 1. The three isolated cellulolytic bacteria grew well at 39 °C on CMC-amended BHM medium under aerobic or facultative anaerobic conditions.

Results of optimization of bacteria growth and CMCase activity are shown in Figure 1 a-d and Figure 2 a-b, respectively. Growth curve of *Escherichia coli* SH13 showed that the best growth was achieved in 39 °C. This isolate had the highest CMCase activity at 39 °C and pH 5.2 in 24 h. Optimal temperature for growth of *Enterobacteriaceae* SH2 was 39 °C. Also, this isolate exhibited the highest enzyme activity at 39 °C and pH 6.2 in 48 h. *Escherichia* sp. SH22 had the highest growth at 39 °C. The highest CMCase activity of *Escherichia* sp. SH22 achieved at 39 °C, pH 5.2 in 48 h. Generally, all isolates grew and produced enzymes at 39 °C better than 25 °C and growth of isolates not affected by pH at 39 °C.

Results of CMCase assay are given in Table 1. The highest and lowest CMCase were observed for isolate SH22 (2.96 unit/mL/min) and SH2 (2.46 unit/mL/min), respectively. 16S rRNA sequence analysis showed that the isolate SH13 had 99% similarity to *Escherichia* spp; isolate SH2 had 94% similarity to bacteria belonging to the family *Enterobacteriaceae*; isolate SH22 had 99% similarity to *Escherichia* spp.

