

Cellulolytic bacteria from migratory ruminants: Isolation, characterization, and application in biomass-to-biogas conversion”

R. Kumar*, O. Zaffar, S. Bhardwaj

Department Of Agricultural Engineering, College of Agriculture Himachal Pradesh Krishi Vishvavidyalay, Palampur 176062 India

*Corresponding author Email: rajinder.kumar226@gmail.com

Journal of Livestock Science (ISSN online 2277-6214) 17: 767-774

Received on 23/8/25; Accepted on 14/9/26; Published on 18/9/26

doi. 10.33259/JLivestSci.2026.767-774

Abstract

The present study was aimed to explore the capability of cellulolytic bacteria, from rumen content of migratory Gaddi sheep and goats of Himachal Pradesh to degrade lignocellulosic agricultural residues and improve the biogas production. A total of eighteen rumen content samples of goats and sheep were collected in sterile container from a slaughter site near Sri Sai University in Palampur. These samples were cultured on carboxymethylcellulose (CMC) agar medium, yielded 40 bacterial isolates, out of which 10 showed cellulolytic activity. Based on cultural, morphological, and biochemical analyses, seven isolates were morphological and biochemically identified as *Escherichia coli* (2 isolates), *Enterobacter* spp. (1), *Pseudomonas* spp. (1), *Micrococcus* spp. (1), and two unidentified Gram-negative bacilli. These cellulolytic bacteria were further demonstrated for enzymatic assays such as β -glucosidase, endoglucanase, and cellobiohydrolase activities and it was observed that *Enterobacter* spp. (SUNG-7) and *Pseudomonas* spp. (SUNG-25) exhibited the highest cellulolytic production. Enzyme optimization production experiments indicated that maximum enzyme production occurred at pH 5, with incubation periods ranging from 72 to 120 hours, using glucose as the preferred carbon source and yeast extract as the optimal nitrogen source. Finally, potential cellulose degrading isolates, (*Enterobacter* spp., SUNG-7 + *Pseudomonas* spp., SUNG-25) were employed for pre-treatment of paddy straw for three-weeks and the biogas production experiment from digested and undigested paddy straw in batch digester was carried out under laboratory condition. The results of biogas production from digested paddy straw with cow dung revealed an increase in biogas production more than 15% with respect to undigested paddy straw. Overall, our findings suggest that cellulolytic bacteria derived from the rumen content can serve as an effective and sustainable approach for pre-treatment of biomass like paddy straw which results in enhancing biogas generation under ambient conditions.

Keywords: Cellulolytic bacteria, *Pseudomonas*, agricultural waste, rumen microbiota, biogas production

Introduction

Agricultural operations in India generate approximately 500-700 million tonnes of lignocellulosic residues annually, including rice and wheat straw, paddy and maize husks, sugarcane bagasse and tops, cotton stalks, and corn cobs. A considerable proportion is used as animal feed, bedding, fuel, or is burned, while an estimated 100-170 million tonnes remain underutilized, representing a substantial renewable biomass resource (Ginni et al., 2021). Efficient conversion of these residues into bioenergy is, however, constrained by the recalcitrant structure of lignocellulose, particularly the complex association of cellulose, hemicellulose, and lignin. Biological pretreatment using lignocellulolytic microorganisms provides an environmentally friendly alternative to conventional physical and chemical methods. Although fungi are well known for lignin degradation, bacterial systems offer advantages such as rapid growth, metabolic versatility, adaptability to fluctuating environmental conditions, and relatively greater potential for genetic manipulation (DeAngelis et al., 2013; Jones et al., 2018). Bacterial lignocellulolytic enzymes, including cellulases, hemicellulases, and lignin-modifying enzymes, therefore have considerable potential for biomass conversion and biofuel production. In particular, microbial consortia can improve lignocellulose degradation through synergistic interactions among different functional groups of microorganisms. Cellulose is primarily degraded through the coordinated action of endoglucanases (EC 3.2.1.4), exoglucanases or cellobiohydrolases (EC 3.2.1.91), and β -glucosidases (EC 3.2.1.21). Endoglucanases cleave internal β -1,4-glycosidic bonds, exoglucanases release cellobiose from cellulose chain ends, and β -glucosidases hydrolyze cellobiose into glucose. These enzymes act synergistically to convert complex cellulose into fermentable sugars, while oxidative enzymes such as lytic polysaccharide monooxygenases and phosphorolytic enzymes may further contribute to cellulose degradation (Behera et al., 2017). Cellulolytic microorganisms naturally occur in soil, compost, decaying plant material, and the gastrointestinal tracts of herbivorous animals and have important applications in biomass decomposition and industrial enzyme production.

