



# Harnessing the potential use of cellulolytic *Klebsiella oxytoca* (M<sub>21</sub>WG) and *Klebsiella* sp. (Z<sub>6</sub>WG) isolated from the guts of termites (*Isoptera*)

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## Abstract

**Background:** For many years, denim-heavy quality cotton twill colored with indigo colors and with a well-worn/faded look has held a lot of appeal. Machine damage, drainage system blockage, and other issues come with the conventional usage of pumice stones for “stone-washing” denims. In view of the abovementioned information, a range of works has been done to investigate the economic prospects of bacterial cellulase enzymes for use in industrial processes, including biopolishing in the textile sector. Ethiopia has excellent termite diversity to isolate bacterial gut-associated cellulase enzymes for biostoning applications. The main purpose of this study was, therefore, to decipher how to isolate and characterize cellulase enzymes from termite (*Isoptera*) gut bacteria with the intention of employing it for biostoning of textiles.

**Purpose:** To use cellulolytic enzymes of *Klebsiella oxytoca* (M21WG) and *Klebsiella* sp. (Z6WG) isolated from termite guts in biostoning of textiles and improving garment quality.

**Methods:** Cellulase enzyme-producing bacteria were isolated and screened from the guts of worker termites sampled from Meki and Zeway termite mounds in the Central Rift Valley region of Ethiopia. Bacterial screening, biochemical, morphological, and 16S rRNA sequence identification techniques were employed to characterize the bacterial strains. In addition, the production, optimization, and purification of the associated cellulase enzymes were employed, and the potential application of the enzymes for biostoning of a textile was demonstrated.

**Result:** The isolated M21WG was found to be 99% identical to the *Klebsiella oxytoca* (MT104573.1) strain, while the isolated Z6WG showed 97.3% identity to the *Klebsiella* sp. strain (MN629242.1). At an ideal pH of 7, a temperature of 37 °C, a 72-h incubation time, and a substrate concentration of 1.5% carboxymethylcellulose sodium, the maximum activity of the crude cellulase extract from these bacteria was assessed. These bacteria produced cellulase enzymes that were moderately efficient. Consequently, it was determined that the cellulase enzymes were effective for biostoning of denim cloth.

**Conclusion:** It was determined that *Klebsiella oxytoca* (M21WG) and *Klebsiella* sp. (Z6WG) could be used as a doorway to better understand harnessing the use of these cellulase-producing bacteria from termite (*Isoptera*) guts. In this

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study, it was also attempted to assess the effectiveness of the two bacterial isolates in biostoning in anticipation of their potential application in the textile realm.

**Keywords:** Bacteria, Cellulolytic, Rift Valley, Termites, Cellulase

## Background

Termites, a type of eusocial insect, have been known to create devastation on lignocellulosic materials such as wood and crops, as well as other man-made buildings (Eggleton, 2010; Nobre and Nunes, 2007). Termites depending on their evolutionary level, both in terms of behavior and anatomy, are divided into higher and lower categories (Ferbianto et al., 2015; Kakkar et al., 2015; Sreena et al., 2015; Sakolvaree and Deevong, 2016; Javaheri-Kermani and Asoodeh, 2019; Oberstet et al., 2020; Kumar and Upadhyay, 2021). However, their commercial potential has been realized in the domain of microbial biotechnology for the exploration of biocatalysts derived from various symbiotic eukaryotes and prokaryotes, in particular, those that harbor the gut compartments of lower termites (Mattéotti et al., 2012; Ali et al., 2019b; Zhou et al., 2019; Arfah et al., 2019). As a social insect, a termite colony consists of three castes, i.e., reproductive, soldier, and worker castes. In their role of cellulose digestion, the worker termites use two sources of cellulolytic enzyme that include cellulase produced by the termite and the gut symbionts (Ferbianto et al., 2015; Egwuatu and Appah, 2018; Sanchez-García et al., 2020; Ye et al., 2019; Zhao et al., 2021; Bezerra et al., 2021).

The symbiotic digestion of lignocellulose in the higher termite family (*Termitidae*) is carried out by an exclusively prokaryotic gut microbiota, which is influenced by microenvironmental factors such as pH, available substrates, and O<sub>2</sub> and H<sub>2</sub> gradients. This has prompted research into the functional roles of specific bacterial taxa in lignocellulose and humus digestion. Due to the thermodynamic inadequacy of acetogenesis in termite gut microbial fermentation, CO<sub>2</sub>-reducing acetogenesis as a “H<sub>2</sub> sink” reaction is one of the most distinctive and perplexing aspects (Upadhyaya et al., 2012; Muwawaet et al., 2016; Mikaelyan et al., 2018; Loh et al., 2021). The associated microorganisms represented by intestinal bacteria have repeatedly been shown to facilitate interactions between trees and wood feeders by supplementing essential nutrients or degrading complex dietary polymers (Sreena et al., 2015; Dar et al., 2021; Ge et al., 2021). Insects can be transiently or permanently infected with gut-associated microbes such as bacterial gut (*Panthoea* and some *Erwinia* species, which assisted in plant cell wall cellulose degradation), fungi, protozoa, and viruses, and this association can be useful or harmful (Kakkar et al., 2015; Bozorov et al., 2019).

Many insects’ ability to subsist on wood, leaves, and detritus has sparked interest in using their gut microbial flora as a bioresource for commercial conversion of low-cost cellulosic material into bioproducts (Li et al., 2021; Nelson et al., 2021). Higher termites, with the exception of some species that harbor cellulolytic amoeba, lack cellulolytic flagellates and instead have a prokaryotic microbiota in their hindgut that aids lignocellulose degradation by contributing bacterial celluloses and hemicellulose that work in tandem with endogenous cellulase secreted by the termites’ gut tissue (Ali et al., 2019a; Tokuda, 2021). Termites, as an insect group, have evolved symbiotic systems that efficiently digest lignocellulose meals, making them a good supply of cellulolytic enzyme (Aueret et al., 2017; Ali et al., 2019b; Nandy et al., 2021).

Of pertinence is to mention that the expression of genes in caste differentiation and cellulose digestion has garnered tremendous research impetus in the niche of termite genomics and proteomics (Scharf and Tartar, 2008; Husseneder et al., 2012). As the termite gut mimics a fermenter chamber (Ngangi et al., 2019), both endogenous cellulases and the cellulases from symbiotic microbes confer excellent lignocellulose-degrading potency to termites (Duan et al., 2017). Needless to say, cellulases represent a group of complex biocatalysts that catalyze the hydrolysis of cellulose to fermentable sugar. The synergistic action of endo-β-glucanase (EC 3.2.1.4), cellobiohydrolase/exo-glucanase (EC 3.2.1.91/EC 3.2.1.176) (Guar et al., 2015; Reffas et al., 2016; Islam and Roy, 2018; Olowomofe et al., 2019; Regmi et al., 2020; Biswas et al., 2020), and β-glucosidase (E.C. 3.2.1.21) is bracketed together with the conversion of cellulose into glucose monomers (Gbenro et al., 2019; Javaheri-Kermani and Asoodeh, 2019; Liu et al., 2019b; Nazir et al., 2021). Bacteria are now being studied extensively for the production of cellulase due to their rapid growth, expression of multi-enzyme complexes, stability at extremes of temperature and pH, lower feedback inhibition, ability to colonize a wide range of environmental niches, and resistance to a variety of environmental stresses (Khatiwada et al., 2016; Rawway et al., 2018; Islam and Roy, 2019; Prabhakar et al., 2021).

The immense thrust received by cellulases over the years may be easily perceived in the context of their wide gamut of applications in the textile and laundry industry (Krik et al., 2002; Sreena et al., 2015; Sakolvaree and Deevong, 2016; Sankarraj and Nallathambi,

2018; Fasiku et al., 2020; Nursyirwani et al., 2020; Kabir and Koh, 2021), paper and pulp industry (Sondhi et al., 2015; Abu-Gharbia et al., 2018; Wang et al., 2020; Sep-ehri et al., 2020), wine-making and brewing (Sojitra et al., 2017, Irshad et al., 2017; Zahra et al., 2020), agriculture (Abdel-Azizet al., 2021; Fatmawati et al., 2021; Tomulescu et al., 2021), animal feed industry (Bharti and Sandhu, 2015; Phuong et al., 2015; Tamilanban et al., 2017; Luo et al., 2013; Ali et al., 2019a; Ali et al., 2019b; Tsegaye et al., 2019, Astolfi et al., 2019, Chauhan, 2020) and pharmaceutical domain (Gupta et al., 2013; Bharti and Sandhu, 2015; Obafemi et al., 2019; Barbosa et al., 2020) as well as in waste management (Gupta et al., 2013; Cui et al., 2014; Mahjabeen et al., 2016; Prasad et al., 2019; Ndlovu et al., 2019; Rehman et al., 2019; Karthika et al., 2020).

At this juncture, it is also worth noting that bacterial cellulase-mediated cellulose hydrolysis is associated to a number of beneficial properties, including increased stability, increased specific activity, and facilitated mass transfer (Maki et al., 2009; Islam and Roy, 2018; Prasad et al., 2019).

In the backdrop of the afore-stated, investigating and using the potential cellulase-producing gut bacteria of termites appears to be an intriguing topic of study. The goal of this research was to characterize cellulase-producing bacteria found in the guts of termites from Ethiopia's Meki and Zeway central Rift Valley, Eastern part of Shawa Zone Oromia Regional State. We attempted to analyze the enzyme's effectiveness in dye leaching after optimizing bacterial culture settings for maximum biocatalyst synthesis and partial purification of the enzyme in anticipation of its possible application in the textile realms.

## Results

In this study, cellulolytic gut bacteria from termites (*Isop-tera*) were isolated from worker termites, screened, and identified based on morphological, biochemical, and genetic properties for potential textile industry application. The soil (mound-building) interface-feeding higher termite samples were obtained in the central Rift Valley, in the eastern part of Shawa Zone, Oromia Regional State, 121.4 km from Addis Ababa, Ethiopia. The high specificity of bacterial populations in the gut compartment, as well as the convergence of higher termite gut arrangements in a homologous region, provided significant evidence of differentiation from other microorganisms.

Bacterial colonies were initially produced on nutrient agar and then cultured on the CMC-Na medium. The bacterial isolates were re-streaked on CMC-Na agar

plates after enrichment to achieve pure colonies, which were then transferred to broth culture for further use and study.

### Screening for cellulolytic isolates

The isolated bacterial strains in this study successfully demonstrated the expected clear zone. The pure colony isolates that were predicted to produce cellulase were cultured on CMC-supplemented agar plates containing Congo red, and the cultures were examined for clear zones surrounding the colonies caused due to the breakdown of CMC.

