Gut-Associated Cellulolytic Bacteria of The Freshwater Apple Snail *Pila Globosa* And Their Role in Cellulose Digestion

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Abstract- *The present study investigates the association between gut-associated bacteria of the freshwater apple snail Pila globosa and their role in lignocellulose digestion. Cellulolytic and hemicellulolytic bacteria were isolated from the gut using carboxymethyl cellulose (CMC) agar medium and subjected to sequential screening based on colony morphology, enzyme activity, and cellulose liquefaction ability. Two promising bacterial isolates, designated PS1 and PS2, were selected for detailed characterization. Submerged fermentation studies were carried out to evaluate extracellular enzyme production, and growth dynamics along with pH variation were monitored over time. Molecular identification based on 16S rRNA gene sequencing revealed that isolate PS1 belonged to the genus Bacillus, while isolate PS2 was affiliated with the genus Klebsiella. Both isolates exhibited the ability to produce cellulolytic and hemicellulolytic enzymes, including CMCase, FPase, and xylanase, with enzyme production closely associated with the active growth phase. Zymogram analysis further confirmed the presence of extracellular enzyme systems involved in cellulose degradation. The results highlight the functional role of gut-associated microbial consortia in facilitating lignocellulose digestion in Pila globosa and suggest the potential of these bacterial isolates as sources of industrially relevant enzymes*

Keywords- Pila globosa, Cellulolytic bacteria, Gut microbiota, Enzymes

I. INTRODUCTION

The digestion of complex plant polysaccharides in herbivorous and detritivorous animals is largely facilitated by symbiotic gut microorganisms. Since the early 20th century, studies on ruminants and insects such as termites have clearly demonstrated the crucial role of gut microbiota in cellulose and hemicellulose degradation. Molluscs, particularly gastropods, have received comparatively less attention despite their ability to thrive on plant-based diets rich in lignocellulosic material (Breznak and Brune 1994; Leschine 1995). Apple snails represent an important group of freshwater gastropods whose feeding ecology suggests the involvement of specialized microbial consortia for efficient digestion (Karasov and Martínez 2007).

Pila globosa, a freshwater apple snail widely distributed in the Indian subcontinent, feeds on aquatic vegetation and decaying plant matter rich in cellulose, hemicellulose, and other complex carbohydrates (Miller 1959). The snail's digestive efficiency cannot be explained solely by host-derived enzymes, indicating a significant contribution from gut-associated microbes. Recent advances in microbial ecology and molecular biology have enabled detailed exploration of gut microbial diversity and their functional roles, particularly through culture-dependent approaches combined with molecular identification techniques such as 16S rRNA gene sequencing (Stackebrandt and Goebel 1994; Song et al., 2018; Kathade et al., 2020).

Microbial enzymes such as cellulases (CMCase and FPase) and xylanases play a pivotal role in the breakdown of plant cell wall components into utilizable sugars. Isolation of gut microbes capable of growing on carboxymethyl cellulose (CMC) agar serves as an effective screening method for identifying potent cellulolytic bacteria. The use of cell-free culture supernatants for enzyme assays allows accurate estimation of extracellular enzyme activity, while zymogram analysis provides insights into enzyme multiplicity and molecular characteristics. Such studies contribute not only to understanding digestive symbiosis but also to identifying novel microbial strains with potential industrial and biotechnological applications (Kim et al., 2015; Aravind et al., 2017; Dar et al., 2017).

The present study was undertaken with the objectives of **(i)** isolating and screening cellulolytic and hemicellulolytic bacteria from the gut of *Pila globosa* using CMC agar medium, **(ii)** identifying the highly active bacterial isolates through 16S rRNA gene sequencing and subsequent sequence analysis, **(iii)** estimating the activities of CMCase, xylanase, and FPase using cell-free culture supernatants, and **(iv)** characterizing enzyme profiles through zymogram analysis following the method of Morag with suitable modifications. This work aims to elucidate the association between *Pila globosa* and its gut microbes in lignocellulose digestion and to explore their enzymatic potential.