The rumen represents a particularly rich natural source of lignocellulolytic microorganisms. Rumen digestion involves a complex microbial community comprising bacteria, fungi, protozoa, and archaea that collectively degrade plant cell walls and convert carbohydrates into volatile fatty acids (VFAs), which serve as major energy sources for ruminants (Henderson et al., 2015; Bhujbal et al., 2022; Mizrahi & Jami, 2021). Among these microorganisms, bacteria play a central role in cellulose and hemicellulose degradation, while fungi and protozoa contribute through physical penetration and enzymatic degradation of plant tissues. The coordinated activity of these microorganisms facilitates carbon, nitrogen, and hydrogen cycling within the rumen ecosystem (Gharechahi et al., 2023; Leahy et al., 2022). Several bacterial genera, including *Bacillus*, *Bacteroides*, *Cellulomonas*, *Clostridium*, *Ruminococcus*, *Streptomyces*, *Acetovibrio*, and *Thermomonospora*, are known for their cellulolytic capabilities. Bacteria are attractive sources of cellulolytic enzymes because of their rapid growth, metabolic diversity, adaptability, and relatively simple cultivation and genetic manipulation. Anaerobic bacteria may also organize cellulolytic and hemicellulolytic enzymes into multi-enzyme complexes such as cellulosomes, thereby facilitating efficient lignocellulose hydrolysis (Malgas et al., 2017). Microbial cellulases have wide industrial applications in the pulp and paper, textile, food, feed, detergent, starch-processing, and biofuel industries (Kuhad et al., 2011; Singh et al., 2019; Ejaz et al., 2021).

Anaerobic digestion (AD) of agricultural residues is an attractive approach for renewable energy generation, and its efficiency can be improved by biological pretreatment with cellulolytic microorganisms. Compared with physical and chemical pretreatments, microbial pretreatment is generally milder, more selective, and less likely to generate inhibitory compounds (Wagner et al., 2018). Ruminants are therefore promising reservoirs for isolating efficient cellulolytic bacteria. The profitability of small ruminant rearing is under threat globally and require new avenue of income (Djimon et al., 2024). Gaddi sheep and goats, which are traditionally managed under migratory conditions in the Himalayan region and consume diverse vegetation, may harbour unique ruminal microbial communities adapted to the degradation of fibrous plant materials (Sharma et al., 2017). Therefore, the present study was undertaken to isolate and characterize cellulolytic bacteria from the rumen contents of Gaddi sheep and goats, evaluate their cellulolytic enzyme-producing potential, and assess their effectiveness in degrading agricultural residues for enhanced biogas production

Materials and Methods

Sample Collection

A total of 18 rumen-content samples were collected aseptically in sterile vials from freshly slaughtered Gaddi sheep and goats near Sri Sai University, Palampur, Himachal Pradesh, India. The samples were transported immediately to the Microbiology Laboratory, Department of Agricultural Engineering, College of Agriculture, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur (32.0981° N, 76.5433° E), for isolation of cellulolytic bacteria. Samples were processed immediately; when immediate processing was not possible, they were stored at 4°C until further analysis.

Isolation of Cellulolytic Bacteria

Sample of rumen content of Gaddi sheep and goat (1,0mL) was suspended with 99 mL 0.85% (w/v) sterile NaCl solution (Normal saline) in a 150 mL conical flask, which was shaken at 120 rpm for 60 min at 37°C. Serial dilutions from 10^{-1} to 10^{-7} were prepared using sterile saline solution. An aliquot of 1000 μ L of each dilution was poured in plates amended with carboxymethylcellulose (CMC) (pH 7.0) containing (g/L) CMC (10.0), KH_2PO_4 (1.0), K_2HPO_4 (1.0), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2), NH_4NO_3 (1.0), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.05), CaCl_2 (0.02), and agar (15.0) (Lo et al. 2019). The plates were incubated at 37°C for 96 h.

