

Research Paper

Isolation of Cellulose Degrading Bacteria from Rumen and Evaluation of Cellulase Production Potential Using Lignocellulosic Substrate

Olyad Erba Urgessa*, Yimeslal Atnafu Sima, Ameha Kebede, Mahafuz Mohammed

School of Biological Sciences and Biotechnology, College of Natural and Computational Sciences, Haramaya University, Ethiopia., P. O. Box 138, Dire Dawa, Ethiopia

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Abstract

The utilization of cheaper substrates reduces cost of cellulase production and there is the need for cellulolytic microbes capable of degrading lignocellulosic materials. Therefore, this study was aimed to isolate and identify bacteria from rumen fluid, and evaluate wheat straw, sorghum straw and bagasse for cellulase production. Rumen fluid were collected from cattle, goat and sheep slaughtered at Haramaya town abattoir. Wheat and sorghum straw, and bagasse were obtained from Haramaya University research plot and Wonji-Shewa Sugar Factory, respectively. One-way analysis of variance (ANOVA) was used for data analysis using SPSS version 20. Microscopic and Biochemical tests of potential cellulase producers were carried on Luria Bertoni Agar enriched with 1% Carboxymethyl cellulose of sodium (CMC-Na). Cellulase production was evaluated using submerged fermentation. The potential CMC degrading isolate (HUG-3b) and (HUS-2a) from goat and sheep rumen fluids, respectively, were identified as *Pseudomonas* sp. and isolate (HUC-4b) from cattle rumen fluid was identified as *Aeromonas* sp. *Pseudomonas* sp from goat rumen produced significantly the highest carboxymethyl-cellulase (CMCase) and filter-paperase (FPase) of 1.54 ± 0.10 U/ml, 1.46 ± 0.09 U/ml from fermentation of 1% w/v Carboxymethyl cellulose used as standard and 1% wheat straw, respectively ($p < 0.05$), whereas, *Pseudomonas* sp. from sheep rumen produced significantly the highest CMCase of 1.81 ± 0.27 U/ml from fermentation of 1% wheat straw ($p < 0.05$) and insignificantly highest FPase of 1.27 ± 0.22 U/ml from fermentation of 1% bagasse ($p > 0.05$). *Aeromonas* sp. produced insignificantly the highest CMCase and FPase of 2.31 ± 0.17 U/ml, 1.50 ± 0.02 U/ml from fermentation of 1% and 2% wheat straw, respectively ($p > 0.05$). It can be concluded that the fermentation of 1% and 2% wheat straw by *Aeromonas* can be utilized for the maximum production of cellulase. Identification of the species or strain of isolate using molecular technique will be recommended.

1. Introduction

Cellulase is an enzyme capable of hydrolyzing β -1, 4-glycosidic bond in cellulose (Ekinci et al., 2002). Cellulose is an abundant natural biopolymer on earth and most dominating agricultural waste. This cellulosic biomass is a renewable and abundant resource with great potential for bioconversion to value-added bio-

products (Sadhu and Maiti, 2013). Cellulases are commonly used in many industrial applications (Cherry et al., 2003; Kuhad et al., 2011; Sadhu and Maiti, 2013).

Cellulases contribute to 8% of the demand in the global enzyme. The cost of using commercial cellulase enzymes is expensive, making it less economical

* Corresponding author, e-mail: olyaderba@gmail.com

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(Kuhad et al., 2011). The demands for more stable, highly active and specific enzymes grow rapidly (Cherry et al., 2003). Therefore, the low production cost and good enzymatic activity increase the value of cellulase for commercial use (Gao et al., 2008). The utilization of cheaper substrates reduces both substrate cost and usage of costly chemicals for the fermentation procedure (Zhang et al., 2006). There are multiple sources of lignocellulosic waste materials from industrial and agricultural processes, e.g., vegetable and fruit peels, sugarcane bagasse, sawdust and agro-residues. The presence of the higher cellulose percentage in many of these wastes makes them cheap sources of substrates for the production of highly demanded cellulase, but the presence of the lignin component slows down the degradation rate (Maki et al., 2011). Thus, cellulolytic microbes capable of degrading lignocellulosic substrates are needed (Kuhad et al., 2011).

