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Comparative expression study of cellulolytic, hemicellulolytic and ligninolyticenzyme coding genes in termite gut bacteria under abiotic stress

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ABSTRACT
Post-harvest burning of wheat stubble is a major concern in North India as well as in the Malwa region of Madhya Pradesh. Termites, an insect of the order Isoptera, are really important decomposers. They break down cellulose, hemicellulose and lignocellulose biomass more efficiently with the help of symbiotic microbial species than other invertebrates. The present study was carried out for the molecular characterization of cellulolytic, hemicellulolytic and ligninolytic enzyme coding genes in potential termite gut bacteria under abiotic stress. In total, 56 bacteria were isolated from the gut of different termites collected from different places of Mandsaur and Neemuch districts of Malwa region of Madhya Pradesh. After various biochemical tests, 12 out of 56 isolates showed cellulolytic, hemicellulolytic and ligninolytic activities under abiotic stressed (high temperature) condition, while among them 5 isolates were recorded with the highest activities. Therefore, in this study we validated the biochemical results in five isolates on high temperature stressed conditions using molecular characterization of selected genes such as cellulase "glycoside hydrolase 6" (GH6), hemicellulase "xylanase A" and ligninase "laccase-like multicopper oxidase" (LMCO) gene expression, through qPCR analysis. Glycoside hydrolase 6 gene expression was found to be highest in Bacillus megaterium MTG19, followed by Bacillus subtilis STG 8 at high temperature (40 to 43 °C). We have also reported xylanase A (Hemicellulase) higher gene expression in Bacillus subtilis STG 8 and Bacillus megaterium MTG 19 at low to high temperature, while laccase-like multicopper oxidase gene expression was found to be high in Streptomyces pseudovenezuelae MTG 17 followed by Bacillus subtilis STG 8 at 37 to 43 °C. This study provides a firm insight about the promising potential activities of cellulase, hemicellulase, and ligninase in bacterial isolates from termites of Malwa region of Madhya Pradesh under abiotic stress conditions which could be a game changer for the management of wheat biomass depolymerization.
Key words:-*Bacillus megaterium, Bacillus subtilis, qPCR, ligninase, cellulase.*

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INTRODUCTION

Lignocellulose products are produced in extremely large quantities every year all around the world. Each year, roughly 109 tonnes of biomass made of lignocellulose are created (1). One of the most plentiful natural resources is this one. Lignocellulose, cellulose, and hemicellulose are its main constituents. The production of renewable energy, particularly biofuels, has a lot of promise thanks to these vast natural resources. The increased lignin contents of these widely accessible materials, however, limit their usage (2). With cellulose and hemicellulose components of the biomass, lignin creates a complicated network that makes lignocellulose biomass depolymerization difficult (3-5). Termites, bees, beetles, and cockroaches are just a few of the creatures that naturally consume lignocellulose biomass. Termites are a complex assemblage of numerous species that can be broadly separated into lower and upper termites (6). They are more effective than otherinvertebrate creatures at degrading lignocellulose biomass (7). In lignocellulose biomass, termites can depolymerize 65-87% hemicellulose and 74-99% cellulose (8). The symbiotic relationship between their gut microbiomes and termite efficiency allows them to consume high lignin-containing lignocellulose biomass (9). The symbiotic microorganisms in termites' intestines actively contribute to the digestion of wood they have consumed (9).

Due to their increased enzymatic activity, bacteria isolated from wood-eating species including termites, bees, beetles, and cockroaches are receiving a lot of interest (10-11). Termite gut bacteria removed about 28% of the lignin from lignocellulose biomass and 65–90% from lignin model compounds (12). Additionally, microorganisms that degrade cellulose andhemicellulose were found in termite guts (11). In

the ecology, there are termite species that number over 2700. For these reasons, termites are seen as potential sources of microbes that degrade lignocellulosic materials. Few studies have been conducted on the isolation and identification of termite-derived lignocellulolytic bacteria, and many more may still be undiscovered. For accurate potency of lignocellulolytic bacteria we have studied the genes encoding for Cellulolytic, Hemi- cellulolytic and Ligninolytic enzymes at their transcription levels under abiotic stress conditions using real-time quantitative polymerase chain reaction (qPCR). Results of the present study will help us better understand molecular mechanisms underlying cellular metabolism. Moreover, these results will serve as a valuable resource for future genetic and genomic studies on studied bacterial specie of the present study.