Screening for Cellulolytic Activity

Screening for cellulolytic activity was performed by using the CMC medium having Congo red dye as indicator to see the halo zone around the bacterial colonies. On culture medium with Carboxymethyl Cellulose (CMC) and Congo red dye (0.5%) followed by 1M NaCl, the zone of cellulase activity was seen surrounding bacterial colonies that produced cellulase. Colonies surrounded by clear halo-evidence of carboxymethyl cellulose hydrolysis-were selected as cellulolytic candidates (Liu et al. 2023).

In CMC medium with Gram's iodine method, the selected bacterial isolates were spot on CMC medium and incubated at $28 \pm 2^\circ\text{C}$ for 72 h, the bacterial isolates that displayed hydrolytic zones (14mm and 12mm) on CMC media were again tested for their capacity to degrade cellulose by this method. The plates were flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 minutes. Selected bacterial isolates were spot on CMC medium and incubated at $28 \pm 2^\circ\text{C}$ for 96 h, the bacterial isolates that displayed hydrolytic zones (14mm and 12mm) on CMC media were again tested for their capacity to degrade cellulose by this method. The plates were flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 minutes. The enzymatic activity was quantified by measuring the hydrolysis zone: the ratio of the halo's diameter to the colony's diameter (clear zone $\div$ colony diameter), consistent with established methods (Gupta et al. 2012).

Morphological and biochemical characterization of isolates

Bacterial colonies demonstrating strong cellulolytic ability on CMC agar were sub-cultured onto standard nutrient media for further examination. Colony morphology and Gram-staining were used to determine cell shape and Gram reaction. To identify isolates at the genus level, a suite of biochemical assays-including Indole, Methyl Red (MR), Voges-Proskauer (VP), citrate utilization, catalase, oxidase, motility, and nitrate reduction tests-were performed routinely

Enzymatic Profiling

Production and collection of crude cellulase enzyme

Isolates that showed positive CMC hydrolysis were inoculated into CMC broth and incubated at 37°C for 24 hours. After incubation, the culture was transferred into centrifuge tubes and spun at 14,000 rpm for 10 minutes at 4°C. The resulting supernatant-containing crude cellulase-was collected and used to assess enzymatic activity. This crude enzyme preparation served as the source for evaluating major cellulolytic enzymes such as: Endoglucanase: Activity measured using CMC substrate, with reducing sugars quantified by the DNS method. β -glucosidase: Determined employing p-nitrophenyl- β -D-glucopyranoside (pNPG) as substrate. Cellobiohydrolase: Estimated via filter paper activity assays. Enzyme activities were tested under varying pH conditions (4.0-8.0), incubation periods (24-144 hours), and different carbon (glucose, sucrose, cellulose) and nitrogen (yeast extract, peptone, ammonium sulfate) sources (Guder and Krishna, 2019).

Substrate Pre-treatment and Biogas Assay

Paddy straw (50 g) was cut into small pieces (3-5 cm), thoroughly washed, and sterilized by autoclaving. The sterilized straw was submerged in 500 mL of a mixed culture of the selected cellulolytic bacterial isolates, *Enterobacter* spp. SUNG-7 and *Pseudomonas* spp. SUNG-25, and kept at ambient temperature for three weeks to facilitate microbial degradation of the lignocellulosic material. Microbial pretreatment of cereal straw has been reported to improve substrate biodegradability and its subsequent utilization during anaerobic digestion (Zhang et al., 2016; Zhong et al., 2016). Following pre-treatment, the bacterially treated paddy straw and untreated paddy straw (control) were separately mixed with 500g of cow dung. Cow dung and water were mixed in a 1:1 ratio by adding 500 mL of water, and biogas slurry was added as inoculum at a 1:10 ratio (w/w). The prepared mixtures were transferred to 2-L batch digesters and subjected to anaerobic digestion at room temperature for four weeks (Fig. 6). Biogas production was monitored daily for the first 20 days and measured using the saline-water displacement method. Zhong et al. (2016) similarly monitored daily biogas production by water displacement during microbial-pretreated straw digestion.