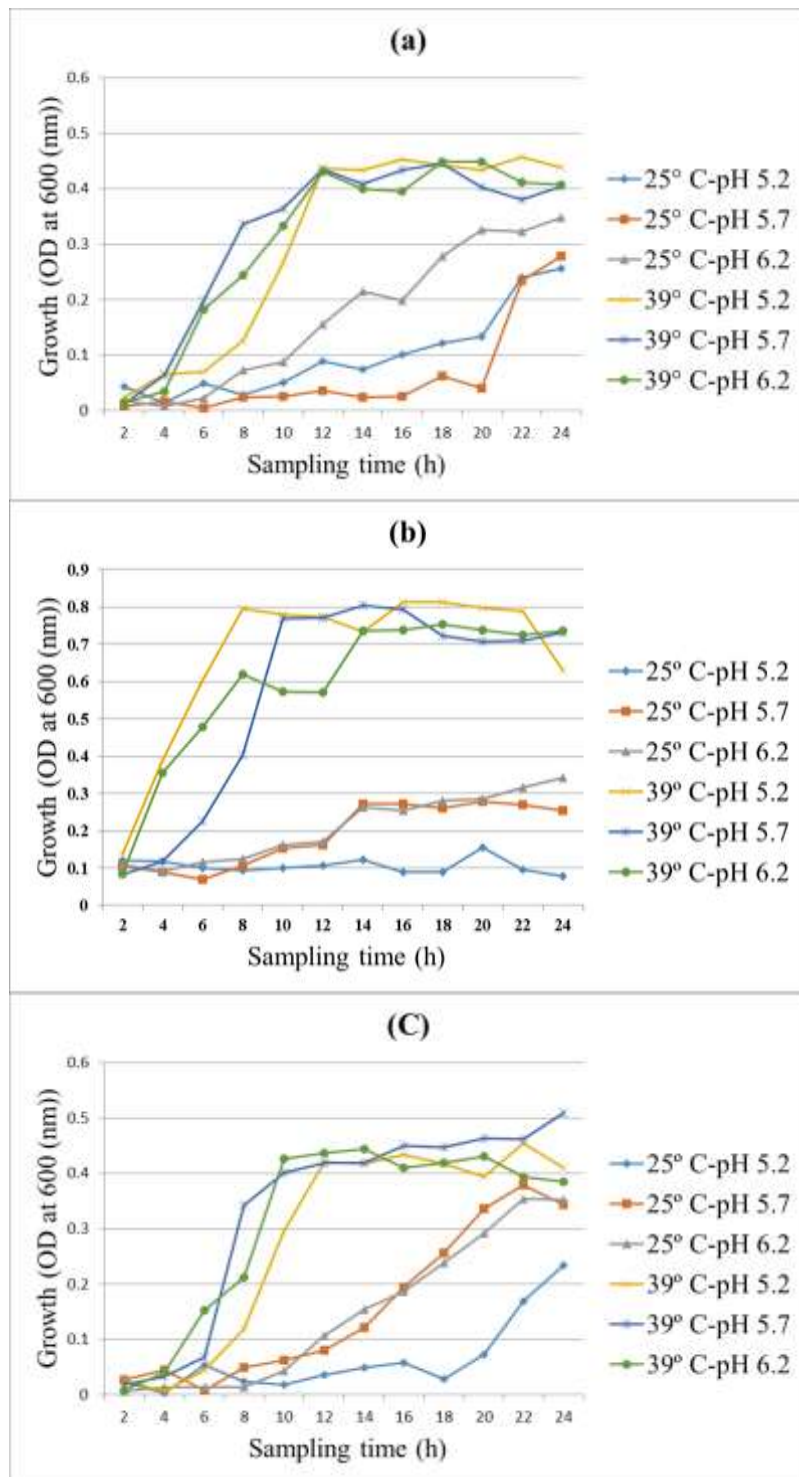


Figure 1: Effect of initial pH and temperature on growth bacteria isolated from gastrointestinal tract of camel. *Escherichia coli* SH13 (a) *Enterobacteriaceae* SH2 (b) *Escherichia coli* SH22