We have previously studied the cellulolytic, hemicellulolytic, and ligninolytic enzymes at biochemical level, now in present investigation further validated the selected genes at transcription levels under various growth conditions using real- time quantitative polymerase chain reaction (qPCR) for more accurate potency of lignocellulolytic bacteria. The current study's findings will help us better comprehend the molecular processes behind cellular metabolism. Additionally, these findings will be an important source for upcoming genetic and genomic research on the examined bacterial species in the current study.

MATERIAL AND METHODS

Preparation of broth culture of potential bacterial Isolates

In previous study after various biochemical tests, 12 out of 56 isolates showed cellulolytic, hemicellulolytic and ligninolytic activities under abiotic stressed (high temperature) condition, among them 5 isolates were recorded with the highest activities. Therefore, in this study we have grown these five isolates in Minimal basal medium composed of Yeast extract (0.5 g/L), KNO_3 (0.5 g/L), NaCl (2 g/L), K_2HPO_4 (2 g/L), $MgSO_4.7H_2O$ (0.05 g/L), $CaCO_3$ (0.02 g/L), $FeSO_4.7H_2O$ (0.01 g/L) amended with 1 % wheat straw powder at 37°C, 40°C, 43°C, 46°C for 24 h. Bacterial cells were harvested from the broth culture by centrifugation at 5,000 rpm for 15 min at 4°C for further steps.

RNA extraction from potential bacteria

RNA extractions were done using liquid nitrogen and Trizol reagent. Bacterial broths were centrifuged at 5,000 rpm for 15 min at 4°C to obtain bacterial pellet. The pellet then kept in liquid nitrogen until further use. After some time, 1 ml Trizol reagent was added into the frozen bacterial pellet and incubated at -20^{0}C for an hour. After which, 200 µl of chloroform was added in the mixture and mixed by gently inverting the tube.

The missed solution was then subjected to centrifuge at 12000 RPM for 15 min at 4^{0}C to obtain three layers. The upper aqueous RNA containing layer was separated in the fresh tube and 500 µl of chilled Isopropyl alcohol (IPA) was added in the tube. The tube was mixed and incubated at -20^{0}C for an hour. The tube was then centrifuged at 10000 RPM for 12 min at 4^{0}C to precipitate RNA. The precipitated RNA was then washed with 75% ethanol and air dried. The air-dried pellet was then dissolved in 20 µl RNase free distilled water and visualized under UV in 1.5% agarose gel electrophoresis.

cDNA preparation and enzymatic quantification through qPCR analysis Complementary strand of RNA was synthesized using Reverse Transcriptase enzyme through RT-PCR (Reverse transcriptase PCR). In this study, cDNA preparation was done using iSCRIPT™ cDNA Synthesis Kit. RT buffer was mixed with oligo dT primer, random primer, reverse transcriptase enzyme, dNTPs, and RNA template. The cycle of the PCR was as follows-Preparatory phase at 25^{0}C for 5min, annealing and extension at 46^{0}C for 20 mins and denaturation of RT at 97^{0}C for 3 min. This settingwas run for 1 cycle only.

qPCR is a specific and highly sensitive technique for gene expression analysis. It uses a fluorescent reporter (either SYBR green or Taqman) to monitor the amount of amplification product produced after every cycle. The quantification of enzymes produced by bacteria were assessed by the quantity of mRNA being produced. qPCR buffer was mixed with specific primers for the respective enzymes' mRNA, and cDNA prepared in the previous step.

qPCR cyclic condition for the GH6 and xylanase A(xynA): The conditions for qPCR were as follows: 10 min for enzyme activation at 95°C, then a cycle of 40 s for denaturation at 95°C, 45s for primer hybridization at 64°C and 30 s for extension at 72°C was carried out 40 times. A final step, 5 min at 72°C, was carried out.

qPCR cyclic condition for the LMCO-gene: The real-time PCR program consisted of 2 min at 50 C for carryover prevention, 5 min at 94 °C for enzyme activation, followed by 5 cycles consisting of 94°C for 15 s, 57–54°C for 15 s, while decreasing the temperature by 0.5°C in every cycle, 72 °C for 30 s, and 30 cycles consisting of 94 °C for 15 s, 54 °C for 15 s and 72 °C for 30 s; the fluorescence signal was measured atthe 72 °C step for both DNA and cDNA (13)

Table 1: Present table showing the earlier established primer sequence which were used in present study.