Results and Discussion

Isolation and Screening

Among the 40 bacterial isolates obtained, 10 observed clear hydrolysis zones on CMC agar plates. The hydrolysis index varied between 1.2 and 2.5. Notably, among the cellulolytic bacterial isolates, SUNG-7 and

SUNG-25 produced the most prominent clearance zones on CMC agar, indicating comparatively greater cellulose-hydrolysing potential. Cellulolytic activity was visualized by flooding the CMC-containing plates with

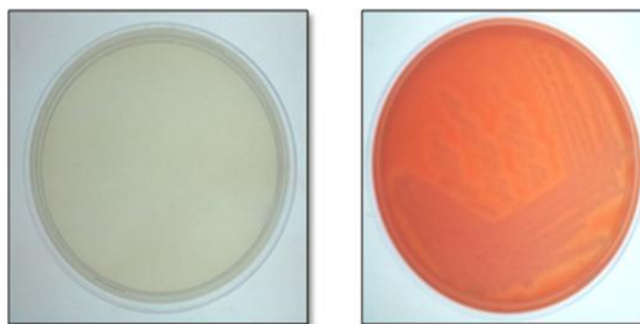


Fig.1: Bacterial isolate showing zone of clearance on CMC medium flooded with congo red (a) Control (b) SUNG -7

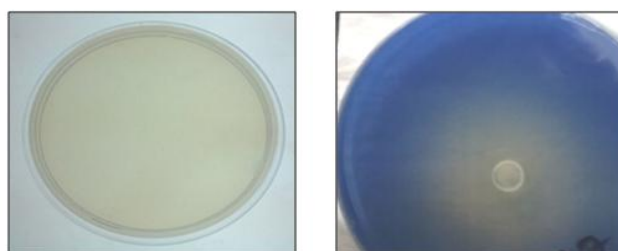


Fig.2: Bacterial isolate showing zone of clearance on CMC medium flooded with Gram's iodine (a) Control (b) SUNG -7

Congo red followed by destaining with 1 M NaCl. The formation of a clear halo around the bacterial colonies was considered indicative of CMC hydrolysis and extracellular cellulase activity (Gupta et al., 2012; Meddeb-Mouelhi et al., 2014). Congo red and iodine-based staining methods have both been widely used for visualizing CMC hydrolysis zones during qualitative screening of cellulolytic microorganisms. The presence of cellulase activity was indicated by the formation of clear zones surrounding the growing colonies of cellulase-producing strains, contrasting against the violet background (Meddeb et al. 2014) (Fig.1 & 2)

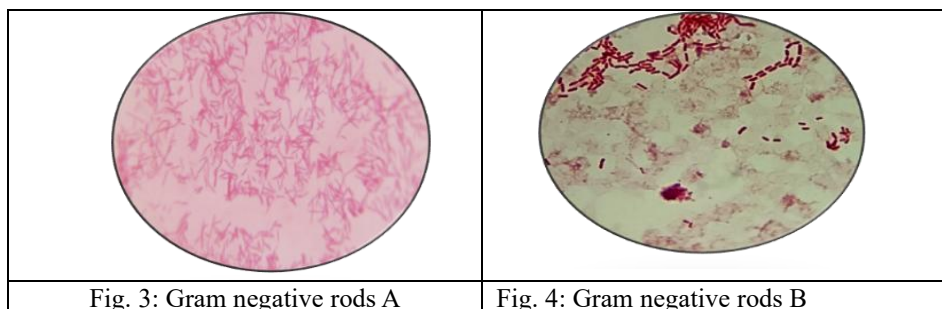
Enzyme Production and Optimization

Cellulase production by the selected isolates was strongly influenced by culture conditions, including pH, incubation period, and the nature of the carbon and nitrogen sources. Maximum cellulolytic activity was observed under mildly acidic conditions (pH 4.5-5.5), which is consistent with reports of cellulolytic bacteria exhibiting optimum enzyme activity around pH 5.0 (Lokapirnasari et al., 2015). Enzyme production also varied with incubation period, with the highest activities of β -glucosidase, endoglucanase and cellobiohydrolase in SUNG-7 and SUNG-25 occurring between 72 and 120 h. Such variation is expected because extracellular cellulase production is dependent on bacterial growth phase, culture conditions and duration of fermentation. Studies on rumen-derived bacteria have similarly demonstrated substantial production of endoglucanase and β -glucosidase, supporting the rumen as an important source of fibrolytic microorganisms and enzymes (Sharma et al., 2017). Carbon and nitrogen sources also influenced enzyme production. Glucose/dextrose supported comparatively high cellulolytic enzyme activities in the selected isolates, while yeast extract and urea were favourable nitrogen sources depending on the bacterial strain. The stimulatory effect of glucose on cellulase production has also been reported in bacterial isolates, including *Pseudomonas*, although the optimum carbon and nitrogen sources vary considerably among strains (Sethi et al., 2013). This strain-dependent response indicates that optimization of nutritional and environmental conditions is important for maximizing cellulase production by promising bacterial isolates.