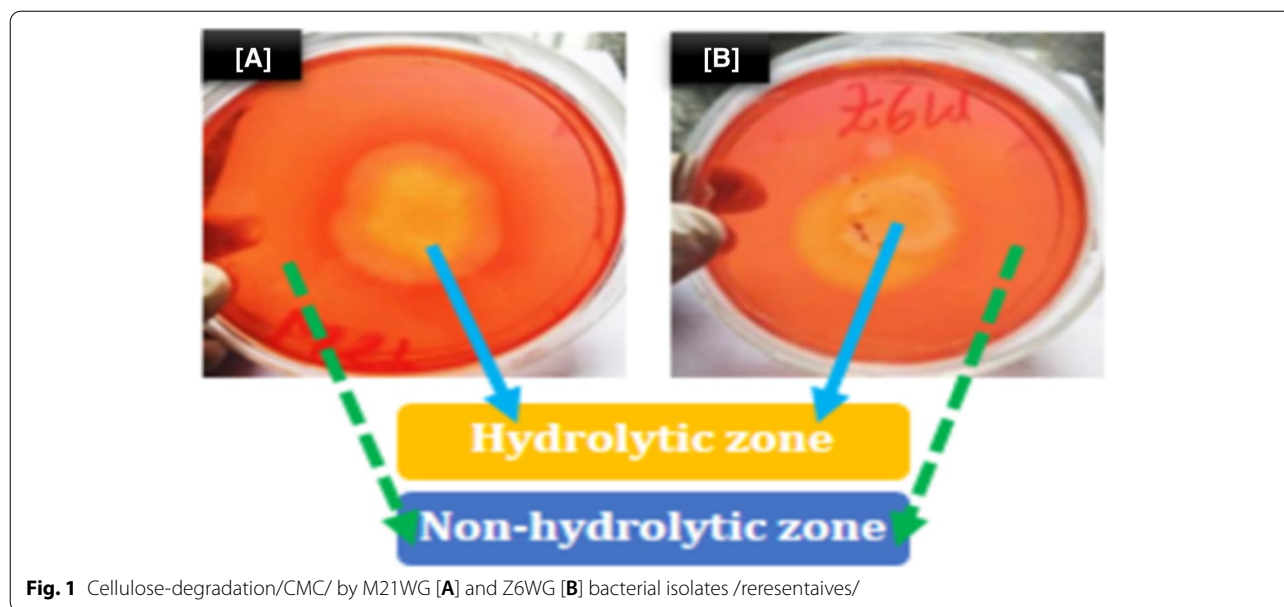
For the evaluation of enzyme efficiency between different bacterial strains, the widths of the clear zones created by colonies were employed. As a result, six of the twenty-seven termite gut isolates ( $M_{21}WG$ ,  $M_{22}WG$ ,  $M_{22}YG$ ,  $Z_5WG$ ,  $Z_6WG$ , and  $Z_6YG$ ) were effective and yielded the effectively positive result indicated below (Fig. 1).

Among them, twenty-seven bacterial isolates, two  $M_{21}WG$ , and  $Z_6WG$  were considered as the most remarkable in their colony characteristics and cellulase producing potential with yellow color hydrolytic zone while red color was non-hydrolytic zone. The diameter of the bacterial colony was 4 mm and the halo  $36 \pm 0.65$  mm and  $29 \pm 0.81$  mm while the diameters of the clear zone were  $32 \pm 0.62$  mm and  $25 \pm 0.73$  mm, respectively, for each bacterium. The maximum halo zone diameter minus bacterial colony/colony diameter ratio was  $8 \pm 0.155$  mm and  $6.25 \pm 0.183$  mm (Table 1). The other remaining bacterial isolates did not show clear zones similar to the control plate and were not considered as cellulase-producing bacterial candidates.

The  $M_{21}WG$  and  $Z_6WG$  cellulolytic bacterial isolates were screened as described above. The isolate  $M_{21}WG$  showed the clearest zone, and it was chosen for further analysis. In general, the presence of a halo zone around the bacterial colony on agar culture plates indicated cellulose hydrolyzing activity (on the basis of zone of clearance after staining the plates with Congo red dye was the highest halo zone). As a result, the bacteria found in termite guts may have the ability to produce cellulase, an essential enzyme that could be used in the cellulose degradation process.

### Morphological characterization of the selected isolates

The best gut bacteria isolates were provisionally identified using morphological and cultural characterization, then purified using the streak plate method and restreaked until a single bacterial colony or pure culture was obtained (Fig. 2). Colony morphological characteristics such as colony formation, color, colony surface roughness and



**Fig. 1** Cellulose-degradation/CMC/ by M21WG [A] and Z6WG [B] bacterial isolates /resentsaives/

microscopic appearance, and cell arrangement were documented for comparative analysis of the selected isolates.

The two selected bacterial isolates, M<sub>21</sub>WG and Z<sub>6</sub>WG, were then analyzed for their biochemical attributes (Table 2). Both of the isolates were positive for glucose and lactose fermentation, as well as indole test. Pertinently, fermentation potency was exhibited to show positive nature.

From Table 2, the bacterial isolates, M<sub>21</sub>WG and Z<sub>6</sub>WG, were negative for gram staining tests. Both were found to be gram-negative (G-) and rod-shaped.

**Biochemical characteristics of isolated bacteria**

The two selected bacterial isolates, M<sub>21</sub>WG and Z<sub>6</sub>WG, were considered for further investigation and their biochemical tests are presented in Table 3. The creation of a bubble in Table 3 suggested that the isolates were catalase-positive and could produce the enzyme cellulase, which basically transformed hydrogen peroxide into water and oxygen. Furthermore, the isolates were found to be negative for motility, indole, and citrate tests. The results of carbohydrate utilization tests are also shown in Table 3.

**Molecular identification of isolates using 16S rRNA sequencing**

The molecular phylogenetic history of the bacterial isolates M<sub>21</sub>WG and Z<sub>6</sub>WG was then determined using 16S rRNA gene sequences. About 1500 bp of 16S rRNA gene was PCR amplified from the contig region from genomic DNA of both M<sub>21</sub>WG and Z<sub>6</sub>WG bacterial isolates. Those unclear (ambiguous) positions were removed for each sequence pair which was called the pair-wise deletion option. The BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) using the online NCBI Genbank showed that M<sub>21</sub>WG isolate has 99% maximum identity with sequences of *Klebsiella oxytoca* strain (MT104573.1), and algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) Z<sub>6</sub>WG isolate has 97.30% similarity with *Klebsiella* sp. strain (MT429262.1).

Also, in this study, the identification of closest relatives via phylogenetic analysis helped to determine the possible affiliation of the isolates. Based on the taxonomic analysis of the 16S rRNA gene sequences, phylogenetic trees were constructed by using multiple sequence alignment programs to understand their genetic and evolutionary relationships (Tamura et al, 2004; Tamura et al, 2021). The isolates were identified based on the percentage similarity with the known species sequences in the database (Fig. 3).

**Table 1** Hydrolytic capacity value of cellulase degrading bacteria

Number	Bacterial isolates	CD (mm)	Halo zone (mm)	CZ (mm)	Hydrolytic capacity $\frac{CZ}{CD}$
1	M <sub>21</sub> WG	4	36 ± 0.65	32 ± 0.62	8 ± 0.155
2	Z <sub>6</sub> WG	4	29 ± 0.81	25 ± 0.73	6.25 ± 0.183

All tests were performed in triplicate described by mean ± SD

CD colony diameter, CZ clear zone, mm millimeter



**Fig. 2** Colony morphology of the isolated bacteria, M<sub>21</sub>WG, and Z<sub>6</sub>WG isolates

**Production of crude cellulase and some factors that affect culture condition for cellulase production**

In this study, the isolates *Klebsiella oxytoca* (M<sub>21</sub>WG) and *Klebsiella* sp. (Z<sub>6</sub>WG) were examined to have a promising potential for cellulase activity of 287.33 ± 0.67 U/mL and 236.67 ± 0.89 U/mL, respectively. The study on the effect of pH on cellulase activity of both enzymes of the isolates has demonstrated that the maximum cellulase production was found to be at values between pH 6 and 8, while the activity of 267 ± 1.38 U/mL was recorded for *Klebsiella oxytoca* (M<sub>21</sub>WG) at pH 7 and 220 ± 1.83 U/mL for *Klebsiella* sp. (Z<sub>6</sub>WG) was recorded at pH 7, as the pH approaches the ideal activity level and the manufacturing activity steadily increased (Fig. 4a).

The influence of temperature on cellulase activity was fairly strong in the temperature range of 35–45 °C for both isolates, with the best activity reported at 37 °C. *Klebsiella oxytoca* M<sub>21</sub>WG enzyme activity was at 231 ± 1.11 U/mL, while *Klebsiella* sp. Z<sub>6</sub>WG enzyme activity was around 203 ± 1.66 U/mL (Fig. 4b). The effect of incubation time on cellulase activity was investigated by incubating the production medium with the test isolate at different time intervals (24–108 h). The highest

cellulase production (257 ± 0.41 U/mL) occurred at 72 h for *Klebsiella oxytoca* M<sub>21</sub>WG, and a relatively lower production enzyme activity of 214 ± 0.85 U/mL occurred at the same incubation period for *Klebsiella* sp. (Fig. 4c).

*Klebsiella oxytoca* (M<sub>21</sub>WG) 242 ± 1.111 U/mL and *Klebsiella* sp. (Z<sub>6</sub>WG) 210 ± 1.36 U/mL had the greatest influence of substrate CMC-Na concentration on cellulase activity in this investigation at a substrate concentration of 1.5%. Cellulase production increased as the initial concentration increased from 0.1 to 1.5%, with further increases in concentration gradually lowering the products. Cellulase production was revealed to be dependent on the carbon sources that supplemented in the culture media (Fig. 4d).

**Partial purification of cellulase enzyme by ammonium sulfate precipitation and dialysis**

The partial purification of cellulase isolated from *Klebsiella oxytoca* (M<sub>21</sub>WG) and *Klebsiella* sp. (Z<sub>6</sub>WG termite guts) had been the difference between crude cellulase, specific activity, protein fold, and yield or

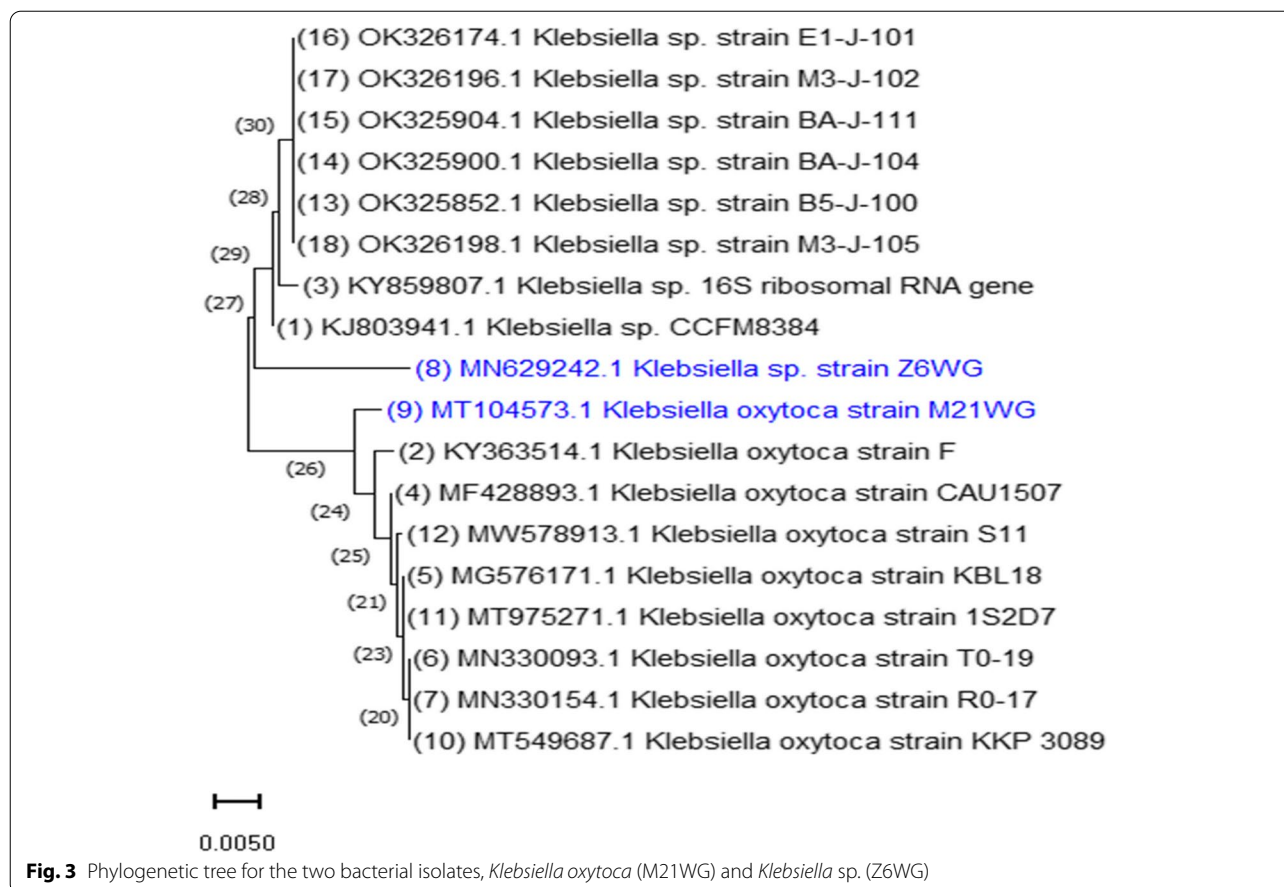
**Table 2** Morphological and cultural characters of the most efficient bacterial isolates, M<sub>21</sub>WG and Z<sub>6</sub>WG

