



OPEN ACCESS

EDITED BY

Sumit Kumar,
Amity University,
India

REVIEWED BY

Vikram Hiren Raval,
University School of Sciences, Gujarat
University, India
Digvijay Verma,
Babasaheb Bhimrao Ambedkar University,
India

*CORRESPONDENCE

Oliyad Jeilu
Oliyad.jeilu.oumer@slu.se

SPECIALTY SECTION

This article was submitted to
Extreme Microbiology,
a section of the journal
Frontiers in Microbiology

RECEIVED 30 September 2022

ACCEPTED 11 November 2022

PUBLISHED 07 December 2022

CITATION

Jeilu O, Simachew A, Alexandersson E,
Johansson E and Gessesse A (2022)
Discovery of novel carbohydrate degrading
enzymes from soda lakes through
functional metagenomics.
Front. Microbiol. 13:1059061.
doi: 10.3389/fmicb.2022.1059061

COPYRIGHT

© 2022 Jeilu, Simachew, Alexandersson,
Johansson and Gessesse. This is an open-
access article distributed under the terms
of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that
the original publication in this journal is
cited, in accordance with accepted
academic practice. No use, distribution or
reproduction is permitted which does not
comply with these terms.

Discovery of novel carbohydrate degrading enzymes from soda lakes through functional metagenomics

Oliyad Jeilu^{1,2*}, Addis Simachew¹, Erik Alexandersson³,
Eva Johansson² and Amare Gessesse^{1,4}

¹Institute of Biotechnology, Addis Ababa University, Addis Ababa, Ethiopia, ²Department of Plant Breeding, Swedish University of Agricultural Sciences, Lomma, Sweden, ³Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Lomma, Sweden, ⁴Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology, Palapye, Botswana

Extremophiles provide a one-of-a-kind source of enzymes with properties that allow them to endure the rigorous industrial conversion of lignocellulose biomass into fermentable sugars. However, the fact that most of these organisms fail to grow under typical culture conditions limits the accessibility to these enzymes. In this study, we employed a functional metagenomics approach to identify carbohydrate-degrading enzymes from Ethiopian soda lakes, which are extreme environments harboring a high microbial diversity. Out of 21,000 clones screened for the five carbohydrate hydrolyzing enzymes, 408 clones were found positive. Cellulase and amylase, gave high hit ratio of 1:75 and 1:280, respectively. A total of 378 genes involved in the degradation of complex carbohydrates were identified by combining high-throughput sequencing of 22 selected clones and bioinformatics analysis using a customized workflow. Around 41% of the annotated genes belonged to the Glycoside Hydrolases (GH). Multiple GHs were identified, indicating the potential to discover novel CAZymes useful for the enzymatic degradation of lignocellulose biomass from the Ethiopian soda Lakes. More than 73% of the annotated GH genes were linked to bacterial origins, with *Halomonas* as the most likely source. Biochemical characterization of the three enzymes from the selected clones (amylase, cellulase, and pectinase) showed that they are active in elevated temperatures, high pH, and high salt concentrations. These properties strongly indicate that the evaluated enzymes have the potential to be used for applications in various industrial processes, particularly in biorefinery for lignocellulose biomass conversion.

KEYWORDS

extremophiles, glycoside hydrolases, lignocellulose biomass, halophiles, soda lakes, CAZymes, functional metagenomics

Introduction

The most abundant bioresource on Earth, lignocellulosic biomass, has an annual global yield of up to 1.3 billion tons (Baruah et al., 2018; Zoghalmi and Paës, 2019). Lignocellulosic biomass is mainly composed of polysaccharides (cellulose and hemicelluloses) and lignin (an aromatic polymer). Hydrolysis of the polysaccharide component of lignocellulosic biomass releases fermentable sugars (Anwar et al., 2014), which can produce renewable energy and chemicals (Abdel-Hamid et al., 2013). Such production can reduce dependence on fossil fuels, decrease greenhouse gas emissions, and mitigate climate change (Kabeyi and Olanrewaju, 2022).

Hydrolysis of lignocellulosic biomass can be done through acid or enzymatic hydrolysis. The acid hydrolysis effectively breaks down the polysaccharides into their monomeric sugars. However, it leads to the generation of inhibitors in subsequent fermentation (Oriez et al., 2019) and contributes to environmental pollution (Jönsson and Martín, 2016). Therefore, enzymatic hydrolysis, often carried out under relatively mild reaction conditions, offers an eco-friendly and efficient method for the hydrolysis of lignocellulosic biomass (Ellilä et al., 2019). In practice, the complete breakdown of lignocellulosic polysaccharides to their monomeric components requires the synergistic action of multiple enzymes, known as Carbohydrate-Active enZymes (CAZymes) (Bredon et al., 2018; Liew et al., 2022).

Industrial processes for lignocellulosic biomass degradation has to operate at high temperatures to increase substrate accessibility. In addition, alkali pretreatment is used to enhance the internal surface area of hemicelluloses while removing acetyl groups and uronic acids (Baruah et al., 2018; Lu et al., 2021; Sun et al., 2021; Verma, 2021). Therefore, enzymes that have activity and stability in an alkaline pH range and/or at high temperatures have a potential for hydrolysis of lignocellulosic biomass (Ariaeenejad et al., 2020; Mamo, 2020). However, most known cellulases and hemicellulases are obtained from mesophilic organisms and have their optimum activity and stability around ambient temperature and in a neutral pH range (Collins et al., 2005; Ben Hmad and Gargouri, 2017). To date, relatively few alkaline active and thermostable cellulases and hemicellulases have been reported (Shuddhodana et al., 2018; Verma, 2021). Furthermore, a significant hurdle to the large-scale conversion of lignocellulose to biofuels is the scarcity of low-cost enzymes capable of effectively depolymerizing biomass (Kern et al., 2013). Thus, there is a high need to search for novel enzymes not only to enhance bioconversion but also to in order to make the process more environmentally friendly and cost-effective (Fongaro et al., 2020; Verma and Satyanarayana, 2020; Verma, 2021). One of the best strategies to obtain such enzymes is to search for lignocellulose polysaccharide degrading enzymes from extreme environments such as soda lakes (Berini et al., 2017a; Cabrera and Blamey, 2018).

Soda lakes are unique poly-extreme environments, mainly characterized by high alkalinity and salinity (Schagerl, 2016) and harbor unique and diverse microbial communities (Lanzén et al., 2013; Sorokin et al., 2015; Grant and Jones, 2016). These microbial

communities from soda lakes provide a one-of-a-kind source of enzymes (Antony et al., 2013) with properties that allow them to endure the rigorous industrial conversion of lignocellulose biomass into fermentable sugars. Cellulases, hemicellulases, and other carbohydrate polymer degrading enzymes produced by microorganisms from these habitats are expected to be active and stable under extreme conditions prevalent in the soda lakes.