Morphological and Biochemical Characterization of the Isolate

The isolate's morphological and biochemical characteristics were examined and compared following the methods outlined by Cappuccino and Sherman (2014). Cell shape and arrangement were observed under a light microscope by performing Gram staining (Fig. 3 A & B). Standard Gram staining and urease tests were performed according to established protocols. Catalase activity was assessed by applying a few drops of 3% (v/v) hydrogen peroxide onto a glass slide containing an overnight-grown culture. Carbohydrate fermentation was evaluated by monitoring acid production, indicated by a color change from red to yellow. To assess

temperature tolerance, the isolate was cultured in nutrient broth and incubated at 37 °C. Based on morphological and biochemical characterization, the following identifications were made:



- *Escherichia coli* (SUNG-1, SUNG-3)
- *Enterobacter* spp. (SUNG-7)
- *Pseudomonas* spp. (SUNG-25)
- *Micrococcus* spp. (SUNG-14)

Two Gram-negative bacilli not conclusively identified (SUNG-12, SUNG-19) (Table1)

Biogas Production using cellulolytic consortium

Biogas production increased progressively with incubation time in both the control and test digesters; however, the test digester consistently produced a higher volume of biogas throughout the experimental period. After 5 days of incubation, accumulated biogas production was 150 mL in the control digester compared with 180 mL in the test digester, representing a 20.0% increase. On the 10th day, biogas production reached 265 mL in the control and 305 mL in the test digester, corresponding to an improvement of 15.09%. A similar trend was observed with further incubation. On the 15th day, the test digester produced 425 mL of biogas compared with 355 mL in the control, showing an increase of approximately 19.7%. By the 20th day, biogas production reached 510 mL in the test digester compared with 440 mL in the control, representing an increase of about 15.9%. Overall, the total accumulated biogas production was 1,420 mL in the test digester compared with 1,210 mL in the control, corresponding to an overall improvement of 17.35%. These results indicate that pretreatment of the agricultural residue with the selected cellulolytic bacterial isolates improved biogas production under laboratory-scale conditions. (Table 2) (Fig. 5). Zhong et al. (2016) reported that pretreating wheat straw with a microbial consortium led to an enhanced biogas yield, achieving a maximum methane production of up to 41%. However, extending the pretreatment duration resulted in a decline in methane yield, attributed to microorganisms consuming the nutrients released from the wheat straw. Similarly, using rumen fluid as a pretreatment agent for wheat straw boosted biogas production by 66.5% and shortened the anaerobic digestion process time by 40% (Zhang et al., 2016a). Additionally, Ali et al. (2017) found that a microbial consortium derived from decomposed sawdust significantly improved methane yield by 72.6% compared to the untreated control.

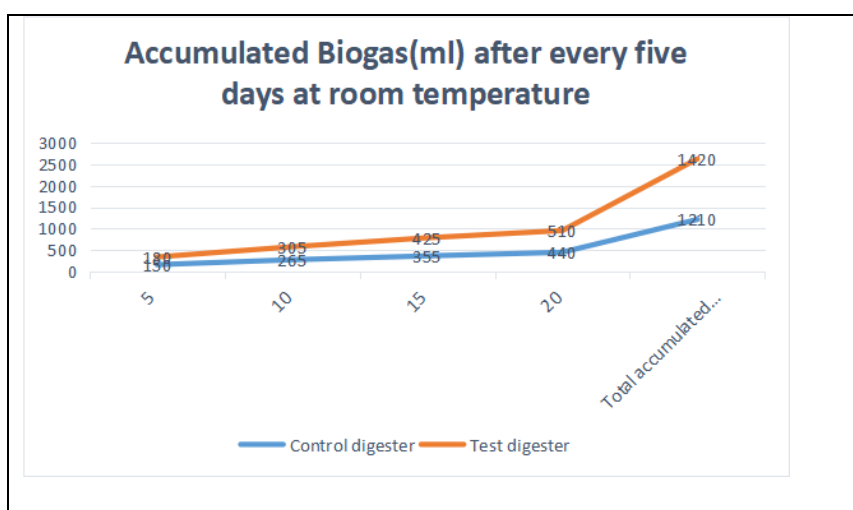


Fig. 5: Biogas production from pretreated paddy straw with isolated consortia.