Gene	Primer	Reference
Glycoside hydrolase 6(GH6)	Cell2F 5'ACCTGCCCGRCCGYGACT 3'	[11]
	Cell2R 5'GAGSGARTCSGGCTCRAT 3'	
laccase-like multicopper oxidase(LMCO)	Cu1AF 5'ACMWCBGTYCAYTGGCAYGG 3'	[12]
	Cu2R 5'GRCTGTGGTACCAGAANGTNCC-3'	
Xylanase A	Fw 5' ACTGATGGGGGCGGTATAGT 3'	Designed through Primer 3 tool
	Rv 5' TGGCGAACCTGTAGTCCAAC 3'	
gyrA internal control	Fw 5' CTGCCGTGAGTGAGTACCCA 3'	[13]
	Rv 5' AACCTGAACCGCACCAACC 3'	

Where R = A or G; Y = C or T; S = G or C; M= A or C; B = G,T or C,

Computational analysis

For the purpose of raw file interpretation Agilent Aria 1.6 was used, and obtained CT values were further converted manually using ΔΔCT method. GraphPad Prism 5 was used for the ANOVA analysis.

RESULTS AND DISCUSSION

Wheat stubble burning after harvest is a significant issue in both North India and the Malwa region of Madhya Pradesh. Termites, an insect belonging to the Isoptera group, play a crucial role in decomposition. With the aid of symbiotic microbial species, they degrade cellulose, hemicellulose, and lignocellulose biomass more effectively than other invertebrates. Termites' symbiotic relationship with their gut microbiota is what gives them this ability. Hence, in the our earlier study, a total of 56 bacteria were found in the guts of various termites that were collected from various locations of Mandsaur and Neemuch districts of Malwa region of Madhya Pradesh. Following a series of biochemical tests, we have reported 12 of the 56 bacterial isolates with potential for cellulolytic, hemicellulolytic, and ligninolytic activity where 5 of the isolates having the greatest activity at abiotic stress (high temperature).

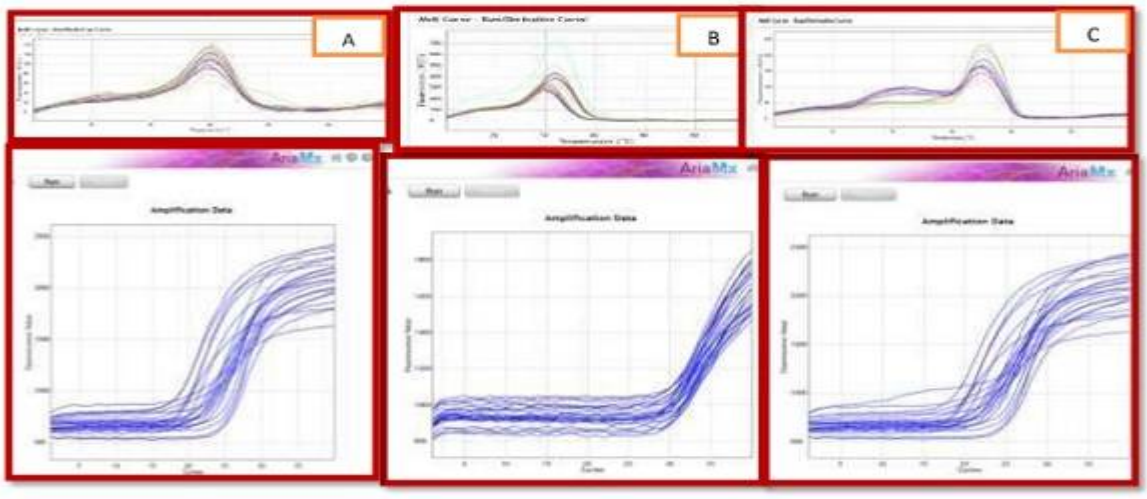

Figure 1: Present figure showing the successful qPCR amplification of glycoside hydrolase 6 (A: Cellulase enzyme), xylanase (B: xynA), and laccase-like multicopper oxidase (C: Ligninase) gene expression in five cultured bacteria at differenttemperature.