II. MATERIALS AND METHODS

Sampling of *Pila globosa* and Dissection

The Medium sized, healthy, freshwater snail, *Pila globosa*(n=6)were collected from Vellayani (lon 76º99´77 E lat 8º43´44 N, Thiruvananthapuram. They were brought to the laboratory, sorted them and were acclimatized for a week to lab. Conditions. The anaesthetized adults of *Pila globosa* (26.88±0.085 gm) were dissected by removing shell with bone cutter. Digestive tract was separated and the surface was sterilized with 70% ethanol.

Isolation and Screening of Microorganisms

The gut contents were collected and homogenized. The samples were spread onto the surface of CMC (carboxymethyl cellulose) agar medium containing the following components (in g L^{-1}): carboxymethyl cellulose (10.0) as the substrate for cellulase (CMCase) activity, peptone (5.0) as a nitrogen source, yeast extract (1.0) as a source of vitamins and growth factors, sodium chloride (5.0) to maintain osmotic balance, dipotassium hydrogen phosphate (1.0) as a buffering and phosphorus source, magnesium sulfate (0.2) as an enzymatic cofactor, and agar (15.0) as the solidifying agent. The medium was prepared in distilled water (1000 ml) and adjusted to a final pH of 7.0 ± 0.2 (Aravind et al., 2017).

16S rRNA Gene Sequencing and Bacterial Identification

otal genomic DNA was extracted from toe clip samples using the DNeasy Blood and Tissue Kit (Qiagen, India). A partial fragment of the mitochondrial 16S rRNA gene was amplified by PCR using universal primers, and the amplified products were purified and sequenced using an ABI capillary sequencing platform. Sequence alignment was performed using BioEdit software (Hall, 1999), and taxonomic identity was confirmed through BLAST searches against the NCBI database (Palumbi et al. 199; Tamura et al., 2011).

Microbial growth and biochemical analysis

Microbial analyses, including Gram staining, motility tests, colony morphology, and growth characteristics, were carried out in detail for the isolated bacterial strains. Growth studies were conducted in liquid medium containing carboxymethyl cellulose (CMC) as the primary carbon source. Four bacterial isolates showing high cellulolytic activity were selected and subjected to fermentative studies in liquid culture for a period of 144 hours. During the incubation period, culture broth samples were collected at 24-hour intervals to monitor changes in pH and growth. Cell-free culture supernatants obtained after centrifugation were used for the estimation of CMCase, xylanase, and FPase activities. Bacterial growth was determined by measuring the optical density of the culture at 600 nm against distilled water as the blank, while the pH of the culture medium collected at each 24-hour interval was measured using a digital pH meter (Miller et al., 1960; Imran et al., 2016).

III. RESULT AND DISCUSSION

Isolation and Screening of Microorganisms

Primary screening of microbial isolates obtained from the gut of *Pila globosa* was carried out on carboxymethyl cellulose (CMC) agar medium. Two bacterial isolates were examined for their colony morphology, including shape, colour, elevation, and margin characteristics. The isolates were subsequently subcultured and incubated at 37 °C for further characterization. Based on preliminary observations, two promising bacterial strains were selected and designated as *PS1* and *PS2* (*PS1 – Pila Strain 1; PS2 – Pila Strain 2*). Secondary screening was performed using CMC agar plates followed by Congo red staining, which revealed distinct zones of clearance around the colonies, indicating cellulase activity. Among the two isolates, strain *PS1* (Fig. 1A) exhibited a significantly larger zone of clearance compared to *PS2*, (Fig. 1B) suggesting higher cellulolytic potential (Fig. 1C). The formation of clear zones following Congo red staining is a well-established qualitative indicator of extracellular cellulase production, as the dye binds specifically to intact cellulose, leaving hydrolysed regions unstained (Teather & Wood, 1982; Kasana et al., 2008). The superior clearance exhibited by strain *PS1* indicates enhanced secretion of endoglucanases (CMCase), highlighting its potential role in cellulose digestion within the gut ecosystem of *Pila globosa*, similar to cellulolytic symbionts reported in other herbivorous invertebrates (Lynd et al., 2002).