Fig. 6: Biogas digester assembly

Table 1: Morphological and biochemical basis identification of efficient cellulolytic bacteria isolate from rumen of gaddi goat

S.N.	Bacterial isolates code	Gram Reaction	Morphology	Motility	O	C	I	MR	VP	Ci	U	H ₂ S	G	M	S	L	R	A	Possible organisms
1	SUNG-3	Gram -ve	Rods	Motile	-	-	-	+	-	-	-	-	+	+	-	+	-	+	<i>Escherichia coli</i>
2	SUNG-5	Gram -ve	Rods	Motile	-	-	-	+	-	-	-	-	+	+	-	+	-	+	<i>Escherichia coli</i>
3	SUNG-7	Gram -ve	Rods	Motile	-	+	-	-	+	+	-	-	+	+	+	-	-	+	<i>Enterobacter spp</i>
4	SUNG-25	Gram -ve	Rods	Motile	+	+	-	-	+	+	-	-	-	-	-	-	-	-	<i>Pseudomonas spp.</i>
5	BAIJ-26	Gram -ve	Rods	Motile	-	+	-	-	-	+	-	-	-	-	-	+	-	-	Gram negative bacilli
6	BAIJ-36	Gram -ve	Rods	Motile	-	+	-	-	-	+	-	-	+	-	-	+	-	-	Gram negative bacilli
7	BAIJ-38	Gram +ve	Cocci	Non-Motile	+	+	-	-	-	+	-	-	-	-	-	-	-	-	<i>Micrococcus</i>

Note: 8O-oxidase; C-catalase; I-indole; MR-methyl red; V-voges Proskauer; Ci- citrate; U-urease; H₂S- hydrogen sulfide; G-Glucose; M- maltose9; S-sucrose; L-lactose; R-rhamnose; A-arabinose

Table 2: Utilization of Efficient isolates to degrade agricultural wastes for biogas production potential in laboratory scale

Incubation Days	Accumulated Biogas(ml) after every five days at room temperature	
	Control digester	Test digester
5	150	180 (20.0%)
10	265	305 (15.09%)
15	355	425 (19.7%)
20	440	510 (15.9)
Total accumulated Biogas(ml)	1210	1420 (17.35%)

Discussion

The present study highlights the rumen contents of migratory Gaddi sheep and goats as a promising source of cellulolytic bacteria for lignocellulosic biomass degradation. Similar studies have demonstrated that the rumen harbours diverse fibrolytic and cellulose-degrading bacteria capable of utilizing complex plant materials (Sharma et al., 2017; Guder and Krishna, 2019; Bhujbal et al., 2022; Gharechahi et al., 2023). Of the 40 bacterial isolates recovered in the present study, 10 exhibited cellulolytic activity on CMC medium, demonstrating variation in cellulose-degrading ability among the culturable isolates (Gupta et al., 2012; Guder and Krishna, 2019). Based on cultural, morphological and biochemical characteristics, seven isolates were tentatively identified as *Escherichia coli*, *Enterobacter* spp., *Pseudomonas* spp., *Micrococcus* spp., and unidentified Gram-negative bacilli. Among the isolates, *Enterobacter* spp. SUNG-7 and *Pseudomonas* spp.

SUNG-25 showed the strongest cellulolytic potential, with comparatively higher β -glucosidase, endoglucanase and cellobiohydrolase activities. The complementary action of these cellulolytic enzymes is important for efficient hydrolysis of cellulose into simpler sugars (Kuhad et al., 2011; Malgas et al., 2017; Ejaz et al., 2021). Maximum enzyme production was observed at pH 5.0 after 72–120 h of incubation, while glucose and yeast extract supported better enzyme production. The influence of pH, incubation period and nutrient sources on bacterial cellulase production has also been reported in earlier studies (Sethi et al., 2013; Lokapirnasari et al., 2015; Sharma et al., 2017).