In present study, we have studied the gene expression of glycoside hydrolase 6 (cellulase), xylanase A (hemicellulase) and laccase-like multicopper oxidase (ligninase) gene in five potent isolates such as *Bacillus* sp. NTG4, *Aneurinibacillus migulanus* MTG 15, *Bacillus subtilis* STG 8, *Streptomyces pseudovenezuelae* MTG 17 and *Bacillus megaterium* MTG 19 using quantitative PCR (qPCR) to molecularly measure the enzyme production for lignin and cellulose, hemicellulose depolymerizing capabilities (Figure 1).

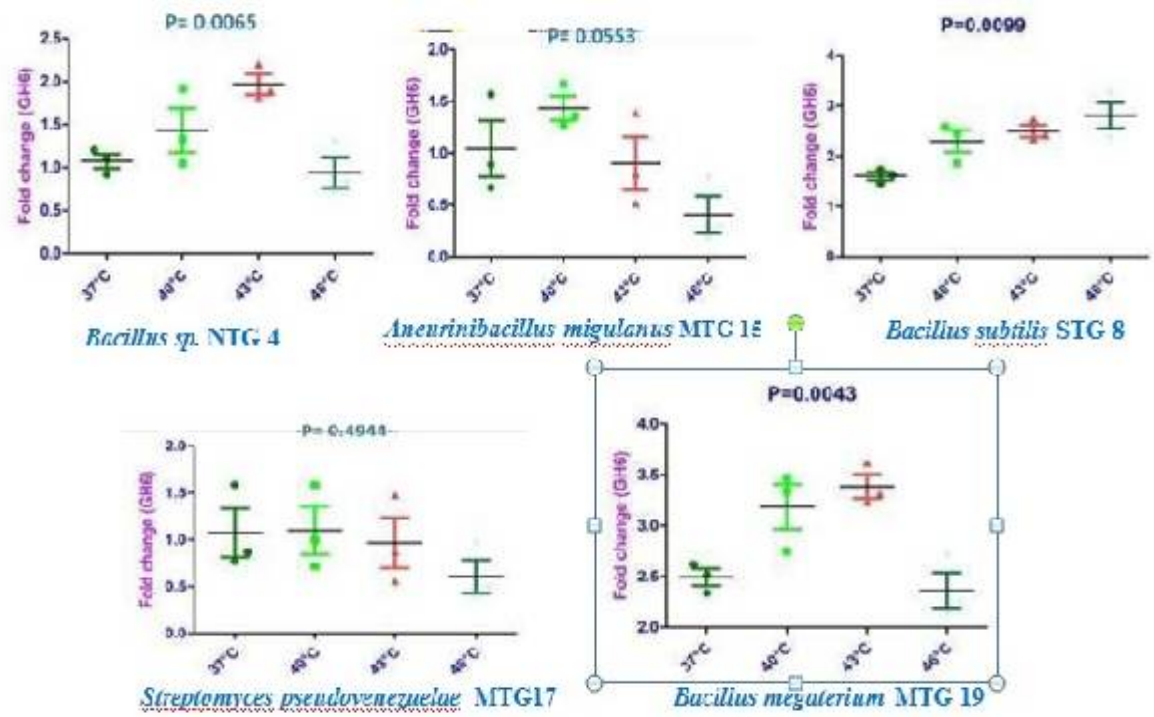

Figure 2: Influence of different temperature on the glycoside hydrolase 6 (Cellulase enzyme) gene expressions in isolated cellulolytic bacterial species grown at different temperature (37°C, 40°C, 43°C, 46°C for 24 h).