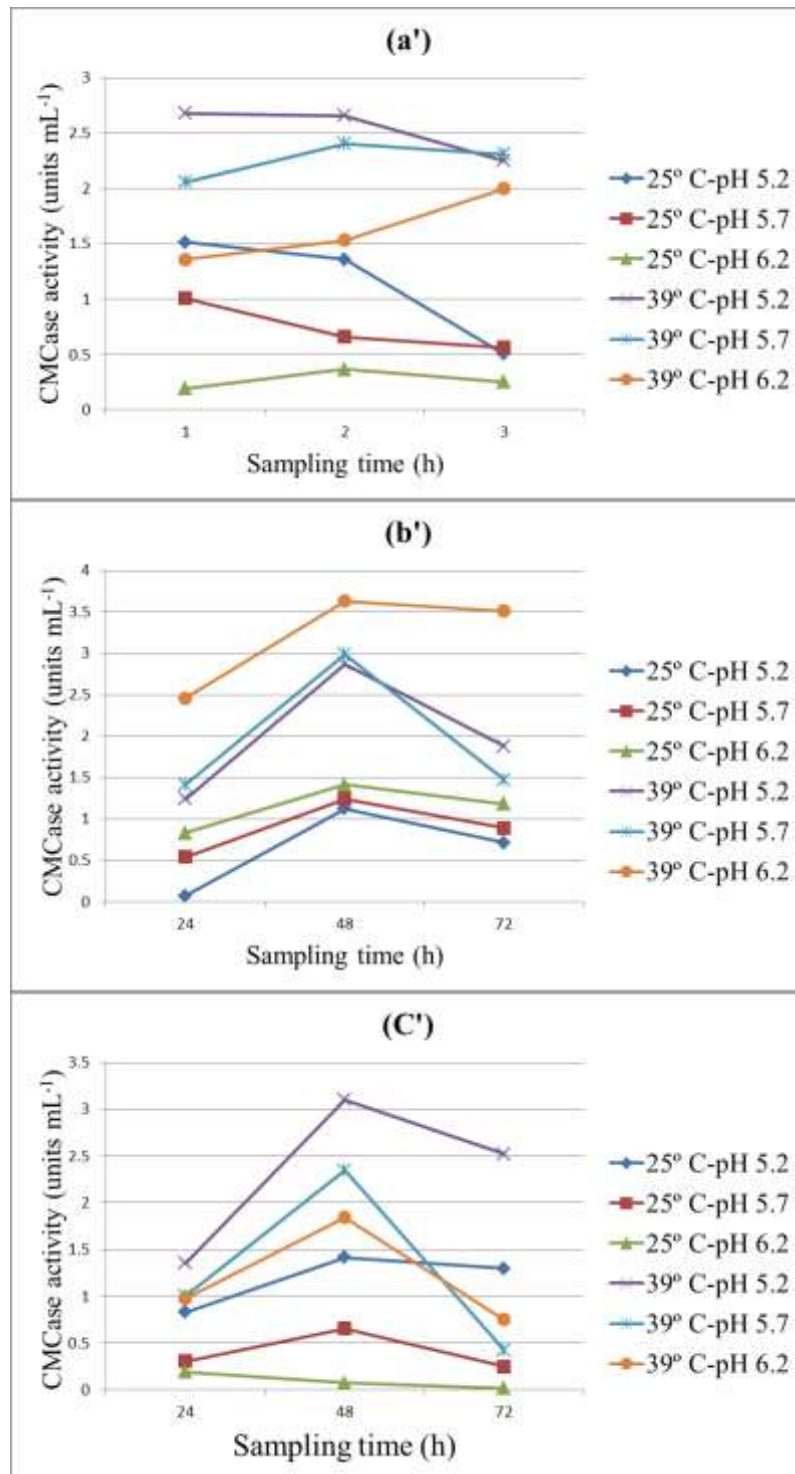


Figure 2. Effect of initial pH and temperature on CMCCase activity of bacteria isolated from gastrointestinal tract of horse. *Escherichia coli* SH13 (a')_ *Enterobacteriaceae* SH2 (b')_ *Escherichia coli* SH22 (c')

Table 1: CMCase activity (units/mL/min) of bacteria isolated from gastrointestinal tract of camel

Isolate no.	Clear zone diameter (cm)	HC*value	CMCase activity (units mL ⁻¹)	GenBank accession no.	Identification	similarity
SH13	2.7	6.25	2.88	<u>MG669346.1</u>	<i>Escherichia coli</i>	99% with <i>Escherichia coli</i> SE11 DNA, complete genome
SH2	1.3	2.6	2.46	<u>MG669345.1</u>	<i>Enterobacteriaceae</i>	94% with <i>Enterobacteriaceae</i> bacterium MB6-2 16S ribosomal RNA gene, partial sequence
SH22	2.3	6.75	2.96	<u>MG669629.1</u>	<i>Escherichia coli</i>	99% with <i>Escherichia coli</i> strain D1, complete genome

*HC (hydrolysis capacity) was calculated from clear zone diameter divided by colony diameter.

In vitro tests

Disappearance of DM, CP, NDF, iNDF and ADF contents were affected by all bacterial isolates ($P < 0.05$), but not OM content ($P > 0.05$). Isolate SH22 showed the highest disappearance of DM and CP (Table 2) and the lowest disappearance of NDF, iNDF and ADF content. Content of CP, NDF and ADF was the same in SH13 and SH2. In addition, there was no significant difference between the amount of NDF and iNDF between isolates SH13 and SH22 ($P > 0.05$). NDF, iNDF and ADF were the highest and disappearance of DM and CP were the lowest in the control group.

Bacterial isolates improved ($P < 0.05$) digestibility of DM, OM, CP, NDF and

ADF of wheat straw compared to control (Table 3). The highest DM, OM and CP digestibility was observed for wheat straw treated with SH22 ($P < 0.05$). Digestibility of NDF and ADF of treated sample with SH13, SH2 and SH22 was not different ($P > 0.05$).

The *in vitro* gas production parameters are shown in Table 4. Potential of gas production (b), truly degraded organic matter (TDOM), microbial biomass and biomass efficiency were affected by microbial processing ($P < 0.05$). Highest TDOM and PF were observed for wheat straw treated with isolates SH2, respectively. Gas production rate was not affected by bacterial treatments ($P > 0.05$).