Although most of the known industrial enzymes are obtained from microorganisms through pure culture isolation and screening (Martin and Vandenberg, 2016), recent estimates show that more than 99% of microorganisms in the environment are uncultivable through conventional microbiological methods (Berini et al., 2017a). Thus, in recent years the advent of metagenomics has provided a powerful tool to access the genetic and metabolic diversity of microorganisms in any environment, bypassing the limitations of the current culture-dependent approaches (Berini et al., 2017a; Ngara and Zhang, 2018; Pabbathi et al., 2021). Functional metagenomics is a method that clones the environmental DNA into a suitable vector to create a library and which is then transformed into a host, such as *E. coli*. This library is then screened for various enzymatic activities (Simon and Daniel, 2011; Coughlan et al., 2015; Lam et al., 2015; Sysoev et al., 2021). Through functional metagenomics, novel biocatalysts, including lipases (Privé et al., 2015), cellulases (Voget et al., 2006), amylases (Vester et al., 2015), and chitinases (Berini et al., 2017b) have been discovered.

Whereas CAZymes from ruminants (Wang et al., 2013, 2019; Shen et al., 2020), lignocellulosic biomass wastes (Montella et al., 2017), and soil (López-Mondéjar et al., 2020), have already been thoroughly explored, investigations of CAZymes produced by microbial communities of soda lakes remain scarce. Thus, the main objective of this study was to search for CAZymes from the Ethiopian Rift Valley soda lakes using functional metagenomics.

Materials and methods

Sampling site and sample collection

Water and sediment samples were collected from three soda lakes in the East African Rift Valley: Lakes Abijata (7°37'0"N, 38°36'0"E), Chitu (7°24'14"N, 38°25'15"E), and Shala (7°25'29"N, 38°36'57"E), using sterile Niskin bottles (Ocean Scientific International Ltd., Hampshire, United Kingdom) and polyethylene bags, respectively. The water samples were filtered within 24 h of sample collection, using a polycarbonate filter membrane (0.22 µm pore size, 47 mm diameter; GE, IL, United States) to harvest the biomass for metagenomic DNA extraction.

Isolation of high molecular weight DNA

Metagenomic DNA was extracted from water and sediment samples according to Øvreås et al. (2003) and Verma and Satyanarayana (2011), respectively, with some modifications

described below. Briefly, about 250 μ l solution of 1 mg/ml Lysozyme and 0.5 mg/ml RNase (Thermo Scientific, Massachusetts, United States) was added to the microbial biomass on the polycarbonate filter membrane and incubated for 15 min at 37°C. The filter was then treated with 10 μ l of 1 mg/ml Proteinase K (Thermo Scientific, Massachusetts, United States) and incubated for 15 min at 37°C. Afterwards, 250 μ l of pre-heated 10% sodium dodecyl sulfate (SDS; *w/v*) was added and incubated for 15 min at 55°C. About 80 μ l of 5 M NaCl and 100 μ l of 1% cetyltrimethylammonium bromide (CTAB; *w/v*) were added and incubated for 10 min at 65°C; followed by the addition of 750 μ l chloroform/isoamyl alcohol (24:1). The mixture was centrifuged at 12,000 $\times g$ for 15 min, and then a 0.6 volume of isopropanol was added to precipitate the aqueous layer. Finally, the mixture was centrifuged at 12000 g for 15 min, and the pellet was washed with 70% (*v/v*) ethanol, dried at room temperature, and dissolved in 10 mM TE buffer (pH 8.0). To extract DNA from sediment samples, about 10 g of sediment was suspended in 13.5 ml of extraction buffer (1%CTAB, *w/v*), 100 mM Tris (pH 8.0), 100 mM NaH₂PO₄ (pH 8.00), 100 mM EDTA, 1.5 M NaCl). Then, 50 μ l of 10 mg/ml proteinase K (Thermo Scientific, Massachusetts, United States) was added, and the mixture was incubated at 37°C for 30 min. After that, 1.5 ml of 20% SDS was added and incubated for 2 h at 65°C. The samples were centrifuged at 4,000 $\times g$ for 20 min at room temperature to separate the sediment remnants from the cell lysates. The cell lysates were mixed with an equal proportion of phenol, chloroform, and isoamyl alcohol (25:24:1) and were centrifuged at 16,000 $\times g$ for 5 min. Then, the DNA in the aqueous layer was precipitated by adding 0.6 volumes of isopropanol and recovered by centrifuging at 16,000 $\times g$ for 10 min. Finally, the pellet was washed with 70% (*v/v*) ethanol, dried by air, and dissolved in 10 mM TE buffer (pH 8.0). The quantification and quality control of extracted DNA were performed using a Nanodrop (Thermo Scientific, Massachusetts, United States) and gel electrophoresis with 0.8% agarose gel (Thermo Scientific, Massachusetts, United States).

Construction of the metagenomic library

The metagenomic libraries were constructed by the CopyControl™ Fosmid Library Production Kit (Epicentre, Madison, United States) according to the manufacturer's protocol. Briefly, the high molecular weight metagenomic DNA was sheared to the appropriate size by passing it through a 200- μ l small-bore pipette tip a few times and then end-repaired using the CopyControl blunt-end repair kit (Epicentre, Madison, United States). The end-repaired DNA was loaded onto a 1% low-melting-point agarose gel for 16–20 h at 35 voltage in 1 \times TAE buffer. The DNA fragments that were run parallel to the 40 kb Fosmid control DNA were excised from the gel without UV light. The DNA from the excised gel was recovered using the GELase protocol (Epicentre, Madison, United States), followed by a

conventional phenol:chloroform:isoamyl alcohol (24:24:1) extraction to remove the enzymes, and the DNA was precipitated using isopropanol. The resultant DNA pellet was resuspended in 10 mM TE (pH 8.0), and the yield and size of the DNA were validated by running an aliquot down the gel. The end-repaired DNA was ligated to the CopyControl pCC1FOS (Epicentre, Madison, United States) vector at a 10:1 vector-to-insert DNA ratio. The ligated DNA was then packaged into the MaxPlax lambda phage heads (Epicentre, Madison, United States). The lambda phage packaged DNA was then infected into *E. coli* cells (EPI100-T1R Plating Strain, Epicentre, Madison, United States), mixed 2–3 times by inverting the tubes and incubated for 30 min at room temperature. The infected cells were then plated on prewarmed Luria-Bertani (LB) agar plates (supplemented with 12.5 μ g/ml Chloramphenicol) and incubated at 37°C for 16–20 h. After the quality control, single colonies were manually transferred to individual wells on a 384-well microtiter plate (ThermoFisher, Madison, United States) with 60 μ l LB medium supplemented with 12.5 μ g/ml chloramphenicol and 20% glycerol. The library was duplicated from each master plate and kept at –80°C for further screening.