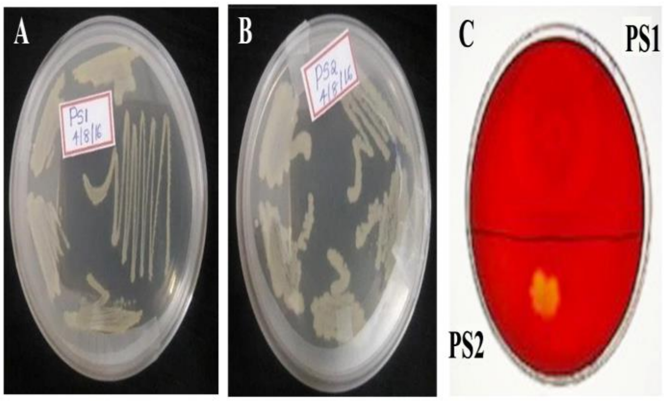

Figure 1- Four isolated strains [A-PS1 and B-PS2,] and cellulolytic activity of PS1and PS2. The clear zone in Congo red staining indicates cellulolytic activity.

The tertiary screening with CMC liquefaction

The two selected bacterial isolates (*PS1* and *PS2*) were further subjected to tertiary screening to evaluate their cellulose-degrading efficiency using the CMC liquefaction assay. Each strain was inoculated into culture tubes containing solidified CMC medium and incubated under controlled conditions. Strain *PS1* demonstrated pronounced cellulolytic activity by converting the solidified CMC medium into a clear and transparent liquid, indicating extensive polymer degradation (Fig. 2A). In contrast, strain *PS2* exhibited minimal liquefaction, reflecting comparatively poor cellulose-degrading capability (Fig. 2B). Liquefaction of solid CMC medium serves as a robust qualitative assay for assessing the overall efficiency of cellulose depolymerization, as it reflects the synergistic action of multiple cellulase components (endoglucanase, exoglucanase, and β-glucosidase) (Beguin & Aubert, 1994). The pronounced liquefaction ability of strain *PS1* suggests its dominance as an active cellulolytic bacterium in the gut of *Pila globosa*, reinforcing the concept of host–microbe associations in facilitating digestion of recalcitrant plant polysaccharides (Flint et al., 2012).

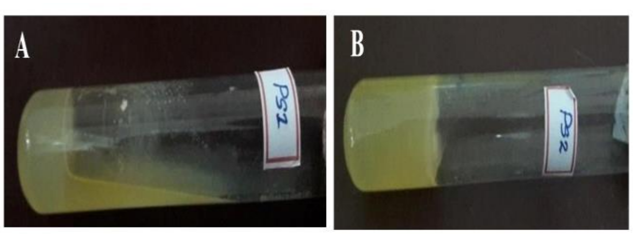

Figure 2- The cellulolytic activity of two isolated strains PS1 and PS2 in culture tubes

Microbial Analysis

Sl.No.	Character	Bacterial Isolates	
		PS1	PS2
1	Colony colour	straw colour	cream
2	Colony margin	seriated	seriated
3	Colony elevation	flat	convex
4	Gram`s staining	+ ive	+ive
5	Motility	moderate	non-motile
6	Shape of cell	rod	rod

Table 1- Microbial analysis of strains (PS1 and PS2 from *P. globosa*)