Colony characterization	Isolated bacterial isolates	
	M <sub>21</sub> WG	Z <sub>6</sub> WG
Colony-forming	Circular	Circular
Colony color	Cream white	White
Colony surface	Smooth	Smooth
<b>Cell characterization</b>		
Gram-reaction	Negative	Negative
Cell arrangement	Rod	Rod

**Table 3** Biochemical characteristics of the two most efficient isolated bacterial isolates, M<sub>21</sub>WG and Z<sub>6</sub>WG

Number	Biochemical tests	Isolated bacterial strain		
		M <sub>21</sub> WG	Z <sub>6</sub> WG	
1	Catalase	+	+	
2	Citrate	-	-	
3	Methyl red	+	+	
4	Indole test	+	+	
5	Motility test	Non-motile	Non-motile	
6	<b>Sugar Fermentation</b>	Glucose	+	+
		Lactose	+	+
7				

“+,” positive: able to utilize carbon source; “-,” negative: unable to utilize carbon source



concentration of salting in and salting out of purification of protein 80% yield of ammonium sulfate precipitation. The specific activity and protein fold while total protein and yield was decreased from the below (Table 4).

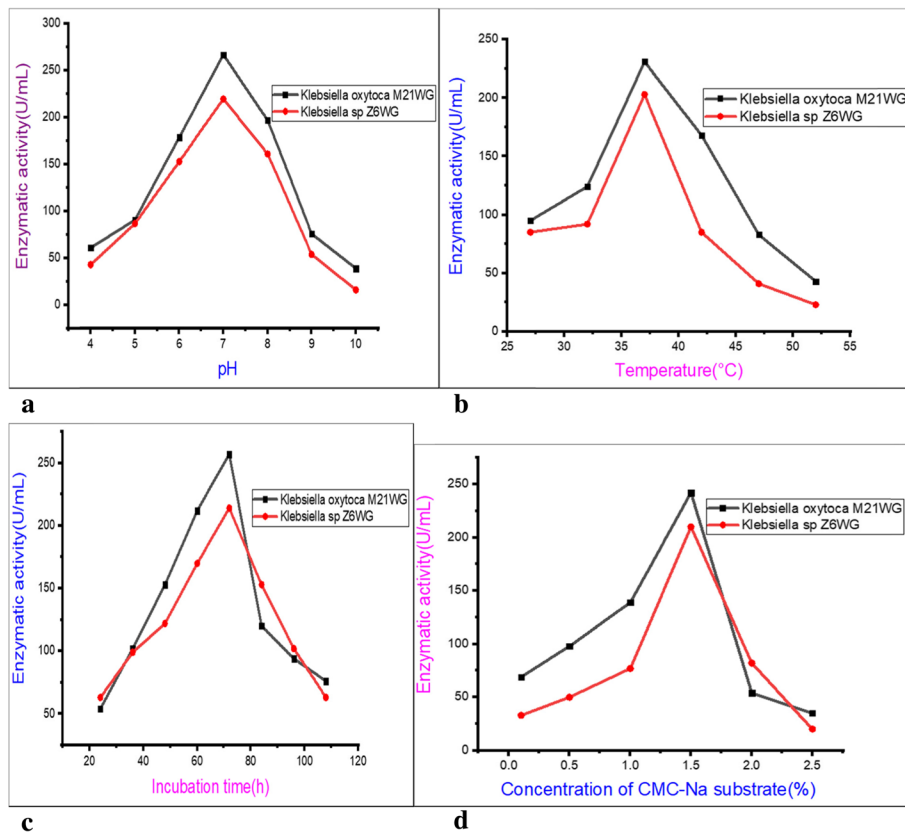
In the enzyme fermentation process, the crude extracts contain different mixtures of proteins and undesirable products as organic acids and other metabolites. So, purification of the required favorable product must take place by different purification methods. The maximum specific activity of crude ( $0.33 \pm 0.27$ ,  $0.32 \pm 0.74$  U/mg), partially purified using ammonium sulfate precipitation ( $7.45 \pm 0.63$ ,  $3.57 \pm 0.57$  U/mg), and dialysis ( $14.12 \pm 0.58$  and  $13.61 \pm 0.67$  U/mg) of *Klebsiella oxytoca* (M<sub>21</sub>WG) and *Klebsiella* sp. (Z<sub>6</sub>WG) estimated.

**Assessment of biostoning potential of the partially purified cellulase for prospective in the textile industry**

Over the years, denim-heavy grade cotton twill, dyed with indigo colors and well-worn look, has churned commendable popularity. Conventional use of pumice stones (with or without oxidizing agent like potassium permanganate) for “stone washing” of denim suffers

from numerous practical snags including impairment of machine parts, blockage of the drainage system, issues of removal of residues on the pumice stones, requisite for a large amount of stones for even small batches, and the possibility of excessive abrasion that may damage the fabric. The stoned denim swatches by the respective cellulase at different dosages and the untreated piece are attesting (visual) the efficacy of the biocatalysts.

The increase in the dosage for both the test cellulase was found to bear a direct correspondence to the gray value for the selected areas in contrast to the untreated counterparts. For cellulase from M<sub>21</sub>WG, a maximal gray value of 142 was obtained for the swatch treated with 4 ECU dosages against 27.1 for the untreated (control) sample while the corresponding values for the biocatalyst from *Klebsiella* sp. Z<sub>6</sub>WG were 150 and 22.8, respectively (Fig. 5a, b). In a similar vein, the corresponding color histograms of the M<sub>21</sub>WG cellulase treatment, the rMean (red), gMean (green), and bMean (blue) values for the untreated samples increased from 52.44, 41.74, and 39.79 (for 1 ECU dosage) to 159.03, 133.52, and 115.37 for 4 ECU treatments, respectively. While for Z<sub>6</sub>WG treatment, the corresponding values were found



**Fig. 4** a Effect of pH, b temperature (°C), c incubation time (h), and d substrate CMC-Na concentration (%) by *Klebsiella oxytoca* M21WG and *Klebsiella sp.* Z6WG on cellulase activity

to escalate from 82.83, 65.82, and 60.06 to 166.78, 144.35, and 127.57, respectively.

It is difficult to control the quantity of abrasion the fabric was exposed to, and acid washing of jeans caused several environmental problems and a novel method of stoning jeans was required to keep jeans charming and comfortable. The biostoning was found to bear cellulolytic enzymes, supporting other previous studies that indicated the applicability in the textile industry.

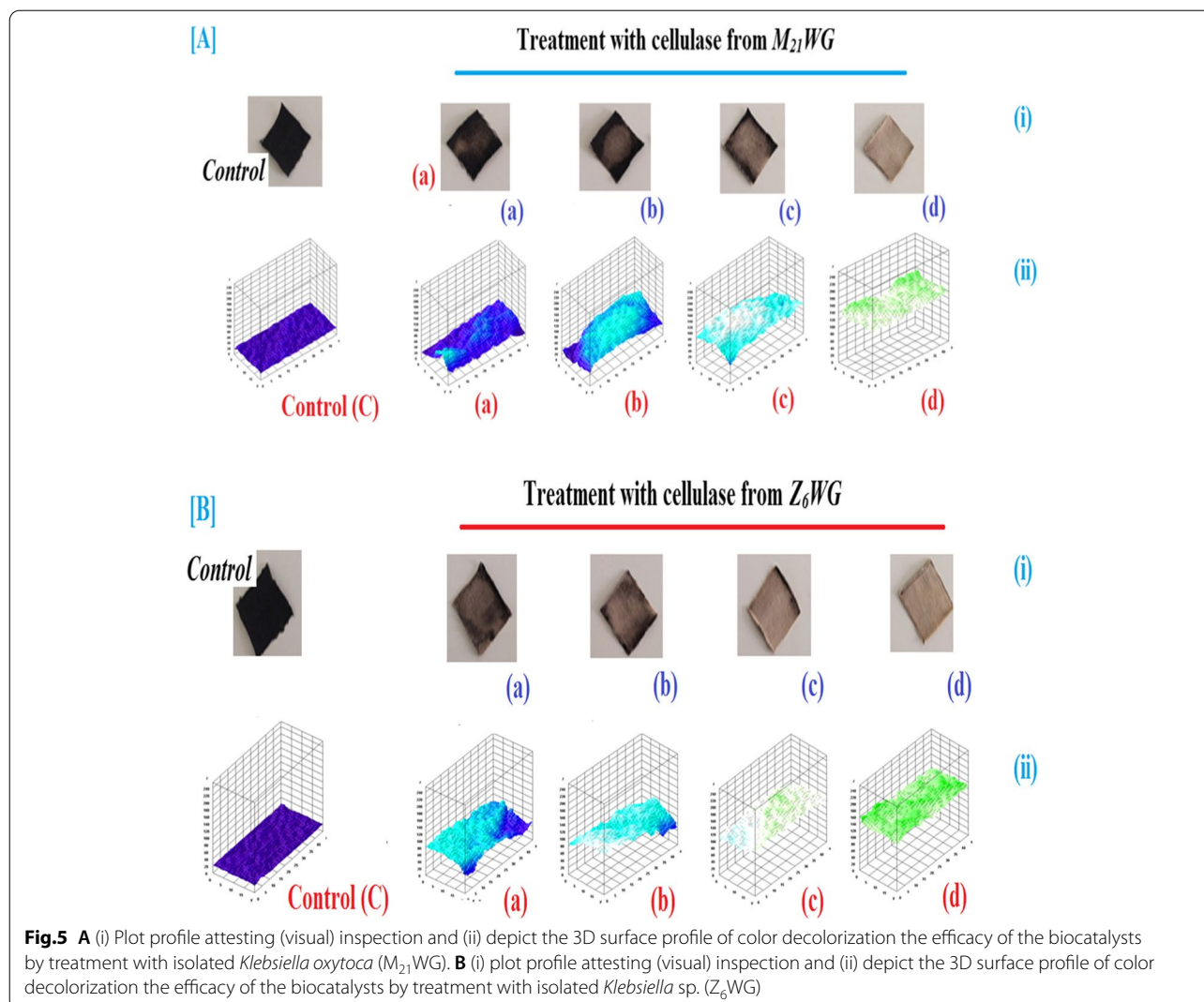
### Discussions

The isolated bacteria were found to allow cellulolytic enzymes, supporting other previous studies that revealed the selected to produce cellulase. The findings of the current investigation revealed a large amount of information about the cellulase-producing bacteria found in termite guts and their roles in the textile industry. Cellulolytic enzymes were found to be allowed by the isolated bacteria, supporting prior research that demonstrated