Cellulase systems, which contain the three enzyme endoglucanase, exoglucanase and β- glucosidase, are present in all cellulose-degrading microorganisms (14). The combined action of these three enzymes fully hydrolyzes cellulose. In this study, we aimed to validate the biochemical findings by employing molecular characterization of glycoside hydrolase 6 (cellulase) gene expressions. Specifically, we utilized qPCR analysis to assess the gene expression levels in five isolates subjected to high temperature challenges. At high temperature (40 to 43°C), *Bacillus megaterium* MTG 19 and *Bacillus subtilis* STG 8 were found to have the highest level of glycoside hydrolase 6 (GH6) gene expressions as shown in figure 2 and table 2. The statistical analysis of variance (ANOVA) was applied on the fold change gene expression values of glycoside hydrolase 6 gene expression for all five isolates, where we found the differential gene expression pattern from *Bacillus species* NTG4, *Bacillus subtilis* STG 8 and *Bacillus megaterium* MTG 19 at different temperature while analysis of variance showed not significant for *Aneurinibacillus migulanus* MTG 15 and *Streptomyces pseudovenezuelae* MTG 17 which indicate constant gene expression pattern in both of the isolates. Overall data of figure 2 and table 2, showed the high cellulolytic gene expression (GH6) in *Bacillus subtilis* STG 8 and *Bacillus megaterium* MTG 19 at low to high temperature (37°C to 46°C). This signifies the useof these strains for the polymerization of biomass of soil.

Hemicellulases are an enzyme class that may disintegrate hemicellulose, a complex carbohydratepresent in plant cell walls. Microorganisms including bacteria and fungus, as well as some animals and insects that eat plant matter produce hemicellulases. Hemicellulases come in a varietyof forms, each with a distinct function and substrate specificity. For instance, xylanases, hemicellulases that degrade xylan, a kind of hemicellulose present in various plant tissues, are hemicellulases. A different kind of hemicellulose, mannans, can also be broken down by mannanases. GH10 family enzyme XynA (Hemicellulases) identified from *Bacillus* sp. was found to degrade both cellulose and xylan (15). In present study we have also reported the highest xylanase A (Hemicellulase) gene expression in *Bacillus subtilis* STG 8 followed by *Bacillus megaterium* MTG 19 at high temperature (40 to 43°C). As shown in figure 3 and table 3. The statistical analysis of variance (ANOVA) was applied on the fold change gene expression values of xylanase A gene expression for all five isolates, where we found the almost constant gene expression pattern in *Bacillus species* NTG4, *Bacillus subtilis* STG 8 ,*Aneurinibacillus migulanus* MTG15, *Streptomyces pseudovenezuelae* MTG17 at low to high temperature (37°C to 46°C). These findings mainly showed the hemicellulase gene expression significance of *Bacillus subtilis* STG 8 and *Bacillus megaterium* MTG 19 at low to high temperature.

Table 2: Glycoside hydrolase 6 (GH6) gene expressions in selected bacterial species grown atdifferent temperature.

S.No.	Isolates	GH6 gene expression at different temperature (qPCR Fold change values average of triplicate)				Analysis of Variance (ANOVA)
1	*Bacillus species* NTG4	37°C	40°C	43°C	46°C	**P=0.0065****
		0.92	1.05	1.89	1.29	
		1.10	1.33	2.20	0.82	
		1.23	1.92	1.81	0.71	
2	*Aneurinibacillus migulanus* MTG15	37°C	40°C	43°C	46°C	P=0.0553
		0.88	1.35	0.51	0.75	
		1.56	1.66	0.79	0.28	
		0.66	1.27	1.38	0.17	
3	*Bacillus subtilis* STG 8	37°C	40°C	43°C	46°C	**P=0.0099****
		1.45	2.58	2.42	2.82	
		1.63	1.86	2.73	2.35	
		1.73	2.45	2.34	3.24	
4	*Streptomyces pseudovenezuelae* MTG17	37°C	40°C	43°C	46°C	P=0.4944
		1.58	0.71	0.55	0.95	
		0.76	0.99	0.86	0.48	
		0.86	1.58	1.47	0.37	
5	*Bacillus megateriu m* MTG 19	37°C	40°C	43°C	46°C	**P=0.0043****
		2.34	3.47	3.31	2.71	
		2.52	2.75	3.62	2.24	
		2.62	3.34	3.23	2.13	