Significant attention has been devoted to the knowledge of cellulase production and the challenges in cellulase research especially in the direction of improving the process economics of various industries (Sukumaran et al., 2005; Kuhad et al., 2011). One way of improving the economics would be to search for new potent microorganisms that have the ability to consume a variety of carbon sources and the potential to produce higher levels of stable cellulases. In line with this, it was reported that rumen of ruminants harbors various microorganisms that are active in cellulose breakdown (Oyeleke and Okusanmi, 2008; Das and Qin, 2012) and thus, it can be an excellent environment for microbial screening for cellulase production. Cellulase research has been concentrated mostly in fungi but there is increasing interest in cellulase production by bacteria due to their higher growth rate and thermo stable and alkali stable properties (Sadhu and Maiti, 2013).

Evaluation and optimization of various carbon sources was conducted by several researchers for cellulase production using bacterial isolates from different samples (Immanuel et al., 2006; Sethi et al., 2013; Liang et al., 2014; Lugani et al., 2015; Nandimath et al., 2016). The concentrations and intervals considered for these optimizations were different. Therefore, this study was aimed at production of cellulase by isolating and identifying potential cellulase producing rumen bacteria, and evaluating and optimizing the cheap carbon source for the production.

2. Material and Methods

2.1. Collection and Preparation of Lignocellulosic Substrates

Wheat and sorghum straws were obtained from Haramaya University research plot and bagasse was collected from Wonji-Shewa Sugar Factory. After washing and drying the samples at 70°C, wheat and sorghum straws were ground in an electric grinder. All samples were stored in polythene bags for subsequent use as fermentation medium (Kapoor et al., 2010).

2.2. Rumen Sample Collection

Fresh rumen fluid of cattle, sheep and goat were collected from Haramaya town slaughterhouse under aseptic conditions following methods of Begum et al. (2013). The collected rumen fluids were filtered through a layer of muslin cloth. Then, the rumen fluid was poured into labeled sterile flasks and kept in a sterilized container at 4°C.

2.3. Isolation of Cellulase Producing Bacteria

Rumen bacteria were isolated directly from each rumen fluid samples by serial dilution technique on Luria Bertoni Agar (LBA) plates, pH 7. One mL of filtered each samples was added to 9 mL of normal saline solution in sterile test tubes and serially diluted up to 10^{-5} . An aliquot of 100 μ L from 10^{-5} diluted test tube was transferred using sterile pipette into LBA (1 g tryptone, 0.5 g yeast extract and 1 g NaCl, 1.5 g agar) and spread using sterile bent glass rod and incubated at 37°C for 48 hrs. After incubation, separated colonies were picked from each plate and separately transferred to fresh Luria Bertani Broth (LBB) and LBA consecutively until pure cultures were obtained (Ibrahim et al., 2007). The pure isolates appeared on the plates were selected and kept on LBA slants at 4°C.

2.4. Screening Cellulase Producing Bacteria

Before the day screening test performed, preserved Luria Bertoni Agar slants (LBA) cultures were inoculated into fresh Luria Bertoni Agar (LBA) and allowed to grow for 24 hr. The LBA medium supplemented with 1% Carboxymethyl Cellulose was used for screening purpose. Single colonies were picked using sterile loop, inoculated and incubated at 35°C for 48 hrs. After incubation, the colonies were screened for cellulase production by flooding the plates with 0.1%

Congo red for 15 min. Then, excess dye was discarded and the plate surface was carefully washed with 1 N saline solution (Shankar and Isaiarasu, 2011). The isolate producing potential cellulase activity was identified by measuring the diameter (mm) of clear zones. The isolates which showed maximum clear zones were kept for bacteriological identification and small scale submerged fermentation.