Functional screening of specific genes in the fosmid library

The library was replicated to LB agar plates supplemented with soluble starch (1%; Sigma-Aldrich, Missouri, United States), carboxyl methylcellulose (1%; CMC, Sigma-Aldrich, Missouri, United States), pectin (1%; Sigma-Aldrich, Missouri, United States), and xylan (1%; Sigma-Aldrich, Missouri, United States), to detect amylase, cellulase, pectinase, and xylanase activities, respectively. Whereas to detect β -glucosidase activity, LB agar plates were supplemented with esculin hydrate (0.1%; Sigma-Aldrich, Missouri, United States) and ferric ammonium citrate (0.25%; Sigma-Aldrich, Missouri, United States). In addition, LB agar plates were supplemented with chloramphenicol (12.5 μ g/ml) and Autoinduction solution (0.2%; Epicentre, Madison, United States). Then, the plates were incubated at 37°C for 2–7 days. For the identification of cellulase and xylanase-positive clones, the method proposed by [Teather and Wood \(1982\)](#) was used, where the colonies were washed off the agar plates with ddH₂O to permit a homogeneous penetration of the staining dye into the medium. Thereafter, the agar plates were stained with 0.2% Congo red solution for 30 min. After the solution was poured off, the agar plates were de-stained up to 3 times for 30 min with 1 M NaCl. Positive clones were detected by forming a yellow halo against a red background. The β -glucosidase activity was detected according to [Eberhart et al. \(1964\)](#) method, where clones exhibiting a black halo around their colonies were selected as positive for β -glucosidase activities. Amylase activity was detected by staining the plates with KI/I₂ solution. A colorless halo surrounded positive colonies on a dark purple background ([Sharma et al., 2010](#)).

Fosmid DNA extraction and sequencing

A total of 22 fosmid clones with positive enzyme activities were selected, cultured and induced to a high copy number using the 500× Copy Control Fosmid Autoinduction Solution (Epicentre, Madison, United States) at 37°C overnight (16–20 h) with 12.5 µg/ml chloramphenicol and vigorous shaking (225–250 rpm). Fosmid DNA was extracted using a GenElute Plasmid Miniprep Kit (Sigma-Aldrich, Missouri, United States) according to the manufacturer's protocol. The quality of extracted DNA was checked using a Nanodrop and sent to BGI Genomics for whole-genome sequencing. The *E. coli* whole genome resequencing was performed using the paired-end 150 bp method using the DNBSEQ™ platform.

Bioinformatics analysis

De-novo assembly

An in-house workflow was developed to analyze the whole genome sequences of the fosmids.¹ Briefly, whole genome sequencing (WGS) raw reads Quality Check (QC) was performed with the FastQC tool (Andrews, 2010), and multiple samples were processed with MultiQC (Ewels et al., 2016). All reads from independent 22 fosmid clones were mapped to the *E. coli* reference genome (EPI100-T1R Plating Strain, Epicenter Biotechnologies) using Bowtie2 (Langmead and Salzberg, 2013) and reads were sorted with SAMtools (Danecek et al., 2021). Reads that did not map to the *E. coli* genome were further used for de-novo assembly with SPAdes v3.15.3 and metaplasmidspades (Bankevich et al., 2012). Further, the CopyControl pCC1FOS (Epicenter Biotechnologies) vector backbone sequences were removed from the assembled contigs. NCBI-Blast-v2.9.0+ and Bedtools-v2.27.1 were used for processing assemblies.

Gene prediction, taxonomy, and functional annotation

Functional annotation of the assembled fosmid contigs were performed using RASTtk (Brettin et al., 2015) webserver, and cross-validation of predicted coding DNA sequence (CDS) using TransDecoder-v5.5.0.² Then, carbohydrate-active enzymes (Cantarel et al., 2009) annotation was conducted using hmmscan (Version 3.3 b2) against CAZy database (version 2021). Kraken2 (version 2.0.8) (Wood et al., 2019) was applied for taxonomic sequence classification using the Minikraken2_v1_8GB database. Finally, KronaTools v2.7.1 (Ondov et al., 2011) was used to visualize taxonomies.

Preparation of cell culture for enzymatic assay

Among the enzymes screened, amylase, cellulase, and pectinase were selected based on their potential application in

complex carbohydrate degradation and their enzymatic activity studied. These positive clones were preliminarily assessed based on their clearing zone diameter, and the top four clones in each enzyme were chosen for further characterization. Positive clones (pectinase, cellulase, and amylase) were inoculated in LB media (50 ml) containing chloramphenicol (12.5 µg/ml) and Autoinduction solution (0.2%), supplemented with 1% of their respective substrates (Pectin/carboxyl methylcellulose/soluble starch). Then, the inoculated media were incubated at 37°C, 200 rpm for 3–5 days, and after incubation, centrifuged at 5,000×g and 4°C for 30 min. The supernatant (crude cell-free extract) was used as a crude enzyme cti fraction for further analysis.

Enzyme assays

Enzyme activity was measured following the dinitrosalicylic acid (DNS) method (Miller, 1959). The standard assay mixture contains 100 µl of the enzyme or crude cell-free extract and 250 µl of the substrate (1% in a final volume of 0.5 ml), and 150 µl McIlvaine buffer. Carboxymethyl cellulose (CMC), pectin, and soluble starch were used as substrates for cellulase, pectinase, and amylase, respectively. The mixture was incubated at 37°C for 15 min, then 750 µl DNS reagent was added, and the samples were boiled at 100°C for 15 min. After cooling down on the ice, the samples were centrifuged at 16,000×g for 2 min to precipitate falling proteins. The samples were transferred to cuvettes, and absorbance was measured at 540 nm (Thermo Scientific). The pH range of the enzyme was determined by measuring standard assay activity between pH 7.0 and 10.5 using 50 mM of appropriate buffers. The temperature range of the enzyme activity was determined by assaying at temperatures between 30 and 75°C. The effect of salt on enzyme activities was investigated by incorporating 0–3M of NaCl into the reaction mixture.

Results

Construction and functional screening of metagenomic libraries

Metagenomic libraries with more than 21,000 clones were generated from three Ethiopian soda lakes (Table 1). At the time of sampling, the salinity of the lakes ranged from 3% (Lake Shala) to 15% (Lake Abijata) and pH from 9.3 (Lake Shala) to 10.0 (Lake Chitu). The number of clones picked from each sample site ranges from 1,440 (Lake Abijata) to 10,368 (Lake Shala; Table 1).

Among all the clones screened (>21,000) for the five hydrolytic enzymes, a total of 408 clones were positive for at least one of the hydrolytic enzymes tested (Figure 1). A relatively high number of clones (281 clones, 1.33% hit ratio) were positive for cellulase, followed by amylase (75 clones, 0.36% hit ratio),

1 <https://github.com/gvarmaslu/WGS-fosmid-project>

2 <http://transdecoder.github.io>

β -glucosidase (20 clones, 0.1% hit ratio), pectinase (15 clones, 0.07% hit ratio) and 12 positives for xylanase (12 clones, 0.06% hit ratio; [Table 2](#)).

Analysis of fosmid insert sequences and detection of carbohydrate-active enzymes

A total of 22 clones that were positive for the enzymes mentioned above were selected and subjected to sequencing. After bioinformatics sequence analysis and assembly, the insert sizes were between 30,230 and 44,461 base pairs. Each library contained clones with average size inserts of approximately 35 kb, yielding

approximately 0.74 GB of the total cloned genomic DNA per library. A total of 1,233 open reading frames (ORFs) were predicted from the fosmid clones ([Table 3](#)). Then, the carbohydrate-active enzyme (CAZy) database was used to annotate the predicted ORFs, resulting in 378 encoding genes with potential functions predicted ([Supplementary Table 1](#)). All the detected genes coding for CAZymes were further assigned to six functional classes: 10 Auxiliary Activities (AAs), 61 Carbohydrate-Binding Modules (CBMs), 24 Carbohydrate Esterases (CEs), 156 Glycoside Hydrolases (GHs), 118 Glycosyl transferases (GTs), and 9 Polysaccharide Lyases (PLs; [Table 3](#)).