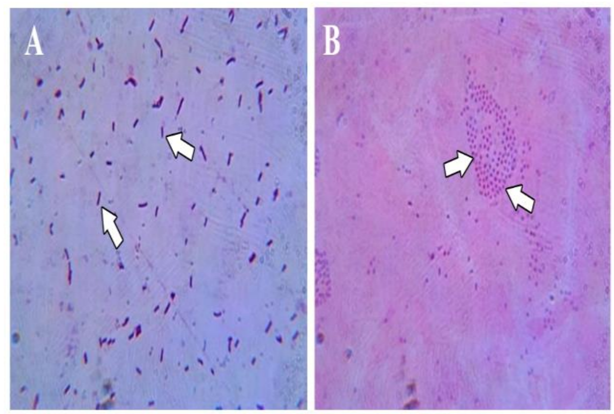

Figure 3- The smear of two isolated strains PS1 and PS2 after Gram's staining

Identification of Bacterial Isolates

The two enzymatically active bacterial isolates were identified through amplification and sequencing of the 16S ribosomal RNA (rRNA) gene. Sequence analysis revealed that isolate *PS1* possessed a partial 16S rRNA gene sequence of approximately 880 bp and showed significant phylogenetic affiliation with members of the genus *Bacillus* (Fig. 3A). Based on sequence similarity and phylogenetic placement, the isolate was designated as *Bacillus* sp. *PS1*. The corresponding 16S rRNA gene sequence was deposited in the NCBI GenBank database under the accession number KY242606.1. Members of the genus *Bacillus* are well known for their ecological versatility, enzyme secretion capacity, and ability to survive under diverse environmental conditions, making them dominant cellulolytic bacteria in various host-associated and free-living ecosystems (Lynd et al., 2002; Sharma et al., 2015).

Similarly, isolate *PS2* yielded a partial 16S rRNA gene sequence of approximately 370 bp. BLAST analysis indicated 100% sequence similarity with species belonging to the genus *Klebsiella*(Fig. 3B). Consequently, the isolate was identified as *Klebsiella* sp. *PS2*, and its 16S rRNA gene sequence was deposited in GenBank under the accession number KY242607.1. The molecular identification confirmed that the cellulolytic and hemicellulolytic activities observed in the isolates were associated with taxonomically distinct bacterial genera. The presence of *Klebsiella* in the gut of *Pila globosa* suggests its potential involvement in hemicellulose and cellulose degradation under anaerobic or facultative anaerobic conditions. The coexistence of phylogenetically distinct genera (*Bacillus* and *Klebsiella*) within the gastrointestinal tract of *Pila globosa* reflects a functionally diverse microbial consortium adapted for efficient degradation of complex plant polymers. Similar microbial diversity has been reported from the digestive tracts of other gastropods and herbivorous invertebrates, highlighting the importance of microbial symbiosis in host nutrition (Cardoso et al., 2012; Flint et al., 2012).

Fermentation in liquid culture medium with the selected 2 bacterial isolates

Bacterial isolates exhibiting larger zones of clearance on CMC agar plates and higher efficiency in liquefying solidified CMC medium were selected for further investigations. Submerged fermentation in liquid CMC medium was carried out to evaluate extracellular enzyme production by the selected isolates. Bacterial growth during fermentation was monitored by measuring the optical density (OD) of the culture at 600 nm, which served as an indirect estimate of cell biomass. Variations in OD values reflected corresponding changes in cell growth under submerged fermentation conditions. The observed decline in OD after reaching peak growth suggests entry into the stationary or decline phase, possibly due to nutrient depletion or accumulation of inhibitory metabolic products (Madigan et al., 2018; Koch, 2007).

Both bacterial isolates, *PS1* and *PS2*, displayed characteristic bacterial growth phases during the incubation period (Fig. 4A). Isolate *PS1* reached maximum growth at 48 hours of incubation, whereas *PS2* exhibited peak growth at 24 hours, followed by a gradual decline in biomass in both strains. The differences in growth kinetics between *PS1* and *PS2* indicate strain-specific metabolic efficiencies and substrate utilization patterns. The delayed peak growth of *PS1* (48 h) compared to *PS2* (24 h) suggests sustained metabolic activity, which may be advantageous for prolonged extracellular enzyme production, as cellulase synthesis is often growth-associated or peaks during late exponential phase (Beguin & Aubert, 1994). The pH of the culture medium showed considerable variation during fermentation, ranging from acidic to alkaline conditions (pH 4–9) (Fig. 4B). The initial pH of the cultures was between 4.95 and 5.91, which progressively increased and reached a maximum value of 9.07 during later stages of incubation, indicating metabolic activity and accumulation of alkaline by-products. The ability of both isolates to tolerate and grow across a wide pH range (4–9) suggests ecological adaptability and supports their potential role as gut symbionts in *Pila globosa*, where fluctuating physicochemical conditions are common (Flint et al., 2012).