2.5. Identification of Potential Cellulase Producing Isolates

The potent cellulase producing isolates were first characterized by Gram stain and then by biochemical tests such as citrate utilization, methyl red test, motility test, starch hydrolysis, indole production, catalase production, oxidase test, urease production and sugar fermentation tests. The results were compared with Bergey's Manual of Determinative Bacteria for identification (Buchanan and Gibbons, 1974). The pure culture of identified isolates were grown on screening medium agar slant individually and stored at 4°C with regular sub-culturing (Immanuel et al., 2006) and maintained as stock culture from which inoculums were prepared.

2.6. Evaluation of Cellulase Production by the Isolate using lignocellulosic substrate

Cellulase production potential of the isolates using CMC, bagasse, and wheat and sorghum straw were evaluated following modified methods of Yang et al. (2014). Submerged fermentation medium of 200 mL was prepared from LB broth medium supplemented with 1% w/v CMC-Na, pH 7. Similarly, the media were prepared by mixing 0.5%, 1%, 1.5%, 2% and 2.5 % w/v of each substrate separately instead of CMC-Na in flasks. Twenty five (25) mL of the liquid medium was transferred to 100 mL capacity flasks and was autoclaved at 121°C for 15 min. After cooling, all the flasks containing different substrates were inoculated with 1 mL of suspension of the isolate. Then, they were incubated in shaker incubator at 40°C, 120 rpm for 48 hours. Afterwards, the cell culture broth was filtered using Whatmann No 1 filter paper and then centrifuged at 3000 rpm for 6 min. Pellet was discarded while supernatant containing cellulase enzyme was taken to assay CMCase and FPase.

2.7. Assay of Cellulase Activity

Method described by Lokapirnasari et al. (2015) with slight modification was used to assay endo-(1, 4)-β-D-glucanase activity. Briefly, 0.5 mL of crude cellulase (supernatant) was mixed with 1 mL substrate (1% CMC-Na in 0.05 M sodium citrate phosphate buffer at pH 7) and it was incubated in a water bath at 50°C for 30 min. Then, 1.5 mL of DNS was added into tube and the mixture was placed in 100 °C water bath for 10 min to stop reaction along with control (containing 0.5 ml enzyme mixed with 1.5 μl DNS and 1 mL substrate, without incubation at 50 °C for 30 min). The tubes were cooled in ice water for 20 min. Absorbance of all the samples was recorded using spectrophotometer at 540 nm.

For total cellulase activity determination, modified method of Majeed et al. (2016) was used. Strip of Whatman No.1 filter paper (1x6 cm) was immersed in 1 mL of 0.05 M sodium citrate buffer of pH 7.0. Then, 0.5 ml of crude cellulase (supernatant) was added and incubated in a water bath at 50°C for 1 hr and the assay continued as described above.

The cellulase activity was determined by using a calibration curve for glucose. One unit of enzyme activity was defined as the amount of enzyme required to form 1 μmol of reducing sugar equivalent to glucose per unit minute under the assay conditions and was calculated from the equation 1 used by Ghose (1987).

$$\frac{\text{Units}}{\text{mL of enzyme}} = \frac{(\mu\text{mol of glucose})(df)}{(V_E)(t)} \quad (1)$$

Where, V_E is the volume of the enzyme used which was 0.5 mL, t is the reaction time taken in min which was 30 min and $df = 1$

μmol of glucose produced was determined from enzyme absorbance and standard curve of glucose (equation 2).

$$Y = 0.0572 X + 0.0079 \text{ in mg/mL} \quad (2)$$

By converting the equation in μmol/ml, it becomes equation 3:

$$Y = (0.0572 X + 0.0079) \times \frac{1000}{180} \text{ in } \mu\text{mol/mL} \quad (3)$$

Where, Y = Optical density measured at 600 nm and X = amount of glucose equivalents liberated from CMC-Na, or filter paper hydrolysis. All the experiments were carried out in triplets.

2.8. Statistical Analysis

Data were analyzed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 20. One-way analysis of variance (ANOVA) was used for analysis. Duncan test was used for evaluating significant difference between mean values of three replications at $p < 0.05$.