**Table 4** The purification step of cellulase isolate from *Klebsiella oxytoca* (M<sub>21</sub>WG) and *Klebsiella sp.* (Z<sub>6</sub>WG)

Enzyme code	Purification steps	Total protein (mg)	Total activity (U)	Specific activity (U/mg)
<i>Klebsiella oxytoca</i> M <sub>21</sub> WG	Crude enzymes	815	270	0.33 ± 0.3
	Ammonium sulfate precipitation	18	134.2	7.45 ± 0.45
	Dialysis	7.3	103	14.12 ± 0.58
<i>Klebsiella sp.</i> Z <sub>6</sub> WG	Crude enzymes	726	236.67	0.32 ± 0.74
	Ammonium sulfate precipitation	30.8	110	3.57 ± 0.53
	Dialysis	7	95.3	13.61 ± 0.69

Values of mean ± SD represent the standard error of replicates  
 mg milligram, U μmol/min/mL, U/mg units per milligram



cellulolytic bacteria utilize cellulose as a carbon and energy source (Dar et al., 2018; Liu et al., 2019a). A distinct halo zone around the microbial colonies is created by the positive bacterial isolate. The cellulose hydrolysis capacity (HC) of the bacterial isolate was determined using colonies that formed distinct halo zones (Phuong et al., 2015; Shinde et al., 2017b; Katiyar et al., 2018).

Egwuatu and Appah (2018) studied the isolation and characterization of filter paper-degrading bacteria from the guts of *Coptotermes formosanus* by biochemical tests *Klebsiella oxytoca* and *Klebsiella terrigena* that is coherent in our investigation. Dar et al. (2018) studied and explored the gut of *Helicoverpa armigera* for cellulose-degrading bacteria and evaluated a potential strain for lignocelluloses biomass (cotton bollworm) deconstruction. *Klebsiella* spMD21 (MG367463) is identified based on 16S rRNA gene sequencing. *Klebsiella* sp. CCFM8384 (KJ803941) in India related coherence to this

finding *Klebsiella* sp. MT429262.1 *Z*<sub>6</sub>WG indicate percent bootstrap values based on 1000 replicates to decide the redundancy of the get sequence with the reference classifications. Dantur et al. (2018) studied that *Klebsiella michiganensis/oxytoca* Kd70 was isolated from the intestine of the larvae of *Diatraea saccharalis* and that independent development of infecting urinary tract in mice and human/animal pathogenicity could have occurred originally from a plant/soil ancestor in Argentina (Dantur et al., 2015). The optimum pH of fermentation for maximum cellulase production was monitored at pH 7 for bacteria species including *Bacillus subtilis* K-18, *Enterobacter* species, *Klebsiella* species, *Pseudomonas* species, and *Serratias* species and able to survive in both alkaline environments and acidic conditions (Chandran et al., Bai et al., 2012, 2021; Shinde et al., 2017a).

The ability of bacteria to grow in a wide range of pH involving extremes pH ranges; the plasma membrane



of microbes might create damage or distracting to the membrane transport protein and also inhibits enzymatic activity.

Cellulose-degrading bacteria from paper and pulp mill dumpsites the ability of bacteria *Klebsiella michiganensis* optimum at pH 7 cellulase activity of 35.6 U/mL/min (Olowomofe et al., 2019). Shinde et al. (2017a) studied the molecular characterization of cellulolytic bacteria derived from termite gut and the optimization of bacterial growth at different temperatures and optimum ranges (30 to 45 °C). The temperature specificity of most isolates grew well between 30 and 40 °C. For example, *Klebsiella variicola* (isolate Z) showed luxuriant growth at 30 °C and other isolates such as *Klebsiella pneumoniae* (isolates B2, C1, C3, and B1 plate). According to Sakthivel et al. (2010), the effect of parameters on cellulase product was maximum after 72 h (0.72 U/mL), pH at 7 (0.80 U/mL), and substrate concentration of CMC 1.0% (1.15 U/mL) for *Corynebacterium lipophiloflavum* isolated from a decaying vegetable. According to the study of Olowomofe et al. (2019), *Klebsiella michiganensis* had optimum cellulase activity after 72 h (38.5 U/mL/min) after 48 h. These isolates recorded the lowest enzyme activity after 96 h. Islam and Roy (2018) studied the effect of carbon source-high substrate specificity by CMC 1.0% (w/v) which showed to be cellulase of *Paenibacillus* sp. C1 has the highest activity against CMC while low activity of *Bacillus* sp. C2, and *Aeromonas* sp. C3 when compared to lactose, rice xylan, wheat bran, xylose, and oat spelt xylan.

The ability to resist on substrate might be useful for many industrial applications. Purwanto (2016) previously studied that the maximum specific activity and protein fold of enzymes at 80% of ammonium sulfate precipitation and dialysis was 0.0107, 0.0160, 1.24, and 1.84 respectively. Among others, cellulases have received considerable interest in the textile industry for mercerization, scouring, bio-polishing, laundering, and biostone finishing (Shah, 2013).

Microorganisms like *Klebsiella*, *Pseudomonas*, *Desulphovibrio*, *Bacillus*, *Sphingomonas*, *Streptococcus*, *Escherichia*, *Aeromonas*, *Proteus*, *Schewanella*, *Alcaligenes*, and *Citrobacter* are among the bacteria that successfully decolorize textile wastewater (Mustafa et al., 2021). The termite (*Coptotermes curvignathus*) gut revealed ligninolytic activity based on the decolorization of all four lignin-like synthetic dyes, including Azure B, phenol red, methylene blue, and Remazol Brilliant Blue (Simol et al., 2021). In the event of bacterial monoculture, the decolorization percentage of bacterial species ranged from percent (C.I. Reactive Red 195 decolorized by *Klebsiella pneumoniae* strain RY10302) to 92% (C.I. Reactive Blue 194 decolorized by *Enterobacter* sp. strain RY11902).

The decolorization percentage for a mixed culture of four bacteria ranged from 58% (C.I. Reactive Blue 19) to 94% (C.I. Reactive Black 1), suggesting that these bacteria could be used to biologically decolorize dyeing wastewater (Imran et al., 2015; Yadav and Chandra, 2015; Liu et al., 2017; Parveen et al., 2017; Gaur et al., 2018; Ozyurek and Bilkay, 2018; Chantarasiri, 2020; Kumar et al., 2021; Mustafa et al., 2021; Sharma et al., 2021; Tahir and Yasmin, 2021). Within 72 h, strain AB-PR achieved maximum decolorization and Cr (VI) removal efficiency of 90 and 89 percent, respectively, whereas individual decolorization of AB-113 by strain AB-PR remained greater than the co-removal of two contaminants. Under saline circumstances, *Klebsiella* sp. strain AB-PR can remove significant amounts of azo dyes as well as heavy metals (Saxena and Gupta, 2021; Emadi et al., 2021; Muhamad et al., 2021; Padhan et al., 2021; Prabhakar et al., 2021).

In the textile industry, cellulase-based solutions such as DeniMax® (Novozymes) and ValumaxA 838 have made it simple and cost-effective to create new hues and finishes (Agrawal, 2017). Cellulase can be utilized for a variety of procedures in the textile industry, including denim finishing, cotton softening, biopolishing, biostone washing, and so on (Singh et al., 2021). According to Bhattacharjee et al. (2019), the reduction of indigo dye takes place under controlled conditions with the presence of hydro-sulfite in caustic soda as the medium. Because the indigo dye is carcinogenic, poisonous, and emits fumes during the activation process, it is exceedingly dangerous to the environment and human health.

## Conclusion

The goal of this study was to harness the use of certain cellulase-producing bacteria from termite (*Isoptera*) guts in Meki and Zeway areas in the Central Rift Valley of Ethiopia. After optimizing the culture parameters of two isolated bacterial strains, *Klebsiella oxytoca* (M<sub>21</sub>WG) and *Klebsiella* sp. (Z<sub>6</sub>WG) for maximum biocatalyst production, it generally consumed less energy, released less chemical into the textile effluents, improved fabric quality, and partially purified the cellulase enzymes. Some environmental conditions influenced the bacteria's ability to produce this enzyme. In general, this study has demonstrated that both isolates have the potential to produce cellulase enzymes for prospective use as environmentally friendly dye-leaching/biostoning product in the textile industry.

## Materials and methods

### Description of the sampling area

The sampling for the study purpose was made from termite castes in Rift Valley, eastern part of Shawa Zone Oromia Regional State 121.4 km away from Addis Ababa,

Ethiopia. Worker termites were sampled from the termite castes found around Meki and Zeway areas in the Central Rift Valley. Meki is the capital of Dugda District, and Zeway is the capital city of Adami Tulu Jido Kombolcha District. The site was purposively selected because termite's caste mound where high-density epigeal occurs and diversity located in farmland that had been under cultivation for a long time and damage the agricultural products (Debelo and Degaga, 2014; Debelo, 2018).

#### Isolation of bacteria from the termite guts

The termites were taken from farmland areas in Ethiopia, where there is a high density of epigeal mounds/nests. Thirty-six healthy worker termites were chosen from the colony and surface sterilized by rubbing the entire body of each termite with a cotton applicator soaked in 70% ethanol, then washing in sterile distilled water and air-drying for 1 min. The head and thorax of an externally sterilized termite were crushed using sterile forceps under sterile conditions. Another set of forceps was sterilized and used to take the termite's complete abdomen away from its body. The abdomen was inserted in 1 mL of phosphate buffer solution after the head and thorax were discarded (PBS). The remaining nine termites went through the same procedure. The bodies were dissected with a sharp blade and a magnifying hand lens after the heads were removed using forceps (Egwuatu and Appeh, 2018).

The intestine was eventually extracted using fine-tipped forceps under the Laminar Air Hood MN20 (Microbiology Safety Cabinet), based on a modest modification of previously described extraction techniques (Phuong et al., 2015; Devaraj and Kesti, 2019). The gut contents were then homogenized in 5 mM sterile phosphate buffer saline (1× PBS), pooled, and transferred to a 1-mL microcentrifuge tube containing PBS solution, where they were centrifuged for 10 s at 4000 rpm at 4 °C. Serial dilutions of the supernatant were performed (Sreeremya et al., 2015).