Enzyme Production by Bacterial Isolates

The bacterial isolate PS1 exhibited significant extracellular cellulolytic and hemicellulolytic activities, as evidenced by the production of CMCase, FPase, and xylanase during submerged fermentation. The maximum CMCase activity (6.796 U ml^{-1}) was recorded at 48 hours of incubation (Fig. 4C). The elevated enzyme activities observed at 48 hours for both isolates suggest that cellulase and hemicellulase production is closely associated with the late exponential growth phase, a phenomenon commonly reported in *Bacillus* species and other cellulolytic bacteria (Beguin & Aubert, 1994; Lynd et al., 2002). The decline in CMCase and xylanase activities after peak production in PS1 may be attributed to enzyme instability, feedback inhibition, or proteolytic degradation under prolonged incubation (Fig. 4D). Similarly, peak FPase and xylanase activities were observed at 48 hours, with activities of 2.833 U ml^{-1} and 0.8118 U ml^{-1}, respectively. Following this peak, CMCase and xylanase activities declined at later incubation periods, whereas FPase activity showed a marginal increase after 96 hours, suggesting differential regulation of cellulase components over time (Fig. 4E). The biphasic FPase activity pattern observed in *PS2* indicates differential regulation of exoglucanase components, possibly due to changes in substrate availability or induction by intermediate hydrolysis products (Wood & Bhat, 1988). Such multiphasic enzyme expression patterns have been reported in cellulolytic microbial systems adapting to complex polysaccharide substrates.

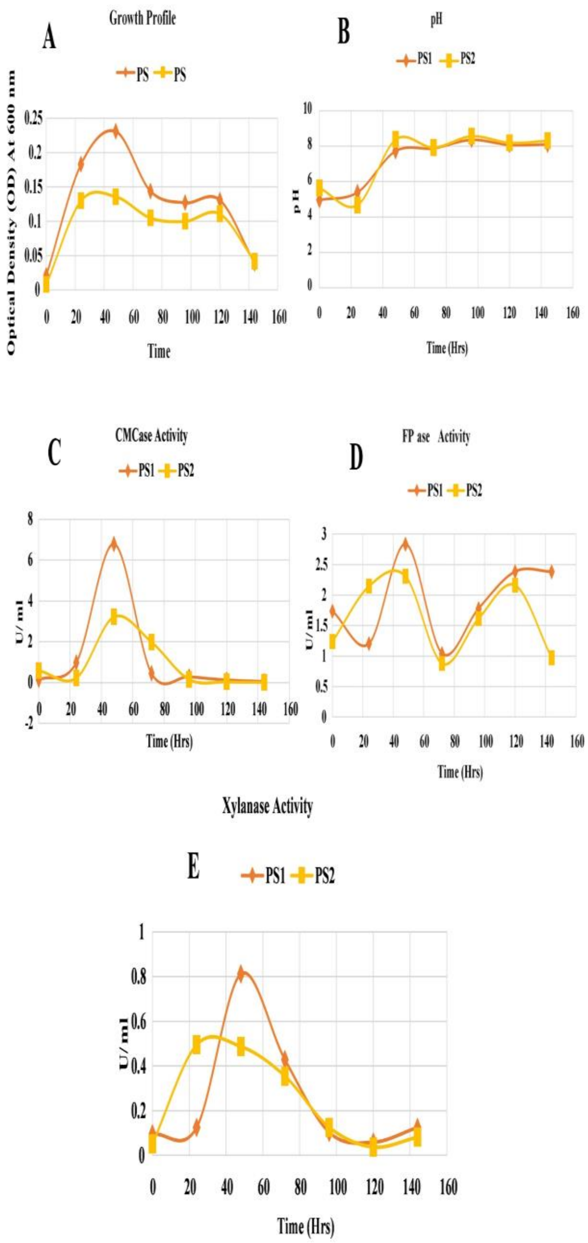

Figure 4. (A) Growth profile of bacterial isolates *PS1* and *PS2*, expressed as optical density (OD) of the culture broth measured at 600 nm with a dilution factor of 10; **(B)** changes in pH of the culture broth of *PS1* and *PS2* over the incubation period; **(C)** CMCase activity of the culture broth of bacterial isolates *PS1* and *PS2* at different time intervals; **(D)** FPase activity of the culture broth of the bacterial isolates during incubation; and **(E)** xylanase activity of the culture broth of *PS1* and *PS2* measured at various time points.