3. Results

3.1. Isolation and Identification of Cellulase Producing Bacteria

A total of 26 bacterial isolates were obtained from goat, sheep and cattle rumen samples. From the 26 isolates, only five isolate showed clear zones. The diameters of clear zones formed by the isolates were shown in Table 1. For each rumen fluid, isolate with the highest clear zone was identified by morphology and biochemical test. Morphology and biochemical characteristics of the isolates were shown in Table 2. Based on biochemical and morphological test results, HUG-3b and HUS-2a isolates were identified to belong to *Pseudomonas* sp. and HUC-4b isolate was identified to belong to *Aeromonas* sp. The indole test demonstrated that HUC-4b isolate has the ability to decompose tryptophan to indole, pyruvic acid and ammonium (NH_4^+). When Kovac's Reagent was mixed with culture broth, the formed indole was reacted with the reagent and the solution turns from yellow to cherry red. This was showed positive result for indole test. Starch hydrolysis test indicated that the isolate has ability to break the starch (amylose and amylopectin) molecules into smaller glucose subunits. When iodine was added to the agar, it reacts with the starch to form a dark brown color and the hydrolyzed area was observed as clear zone around the bacterial growth showing positive for starch hydrolysis (Table 2).

Table 1: Top five cellulase producing bacterial isolates with zone of inhibition on Congo red stained CMC agar plate

S. No	Samples	Colony code	zone of hydrolysis diameter (mm)
1	Goat rumen fluid	HUG-2b	17.5
2	Goat rumen fluid	HUG-3b	18
3	Goat rumen fluid	HUG-5a	12
4	Sheep rumen fluid	HUS-2a	16.5
5	Cattle rumen fluid	HUC-4b	12

3.2. Evaluation of Cellulase Production by the Isolate using lignocellulosic substrate

The evaluation and optimization of concentration of carbon source has been carried out by taking five levels of concentrations of wheat straw, sorghum straw and bagasse. The results were shown in Figure 1(a), Figure 1(b), Figure 2(a), Figure 2(b), Figure 3(a) and Figure 3(b).

Pseudomonas sp. (HUG-3b) from goat rumen produced significantly the highest ($p < 0.05$) CMCCase from 1% Carboxymethyl cellulose used as standard. Next to the standard (1.54 ± 0.10 U/mL), the highest CMCCase activity (1.36 ± 0.21 U/mL) was produced at 1% wheat straw content in fermentation medium (Figure 1(a)). At 1% substrate content in fermentation medium, the isolate produced significantly the highest ($p < 0.05$) FPase activity (1.46 ± 0.09 U/ml) from wheat straw as compared to other substrate including 1% Carboxymethyl cellulose used as standard (Figure 1(b)). *Pseudomonas* sp. (HUS-3b) from sheep rumen produced significantly the highest ($p < 0.05$) CMCCase activity (1.81 ± 0.27 U/mL) at 1% wheat straw content in fermentation medium as compared to other substrates including 1% Carboxymethyl cellulose used as standard (Figure 2(a)). At 1% substrate content in fermentation medium, the isolate produced the highest FPase activity (1.27 ± 0.22 U/ml) in 1% bagasse content in fermentation medium. But, it was not significantly different ($p > 0.05$) compared to other substrates and concentrations including 1% Carboxymethyl cellulose used as standard (Figure 2(b)).

Aeromonas sp. (HUC-4b) from rumen cattle produced the highest CMCCase activity (2.31 ± 0.17 U/mL) in fermentation medium containing 1% wheat straw, but the value is not significantly ($p > 0.05$) different from that produced from wheat straw at 1.5 and 2% w/v (Figure 3(a)). The highest FPase activity of 1.5 ± 0.02 U/mL was produced at 2% wheat straw content in the medium and the value is not significantly different from that of 1% standard used (Figure 3(b)).

Table 2: Morphology and biochemical characteristics of the isolates with maximum clear zone.