Glycoside hydrolase enzymes

GHs were the most abundant class, representing 40% of the identified carbohydrate-degrading enzymes ([Table 4](#); [Supplementary Table 1](#)). These GHs were categorized into 38 GH families. The most abundant GH families were GH2, GH3, GH5, GH12, GH13, and GH28. About 10% of the GH families belonged to the GH3 family, which encodes β -glucosidase (EC 3.2.1.21), and N-acetyl- β -D-glucosaminidases (EC 3.2.1.30). The GH5 family representing Cellulase (EC 3.2.1.4) were the second most abundant GH family, representing 8% of the total families. About 5% of the GH families were found to belong to the GH28, a family

TABLE 1 Physical parameters of the studied soda lakes of Ethiopia with the number of metagenomics libraries constructed.

Sample site	pH	Salinity (%)	Number of clones
Abijata	9.5	15	1,440
Chitu	10	6	9,216
Shala	9.3	3	10,368
Total			21,024

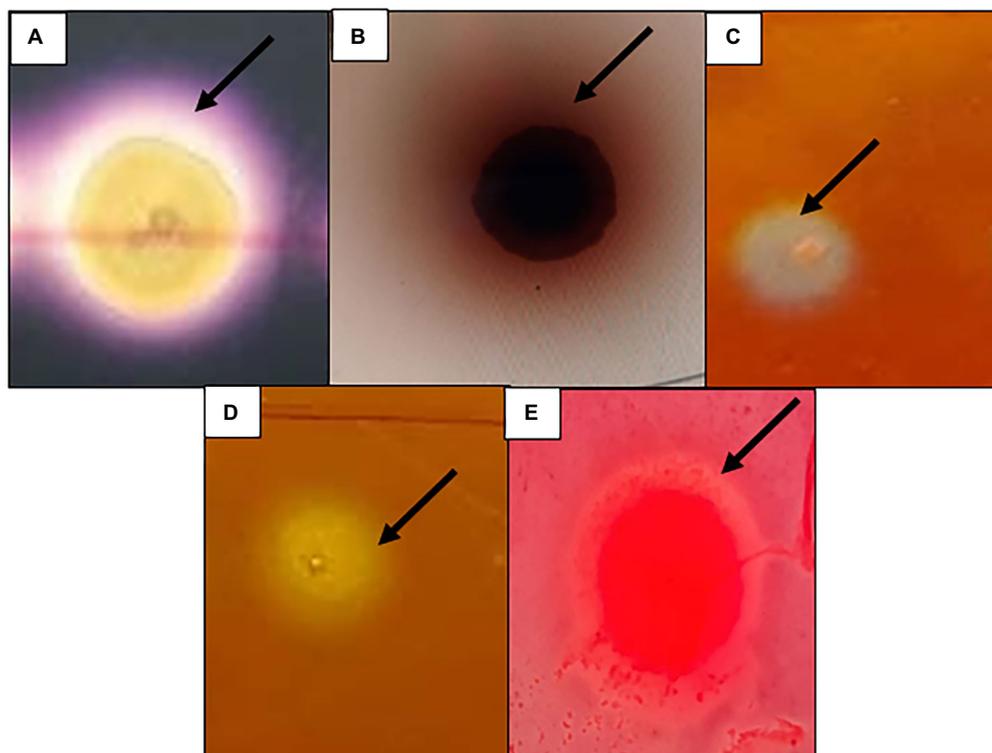


FIGURE 1
Functional screening of the metagenomics library constructed from Ethiopian soda lakes showing positive for (A) Amylase, (B) β -glucosidase, (C) Cellulase, (D) Pectinase, (E) Xylanase.

that contains pectinase polygalacturonase enzymes. Starch degrading enzymes such as alpha-amylases (GH13) were also abundant (4%; Figure 2).

Tracking the microbial sources of the annotated GH genes

The microbial source of the annotated GH genes were predicted, and more than 73% of the genes aligned to bacterial sources. However, there were no organism hits for about 27% of the annotated GH genes. Most of the bacterial genes at the class level belonged to the *Gammaproteobacterial* and at the genera level to *Halomonas* (Supplementary Figure 1).

TABLE 2 Hit ratios of the screened hydrolytic enzymes.

Enzyme	Hit ratio
Amylase	1:280
B-glucosidase	1:1051
Cellulase	1: 75
Pectinase	1:1402
Xylanase	1: 1752

Expression and characterization of selected enzymes

Cellulase

Further studies on the effect of pH, temperature, and salt concentration on the cellulase activities of the selected clones (F8, F9, F17, and F22) showed that the clone F8 had its optimum cellulase activity at pH 9.5, whereas clones F17 and F9 showed the optimum activity at pH 8.5 (Figure 3A). All clones showed their optimum cellulase activity at 37°C (Figure 3B). The optimum cellulase activities for all clones were at 0M NaCl, while the clone F17 maintained 75% of the cellulase activity up to 3M salt concentration (Figure 3C).

Amylase

Studies on the effect of pH, temperature and salinity on the amylase activity of four screened fosmid clones (F13, F15, F17, and F22) showed that all four clones, except for F22, had the maximum amylase activity at pH 8.5 (Figure 4A), and that the clones F17, F13, and F15 showed the optimum amylase activity at 55, 50, and 45°C, respectively (Figure 4B). Furthermore, these amylase-positive clones showed increasing relative amylase activity with increased salinity (Figure 4C).

TABLE 3 The DNA Insert size of each fosmid in base pairs, the number of ORFs, and detected CAZyme classes.

Fosmid clones	Contig size (bp)	GC (%)	ORFs	AAs	CBMs	CEs	GHs	GTs	PL	Total CAZYmes
F1	39,939	59.81	77	0	0	5	5	0	0	10
F2	33,919	44.52	34	0	0	0	10	17	0	28
F3	30,230	53.28	34	0	1	0	0	0	0	1
F4	43,585	63.87	75	0	1	1	2	5	0	9
F6	31,316	52.00	36	2	15	4	27	11	1	60
F8	36,493	47.93	39	0	3	1	11	9	1	25
F9	36,313	57.36	67	1	2	1	7	4	2	17
F10	44,461	64.38	79	0	1	1	5	2	0	10
F11	31,461	64.19	72	0	0	0	1	0	0	1
F13	37,445	65.37	83	0	2	1	6	4	0	13
F14	36,784	60.12	68	0	1	2	5	6	0	14
F15	42,189	65.68	87	1	2	0	4	6	3	16
F16	33,691	49.63	39	0	1	1	4	1	0	7
F17	35,026	58.60	58	0	5	2	7	4	0	18
F18	35,903	56.98	68	0	3	0	8	4	1	16
F19	35,357	51.12	44	0	0	1	9	7	0	17
F20	31,419	65.25	72	0	3	0	3	7	0	13
F21	33,565	49.65	39	0	1	1	4	7	0	13
F22	35,225	52.03	40	1	3	4	14	6	0	28
F23	36,297	47.81	28	1	15	1	18	6	1	42
F24	38,076	53.27	40	0	0	0	3	9	0	16
F25	30,893	52.05	54	0	0	1	2	2	0	5
Total			1,233	10	61	27	156	117	9	378

ORFs, open reading frames; AAs, auxiliary activities; CBMs, carbohydrate-binding modules; CEs, carbohydrate esterases; GHs, glycoside hydrolases; GTs, Glycosyl transferases; PLs, polysaccharide lyases

TABLE 4 Carbohydrate degrading enzymes detected in the fosmid clones (the complete list in Supplementary Table).