Pretreatment of paddy straw with SUNG-7 and SUNG-25 for three weeks resulted in more than 15% improvement in biogas production during subsequent anaerobic digestion. This increase may be attributed to partial hydrolysis of the cellulosic fraction, thereby improving substrate accessibility to the anaerobic microbial community. Similar improvements in the anaerobic digestion of lignocellulosic residues following rumen-microbial or microbial-consortium pretreatment have been reported previously (Zhang et al., 2016; Zhong et al., 2016; Wagner et al., 2018). Biological pretreatment is particularly attractive because it can operate under relatively mild conditions with lower chemical and energy requirements (Wagner et al., 2018). Overall, the findings demonstrate the potential of rumen-derived cellulolytic bacteria for biological pretreatment of agricultural residues and enhancement of biogas production. However, molecular identification and biosafety assessment of the promising isolates, together with direct methane quantification and validation at larger digester scales, are required before practical application.

Conclusion

Cellulolytic bacterial strains isolated from the rumen of migratory small ruminants, particularly *Enterobacter* and *Pseudomonas* spp., showed promising potential for the biological conversion of lignocellulosic agricultural residues. Pretreatment of paddy straw with these isolates improved its subsequent utilization during anaerobic digestion, resulting in enhanced biogas production. These findings suggest that rumen-derived cellulolytic bacteria could provide a simple and environmentally sustainable approach for improving agricultural residue management while supporting renewable energy production.

Acknowledgement

The authors thank the staff of CSKHPKV, Palampur, and AICRP on EAAI (ICAR-08-13 Scheme) for supporting this research.

Conflict of interest

On behalf of all authors, the corresponding author declares that there is no conflict of interest.

References

- 1) Ali, S.S., Abomohra, A.E., & Sun, J. 2017. Effective bio-pretreatment of sawdust waste with a novel microbial consortium for enhanced biomethanation. *Bioresource Technology* 238: 425–432.
- 2) Behera, B.C., Sethi, B.K., Mishra, R.R., Dutta, S.K., & Thatoi, H.N. 2017. Microbial cellulases–Diversity & biotechnology with reference to mangrove environment: A review. *Journal of Genetic Engineering and Biotechnology* 15(1): 197–210. doi: 10.1016/j.jgeb.2016.12.001.
- 3) Bhujbal, S.K., Ghosh, P., Vijay, V.K., Rathour, R., Kumar, M., Singh, L., & Kapley, A. 2022. Biotechnological potential of rumen microbiota for sustainable bioconversion of lignocellulosic waste to biofuels and value-added products. *Science of the Total Environment* 814: 152773.
- 4) Cappuccino, J.G., & Sherman, N. 2014. *Microbiology: A Laboratory Manual*. Pearson Higher Education.
- 5) DeAngelis, K.M., Sharma, D., Varney, R., Simmons, B., Isern, N.G., Markillie, L.M., Nicora, C., Norbeck, A.D., Taylor, R.C., Aldrich, J.T., & Robinson, E.W. 2013. Evidence supporting dissimilatory and 6) assimilatory lignin degradation in *Enterobacter lignolyticus* SCF1. *Frontiers in Microbiology* 4: 280.
- 6) Djimon, A.M., Offoumon, O.T.L.F., Worogo, H.S.S., Worogo, J.S.B.S., Alabi, C.A.D., Idrissou, Y., Soulé, F., Alimi, N., Yacoubou, A.M., Assan, A.S. 2024. Profitability of sheep farming in Benin. *Journal of Livestock Science* 15: 267–275 doi: 10.33259/JLivestSci.2024.267-275
- 7) Ejaz, U., Sohail, M., Ghanemi, A. 2021. Cellulases: From bioactivity to a variety of industrial applications. *Biomimetics* 6(3): 44. doi: 10.3390/biomimetics6030044.
- 8) Gharechahi, J., Vahidi, M.F., Sharifi, G., Ariaeenejad, S., Ding, X.Z., Han, J.L., & Salekdeh, G.H. 2023. Lignocellulose degradation by rumen bacterial communities: New insights from metagenome analyses. *Environmental Research* 229: 115925.
- 9) Ginni, G., Kavitha, S., Kannah, R.Y., Bhatia, S.K., Kumar, A., Rajkumar, M., Kumar, G., Pugazhendhi, A., & Chi, N.T.L. 2021. Valorization of agricultural residues: Different biorefinery routes. *Journal of Environmental Chemical Engineering* 9(4): 105435.
- 10) Guder, D.G., & Krishna, M.S.R. 2019. Isolation and characterization of potential cellulose degrading bacteria from sheep rumen. *Journal of Pure and Applied Microbiology* 13(3): 1831–1839. doi: 10.22207/JPAM.13.3.60.