By adding 0.9 mL of phosphate buffer solution to another sterile microcentrifuge tube and transferring 0.1 mL of the original homogenate into it, ten-fold serial dilutions of termite abdomen homogenate were generated, mixing carefully after each transfer. Using sterile disposable pipettes, those three 10-fold serial dilutions were done to obtain concentrations of  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-6}$  using sterile disposable pipettes (Van-Dexter and Boopathy, 2019). Then, the diluted termite gut contents were inoculated in Nutrient Broth agar (Nutrient Broth 9 g/L and agar 15 g/L) and CMC medium (NaNO<sub>3</sub> 2.5 g; KH<sub>2</sub>PO<sub>4</sub> 2 g; MgSO<sub>4</sub> 0.2 g; NaCl 0.2 g; yeast extract 0.2 g; CaCl<sub>2</sub>·6H<sub>2</sub>O 0.1 g in a liter) containing 0.5% carboxymethyl cellulose (CMC) as a

sole carbon source; the pH of the medium is adjusted to  $\pm 7.2$  with 1 M NaOH and HCl. The sterilization of the prepared medium took 15 min in an autoclave at 121 °C. The medium was sterilized before being placed onto sterile Petri dishes and allowed to solidify. The termite gut bacteria were then cultured in this culture media, and plates were incubated at 37 °C for 48 h before being stored at 4 °C with some modifications (Ali et al., 2019a; Peristiwatiet al., 2018).

#### Screening of cellulolytic bacteria

Cellulolytic bacteria were screened from among the pure colonies using 1% carboxymethyl cellulose (CMC)-supplemented agar plates based on the methodology reported by Sharma et al. (2015). Post-incubation at 30 °C for 24 h, the plates were flooded with 0.1% Congo red for 15 min, followed by de-staining with 1 M NaCl solution for 15 min and measurement of the clear zones, attesting the CMC hydrolysis (Phuong et al., 2015; Javaheri-Kermani and Asoodeh, 2019) based on the following equations:

$$\text{Diameter of clear zone} = \text{Diameter of halo zone} - \text{Diameter of colony} \quad (1)$$

$$\text{Hydrolytic capacity} = \frac{\text{Diameter of clear zone (CZ)}}{\text{Diameter of bacterial colony (CD)}} \quad (2)$$

#### Morphological and biochemical characteristics of the bacterial isolates

Morphological features were determined using standard methods. Pertinently, the bacterial isolates from the termite guts were able to grow under aerobic conditions. The biochemical tests encompassed by means of catalase activity test, urease test, citrate test, indole test, motility test, and methyl red test, fermentation tests for glucose and lactose. This procedure was repeated across treatments for each pure colony which served as biological replicates. The various reagents for these tests were procured from Hi-Media (Waghmare et al., 2014; Guder and Krishna, 2019).

#### Molecular identification of bacterial isolates by 16S rRNA sequencing

Based on the initial screening results, the genomic DNA (gDNA) of two potential cellulase-producing bacteria designated as M<sub>21</sub>WG and Z<sub>6</sub>WG was extracted, and their 16S rRNA genes were PCR amplified by using universal bacterial-specific primers E9F (5'-GAGTTTGATCCTGGCTCAG-3') and U1510R (5'-GGTACCTTGTACGACTT-3') (Farrelly et al.,

1995). Finally, the PCR products were sent to YAAZH Xenomics, Coimbatore, India, for Sanger sequence; the obtained sequence data was compared with known sequences in the Genbank using the basic local alignment search tool of the national center for biotechnology information (NCBI) (Saitou and Nei, 1987; Felsenstein, 1985; Kimura, 1980; Kovtunovych et al., 2003; Younis et al., 2017).

### Cellulase assay

Post-plotting of the standard glucose concentration curve, the cellulase (CMCase) activity was quantified based on the DNS method (Lowry, 1951).

Yan et al. (2019) encompassed a protocol of the addition of 0.5 mL of crude enzyme solution to 1.5 mL of 1% CMC-Na solution, followed by incubation of the enzyme-substrate mixture, post-shaking well at 50 °C for 30 min in a water bath and, the addition of 1.5 mL of DNS reagent and shaking, boiling for around 10 min, subsequent cooling, and eventual recording of absorbance value at 540 nm in the UV-visible spectrophotometer (Biochrom, England). The following equation was used to calculate the CMCase activity:

$$\text{CMCase or Cellulase activity} \left( \frac{\text{U}}{\text{mL}} \right) = \frac{A \times 1000}{30 \times 0.5} \quad (3)$$

where  $A$  is the glucose content from the sample (mg/mL), 30 refers to 30 min of the reaction time, 1000 is the dilution factor, and 0.5 indicates the volume of enzyme used in the analysis.

### Determination of optimal process parameters for cellulase production

Process parameters such as pH, temperature, incubation time, and substrate concentration were optimized for maximal cellulase production by the two potential isolates M<sub>21</sub>WG and Z<sub>6</sub>WG. For the determination of the optimum pH and temperature, the two isolates were cultured in respective 250 mL Erlenmeyer flasks containing CMC-Na broth under a range of pH (4.0–10.0), temperature (27–52 °C), incubation time (24–108 h), and substrate concentration (0.1–2.5%) carbon source at 120 rpm for 3 days (Javaheri-Kermani and Asoodeh, 2019; Sakolvaree and Deevong, 2016; Khatiwada et al., 2016).

### Partial purification of cellulase and produced under optimal conditions

Post-culturing of the bacterial isolates under the respective optimal conditions, as determined in the previous

step, we resorted to partial purification of the crude enzyme. The crude enzyme, corresponding to each of the isolates, was harvested post centrifugation of the respective culture at 10,000 rpm for 20 min to eliminate cells and residual medium; 100 mL of crude enzyme-rich culture supernatant was subjected to overnight precipitation with ammonium sulfate (80% saturation) at 4 °C followed by centrifugation for 20 min at 4 °C. This was followed by re-suspension of the pellet in 100 mM phosphate buffer (pH 7.0) and overnight dialysis against the sample buffer using a cellulose membrane tube (12 kDa cutoff). The partially purified enzyme was used for subsequent enzyme assay by Biochrom, England (Potprommanee et al., 2017).

### Assessment of biostoning potency of the partially purified cellulase for prospective in the textile industry

The purpose of this investigation was to see how effective partly purified cellulase from the selected bacterial isolates M<sub>21</sub>WG and Z<sub>6</sub>WG were at bleaching denim fabric. In 250-mL Erlenmeyer flasks with 10 steel balls, the textile samples (in three repetitions) were treated (enzymomechano treatment) with the partly purified enzyme (harvested from each of the isolates) (diameter 0.7 cm). Denim swatches were immersed in 20 mL of 100 mM sodium phosphate buffer (pH 7) at a solid: liquid ratio of 1:10 and incubated at 40 °C for 1 h at 250 rpm. An HP Scanjet G4050 scanner was used to scan the control and treated denim swatches at 300 dpi resolution.

The Fiji (Win 32) software was used to analyze the BMP images that were captured as a result of the scanning. Demarcate a rectangular area (50 w × 20 h) on each of the denim images. The image of each of the denim swatch, plot profile, 3D surface plot, and color histogram (RGB) were generated, and the results were analyzed to assess the bleaching potency of the enzyme, isolated from each of the isolates some modified (Gautam and Sharma, 2012; Costa et al., 2021).

### Abbreviations

BLAST: Basic Local Alignment and Search Tool; 16S rRNA: 16 small ribosomal ribonucleic acid; M<sub>21</sub>WG: Bacterial isolate from Meki termite gut white color number 21; Z<sub>6</sub>WG: Bacterial isolate from Zeway termite gut cream white color number 6; NCBI: National Center for Biotechnology Information.

### Acknowledgements

We would like to extend our special thanks for the samples and meta-data collected from Meki and Zeway area, Central Rift Valley region of Ethiopia through the assistance of the local community in the areas. Addis Ababa Science and Technology University is also appreciated for offering a limited funding for the laboratory experiments.

### Authors' contributions

All authors carried out the collection of samples conducted from Meki and Zeway in Central Rift Valley, eastern part of Shawa Zone Oromia Regional State, and 90 km away from Addis Ababa, Ethiopia. GamachisKorsa,

Rocktotpalkonwarh, Chandranmasi, and MesfinTafesse, carried out harnessing cellulolytic gut bacteria from termites (*Isoptera*) of Central Rift Valley of Ethiopia for prospective textile industry application. All authors have approved the latest version of the manuscript and agree to be held accountable for the content therein.

#### Availability of data and materials

The datasets used or analyzed during the preparation of the manuscript are available from the corresponding author on reasonable request.

#### Declarations

#### Ethics approval and consent to participate

Not applicable

#### Consent for publication

Not applicable

#### Competing interests

The authors declare that they have no competing interests.

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Received: 17 October 2021 Accepted: 30 December 2021