GH family	Fosmid	Hit accession	Identity (%)	Predicted enzyme
GH3	F17	ATH78710.1	96.8	β -glucosidase
GH103	F24	AVI63997.1	86.4	Peptidoglycan lytic
	F11	AXY42412.1	85.5	transglycosylase
GH13	F15	AHO18837.1	69.0	α -amylase
GH73	F19	AZU02762.1	51.9	Lysozyme
GH47	F13	CBX90714.1	47.8	α -mannosidase
GH2	F15	ACR62057.1	47.5	β -galactosidase
GH3	F9	SQK97389.1	46.3	β -glucosidase
GH13	F13	QTJ41571.1	46.0	α -amylase
GH78	4F4	ACT97502.1	43.0	α -L-rhamnosidase
GH43	F14	BBI53471.1	42.0	β -xylosidase
	GH3	F22	SQK97389.1	40.7
F6		SQK97389.1	40.5	
GH5	F8	QXC62151.1	40.4	Cellulase
GH38	F1	QJS43890.1	40.1	α -mannosidase
GH140	F22	QJW98933.1	39.6	β -1,2-apiosidase
	F6			
GH53	F22	QKX57828.1	39.5	Endo- β -1,4-galactanase
GH53	F6			
GH55	F25	ATI54262.1	39.4	Exo- β -1,3-glucanase
GH5	F25	QXC62151.1	39.4	Cellulase
GH103	F20	BCX45957.1	38.1	Peptidoglycan lytic transglycosylase
GH92	F17	AGB27560.1	38.1	α -1,2-mannosidase
GH51	F17	QKX56076.1	37.9	Endoglucanase
GH5	F10	QXC62151.1	37.6	Cellulase
GH51	F19	QKX56076.1	37.4	Endoglucanase
GH55	F14	AOW25396.1	36.7	Exo- β -1,3-glucanase
GH5	F23	QXC62151.1	36.0	Cellulase
GH99	F13	CAZ29389.1	36.0	α -1,2-mannosidase
GH3	F10	SQK97389.1	35.8	β -glucosidase
GH37	F19	AWO98658.1	35.4	α -trehalase
GH5	F9	QXC62151.1	35.4	Cellulase
GH23	F9	APT58734.1	35.4	Lysozyme
GH51	F8	QKX56076.1	35.0	Endoglucanase

EC, Enzyme commission number.

Pectinase

Studies on the pectinase activity of four fosmid clones (F2, F6, F18, and F23) showed that the clones F18 and F23 had their optimum pectinase activity at pH 9.5 (Figure 5A) and that all four clones had optimum pectinase activity at 65°C (Figure 5B). When different salt concentrations were added, clone F18 showed enhanced pectinase activity, while others retained more than 75% of the pectinase activity up to 3M salt concentrations (Figure 5C).

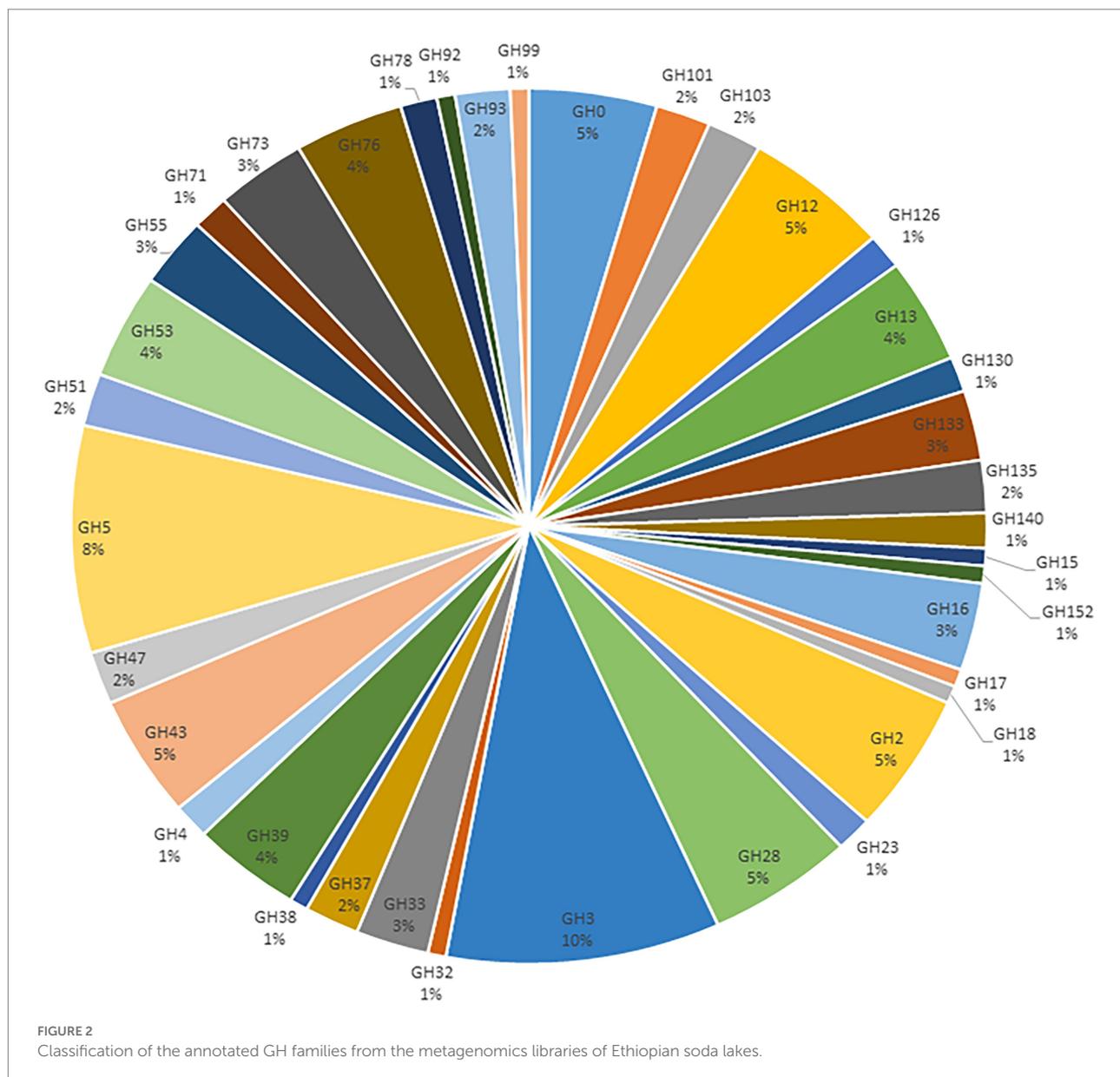
Discussion

Despite the potential of soda lakes as sources of unique extremozymes, only a few enzymes have so far been identified

from these ecosystems. Previously, carbohydrate-degrading enzymes, i.e., xylanase (Gessesse and Mamo, 1998), amylases (Martins et al., 2001), and cellulase (Minig et al., 2009), have been discovered from Ethiopian soda lakes. However, these studies have been based on conventional culture-dependent approaches, in which most microorganisms are unable to grow, thereby limiting the identification of novel enzymes (Culligan et al., 2014). In this study of the Ethiopian soda lakes, we used a functional metagenomics approach to discover carbohydrate-degrading enzymes (CAZymes) to circumvent this limitation.