In contrast, the bacterial isolate PS2 exhibited a distinct pattern of enzyme production. The highest CMCase and FPase activities were recorded at 48 hours, with values of 3.232 U ml^{-1} and 2.309 U ml^{-1}, respectively. Notably, FPase activity in *PS2* displayed a biphasic pattern, with two distinct activity peaks observed at 48 and 120 hours of incubation. The maximum xylanase activity for PS2 (0.494 U ml^{-1}) was detected at 24 hours, indicating early induction of hemicellulolytic enzymes in this isolate. Although *Pila globosa* itself is not known to produce high levels of endogenous cellulolytic enzymes, the presence of efficient cellulose-degrading bacteria in its gastrointestinal tract compensates for this limitation. The observed enzyme activities strongly support the role of gut-associated microbial consortia in facilitating cellulose digestion in *P. globosa*, particularly within the intestinal region where extensive breakdown of plant material occurs (Karasov & Martínez del Rio, 2007). Recent studies have demonstrated that different regions of the gastrointestinal tract of pulmonate snails harbor diverse and functionally specialized bacterial communities, which contribute to the digestion of recalcitrant plant polymers (Cardoso et al., 2012; Pawar et al., 2015). The functional diversity observed between isolates *PS1* and *PS2* further highlights the ecological complexity and metabolic specialization of gut microbiota in *P. globosa*.

IV. CONCLUSION

The present study demonstrates that the freshwater apple snail *Pila globosa* harbours functionally diverse gut-associated bacteria that significantly contribute to lignocellulose digestion. Two cellulolytic isolates, identified as *Bacillus* sp. PS1 and *Klebsiella* sp. PS2, exhibited distinct growth dynamics and enzyme production profiles. Among them, PS1 showed superior cellulolytic efficiency with higher CMCase, FPase, and xylanase activities, particularly during the late exponential growth phase. Zymogram analysis confirmed the presence of active extracellular cellulase systems. These findings highlight the importance of microbial symbiosis in the digestive physiology of *P. globosa* and suggest that its gut microbiota represents a promising source of industrially relevant lignocellulose-degrading enzymes

REFERENCES

[1] Akpanudo, N. W., & Olabemiwo, O. M. (2024). Synthesis and characterization of silver nanoparticles and nanocomposites using Echinochloa pyramidalis (Antelope grass) plant parts and the application of the nanocomposite for the removal of PAHs

[2] from bitumen seepage water. *South African Journal of Chemical Engineering*, *47*(1), 98-110.

[3] Aravind, K. K., Sandeep, S., & Subramaniyan, S. (2017). Analysis of Microbes and their Enzyme in *Achatina fulica*. *International Journal of Science and Research*, 6(3), 1897-1903.

[4] Beguin, P., & Aubert, J. P. (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews*, **13**, 25–58.

[5] Beguin, P., & Aubert, J. P. (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews*, **13**, 25–58.

[6] Beguin, P., & Aubert, J. P. (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews*, **13**, 25–58.

[7] Breznak, J. A., & Brune, A. (1994). Role of microorganisms in the digestion of lignocellulose by termites. *Annual Review of Entomology*, **39**, 453–487.

[8] Cardoso, A. M., Cavalcante, J. J., Cantão, M. E., Thompson, C. E., Flatschart, R. B., Glogauer, A., … Thompson, F. L. (2012). Metagenomic analysis of the microbiota associated with the digestive tract of the land snail *Achatina fulica*. *PLoS ONE*, **7**, e33467.

[9] Cardoso, A. M., Cavalcante, J. J., Cantão, M. E., Thompson, C. E., Flatschart, R. B., Glogauer, A., & Thompson, F. L. (2012). Metagenomic analysis of the microbiota associated with the digestive tract of the land snail *Achatina fulica*. *PLoS ONE*, **7**, e33467.

[10] Dar, M. A., Pawar, K. D., & Pandit, R. S. (2017). Gut microbiome analysis of snails: a biotechnological approach. *Organismal and Molecular Malacology/Ed. S. Ray. InTech*, 189-217.

[11] Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, **3**, 289–306.

[12] Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, **3**, 289–306.

[13] Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, **3**, 289–306.