Published online: 16 February 2022

#### References

- Abdel-Aziz SH, Ibrahim AM, Guirgis AA, Dawwam GE, Elsabayt ZE (2021) Isolation and screening of cellulase producing bacteria isolated from soil. Benha. *J Appl Sci* 6(3):207–213. <http://bjas.journals.ekb.eg>
- Abu-Gharbia MA, El-Sawy NM, Nasr AM, Zedan LA (2018) Isolation, optimization and characterization of cellulases and hemicellulases from *Bacillus cereus* LAZ 518 isolated from cow dung using corn cobs as lignocellulosic waste. *J Pharm Appl Chem* 4(1):1–3. <https://doi.org/10.21608/JPAC.2018.202329>
- Agrawal BJ (2017) Biostoning of denim an environmental friendly approach. *Current Trend Biomed EngBiosci* 3(3):45–47. <https://doi.org/10.19080/CTBEB.2017.03.555612>
- Ali HR, Hemeda NF, Abdelalim YF (2019a) Symbiotic cellulolytic bacteria from the gut of the subterranean termite *Pseudotermes hyposoma* Desneux and their role in cellulose digestion. *AMB Expr* 9(1):111. <https://doi.org/10.1186/s13568-019-0830-5>
- Ali SS, Al-Tohamy R, Sun J, Wu J, Huizi L (2019b) Screening and construction of a novel microbial consortium SSA-6 enriched from the gut symbionts of wood-feeding termite, *Coptotermes formosanus* and its biomass-based biorefineries. *Fuel* 236:1128–1145. <https://doi.org/10.1016/j.fuel.2018.08.117>
- Arfah RA, Natsir H, Atifah N, Zarkoni TR, Mahmud M (2019) Isolation and characterization of soil termites (*Macrotermes gilvus*) cellulolytic bacteria and activity determination of cellulase enzyme on newsprint substrates. *J Phys IOP Conf Series* 1341(3):032037. <https://doi.org/10.1088/1742-6596/1341/3/032037>
- Astolfi V, Astolfi AL, Mazutti MA, Rigo E, Di Luccio M, Camargo AF, Dalastra C, Kubeneck S, Fongaro G, Treichel H (2019) Cellulolytic enzyme production from agricultural residues for biofuel purpose on circular economy approach. *Bioprocess Biosys Eng* 42(5):677–685. <https://doi.org/10.1007/s00449-019-02072-2>
- 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>
- Bai S, Kumar MR, Kumar DM, Balashanmugam P, Kumaran MB, Kalaichelvan PT (2012) Cellulase production by *Bacillus subtilis* isolated from cow dung. *Arch Appl Sci Res* 4(1):269–79. <http://scholarsresearchlibrary.com/archives.html>
- Barbosa KL, dos Santos Malta VR, Machado SS, Junior GA, da Silva AP, Almeida RM, da Luz JM (2020) Bacterial cellulase from the intestinal tract of the sugarcane borer. *Int J Biol Macromol* 15(161):441–448. <https://doi.org/10.1016/j.ijbiomac.2020.06.042>
- Bezerra FI, DeSouza O, Ribeiro GC, Mendes M (2021) A new primitive termite (*Isoptera*) from the Crato Formation, Araripe Basin, Early Cretaceous of South America. *Am J Earth Sci* 109:103260. <https://doi.org/10.1016/j.jsames.2021.103260>
- Bharti P, Sandhu RK (2015) Partial purification of cellulase produced by a bacterium isolated from wood decompost. *Int J Pure App Biosci*. 3(4):208–215. [www.ijpab.com](http://www.ijpab.com)
- Bhattacharjee M, Dhar AK, Islam MM, Rashid MA (2019) Development of washing effects on reactive dyed denim fabrics: a value-added approach of denim wash. *Int J Text Sci* 8(2):41–48. <https://doi.org/10.5923/j.textile.20190802.03>
- Biswas S, Al Saber M, Tripty IA, Karim MA, Islam MA, Hasan MS, Alam AR, Jahid MI, Hasan MN (2020) Molecular characterization of cellulolytic (endo-and exoglucanase) bacteria from the largest mangrove forest (*Sundarbans*), Bangladesh. *Ann Microbiol* 70(1):1–1. <https://doi.org/10.1186/s13213-020-01606-4>
- Bozorov TA, Rasulov BA, Zhang D (2019) Characterization of the gut microbiota of invasive *Agrilus mali* Matsumura (Coleoptera: Buprestidae) using high-throughput sequencing: uncovering plant cell-wall degrading bacteria. *Sci Rep* 9:4923. <https://doi.org/10.1038/s41598-019-41368-x>
- Chandranmasi GG, Tafesse M (2021) Isolation, screening, characterization, and identification of alkaline protease producing bacteria from leather industry effluent. *Ann Microbiol* 71. <https://doi.org/10.1186/s13213-021-01631-x>
- Chantarasiri A (2020) *Klebsiella* and *Enterobacter* isolated from mangrove wetland soils in Thailand and their application in biological decolorization of textile reactive dyes. *Int J Environ Res Public Health* 17:7531. <https://doi.org/10.3390/ijerph17207531>
- Chauhan PS (2020) Role of various bacterial enzymes in complete depolymerization of lignin: a review. *Biocatalys Agric Biotechnol* 23:101498. <https://doi.org/10.1016/j.bcab.2020.101498>
- Costa FN, de Souza Lima J, Valério A, de Souza AA, de Oliveira D (2021) Utilization of montmorillonite in biostoning process as a strategy for effluent reuse. *J Chem Technol Biotechnol* 96(4): 890–898. <https://doi.org/10.1002/jctb.6597>
- Cui D, Li G, Zhao M, Han S (2014) Decolorization of azo dyes by a newly isolated *Klebsiella* sp. strain Y3 and effects of various factors on biodegradation. *Biotechnol Biotechnol Equip* 28(3):478–86. <https://doi.org/10.1080/13102818.2014.926053>
- Dantur KI, Chalfoun NR, Claps MP, Tórtora ML, Silva C, Jure Á, Porcel N, Bianco MI, Vojnov A, Castagnaro AP, Welin B (2018) The endophytic strain *Klebsiellamichiganensis* Kd70 lacks pathogenic island-like regions in its genome and is incapable of infecting the urinary tract in mice. *Front Microbiol* 9:1548. <https://doi.org/10.3389/fmicb.2018.01548>
- Dantur KI, Enrique R, Welin B, Castagnaro AP (2015) Isolation of cellulolytic bacteria from the intestine of *Diatraea saccharalis* larvae and evaluation of their capacity to degrade sugarcane biomass. *AMB Expr* 5(1):15. <https://doi.org/10.1186/s13568-015-0101-z>
- Dar MA, Shaikh AA, Pawar KD, Pandit RS (2018) Exploring the gut of *Helicoverpa armigera* for cellulose degrading bacteria and evaluation of a potential strain for lignocellulosic biomass deconstruction. *Process Biochem* 73:142–53. <https://doi.org/10.1016/j.procbio.2018.08.001>
- Dar MA, Shaikh AF, Pawar KD, Xie R, Sun J, Kandasamy S, Pandit RS (2021) Evaluation of cellulose degrading bacteria isolated from the gut system of cotton bollworm, *Helicoverpa armigera* and their potential values in biomass conversion. *Peer J* 9:e11254. <https://doi.org/10.7717/peerj.11254>
- Debelo DG (2018) Faunal survey of the termites of the genus *Macrotermes* (*Isoptera: Termitidae*) of Ethiopia. *J Entomol Nematol* 10(7):50–64. <https://doi.org/10.5897/JEN2018.0216>
- Debelo DG, Degaga EG (2014) Termite species composition in the central rift valley of Ethiopia. *North America J Agr Biol* 5(3):123–34. <https://www.internationalscholarsjournals.org>

- Duan J, Liu J, Ma X, Zhang Y, Wang X, Zhao K (2017) Isolation, identification, and expression of microbial cellulases from the gut of *Odontotermes formosanus*. *J Microbiol Biotechnol* 27(1):122–9. <https://doi.org/10.4014/jmb.1604.04040>
- Eggleton P (2010) An introduction to termites: biology, taxonomy and functional morphology. In: *Biology of termites: a modern synthesis*. Springer, Dordrecht, pp 1–26. [https://link.springer.com/chapter/10.1007/978-90-481-3977-4\\_1](https://link.springer.com/chapter/10.1007/978-90-481-3977-4_1)
- Egwuatu TF, Appah OG (2018) Isolation and characterization of filter paper degrading bacteria from the guts of *Coptotermes formosanus*. *J Bioremed Biodegrad* 9:440. <https://doi.org/10.4172/2155-6199.1000440>
- Emadi Z, Sadeghi M, Forouzandeh S, Sadeghi R, Mohammadi-Moghadam F (2021) Simultaneous decolorization/degradation of AB-113 and chromium (VI) removal by a salt-tolerant *Klebsiella* sp. AB-PR and detoxification of biotransformed metabolites. *Int J Environ Sci Technol* 1–8. <https://doi.org/10.1007/s13762-021-03360-9>
- Farrelly V, Rainey FA, Stackebrandt E (1995) Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. *Appl Environ Microbiol* 61(7):2798–801. <https://aem.asm.org/content/aem/61/7/2798.full.pdf>
- Fasiku SA, Ogunisola OF, Fakunle A, Olanbiwoninu AA (2020) Isolation of bacteria with potential of producing extracellular enzymes (amylase, cellulase and protease) from soil samples. *J Adv Microbiol* 21–6. <https://doi.org/10.9734/jamb/2020/v20i330224>
- Fatmawati NV, Ketbot P, Phitsuvan P, Waonukul R, Tachaapaikoon C, Kosugi A, Ratanakhanokchai K, Pason P (2021) Efficient biological pretreatment and bioconversion of corn cob by the sequential application of a *Bacillus firmus* K-1 cellulase-free xylanolytic enzyme and commercial cellulases. *Appl Microbiol Biotechnol* 105:4589–4598. <https://doi.org/10.1007/s00253-021-11308-9>
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39(4):783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- Ferbiyanto A, Rusmana I, Raffudin R (2015) *c Macrotermes gilvus*. *Hayati J Biosci* 22:197–200. <https://doi.org/10.1016/j.hjb.2015.07.001>
- Gaur N, Narasimhulu K, Setty YP (2018) Extraction of ligninolytic enzymes from novel *Klebsiella pneumoniae* strains and its application in wastewater treatment. *Appl Water Sci* 8:111. <https://doi.org/10.1007/s13201-018-0758-y>
- Gaur R, Tiwari S (2015) Isolation, production, purification and characterization of an organic-solvent-thermostable alkalophilic cellulase from *Bacillus vallismortis* RG-07. *BMC Biotechnol* 15(1):1–2. <https://doi.org/10.1186/s12896-015-0129-9>
- Gautam R, Sharma J (2012) Optimization, purification of cellulase produced from *Bacillus subtilis* subsp. inaquosorum under solid state fermentation and its potential applications in denim industry. *Int J Sci Res* 3(1759–63). [www.ijsr.net](http://www.ijsr.net)
- Gbenro T J, Kamaraj M, Adesanmi A (2019) Isolation and identification of cellulolytic bacteria from wood industry soil in Kancheepuram, Chennai. *Int J Adv Sci Res Eng* 5(7):144–49. <https://doi.org/10.31695/IJASRE.2019.33420>
- Ge S-X, Shi F-M, Pei J-H, Hou Z-H, Zong S-X, Ren L-L (2021) Gut bacteria associated with *Monochamus saltuarius* (Coleoptera: Cerambycidae) and their possible roles in host plant adaptations. *Front Microbiol* 12:687211. <https://doi.org/10.3389/fmicb.2021.687211>
- Guder DG, Krishna MSR (2019) Isolation and characterization of potential cellulose degrading bacteria from sheep rumen. *J Pure Appl Microbiol* 13(3):1831–1839
- Gupta R, Mehta G, Deswal D, Sharma S, Jain KK, Kuhad RC, Singh A (2013) Cellulases and their Biotechnological applications. Springer India *Biotechnol Environ Mgt Resour Recov* 89–106. [https://doi.org/10.1007/978-81-322-0876-1\\_6](https://doi.org/10.1007/978-81-322-0876-1_6)
- Husseneder C, McGregor C, Lang RP, Collier R, Delatte J (2012) Transcriptome profiling of female alates and egg-laying queens of the *Formosan subteranean* termite. *Compar Biochem Physiol Part D Genomics and Proteom* 7(1):14–27. <https://doi.org/10.1016/j.cbd.2011.10.002>
- Imran M, Crowley DE, Khalid A, Hussain S, Mumtaz MW, Arshad M (2015) Microbial biotechnology for decolorization of textile wastewaters. *Rev Environ Sci Bio/Technol* 14(1):73–92. <https://doi.org/10.1007/s11157-014-9344-4>
- Irshad M, Murtza A, Zafar M, Bhatti KH, Rehman A, Anwar Z (2017) Chitosan immobilized pectinolytics with novel catalytic features and fruit juice clarification potentialities. *Int J Biol Macromol* 104:242–250. <https://doi.org/10.1016/j.jbiomac.2017.06.027>
- Islam F, Roy N (2018) Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses. *BMC Res Note* 11(1):445. <https://doi.org/10.1186/s13104-018-3558-4>
- Islam F, Roy N (2019) Isolation and characterization of cellulase-producing bacteria from sugar industry waste. *America J BioSci* 16–24. <https://doi.org/10.11648/j.ajbio.20190701.13>
- Javaheri-Kermani M, Asoodeh A (2019) A novel beta-1, 4 glucanase produced by symbiotic *Bacillus* sp. CF96 isolated from termite (*Anacanthotermes*). *Int J Biol Macromol* 131:752–9. <https://doi.org/10.1016/j.jbiomac.2019.03.124>
- Kabir SM, Koh J (2021) Sustainable textile processing by enzyme applications. In *Biodegrad*. Intech Open 1–28. <https://doi.org/10.5772/intechopen.97198>
- Kakkar N, Sanjeev KG, Baljeet SS (2015) Studies on cellulolytic activity and structure of symbiotic bacterial community in *Odontotermes parvidens* guts. *Int J Curr Microbiol App Sci* 4(10):310–5. <http://www.ijcmas.com>
- Karthika A, Seenivasagan R, Kasimani R, Babalola OO, Vasanthy M (2020) Cellulolytic bacteria isolation, screening and optimization of enzyme production from vermin compost of paper cup waste. *Waste Mgt* 116:58–65. <https://doi.org/10.1016/j.wasman.2020.06.036>
- Katiyar P, Srivastava SK, Tyagi VK (2018) Measurement of cellulolytic potential of cellulase producing bacteria. *Annual Res Rev Biol* 1–9
- Khatiwada P, Ahmed J, Sohag MH, Islam K, Azad AK (2016) Isolation, screening and characterization of cellulase producing bacterial isolates from municipal solid wastes and rice straw wastes. *J Biopr Biotech* 6(280):2. <https://doi.org/10.4172/2155-9821.1000280>
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16(2):111–20. <https://doi.org/10.1007/bf01731581>
- Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. *Curr Opin Biotechnol* 13(4):345–51. [https://doi.org/10.1016/S0958-1669\(02\)00328-2](https://doi.org/10.1016/S0958-1669(02)00328-2)
- Kovtunovych G, Lytvynenko T, Negrutskaya V, Lar O, Brisse S, Kozyrovska N (2003) Identification of *Klebsiella oxytoca* using a specific PCR assay targeting the polygalacturonase pglX gene. *Res Microbiol* 154(8):587–592. [https://doi.org/10.1016/s0923-2508\(03\)00148-7](https://doi.org/10.1016/s0923-2508(03)00148-7)
- Kumar S, Upadhyay RK (2021) Anti-termite potential of various bio-organic constituents with special reference to family Asteraceae. *World J Pharm Res* 10(3):1109–49. [www.wjpr.net](http://www.wjpr.net)
- Kumar V, Umrao PD, Kaistha SD (2021) Amelioration of disulfonated acid red and hexavalent chromium phytotoxic effects on *Triticum aestivum* using bioremediating and plant growth-promoting *Klebsiella pneumoniae* SK1. *J Pure Appl Microbiol* 15(3):1301–1312. <https://doi.org/10.22207/JPAM.15.3.20>
- Li J, Chen S, Tao X, Shao T (2021) Gut microbiota of *Ostriniana ubialis* larvae degrade maize cellulose. *Res Sq*. <https://doi.org/10.21203/rs.3.rs-589374/v1>
- Liu H, Zeng L, Jin Y, Nie K, Deng L, Wang F (2019a) Effect of different carbon sources on cellulase production by marine strain *Microbulbifer hydrolyticus* IRE-31-192. *Appl Biochem Biotechnol* 188(3):741–749. <https://doi.org/10.1038/s41396-018-0255-1>
- Liu N, Li H, Chevrette MG, Zhang L, Cao L, Zhou H, Zhou X, Zhou Z, Pope PB, Currie CR, Huang Y (2019b) Functional metagenomics reveals abundant polysaccharide degrading gene clusters and cellobiose utilization pathways within gut microbiota of a wood-feeding higher termite. *J ISME* 13(1):104–17. <https://doi.org/10.1038/s41396-018-0255-1>
- Liu Y, Huang L, Guo W, Jia L, Fu Y, Gui S, Lu F (2017) Cloning, expression, and characterization of a thermostable and pH-stable laccase from *Klebsiella pneumoniae* and its application to dye decolorization. *Proc Biochem* 53:125–34. <https://doi.org/10.1016/j.procbio.2016.11.015>
- Loh HQ, Hervé V, Brune A (2021) Metabolic potential for reductive acetogenesis and a novel energy-converting [NiFe] hydrogenase in Bathyarchaea from termite guts a genome-centric analysis. *Front Microbiol* 11:635786. <https://doi.org/10.3389/fmicb.2020.635786>
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–75. <http://www.jbc.org/content/193/1/265.citation>