Table 2: Effects of bacteria isolated from gastrointestinal tract of camel on the disappearance of dry matter *in vitro* and changing chemical composition (%) of wheat straw

Treatments	DM*	OM†	CP‡	NDF§	iNDF¶	ADF**
Raw wheat straw	-	93	3.9	80	43	56.98
Wheat straw (control)	17.57 ^c	91.29	2.921 ^c	81.66 ^a	43.37 ^a	57.16 ^a
Wheat straw +SH13	19.49 ^b	91.22	6.543 ^b	77.85 ^{bc}	33.12 ^c	50.58 ^b
Wheat straw +SH2	21.16 ^a	91.56	7.002 ^b	79.10 ^b	37.39 ^b	51.13 ^b
Wheat straw +SH22	22.14 ^a	91.36	7.760 ^a	76.44 ^c	32.73 ^c	49.70 ^b
SEM	0.546	0.314	0.226	0.676	0.747	0.841
P-value	0.0001	0.878	<.0001	0.0004	<.0001	<.0001

*Disappeared dry matter; †Organic matter; ‡Crude protein; §Neutral detergent fibre; ¶Indigestible neutral detergent fibre; **Acid detergent fibre; ††Standard error of means; ^{a,b,c}In each column, values with different letters are significantly different ($P < 0.05$).

Table 3: Effects of bacteria isolated from gastrointestinal tract of camel on *in vitro* nutrient digestibility (%) of wheat straw

Treatments	DM*	OM†	CP‡	NDF§	ADF¶
Wheat straw (control)	34.64 ^c	33.52 ^c	37.09 ^c	33.57 ^b	32.82 ^b
Wheat straw +SH13	39.10 ^b	39.55 ^b	47.21 ^a	40.60 ^a	39.93 ^a
Wheat straw +SH2	42.18 ^a	43.13 ^a	43.41 ^b	39.84 ^a	38.94 ^a
Wheat straw +SH22	42.61 ^a	42.90 ^a	48.83 ^a	39.75 ^a	38.56 ^a
SEM**	0.608	0.761	0.918	0.704	0.774
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*Dry matter; †Organic matter; ‡Crude protein; §Neutral detergent fibre; ¶Acid detergent fibre; **Standard error of means; In each column, values with different letters are significantly different ($P < 0.05$).

Table 4: Effects of bacteria isolated from gastrointestinal tract of camel on gas production parameters of wheat straw

Treatments	b*	c†	TDOM‡	pf§	Biomass	Biomass Efficiency
Wheat straw (control)	31.09±0.619 ^c	0.0323±0.0015	135.3 ^c	9.106 ^b	102.6 ^d	0.758 ^b
Wheat straw +SH13	40.77±1.309 ^a	0.0330±0.0025	166.3 ^a	8.761 ^b	125.8 ^b	0.747 ^b
Wheat straw +SH2	35.54±0.842 ^b	0.0302±0.0016	167.7 ^a	10.28 ^a	131.7 ^a	0.785 ^a
Wheat straw +SH22	31.50±0.638 ^c	0.0307±0.0014	152.8 ^b	10.45 ^a	120.6 ^c	0.789 ^a
SEM¶	1.156	0.0023	2.330	0.254	1.614	0.006
P-value	0.0002	0.8057	<.0001	0.0009	<.0001	0.0013

*Potential of gas production; †Rate of gas production; ‡Truly degraded organic matter; §Partitioning factor; ¶Standard error of means; ^{a,b,c,d}In each column, values with different letters are significantly different (P<0.05).

Discussion

In the current study, three cellulolytic isolates were investigated, all identified by 16S rRNA gene sequence analysis as belonging to the family of *Enterobacteriaceae* (SH13 and SH22 were presumptively identified as belonging to the genus *Escherichia*). Pang et al. (2017) isolated an *E. coli* strain with cellulose and hemicellulose-degrading activity from Inner Mongolia bovine rumen. Harsini et al. (2019) isolated *Enterobacter cloacae* L2 and *E. coli* Z2 from gastrointestinal tract of Arabian Horse whose CMCase activity was 2.05 and 2.43, respectively. Lokapirnasari et al. (2015) isolated *E. cloacae* WPL 214 from bovine rumen fluid produced cellulose enzyme, that indicated that *E. cloacae* WPL 214 can be used to hydrolyze fibrous feeding material. Cellulase enzyme of *E. cloacae* WPL 214 had endoglucanase activity of 0.09 U/ml, exoglucanase of 0.13 U/ml/min, and cellobiase of 0.10 U/ml/min at 35 °C and optimum pH 5. Rezaei et al. (2009) demonstrated that *Enterobacter cloacae* could be used as the sole microorganism to accomplish cellulose degradation. *Enterobacter cloacae* has been found to have endo 1,4-D-glucanase activity. Therefore, it is able to degrade cellulose (Sami et al, 2008).