[14] Imran, M., Saida, B., Ghadi, S. C., Verma, P., & Shouche, Y. S. (2016). The gut-associated Klebsiella sp. of the apple snail produces multiple polysaccharide degrading enzymes. *Current Science*, 2170-2172.

[15] Karasov, W. H., & Martínez del Rio, C. (2007). *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins*. Princeton University Press.

[16] Karasov, W. H., & Martínez del Rio, C. (2007). *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins*. Princeton University Press.

[17] Karnaouri, A., Jalvo, B., Moritz, P., Matsakas, L., Rova, U., Hoofft, O., ... & Christakopoulos, P. (2020). Lytic polysaccharide monooxygenase-assisted preparation of oxidized-cellulose nanocrystals with a high carboxyl content from the tunic of marine invertebrate Ciona intestinalis. *ACS Sustainable Chemistry & Engineering*, *8*(50), 18400-18412.

[18] Kasana, R. C., Salwan, R., Dhar, H., Dutt, S., & Gulati, A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using Gram’s iodine. *Current Microbiology*, **57**, 503–507.

[19] Kathade, S., Aswani, M., & Nirichan, B. (2020). Probiotic characterization and cholesterol assimilation ability of Pichia kudriavzevii isolated from the gut of the edible freshwater snail “Pila globosa”. *Egyptian Journal of Aquatic Biology and Fisheries*, *24*(7-Special issue), 23-39.

[20] Kim, J. Y., Yoon, S. M., & Kim, Y. S. (2015). Cellulase activity of symbiotic bacteria from snails, Achatina fulica. *Journal of the Korean Wood Science and Technology*, *43*(5), 628-640.

[21] Koch, A. L. (2007). Growth measurement. In *Methods for General and Molecular Microbiology* (3rd ed.). ASM Press.

[22] Leschine, S. B. (1995). Cellulose degradation in anaerobic environments. *Annual Review of Microbiology*, **49**, 399–426.

[23] Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, **66**, 506–577.

[24] Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, **66**, 506–577.

[25] Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, **66**, 506–577.

[26] Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, **66**, 506–577.

[27] Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). *Brock Biology of Microorganisms* (15th ed.). Pearson.

[28] Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, **31**, 426–428.

[29] Miller, G. L., Blum, R., Glennon, W. E., & Burton, A. L. (1960). Measurement of carboxymethylcellulase activity. *Analytical Biochemistry*, *1*(2), 127-132.

[30] Morag, E., et al. (1991). Zymogram analysis of cellulolytic enzymes. *Applied and Environmental Microbiology*, **57**, 2850–2856.

[31] Palumbi, S.R., A. Martin, S. Romano, W.O. McMillan, L. Stice, and G. Grabowski. 1991. The Simple Fool’s Guide to PCR. Department of Zoology, University of Hawaii, Honolulu, Hawaii.

[32] Pawar, K. D., Banskar, S., Rane, S. D., Charan, S. S., Kulkarni, G. J., Sawant, S. S., … Shouche, Y. S. (2015). Bacterial diversity in different regions of gastrointestinal tract of *Perionyx excavatus*. *PLoS ONE*, **10**, e0127285.

[33] Sharma, A., Tewari, R., Rana, S. S., Soni, R., & Soni, S. K. (2015). Cellulases: classification, methods of determination and industrial applications. *Applied Biochemistry and Biotechnology*, **179**, 1346–1380.

[34] Song, H., Yu, Z. L., Yang, M. J., Zhang, T., & Wang, H. Y. (2018). Analysis of microbial abundance and community composition in esophagus and intestinal tract of wild veined rapa whelk (Rapana venosa) by 16S rRNA gene sequencing. *The Journal of General and Applied Microbiology*, *64*(4), 158-166.

[35] Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in bacterial systematics. *International Journal of Systematic Bacteriology*, **44**, 846–849.

[36] Teather, R. M., & Wood, P. J. (1982). Use of Congo red–polysaccharide interactions in enumeration and characterization of cellulolytic bacteria. *Applied and Environmental Microbiology*, **43**, 777–780.

[37] Wood, T. M., & Bhat, K. M. (1988). Methods for measuring cellulase activities. *Methods in Enzymology*, **160**, 87–112.