- Luo W, Wang J, Liu XB, Li H, Pan H, Gu Q, Yu X (2013) A facile and efficient pretreatment of corncob for bioproduction of butanol. *Biores Technol* 140:86–9. <https://doi.org/10.1016/j.biortech.2013.04.063>
- Mahjabeen F, Khan S, Choudhury N, Hossain MM, Khan TT (2016) Isolation of cellulolytic bacteria from soil, identification by 16S rRNA gene sequencing and characterization of cellulase. *Bangladesh J Microbiol* 33(1–2):17–22. <https://doi.org/10.3329/bjmv.v33i1.39598>
- Maki M, Leung KT, Qin W (2009) The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. *Int J Biol Sci* 5(5):500. <https://dx.doi.org/10.7150%2Fijbs.5.500>
- Mattéotti C, Bauwens J, Brasseur C, Tarayre C, Thonart P, Destain J, Francis F, Haubruge E, De Pauw E, Portetelle D, Vandenberg M (2012) Identification and characterization of a new xylanase from Gram positive bacteria isolated from termite gut (*Reticulitermesantonensis*). *Protein Expr Pur* 83(2):117–27. <https://doi.org/10.1016/j.pep.2012.03.009>
- Mikaelyan A, Meuser K, Brune A (2018). Microenvironmental heterogeneity of gut compartments drives bacterial community structure in wood and humus-feeding higher termites. *FEMS Microbiol Ecol* 93(1). <https://doi.org/10.1093/femsec/fiw210>
- Muhamad MH, Abdullah NLM, Hasan HA, Bakar SN, Kurniawan SB (2021) A hybrid treatment system for water contaminated with pentachlorophenol: removal performance and bacterial community composition. *J Water Pro Eng* 43:102243. <https://doi.org/10.1016/j.jwpe.2021.102243>
- Mustafa G, Zahid MT, Ali S, Abbas SZ, Rafatullah M (2021) Biodegradation and discoloration of disperse blue-284 textile dye by *Klebsiella pneumoniae* GM-04 bacterial isolate. *J King Saud Univ-Sci* 33(4):101442. <https://doi.org/10.1016/j.jksus.2021.101442>
- Muwamba EM, Budambula NLM, Osiamo ZL, Boga HI, Makonde HM (2016) Isolation and characterization of some gut microbial symbionts from fungus-cultivating termites (*Macrotermes* and *Odontotermes* spp.). *African J Microbiol Res* 10(26):994–1004
- Nandy G, Chakraborti M, Shee A, Aditya G, Acharya K (2021) Gut microbiota from lower groups of animals: an upcoming source for cellulolytic enzymes with industrial potentials. *Biointerface Res Appl Chem* 11(5):13614–13637. <https://doi.org/10.33263/BRIAC.11.1361413637>
- Nazir S, Irfan M, Nadeem M, Shakir HA, Khan M, Ali S, Syed Q (2021) Utilization of *Bombyx mori* seed pods: a novel substrate for cellulase production through solid state fermentation using response surface methodology. *Punjab Univ J Zool* 34:213–219. <https://doi.org/10.17582/journal.pujz/2019.34.2.213.219>
- Ndlovu TM, Van Wyk JP (2019) Isolation of cellulase enzyme from brown garden snail (*Cornuaspersum*) for the saccharification of waste paper material. *Method X*. 6:1030–1035. <https://doi.org/10.1016/j.mex.2019.04.019>
- Nelson K, Muge E, Wamalwa B (2021) Cellulolytic *Bacillus* species isolated from the gut of the desert locust *Schistocerca gregaria*. *Sci Afr* 11:e00665. ISSN 2468–2276. <https://doi.org/10.1016/j.sciaf.2020.e00665>
- Ngangi J, Moko EM, Rahardyan D (2019) Cellulase induction enzymes characteristics of hindguts of endemic termites of North Sulawesi. *J Phys IOP Conf Series* 1317(1):012076. <https://doi.org/10.1088/1742-6596/1317/1/012076>
- Nobre T, Nunes L (2007) Non-traditional approaches to subterranean termite control in buildings. *Wood Mater Sci Eng*. 2(3–4):147–56. <https://doi.org/10.1080/17480270801945413>
- Nursyirwani N, Feliatra F, Tanjung A, Harjuni F (2020) Isolation of cellulolytic bacteria from mangrove sediment in Dumai marine station Riau and the antibacterial activity against pathogens. *Earth Environ Sci: IOP Conf Series* 430(1):012012. <https://doi.org/10.1088/1755-1315/430/1/012012>
- Obafemi YD, Ajayi AA, Taiwo OS, Olorunsola SJ, Isibor PO (2019) Isolation of polygalacturonase-producing bacterial strain from tomatoes (*Lycopersicon esculentum* Mill). *Int J Microbiol* 15:2019. <https://doi.org/10.1155/2019/7505606>
- Oberst S, Lai JC, Martin R, Halkon BJ, Saadatfar M, Evans TA (2020) Revisiting stigmergy in light of multi-functional, biogenic, termite structures as communication channel. *J Comp Str Biotechnol* 18:2522. <http://creativecommons.org/licenses/by/4.0/>
- Olowomofe TO, Babalola TF, Oluylide OO, Adeyanju A (2019) Isolation, screening and molecular identification of cellulose-degrading bacteria from paper and pulp mill dumpsites. *Front Environ Microbiol* 5(3):77. <https://doi.org/10.11648/j.fem.20190503.12>
- Ozyurek SB, Bilkay IS (2018) Biodegradation of petroleum by *Klebsiella pneumoniae* isolated from drilling fluid. *Int J Environ Sci Technol* 15:2107–2116. <https://doi.org/10.1007/s13762-017-1581-y>
- Padhan B, Poddar K, Sarkar D, Sarkar A (2021) Production, purification, and process optimization of intracellular pigment from novel psychrotolerant *Paenibacillus* sp. BPW19. *Biotechnol Report* 29:e00592. <https://doi.org/10.1016/j.btre.2021.e00592>
- Parveen T, Pandey C, Hai HA (2017) Enhancement of production of cellulases from UV (Ultraviolet light) treated *Bacillus subtilis*. *Int J Sci Res* 6(9):1580–7. [https://www.ijsr.net/search\\_index\\_results\\_paperid.php?id=ART20176960](https://www.ijsr.net/search_index_results_paperid.php?id=ART20176960)
- Peristiwati NYS, Herlini H (2018) Isolation and identification of cellulolytic bacteria from termites gut (*Cryptotermes* sp.). *J Phys: IOP Conf Series* 1013:012173. <https://doi.org/10.1088/1742-6596/1013/1/012173>
- Phuong NT, Na NTL, Thang ND (2015) Isolation of cellulose degrading bacteria from the gut of the termite *Coptotermes gestroi*. *Asian J Microbiol Biotech Environ Sci* 17(4):931–936. [https://www.researchgate.net/profile/Phuong\\_Nguyen283/publication/298824415](https://www.researchgate.net/profile/Phuong_Nguyen283/publication/298824415)
- Potprommanee L, Wang XQ, Han YJ, Nyobe D, Peng YP, Huang Q, Liu JY, Liao YL, Chang KL (2017) Characterization of a thermophilic cellulase from *Geobacillus* sp. HTA426, an efficient cellulase-producer on alkali pretreated of lignocellulosic biomass. *PLOS ONE* 12(4):e0175004. <https://doi.org/10.1371/journal.pone.0175004>
- Prabhakar Y, Gupta A, Kaushik A (2021) Using indigenous bacterial isolate *Nesterekoniala cusekhoensis* for removal of azo dyes: a low-cost ecofriendly approach for bioremediation of textile wastewaters. *Environ. Dev Sustain* 24:1–24. <https://doi.org/10.1007/s10668-021-01661-0>
- Prasad RK, Chatterjee S, Mazumder PB, Sharma S, Datta S, Vairale MG, Dwivedi SK (2019) Study on cellulase (B-1, 4-endoglucanase) activity of gut bacteria of *Sitophilus oryzae* in cellulosic waste biodegradation. *Biores Technol Report* 7:100274. <https://doi.org/10.1016/j.btre.2019.100274>
- Purwanto MGM (2016) The role and efficiency of ammonium sulfate precipitation in purification process of papain crude extract. *Pro Chem* 18:127–131. <https://doi.org/10.1016/j.proche.2016.01.020>
- Rawway M, Ali SG, Badawy AS (2018) Isolation and identification of cellulose degrading bacteria from different sources at Assiut Governorate (Upper Egypt). *Int J Ecol Health Environ* 6:15. <https://doi.org/10.18576/jehe/060103>
- Reffas FZ, Missouri A, Nourine Z, Tifrit A, Daouadji KL, Tayeb S, Abbouni B (2016) Optimization of cellulase production by bacterial strains, isolated from the soils of the North West of Algeria. *Der Pharmacia Lett* 8:42–8. <http://scholarsresearchlibrary.com/archive.html>
- Regmi S, Choi YS, Kim YK, Khan MM, Lee SH, Choi YH, Cho SS, Jin YY, Yoo JC, Suh JW (2020) Industrial attributes of β-glucanase produced by *Bacillus* sp. CSB55 and its potential application as bioindustrial catalyst. *Bioproc Biosyst Eng* 43(2):249–59. <https://doi.org/10.1007/s00449-019-02221-7>
- Rehman MM, Zeeshan M, Shaker K. et al (2019) Effect of micro-crystalline cellulose particles on mechanical properties of alkaline treated jute fabric reinforced green epoxy composite. *Cellulose* 26:9057–9069. <https://doi.org/10.1007/s10570-019-02679-4>
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4(4):406–25. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Sakolvaree J, Deevong P (2016) Isolation and characterization of cellulase producing bacteria from the gut of a higher termite, *Termespropinquus*. Proceedings of the Burapha University International Conference. Bangsaeen, Chonburi, Thailand. 28–29. <http://www.buuconference.buu.ac.th>
- Sakthivel M, Karthikeyan N, Jayaveny R, Palani P (2010) Optimization of culture conditions for the production of extracellular cellulase from *Corynebacterium lipophilum*. *J Ecobiotechnol* 2:06–13. <http://journal-ecobiotechnology.com>
- Sánchez-García A, Peñalver E, Delclòs X, Engel MS (2020) Early Cretaceous termites in amber from northern Spain (*Isoptera*). *Cret Res*. 110:104385. <https://doi.org/10.1016/j.cretres.2020.104385>
- Sankarraj N, Nallathambi G (2018) Enzymatic biopolishing of cotton fabric with free/immobilized cellulase. *Carbohydr Polym* 191:95–102. <https://doi.org/10.1016/j.carbpol.2018.02.067>
- Saxena A, Gupta S (2021) Bioefficacies of microbes for mitigation of Azo dyes in textile industry effluent: a review. *Bio Res* 15 (4):9858–81. 9865. <https://doi.org/10.15376/biores.15.4.Saxena>