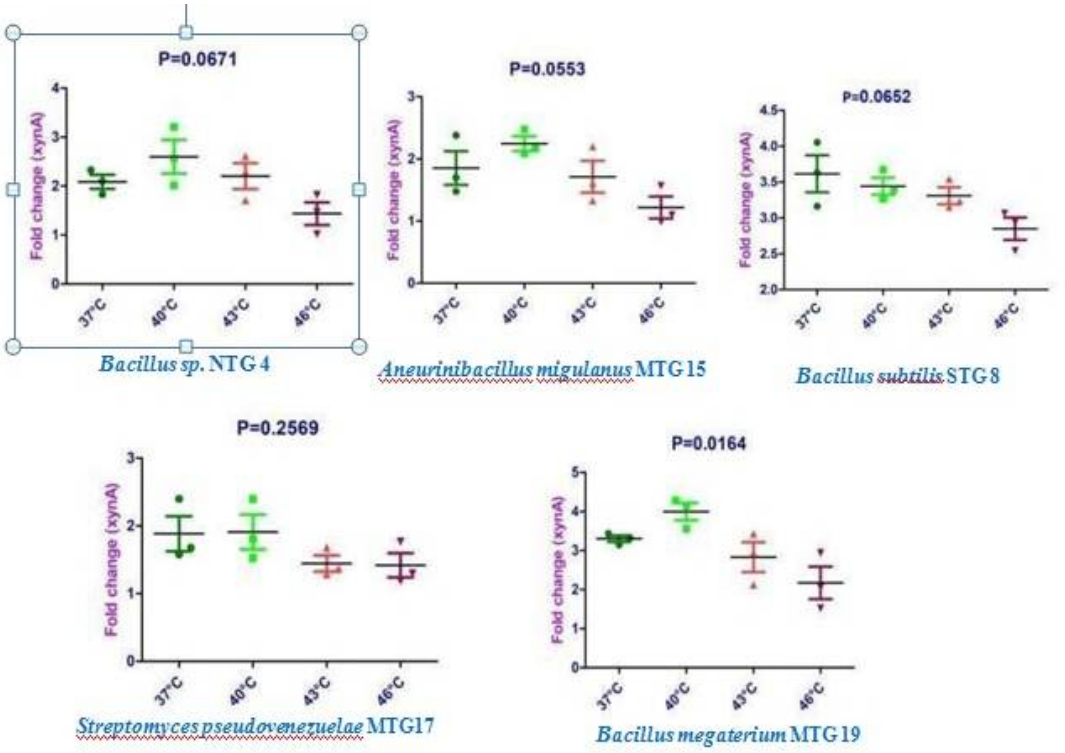

Figure 3: Influence of different temperature on the hemicellulase gene xyn A expression in isolated hemicellulolytic bacterial species grown at different temperature (37°C, 40°C, 43°C, 46°Cfor 24 h).

Table 3: xylanase (xynA) gene expression in selected bacterial species grown at different temperature.

S. N.	Isolates	Xylanase (xynA) gene expression at different temperature (qPCR Fold change values average of triplicate)				Analysis of Variance (ANOVA)
1	*Bacillus species* NTG4	37°C	40°C	43°C	46°C	**P=0.671**
		2.33	2.02	2.29	1.47	
		2.1	3.21	2.61	1.02	
		1.83	2.57	1.71	1.82	
2	*Aneurinibacillus migulanus* MTG15	37°C	40°C	43°C	46°C	P=0.0553
		1.69	2.16	1.32	1.56	
		2.37	2.47	1.60	1.09	
		1.47	2.08	2.19	0.98	
3	*Bacillus subtilis* STG 8	37°C	40°C	43°C	46°C	**P=0.0652**
		3.63	3.39	3.24	3.06	
		3.16	3.67	3.54	2.94	
		4.05	3.26	3.15	2.54	
4	*Streptomyces pseudovenezuelae* MTG17	37°C	40°C	43°C	46°C	P=0.2569
		2.399	1.529	1.369	1.769	
		1.579	1.809	1.679	1.299	
		1.679	2.399	1.289	1.189	
5	*Bacillus megaterium* MTG 19	37°C	40°C	43°C	46°C	**P=0.0164***
		3.151	4.281	2.121	1.521	
		3.331	3.561	3.431	2.051	
		3.431	4.151	2.941	2.941	

The lignin-degrading enzymes can be divided into two main groups such as lignin- degrading auxiliary enzymes and lignin modifying enzymes. In present study we have screened the lignin degrading auxiliary enzyme "laccase-like multicopper oxidase gene" successfully in selected bacterial isolates. This enzyme enables the process of lignin degradation through the sequential action of several proteins that may include oxidative H_2O_2 (14). This study found *Streptomyces pseudovenezuelae* MTG 17 and *Bacillus subtilis* STG 8 with highest levels of laccase-like multicopper oxidase gene expression (Figure 4 & table 4) at low to higher temperature. This gene expression study further provide the firm evidence of our earlier produced biochemical data, in which we have found the five isolates with cellulolytic and ligninolytic potential under different abiotic stressed conditions.