In the present study, results indicated that changes in the pH cannot affect the growth of cellulolytic bacteria at 39 °C. The effect of pH on the growth of cellulolytic bacteria was studied and optimum pH for the growth was found between 7 to 7.5 (Hethener et al., 1992, Balamurugan et al, 2011,

Maruthamalai et al., 2012; Bholay et al., 2014). The temperature specificity of isolates showed that all of them grew at the three specified temperatures. However, growth and enzyme production were better at 39 °C than at 25 °C. There have been diverse reports on the optimal initial pH and temperature for cellulolytic enzyme production by *Paenibacillus* sp. In a previous study, *P. curdlanolyticus* B-6 was grown for enzyme production at pH 7.0 and 37 °C (Waeonukul et al. 2009). Furthermore, it was reported that the optimal initial pH and temperature for CMCase production by *P. polymyxa* were pH 5.5 and 37 °C, respectively (Kumar et al. 2012). Yoon et al. accounted that the optimal growth temperature for *P. terrae* was 30 °C. Liang et al (2014) reported the best incubation conditions of *Paenibacillus terrae* ME27-1 were pH 8.0 and 28 °C. The CMCase activity declined when the initial pH and incubation temperature were not optimal. In a previous study by Shinde et al (2017) *Enterobacter cloacae* and *Enterobacter aerogens* showed optimum growth at 35 °C. Also, Shinde et al (2017) reported that *Enterobacter* species were able to survive both acidic and alkaline environments.

Disappearance of dry matter is caused by these bacteria. Bacterial treatments increased CP and decreased NDF, iNDF and ADF content. *Escherichia* sp SH22 had the most enzymatic activity and the greatest impact on chemical composition of wheat straw. Consistent with current results,

Harsini et al. (2019) demonstrated that cellulolytic bacteria were isolated from the gastrointestinal tract of the Arabian horse, increased CP and decreased NDF, iNDF and ADF content. Also *P. polymyxa* L11 had the most enzymatic activity and the greatest impact on chemical composition of wheat straw. Most probably cellulose was partially degraded during the process. More enzymatic activity leads to more degradability and the chemical composition of wheat straw is more affected. Azizi-Shotorkhoft et al. (2016) reported that processing of wheat straw with bacterial isolates from the termite gut resulted in a higher DM loss. Similar to the study by Azizi-Shotorkhoft et al. (2016), the CP content of wheat straw increased when treated with *Ochrobactrum intermedium*. Additionally, Borji (2003) observed a 42% increase in CP content for wheat straw processed with bacteria isolated from the termite gut. Bioprocessing of wheat straw with *P. brevispora* leads to increase in crude protein and decrease in crude fibre (Arora & Sharma, 2011). Azizi-Shotorkhoft et al. (2016) reported no differences in NDF content of wheat straw when treated with bacterial isolates from the termite gut. Generally, decrease in NDF, iNDF and ADF content and increase in DM disappearance and CP content in wheat straw, treated with bacterial isolates, indicated that these isolates could use wheat straw as a carbon source, grow, and release more enzymes. The combination of bacterial mass and secreted cellular and extracellular enzymes by bacteria is the main reason for the increased CP content. On the other hand, an increase in protein due to an increase in microbial biomass may have reduced the NDF fraction and its constituents.

The result of the present study showed that bacterial isolates improved *in vitro* digestibility of DM, OM, CP, NDF and ADF. During processing of wheat straw with isolated bacteria, free sugars will be available for the rumen microbiota (Okano

et al., 2009), fibre content will be reduced and nutrient digestibility will be increased (Van Soest, 1994). Consistent with our results, Harsini et al. (2019) reported that processing of wheat straw by isolated bacteria from the gastrointestinal tract of the Arabian horse improved *in vitro* nutrient digestibility. Harsini et al. (2019) used *Paenibacillus polymyxa* L1, *Paenibacillus polymyxa* L12, *Enterobacter cloacae* L2 and *Escherichia coli* Z2, and reported that *Paenibacillus polymyxa* L12 showed the highest digestibility compared to control.