The DNA extracted from the Ethiopian soda lakes was used to construct metagenomic libraries, which generated clones that produced carbohydrate-degrading enzymes. The hit ratios obtained for the hydrolytic enzymes, especially cellulase and amylase, were high (1:75 and 1:280, respectively) compared to many previous reports in other environments (Ferrer et al., 2012; Nguyen et al., 2012; Liu et al., 2015; Wang et al., 2015; Maruthamuthu et al., 2016). The functional screening approach has previously been reported to be superior in finding genes producing functional products, which increases the potential to uncover entirely new classes of enzymes that lack homologies to previously identified sequences (Culligan et al., 2014; Berini et al., 2017a). The high hit ratios of this study might largely be attributed to the source of the metagenome, i.e., the abundance and diversity of microorganisms producing the different carbohydrate-degrading enzymes in the Ethiopian soda lakes. The Ethiopian soda lakes are characterized by high primary production due to the presence of a dense population of haloalkaliphilic cyanobacteria (Wood et al., 1984; Grant and Sorokin, 2011), which in turn support a diverse group of heterotrophic prokaryotes (Lanzén et al., 2013; Grant and Jones, 2016). Since some of the heterotrophic microorganisms are involved in nutrient recycling released by dead cells, many of them are expected to produce different hydrolytic enzymes, including those involved in the hydrolysis of complex carbohydrates, such as cellulose, hemicellulose, pectin and starch (Jones and Grant, 1999; Sorokin and Kuenen, 2005; Sorokin et al., 2014). The presence of a diverse group of heterotrophs producing the above enzymes might, in turn, lead to an observed high hit ratio.

The complete breakdown of lignocellulosic polymers requires the synergistic action of multiple CAZymes (Bredon et al., 2018; Liew et al., 2022), i.e., mainly consisting of the Glycoside Hydrolases (GHs) families such as cellulase, xylanase, hemicellulase, and pectinase (Rastogi and Shrivastava, 2020; Shrivastava, 2020). About 41% of the CAZymes database annotated genes obtained from the Ethiopian soda lakes have been shown to belong to GHs. GHs are the best-described families of carbohydrate-degrading enzymes due to their high prevalence and broad distribution across genomes (Berlemont and Martiny, 2015; Stewart et al., 2018; Wang et al., 2019). Microorganisms in soda lakes degrade complex polysaccharides, releasing short metabolizable oligosaccharides into the lake environment. Detecting multiple GH enzymes, including GH5, GH3, GH13, GH43, and GH28, indicates the relevance of these enzymes to the ecosystems of the soda lakes and their carbon cycle. The short

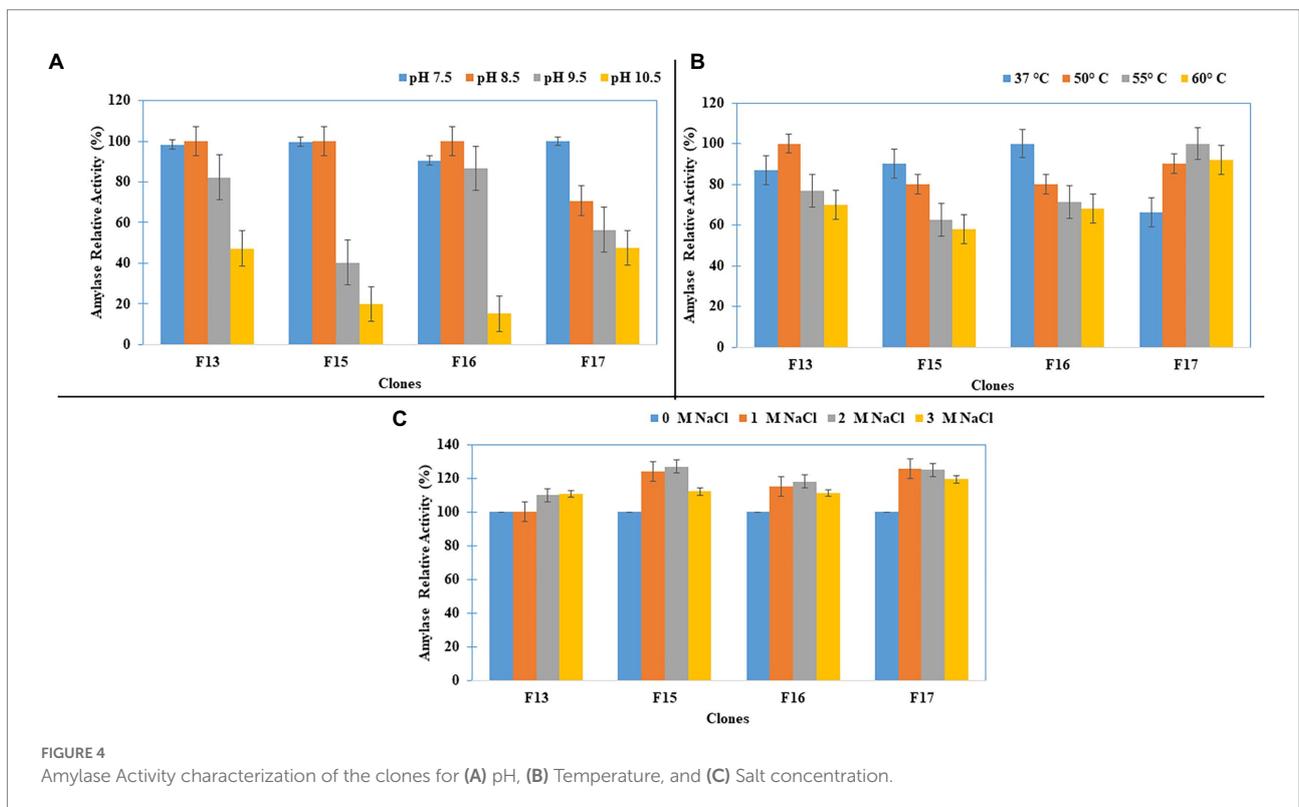
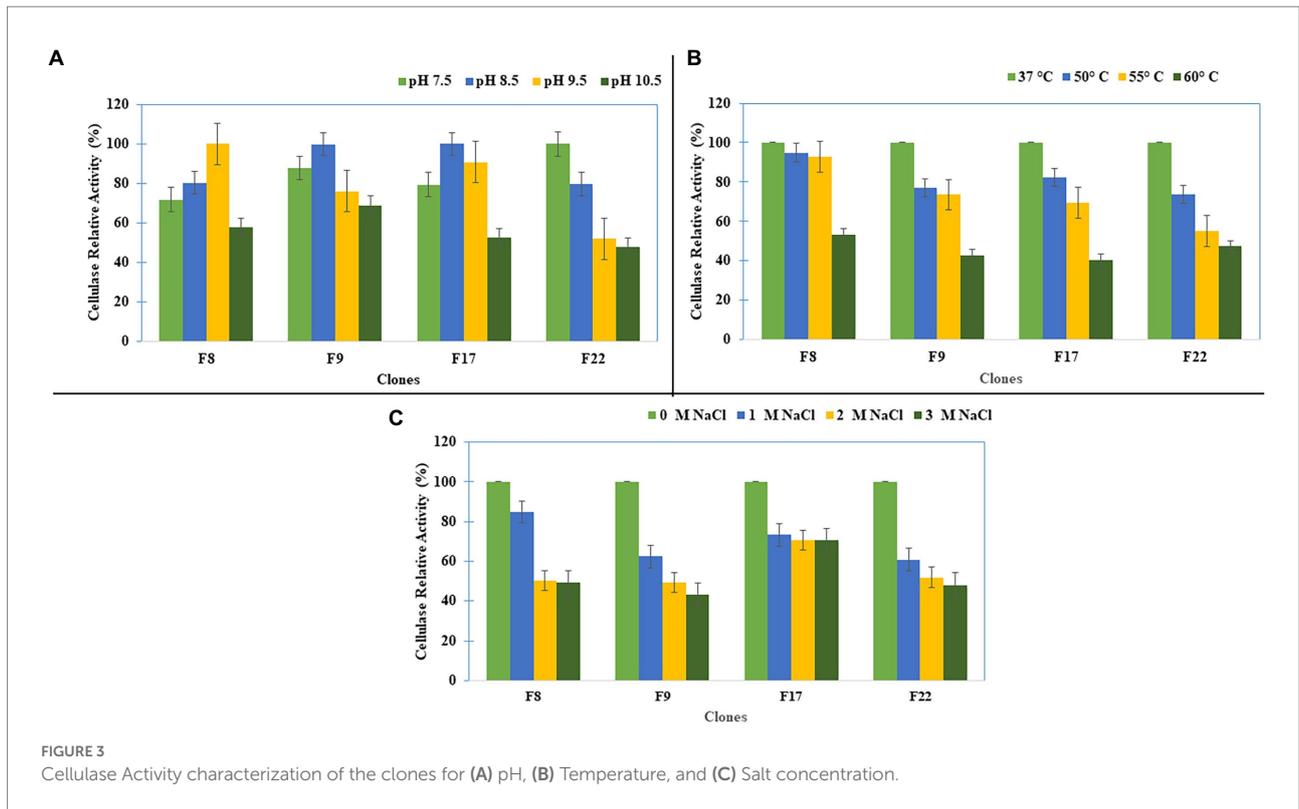