Characteristics	bacterial isolates		
	HUG-3b	HUS-2a	HUC-4b
Gram staining	Negative	Negative	Negative
Morphology	Rod	Rod	Rod
Motility test	Positive	Positive	Positive
Glucose fermentation	Positive	Positive	Positive
Lactose fermentation	Positive	Positive	Positive
Sucrose fermentation	Positive	Positive	Positive
Mannitol fermentation	Positive	Positive	Positive
Dextrose fermentation	Positive	Positive	Positive
Arabinose fermentation	Positive	Positive	Positive
Gas formation	Negative	Negative	Negative
Citrate utilization	Positive	Positive	Negative
Urease test	Positive	Positive	Positive
Indole test	Negative	Negative	Positive
Catalase test	Positive	Positive	Positive
Starch hydrolysis test	Negative	Negative	Positive
Oxidase test	Positive	Positive	Positive
Methyl red test	Negative	Negative	Positive

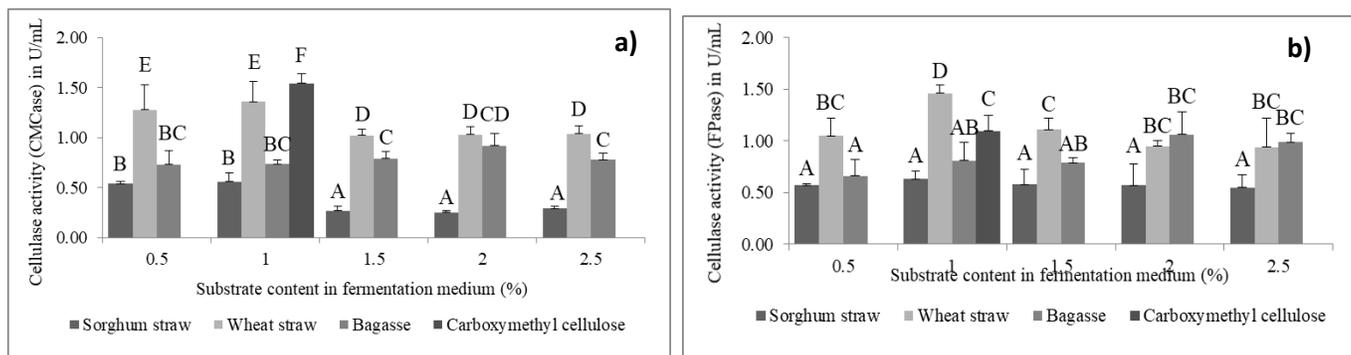


Figure 1: The effect of substrate concentration on cellulase production by *Pseudomonas* sp. (HUG-3b). Enzyme activity of cellulase extract determined using CMC as substrate (a). Enzyme activity of cellulase extract determined using Filter paper as substrate (b). 1% CMC in fermentation medium used as standard. Error bars represent Standard deviation. Letters compare means of tree triplicates cellulase activities of substrates across all concentrations level at $p < 0.05$.

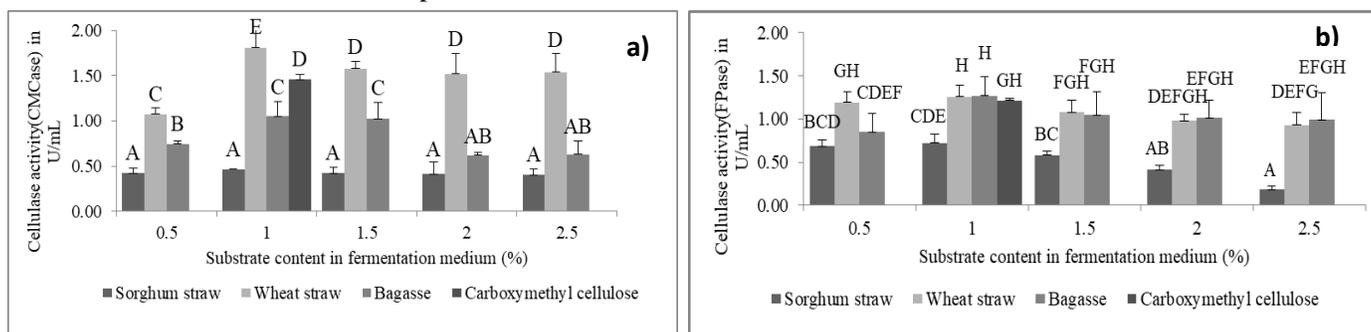


Figure 2: The effect of substrate concentration on cellulase production by *Pseudomonas* sp. (HUS-2a). Enzyme activity of cellulase extract determined using CMC as substrate (a). Enzyme activity of cellulase extract determined using Filter paper as substrate (b). 1% CMC in fermentation medium used as standard. Error bars represent Standard deviation. Letters compare means of tree triplicates cellulase activities of substrates across all concentrations level at $p < 0.05$.