The dry matter digestibility of wheat and barley straw was treated with *Enterobacter cloacae*, *Ochrobactrum anthropi* and *Bacillus sphaericus*, which were isolated from termite gut Borji (2003). The processing period and type on lignocellulosic material source affect the digestibility of by-products (Borji 2003). The bacterial treatments enhanced DM, OM and ADF digestibility of wheat straw in study by Azizi-Shotorkhoft et al, (2016). Utilization of enzymatic extracts isolated from white-rot fungi enhanced the *in vitro* degradation of wheat straw (Rodrigues et al, 2008).

Treatment with bacterial isolates improved gas production, microbial biomass, and the TDOM of wheat straw. Similar results were also obtained by Harsini et al, (2019). Increasing the availability of substrate for production of microbial proteins may be one reason for increased gas production (Atasoglu et al, 1998). It is revealed that bioprocessing of wheat straw provided sufficient nutrients to support microbial protein synthesis by rumen microorganisms. Partitioning factor, microbial biomass and microbial biomass efficiency were higher for SH2 isolate. In fact, this treatment increased the fermentation and produced less gas and more microbial protein.

Three bacteria (SH2, SH13, and SH22) with cellulolytic activity were isolated from the foregut of the dromedary camel. These three bacterial isolates decreased acid

detergent fibre and neutral detergent fibre of wheat straw, increased digestibility of dry matter, organic matter, crude protein, acid

detergent fibre and neutral detergent fibre, and enhanced nutrition value of wheat straw.

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Conflict of interest

The authors declare that they have no conflict of interest.

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عمل آوری مواد لیگنوسلولزی با باکتری‌های سلولولیتیک جدا شده از شکمبه شتر تک کوهانه

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چکیده

محصولات فرعی کشاورزی اغلب برای تغذیه دام استفاده می‌شوند، اما بیش‌تر ارزش غذایی پایینی دارند. این مطالعه به منظور بهبود ارزش تغذیه‌ای کاه گندم با استفاده از باکتری‌های سلولولیتیک جدا شده از دستگاه گوارش شتر تک‌کوهانه انجام شد. مایع شکمبه از چهار شتر دارای فیستولای شکمبه‌ای جمع‌آوری شد. باکتری‌های تجزیه‌کننده سلولز با استفاده از محیط اصلاح شده با کربوکسی متیل سلولز (CMC) جداسازی شدند. فعالیت کربوکسی متیل سلولاز (CMCase) با اندازه‌گیری آزادسازی قندهای احیاکننده تعیین شد. pH و دمای بهینه برای رشد و فعالیت CMCase جدایه‌ها تعیین شد. سه جدایه (SH13، SH2 و SH22) حداکثر ظرفیت هیدرولیز را نشان دادند و برای بررسی بیشتر انتخاب شدند. تجزیه و تحلیل توالی ژن *S rRNA* این جدایه‌ها نشان داد که جدایه‌ها به ترتیب ۹۹، ۹۴ و ۹۸ درصد شباهت با *شریشیا کلی*، *انتروباکتریاسه* و *شریشیا کلی* داشتند. کاه گندم با باکتری‌های جدا شده در محیط مایع به مدت ۶ هفته انکوبه شد. نتایج نشان داد که تلقیح با ایزوله‌های SH22 منجر به بیش‌ترین ناپدید شدن ماده خشک و محتوای پروتئین خام و کم‌ترین مقدار فیبر نامحلول در شوینده خنثی (NDF)، iNDF و فیبر نامحلول در شوینده اسیدی (ADF) شد. تیمار با ایزوله SH13 بیش‌ترین قابلیت هضم NDF و ADF را داشت و تیمار با SH2 دارای بالاترین ماده آلی تجزیه شده واقعی (TDOM) و فاکتور بخش‌بندی (PF) بود. داده‌های ما نشان می‌دهد که شاید باکتری‌های جدا شده از دستگاه گوارش شتر بتوانند قابلیت هضم و ارزش غذایی کاه گندم را افزایش دهند.

کلمات کلیدی: شتر، باکتری سلولولیتیک، قابلیت هضم، کاه گندم

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