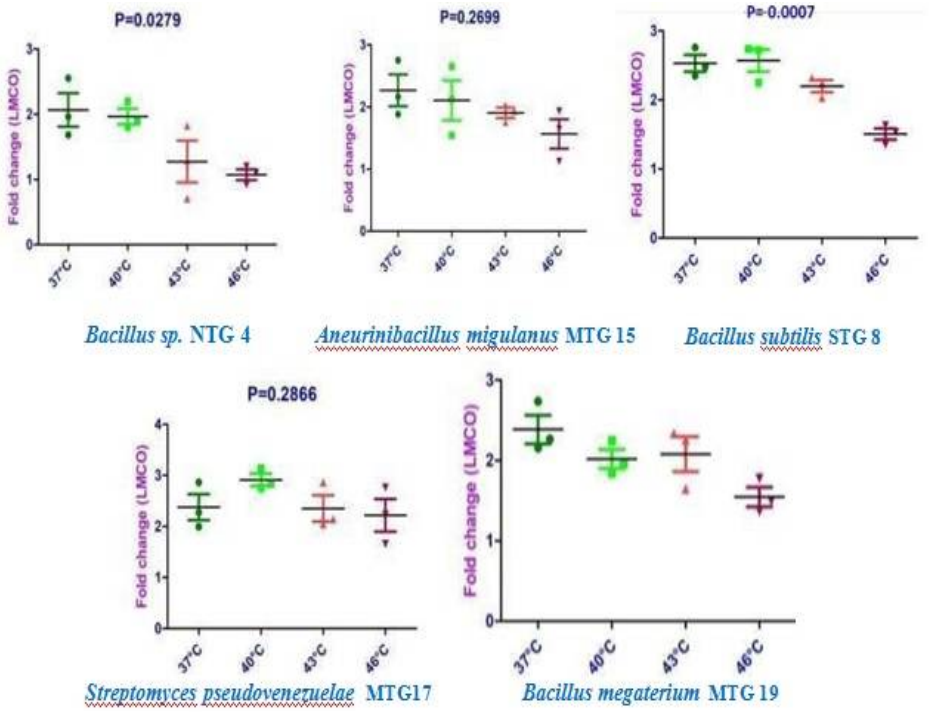

Figure 4: Influence of different temperature on the laccase-like multicopper oxidase (ligninase enzyme) gene expression in isolated ligninolytic bacterial species grown at different temperature (37°C, 40°C, 43°C, 46°Cfor 24 h).

Table 4: Laccase-like multicopper oxidase (LMCO) gene expression in selected bacterial speciesgrown at different temperature.

S.No.	Isolates	LMCO gene expression at different temperature (qPCRFold change values average of triplicate)				Analysis of Variance (ANOVA)
1	*Bacillus species* NTG4	37°C	40°C	43°C	46°C	P=0.0279*
		1.68	1.89	1.29	0.92	
		1.96	2.28	0.82	1.15	
		2.55	1.81	0.71	1.27	
2	*Aneurinibacillus migulanus* MTG15	37°C	40°C	43°C	46°C	P=0.2699
		1.88	2.12	1.75	1.12	
		2.16	2.65	1.93	1.93	
		2.75	1.54	2.03	1.64	
3	*Bacillus subtilis* STG 8	37°C	40°C	43°C	46°C	P=0.0007***
		2.48	2.72	2.32	1.35	
		2.76	2.25	2.03	1.53	
		2.35	2.74	2.24	1.63	
4	*Streptomyces pseudovenezuelae*MTG17	37°C	40°C	43°C	46°C	P=0.2866
		1.99	2.83	1.86	2.23	
		2.23	3.14	2.04	2.76	
		2.86	2.75	2.14	1.65	
4	*Bacillus megaterium* MTG 19	37°C	40°C	43°C	46°C	P=0.0401*
		2.73	1.96	2.33	1.49	
		2.26	1.84	1.64	1.77	
		2.15	2.24	2.25	1.36	