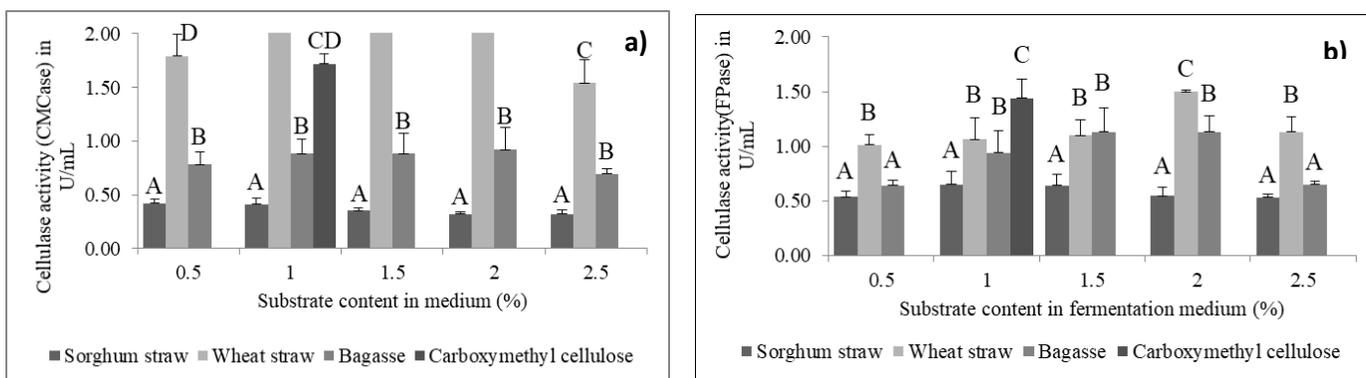


Figure 3: The effect of substrate concentration on cellulase production by *Aeromonas* sp. (HUC-4b). Enzyme activity of cellulase extract determined using CMC as substrate (a). Enzyme activity of cellulase extract determined using Filter paper as substrate (b). 1% CMC in fermentation medium used as standard. Error bars represent Standard deviation. Letters compare means of tree triplicates cellulase activities of substrates across all concentrations level at $p < 0.05$.

4. Discussion

4.1. Isolation and Identification of Cellulase Producing Bacteria

In this study, a total of 26 isolate colonies from goat, sheep and cow rumen were tested for CMC-degrading activities. Thirteen isolates were able to grow on CMC-containing medium, from which only five isolates showed clear zones (Table1). In line with this, Pinheiro et al. (2019) reported that the isolates from gastrointestinal tract of giant land snail were able to grow on CMC as their sole carbon sources, but did not display visible degradation halos for CMCCase. Using CMC-Na as the sole carbon source in selective media, followed by the Congo red staining method for preliminary isolation of cellulolytic bacterium, is widely considered to be the best method for preliminary screening of cellulolytic bacteria because it is simple and fast (Yang et al., 2014). A review paper by Sandhu and Maiti (2013) concluded that the diameter of the clearing zone may not accurately reflect the true cellulase activity.