metabolizable oligosaccharides produced are fed into the core carbohydrate metabolic pathways, providing energy and precursor metabolites for other pathways (Salam, 2018).

Among the components of lignocellulosic biomass, cellulose is the most prevalent biopolymer on the planet (Lakhundi et al., 2015). In nature, microorganisms involved in nutrient recycling in the ecosystem, including soda lakes, enzymatically hydrolyze cellulose to its monomeric unit and use it as a nutrient for growth. Different cellulase enzymes are required to achieve complete hydrolysis of cellulose, the main ones being endoglucanases and β -glucosidases (Tiwari et al., 2016). In this study, about 10 and 8% of the GHs families belonged to GH3 and GH5, respectively. The GH3 and GH5 families possess β -glucosidase (EC 3.2.1.21) and endo-1,4-glucanase (EC 3.2.1.4) activities, respectively. The GH5 enzymes are involved in the hydrolysis of cellulose into cellobiose

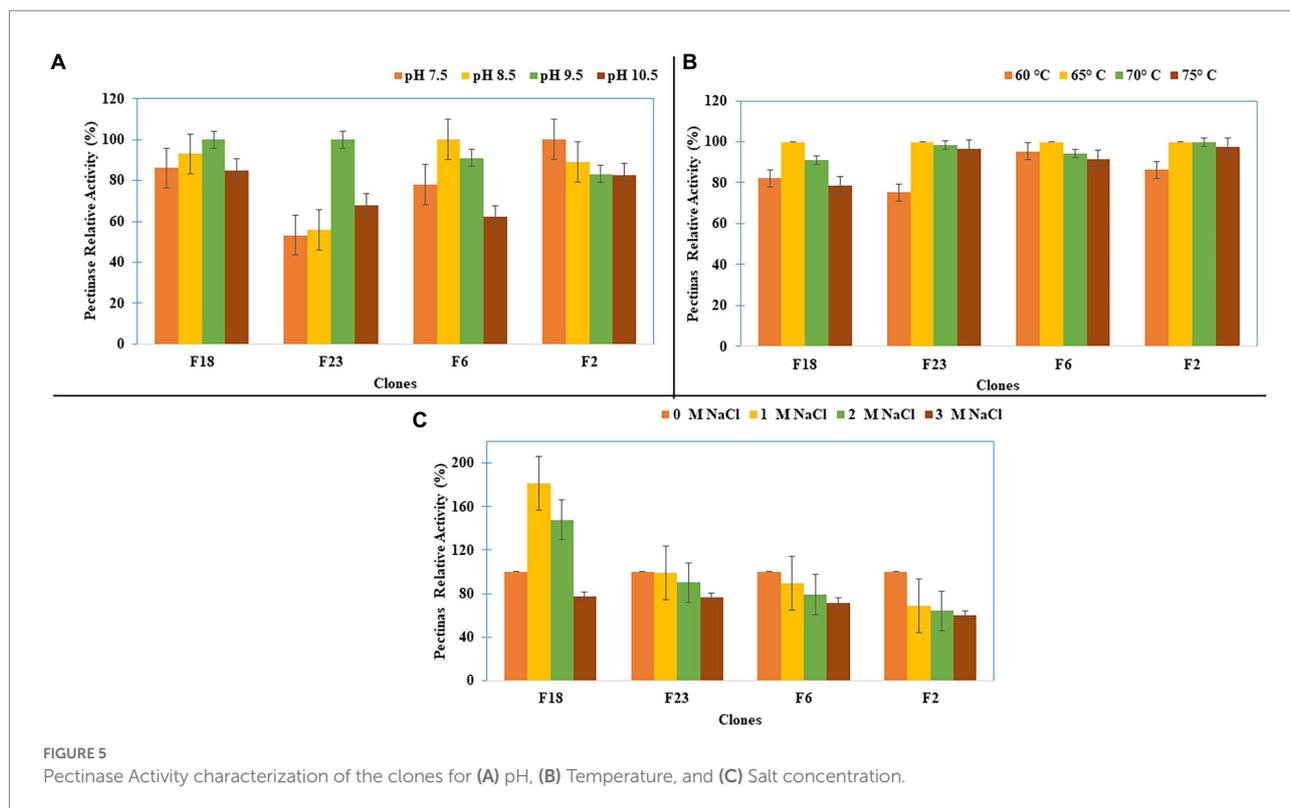
(Wang et al., 2019), while GH3 enzymes break down cellobiose into glucose (Agirre et al., 2016). Members of the GH5 superfamily are extensively dispersed across archaea, bacteria, and eukaryotes, and various enzyme functions related to biomass conversion have been discovered in this superfamily (Mohammadi et al., 2022). In cellulose biomass degradation, β -glucosidase (GH3) activity is regarded as the rate-limiting factor, reducing cellobiose inhibition on endoglucanases and permitting more effective cellulolytic enzyme action (Tiwari et al., 2017; Zhang et al., 2017).