CONCLUSION

The present gene expression study provide baseline data for clear understanding of the exciting prospective activities of cellulase, hemicellulase ,and ligninase in bacterial isolates of termites gut, collected from Mandsaur and Neemuch districts of the Malwa region of Madhya Pradesh. We have successfully characterized the bacterial isolates such as *Bacillus megaterium* MTG 19, *Streptomyces pseudovenezuelae* MTG 17 and *Bacillus subtilis* STG 8 for the decomposition of the wheat straw under adverse climatic conditions.

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References

1. Dahmen, N.; Lewandowski, I.; Zibek, S.; Weidtmann, A. (2018). Integrated Lignocellulosic Value Chains in a Growing Bioeconomy: Status Quo and Perspectives. *GCB Bioenergy* , *11*, gcbb.12586.
2. Nasehi M, Torbatinejad NM, Zerehdaran S, Safaei AR (2014) Effect of (Pleurotusflorida) Fungi on chemical composition and rumen degradability of wheat and barley straw. Iran j applanimsci 4(2):257– 261.
3. Studer MH, Demartini JD, Davis MF, Sykes RW, Davison B, Keller M (2011) Lignin content in natural Populus variants affects sugar release. Proc Natl AcadSci U S A 1–6. https://doi.org/10.1073/pnas. 10092521081073/pnas.1009252108.
4. De Martini JD, Pattathil S, Miller JS, Li H, Hahn MG Wyman CE (2013) Investigating plant cell wall components that affect biomass recalcitrance in poplar and switch grass. Energy Environ Sci 6(3):898–909.
5. Sun Q, Foston M, Meng X, Sawada D, Pingali SV, O'Neill HM, Li H, Wyman CE, Langan P, Ragauskas AJ, Kumar R (2014) Effect of lignin content on changes occurring in poplar cellulose ultrastructure during dilute acid pretreatment. Biotechnol Biofuels 7(1):150. https://doi.org/10.1186/s13068-014-0150-6.
6. Inward D, Beccaloni G, Eggleton P (2007) Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. Biol Lett 3(3):331– 335.
7. Ulyshen MD (2016) Wood decomposition as influenced by invertebrates. Biol Rev 91(1):70–85.
8. Brune, A (2014) Symbiotic digestion of lignocellulose in termite guts. Nature Nat Rev Microbiol 12(3):168–180.
9. Auer L, Lazuka A, Sillam-Dussès D, Miambi E, O'Donohue M, Hernandez-Raquet G (2017) Uncovering the potential of termite gut microbiome for lignocellulose bioconversion in anaerobic batch bioreactors. Front Microbiol 8:2623. https://doi.org/10.3389/fmicb. 2017.02623.
10. Anjum SI, Shah AH, Aurongzeb M, Kori J, Azim MK, Ansari MJ, Bin L (2017) Characterization of gut bacterial flora of *Apis mellifera* from North-West Pakistan. Saudi J Biol Sci 25(2):388–392.
11. Tsegaye, B., Balomajumder, C., & Roy, P. (2019). Isolation and characterization of novel lignolytic, cellulolytic,

and hemicellulolytic bacteria from wood-feeding termite *Cryptotermes brevis*. *International Microbiology*, *22*, 29-39.
12. Kato K, Kozaki S, Sakuranaga M (1998) Degradation of lignin compounds by bacteria from termite guts. Biotechnol Lett 20(5):459–462
13. Moreno, B., & Benitez, E. (2016). Impact of agricultural management on bacterial laccase-encoding genes with possible implications for soil carbon storage in semi-arid Mediterranean olive farming.PeerJ, 4, e2257.
14. Wei Xie, Qi Yu, Ruiqin Zhang, et al. (2021) Structure-function relationship of bifunctionalXynA. *Authorea.* DOI: 10.22541/au.160970138.89944623/v1.
15. Janusz, A. Pawlik, J. Sulej, U.S. Burek, A.J. Wilkołazka, A. Paszczynski.((2017).Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution FEMS Microbiology Reviews fux049, 41 pp. 941-962.

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