The maximum clear zone forming isolates, HUG-3b, HUS-2a and HUC-4b from goat, sheep and cattle rumen, respectively, were characterized by morphological and biochemical tests (Table 2). Based on the microscopic results and comparing biochemical test character with Bergey's manual of determinative bacteriology, the isolate was identified as the *Pseudomonas* sp. (HUG-3b, HUS-2a), and *Aeromonas* sp. (HUC-4b). Several studies conducted on screening

cellulolytic bacteria from different environments, such as rumen of Korean native goat (Min et al., 1994), rumen of cow, sheep, and goat (Oyeleke and Okusanmi, 2008), gut of the *Ctenopharyngodon idellus* (Jiang et al., 2011), paper mill sludges (Maki et al., 2011), rumen fluid of goat (Begum et al., 2013), soil (Sethi et al., 2013), rumen of inner Mongolia sheep (Liu et al., 2014), cecum part of intestine of Tibetan pig (Yang et al., 2014), rumen fluid waste of bovine abattoir (Lokapinasari et al., 2015), rumen of *Labeo rohita* (Majeed et al., 2016), molasses (Islam and Roy, 2018) and dumping sites (Chakraborty et al., 2019). In these studies, a high cellulolytic degrading isolates were identified as *Actinomyces* sp., *Pseudomonas* sp., *Aeromonas* sp., *Paenibacillus*, *Paracoccus pantotrophus*, *Pseudomonas fluorescens*, *Fibrobacter succinogenes*, *Bacillus subtilis* BY-2, *Enterobacter cloacae*, *Aeromonas bestiarum*, *Aeromonas* sp., and *Aeromonas hydrophila* ASM-S32, respectively.

4.2. Evaluation of Cellulase Production by the Isolate using Lignocellulosic Substrate

The evaluation and optimization of concentration of carbon source has been carried out by taking five levels of concentrations of wheat straw, sorghum straw and bagasse were evaluated. *Pseudomonas* sp. (HUG-3b) from goat rumen produced the highest CMCCase in fermentation medium containing wheat straw at all levels of concentrations as compared to sorghum straw and bagasse. The isolate produced significantly the highest FPase (1.46 ± 0.09 U/mL) in fermentation

medium containing 1% w/v wheat straw as compared to that produced in other concentration of substrates including the standard (Figure 1 (a) and (b)). The higher cellulase production from wheat straw is may be because of relatively higher cellulose content in wheat straw as cellulases are inducible enzymes requiring inducer substrate containing cellulose. The highest CMCCase (1.36 ± 0.21 U/mL) was produced at 1% wheat straw content in fermentation medium. But, it was significantly lower as compared to that produced in 1% Carboxymethyl cellulose used as standard (1.54 ± 0.10 U/mL). This is because of carboxymethyl cellulase is easily soluble and good substrate inducer for endocellulase production (CMCase) by the isolate. Evaluating different carbon sources for production of cellulase, Min et al. (1994) reported the best CMCCase of 0.06 U/mL produced from fermentation of CMC substrate by *Actinomyces* sp. isolated from rumen of Korea goat.

For *Pseudomonas* sp. (HUS-2a) from sheep, the highest CMCCase was produced in fermentation medium containing wheat straw at all levels of concentrations (Figure 2(a)). The highest CMCCase (1.81 U/mL) was produced in 1% wheat straw containing fermentation medium. The isolate produced the highest FPase (1.27 ± 0.22 U/mL) in 1% bagasse (Figure 2(b)). This is may be because of the feeding habit of the sheep on fiber foodstuff from which the rumen was collected and bacteria was isolated. In previous study, Liu et al. (2014) reported significantly higher CMCCase of 62.5 ± 3.0 U/mL and FPase of 30.8 ± 5.4 U/mL produced from fermentation of filter paper by *Fibrobacter succinogenes* isolated from Inner Mongolia sheep.

Aeromonas sp. (HUC-4b) from cattle rumen produced the highest CMCCase in fermentation medium containing wheat straw at all levels of concentrations (Figure 3(a)). The highest CMCCase of 2.31 ± 0.17 U/mL was produced at 1% wheat straw. Significantly, the highest FPase (1.5 ± 0.02 U/mL) was produced by the isolate at 2% w/v wheat straw (Figure 3(b)). Evaluating the various carbon sources at 1% w/v, Islam and Roy (2018) reported 0.5 U/mL of CMCCase produced from fermentation of CMC substrate by *Aeromonas* sp. isolated from molasses which is significantly less than the present study result for CMC substrate (1.72 ± 0.09 U/mL) at the same level concentration. In another study,