- Scharf ME, Tartar A (2008) Termite digestive as sources for novel lignocelluloses. *Biofuel Bioprod Bioref* 2(6):540–52. <https://doi.org/10.1002/bbb.107>
- Sepehri A, Sarrafzadeh MH, Avateffazeli M (2020) Interaction between *Chlorella vulgaris* and nitrifying enriched activated sludge in the treatment of wastewater with low C/N ratio. *J Clean Prod* 247:119164. <https://doi.org/10.1016/j.jclepro.2019.119164>
- Shah SR (2013) Chemistry and application of cellulase in textile wet processing. *Res J Eng Sci* ISSN 2278:9472
- Sharma D, Joshi B, Bhatt MR, Joshi J, Malla R, Bhattarai T, Sreerama L (2015) Isolation of cellulolytic organisms from the gut contents of termites native to Nepal and their utility in saccharification and fermentation of lignocellulosic biomass. *J Biomas Biofuel* 2:11–20. <https://doi.org/10.11159/jbb.2015.002>
- Sharma S, Setia H, Toor AP (2021) Assessing the bioremediation potential of indigenously isolated *Klebsiella* sp. WAH1 for diclofenac sodium: optimization, toxicity and metabolic pathway studies. *World J Microbiol Biotechnol* 37(2):33. <https://doi.org/10.1007/s11274-021-02998-4>
- Shinde VS, Agrawal T, Kotasthane AS (2017a) Molecular characterization of cellulolytic bacteria derived from termite gut and optimization of cellulase production. *Int J Curr Microbiol Appl Sci* 6(10):2474–92. <https://doi.org/10.20546/jcm.2017.6.10.292>
- Shinde VS, Shinde RM, Agrawal T, Kotasthane AS (2017b) Extracellular cellulase activity of termite gut bacteria isolated from different geographical locations of Chhattisgarh (C.G.). India. *Plant Arch* 17(1):601–607
- Simol CF, Chubo JK, King PJH, Ong KH, Chew C, NAWI K (2021) Qualitative and Molecular Screening of Potential Lignolytic Microbes from Termite (*Coptotermes curvignathus*) Gut. *Borneo J Res Sci Tech* 11(1):35–42
- Singh A, Bajar S, Devi A, Pant D (2021) An overview on the recent developments in fungal cellulase production and their industrial applications. *Biores Technol Report* 14:100652. <https://doi.org/10.1016/j.biteb.2021.100652>
- Sojitra UV, Nadar SS, Rathod VK (2017) Immobilization of pectinase onto chitosan magnetic nanoparticles by macromolecular cross-linker. *Carbohydr Polym* 157:677–685. <https://doi.org/10.1016/j.carbpol.2016.10.018>
- Sondhi S, Sharma P, George N, Chauhan PS, Puri N, Gupta N (2015) An extracellular thermo-alkali-stable laccase from *Bacillus tequilensis* SN4, with a potential to biobleach softwood pulp. *3 Biotechnol* 5(2):175–85. <https://doi.org/10.1007/s13205-014-0207-z>
- Sreena CP, Resna NK, Sebastian D (2015) Isolation and characterization of cellulase producing bacteria from the gut of termites (*Odontotermes* and *Heterotermes* species). *Int J Biotechnol* 9:1–10. <https://doi.org/10.9734/BBJ/2015/20001>
- Tahir U, Yasmin A (2021) Decolorization and discovery of metabolic pathway for the degradation of Mordant Black 11 dye by *Klebsiella* sp. MB398. *Brazilian J Microbiol* 52(2):761–71. <https://doi.org/10.1007/s42770-021-00470-x>
- Tamilanban R, Velayudhan SS, Rajadas SE, Harshavardhan S (2017) Purification and characterization of an extracellular cellulase produced using alkali pretreated rice straw by *Stenotrophomonas maltophilia*. *Int J Biol Res* 2:2455–6548. [www.biologyjournal.in](http://www.biologyjournal.in)
- Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceed Nat Acad Sci* 101(30):11030–11035. <https://www.pnas.org/cgi/doi/10.1073/pnas.0404206101>
- Tamura K, Stecher G, Kumar S (2021) MEGA 11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol* <https://doi.org/10.1093/molbev/msab120>
- Tokuda G (2021) Origin of symbiotic gut spirochetes as key players in the nutrition of termites. *Environ Microbiol* 23(8):4092–4097. <https://doi.org/10.1111/1462-2920.15625>
- Tomulescu C, Moscovici M, Lupescu I, Stoica RM, Vamanu A (2021) A review: *Klebsiella pneumoniae*, *Klebsiella oxytoca* and biotechnology. *Rom Biotechnol Lett* 26(3): 2567–2586. <https://doi.org/10.25083/rbl/26.3/2567.2586>
- Tsegaye B, Balomajumder C, Roy P (2019) Isolation and characterization of novel lignolytic, cellulolytic, and hemicellulolytic bacteria from wood-feeding termite *Cryptotermes brevis*. *Int Microbiol* 22(1):29–39. <https://doi.org/10.1007/s10123-018-0024-z>
- Upadhyaya SK, Manandhar A, Mainali H, Pokhrel AR, Rijal A, Pradhan B, Koirala B (2012) Isolation and characterization of cellulolytic bacteria from gut of termite. In *Rentech Symp Comp* 1(4):14–18
- Van Dexter S, Boopathy R (2019) Biodegradation of phenol by *Acinetobacter taylorii* isolated from the gut of the termite. *Environ Sci Pollut Res* 26(33):34067–34072. <https://doi.org/10.1007/s11356-018-3292-4>
- Vishal Devaraj, Sheetal S Kesti, (2019) Isolation and Molecular Characterization of Termite GUT Microflora. *Int J Sci Res Biol Sci* 6(3):41–49
- Waghmare PR, Kshirsagar SD, Saratale RG, Govindwar SP, Saratale GD (2014) Production and characterization of cellulolytic enzymes by isolated *Klebsiella* sp. PRW-1 using agricultural waste biomass. *Emir J Food Agri* 26(1):44–59. <https://doi.org/10.9755/efja.v26i1.15296>
- Wang Q, Fu X, Liu S, Ji X, Wang Y, He H, Yang G, Chen J (2020) Understanding the effect of depth refining on upgrading of dissolving pulp during cellulase treatment. *Ind Crops Prod* 144:112032. <https://doi.org/10.1016/j.indcrop.2019.112032>
- Yadav S, Chandra R (2015) Syntrophic co culture of *Bacillus subtilis* and *Klebsiella pneumoniae* for degradation of kraft lignin discharged from rayon grade pulp industry. *J Environ Sci* 33:229–228. <https://doi.org/10.1016/j.jes.2015.01.018>
- Yan S, Sun X, Zhang W, Zhu L (2019) Isolation, identification and cellulase-producing condition optimization of *Bacillus subtilis* Q3 strain. In *AIP Conf Proc* 2110(1):020004. <https://doi.org/10.1063/1.5110798>
- Ye C, Rasheed H, Ran Y, Yang X, Xing L, Su X (2019) Transcriptome changes reveal the genetic mechanisms of the reproductive plasticity of workers in lower termites. *BMC Genom* 20(1):702. <https://doi.org/10.1186/s12864-019-6037-y>
- Younis AI, Elbially AI, Abo Remila EM, Ammar AM (2017) Molecular detection of genus *Klebsiella* species and genotypic identification of *Klebsiella pneumoniae* and *Klebsiella oxytoca* by duplex polymerase chain reaction in poultry. *Glob Veter* 18(3):234–241. <https://doi.org/10.5829/idosi.gv.2017.234.241>
- Zahra T, Irfan M, Nadeem M, Ghazanfar M, Ahmad Q, Ali S, Siddique F, Yasmeen Z, Franco M (2020) Cellulase production by *Trichoderma viride* in submerged fermentation using response surface methodology. *Punjab Univ J Zool* 35(2): 223–8. <https://doi.org/10.17582/journal.pujz/2020.35.2.223.228>
- Zhao Z, Shih C, Gao T, Ren D (2021) Termite communities and their early evolution and ecology trapped in Cretaceous amber. *Cret Res* 117:104612. <https://doi.org/10.1016/j.cretres.2020.104612>
- Zhou J, Duan J, Gao M, Wang Y, Wang X, Zhao K (2019) Diversity, roles, and Biotechnological applications of symbiotic microorganisms in the gut of termite. *Cur Microbiol* 76(6):755–61. <https://doi.org/10.1007/s00284-018-1502-4>

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