- 11) Gupta, P., Samant, K., & Sahu, A. 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology* 2012: 578925. doi: 10.1155/2012/578925.
- 12) Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., & Janssen, P.H. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports* 5: 14567.
- 13) Jones, J.A., Kerr, R.G., Haltli, B.A., & Tinto, W.F. 2018. Temperature and pH effect on glucose production from pretreated bagasse by a novel species of *Citrobacter* and other bacteria. *Heliyon* 4(6).
- 14) Kuhad, R.C., Gupta, R., & Singh, A. 2011. Microbial cellulases and their industrial applications. *Enzyme Research* 2011: 280696. doi: 10.4061/2011/280696.
- 15) Leahy, S.C., Janssen, P.H., Attwood, G.T., Mackie, R.I., McAllister, T.A., Kelly, W.J. 2022. Electron flow: Key to mitigating ruminant methanogenesis. *Trends in Microbiology* 30(3): 209–212. doi: 10.1016/j.tim.2021.12.005.
- 16) Liu, Q., Li, L., Chen, Y., Wang, S., Xue, L., Meng, W., Jiang, J., & Cao, X. 2023. Diversity of endophytic microbes in *Taxus yunnanensis* and their potential for plant growth promotion and taxane accumulation. *Microorganisms* 11(7): 1645. doi: 10.3390/microorganisms11071645.
- 17) Lokapirnasari, W.P., Nazar, D.S., Nurhajati, T., Supranianondo, K., Yulianto, A.B. 2015. Production and assay of cellulolytic enzyme activity of *Enterobacter cloacae* WPL 214 isolated from bovine rumen fluid waste of Surabaya abattoir, Indonesia. *Veterinary World* 8(3): 367–371. doi: 10.14202/vetworld.2015.367-371.
- 18) Malgas, S., Thoresen, M., van Dyk, J.S., Pletschke, B.I. 2017. Time dependence of enzyme synergism during the degradation of model and natural lignocellulosic substrates. *Enzyme and Microbial Technology* 103: 1–11. doi: 10.1016/j.enzmitec.2017.04.007.
- 19) Meddeb-Mouelhi, F., Moisan, J.K., & Beauregard, M. 2014. A comparison of plate assay methods for detecting extracellular cellulase and xylanase activity. *Enzyme and Microbial Technology* 66: 16–19. doi: 10.1016/j.enzmitec.2014.07.004.
- 20) Mizrahi, I., & Jami, E. 2021. A method to the madness: Disentangling the individual forces that shape the rumen microbiome. *EMBO Reports* 22(2): e52269.
- 21) Sethi, S., Datta, A., Gupta, B.L., & Gupta, S. 2013. Optimization of cellulase production from bacteria isolated from soil. *ISRN Biotechnology* 2013: 985685. doi: 10.5402/2013/985685.
- 22) Sharma SA, Sharma D, Rana K, Kanwar SS, Mal G, & Singh B, 2017. Isolation and characterization of fibrolytic enzymes from sheep rumen bacteria. *International Journal of Probiotics & Prebiotics* 12(4).
- 23) Singh, N., Devi, A., Bishnoi, M.B., Jaryal, R., Dahiya, A., Tashyrev, O., & Hovorukha, V. 2019. Overview of the process of enzymatic transformation of biomass. In: Biernat, K. (Ed.), *Elements of Bioeconomy*. IntechOpen. doi: 10.5772/intechopen.85036.
- 24) Wagner, A.O., Lackner, N., Mutschlechner, M., Prem, E.M., Markt, R., & Illmer, P. 2018. Biological pretreatment strategies for second-generation lignocellulosic resources to enhance biogas production. *Energies* 11(7): 1797. doi: 10.3390/en11071797.
- 25) Zhang, H., Zhang, P., Ye, J., Wu, Y., Fang, W., Gou, X., & Zeng, G. 2016. Improvement of methane production from rice straw with rumen fluid pretreatment: A feasibility study. *International Biodeterioration & Biodegradation* 113: 9–16.
- 26) Zhong, C., Wang, C., Wang, F., Jia, H., Wei, P., & Zhao, Y. 2016. Enhanced biogas production from wheat straw with the application of synergistic microbial consortium pretreatment. *RSC Advances* 6(65): 60187–60195.