Chakraborty et al. (2019) reported significantly greater CMCCase of 32.8 U/mL from fermentation of 1% w/v CM by *Aeromonas hydrophila* ASM-S32 isolated from dumping site compared to any cellulase activity values obtained in this study. For bagasse, the highest FPase of $1.13 \pm 0.02 \pm 0.22$ or 0.15 U/mL was produced at 1.5 and 2% w/v substrate content in fermentation medium. In previous similar study, the concentrations of bagasse substrate (0.5, 1, 1.5, 2, and 2.5% w/v) were evaluated for cellulase production by *Aeromonas bestiarum* (KF625168) isolated from the gut of *labeo rohita* and 3.766 U/mL of FPase was produced at 2.5% (Majeed et al., 2016).

The study results showed that the *Pseudomonas* sp. (HUG-3b) and (HUS-2a), and *Aeromonas* sp.(HUC-4b) exhibited different responses, and the best unit of CMCCase enzyme activity was obtained from wheat straw for all isolate and assay types as compared to sorghum straw and bagasse. Evaluating different agricultural wastes such as sugarcane bagasse, wheat straw, rice husk, defatted soybean meal, corn cobs and wheat bran at 2% w/v for CMCCase production by *Bacillus subtilis* BS06, Arshad et al. (2017) reported a different value that of all these tested substrates, maximum CMCCase (8.0 ± 0.32 IU) production was from submerged fermentation of sugarcane bagasse.

In previous studies, the optimization of concentration of carbon source has been carried out by several researchers for different microbe or microbe isolated and selected from different sample. Immanuel et al. (2006) evaluated the concentrations of coir substrate (0.5, 0.1, 1.5 and 2% w/v) for cellulase production by *Cellulomonas*, *Bacillus* and *Micrococcus* spp isolated from coir retting effluents and found that the endoglucanase enzyme activity was more at 1.5% . Sethi et al. (2013) evaluated the effect of glucose, lactose, and sucrose at the concentration of (1, 2, 3, 4, 5% w/v) for cellulase production by *Pseudomonas fluorescens* isolated from soil and obtained the maximum CMCCase of 1.5, 0.4, 0.7 U/mL at 5%, respectively for all substrates. Liang et al. (2014) evaluated the effect of wheat bran concentration of (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 % w/v) for cellulase production by aerobic *Paenibacillus terrae* isolated from soil and obtained the maximum CMCCase of 1.4 U/mL at 5%. Lugani et al. (2015) evaluated the effect of CMC concentration of (0.5, 1,

1.5, 2, 2.5% w/v) for cellulase production by *Bacillus* sp. Y3 isolated from cow dung soil and obtained the maximum FPase and CMCase activity of 3.78 and 4.52 U/mL, respectively at 1%.

Comparison between the values of this study and the previous ones, there are credible variations among them. Some of the values in the previous studies are nearly the same; others are very low and still others very high. According to Liu et al. (2014), it is not very consistent to compare the cellulose degradation rates and cellulase activities because of different strains of microbial sp. involved, different kinds of cellulose sources and different procedures of activity determination methods.

5. Conclusions

The study was conducted to isolate effective cellulase producing bacteria from goat, sheep and cattle rumen fluid. The diameter of halo zone on CMC agar plate showed that HUG-3b, HUS-2a and HUC-4b from goat, sheep and cattle rumen, respectively, were potential cellulase producers. Based on the biochemical

and morphological characteristics, these strains were identified to be *Pseudomonas* sp. (HUG-3b, HUS-2a), and *Aeromonas* sp. (HUC-4b). Maximum cellulase activity was found different for three isolated bacterial sp. The study results revealed that the optimum production of cellulase by the three isolates was in 1 % wheat straw containing fermentation medium under studied condition. It can be concluded from the study that the *Aeromonas* sp. (HUC-4b) had the best cellulase degrading ability, and 1% and 2% wheat straw was the best lignocellulosic substrate that can be utilized for production of cellulase. Further molecular technique identification of the isolated and selected bacterial sp. will be required.

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