Among the hemicellulase enzymes, GH43 was the most abundant family in the present study. Previous studies have reported the enzyme activities of GH43 to be β -xylosidase (EC 3.2.1.37), xylanase (EC 3.2.1.8) and exo- β -1,3-galactanase (EC 3.2.1.145; (Shrivastava, 2020), which are enzymes contributing to



the degradation of xylan (the main component of hemicellulose) to xylose monomers (Houfani et al., 2021). One major challenge in producing biofuels from lignocellulosic biomass by enzymatic

hydrolysis is that hemicellulose and lignin create a protective barrier surrounding the cellulose. Furthermore, the crystalline form of cellulose renders it insoluble and resistant to enzyme



degradation (Zoghلامي and Paës, 2019; Reichart et al., 2021). Thus, the removal of hemicellulose may result in increased access of cellulase to cellulose (Peng et al., 2015). Previous studies have shown that for hemicellulose degradation, the numerous GHs enzymes operating at various levels of the hemicellulolytic matrix are needed (Horn et al., 2012; Andlar et al., 2018). In this context, the breakdown of hemicelluloses by a monofunctional hemicellulase enzyme is uneconomical. However, the multifunctional hemicellulase activity of GH43 (primarily in the xylan component) can minimize enzyme costs and is essential for the complete hydrolysis of lignocellulosic biomass (Limsakul et al., 2021).

About 5% of GHs belonged to the family of GH28. The GH28 families have been shown to encode polygalacturonase, which acts on the 1,4-glycosidic bond, and plays a crucial part in pectin digestion (Zhao et al., 2014). The architectural characteristics of cell walls, which have been represented as a cellulose-hemicellulose network embedded in a pectin matrix, imply that pectins may hide cellulose and/or hemicellulose, preventing degradative enzymes from attacking them (Xiao and Anderson, 2013). Thus, pectin degradation is needed for the complete degradation of lignocellulose biomass. A recent study has shown that some haloalkaliphile *Bacteroidota* and *Clostridia* from soda lakes utilize pectin as a substrate (Sorokin et al., 2014).

Furthermore, approximately 4% of the GHs in this investigation belonged to GH13, which encodes amylases. The Ethiopian soda lakes are known to have one of the highest primary

productivity of any natural habitat due to the thick biomass of cyanobacteria (Faris, 2017), which frequently accumulate starch (10–50%) as part of their biomass (Möllers et al., 2014). Therefore, these heterotrophic microorganisms are known to produce abundant hydrolytic enzymes, such as amylases, to allow the breakdown of starch (Sorokin et al., 2014).

Soda lakes are a unique source of microorganisms that thrive in high alkalinity and salinity (Sorokin et al., 2014). These microorganisms produce either haloalkaliphilic or haloalkali-tolerant hydrolytic enzymes. The haloalkaliphilic enzymes usually show optimal activity in high alkaline and saline ranges. On the other hand, the haloalkali-tolerant enzymes may work best at a neutral pH and without salt. Still, these enzymes are active and stable in the high alkalinity and salinity range (DasSarma and DasSarma, 2015). The cellulases obtained in this study were active and stable in alkaline conditions, high temperatures and salt concentrations, indicating these enzymes are haloalkali-tolerant. This is contrary to the majority of reported cellulases, which demonstrated limited activity in these multi-extreme circumstances, such as in the presence of alkaline and salinity, and just a few alkaliphilic and halotolerant cellulases have been identified (Zhao et al., 2012; Garg et al., 2016). The conversion of lignocellulosic biomass usually occurs at high temperatures in the presence of alkaline conditions (Zhu et al., 2020). The production of salts arises from the neutralization of these alkalines. These salts must be eliminated, which consumes tons of water and energy, for subsequent processes to continue. However, these extreme

conditions affect the activity, and the stability of most known enzymes could be seriously affected (Sweeney and Xu, 2012). Therefore, enzymes that are stable in these extreme reaction conditions and in the presence of salts or tolerant to them are in great demand during downstream processes. Thus, the halo- and alkali-stable cellulases and hemicellulases identified in this study have the potential to hydrolyze lignocellulosic biomass for different industrial applications efficiently. In addition, the halotolerant nature of these enzymes offers an additional advantage because of their resistance to inactivation by salt residues that may result from pretreatment procedures (Zhang et al., 2011).

Furthermore, the biochemical characterization of the studied pectinase also showed high performance under alkaline, thermophilic, and halophilic conditions. There are several reports on alkaline and thermostable pectinase enzymes from various geographic locations and sources, particularly from agro- and industrial-wastes (Khatri et al., 2015; Oumer and Abate, 2017; Yu et al., 2017; Thakur et al., 2021). Regarding soda lakes, only two occurrences of pectin breakdown have thus far been described, both of which describe unique species of the phylum *Bacteroidota* belonging to the genera *Alkaliflexus* and *Natronoflexus* (Sorokin et al., 2014). At the same time, these microorganisms are low-salt tolerant saccharolytic fermentative alkaliphiles capable of hydrolyzing pectin. Thus, the poly-extreme nature of the pectinases in this study would make them ideal candidates for various industrial applications, particularly biomass degradation, retting and degumming of plant fibers, coffee and tea fermentation, and bio-scouring of cotton, all of which require extremes of temperature, pH, and ionic strength.

Halotolerant α -amylases have received considerably less consideration. However, most are thermostable and generate oligosaccharides in low-water or nonaqueous solvents, where hydrolytic processes are blocked (Masasa et al., 2022). This study's amylases had optimum activity in the alkalinity range and at high temperatures. In addition, salinity enhanced the amylase activity of some clones, suggesting that these amylases are poly-extremophilic. Previously, haloalkaliphilic amylases were reported from various environments (Chakraborty et al., 2011; Moshfegh et al., 2013), including soda lakes in Ethiopia (Martins et al., 2001); however, the vast majority of these studies were from bacterial origins. Polyextremophilic α -amylase are extremely useful for the textile, food, brewing, and distilling industries, as has been argued in previous publications (Patel and Saraf, 2015).

In this study, most of the GH genes identified were found to likely originate from bacteria belonging to the genus of *Halomonas*, which corresponds to *Halomonas* as one of the most abundant genera in our previous work on the microbial diversity of Ethiopian lakes using amplicon metagenomics (Jeilu et al., 2022). *Halomonas* have, in previous investigations, been reported to be one of the most abundant prokaryotes in soda lakes (Humayoun et al., 2003; Dimitriu et al., 2008; Asao et al., 2011;

Lanzén et al., 2013; Sorokin et al., 2014), responsible for the biogeochemical cycling and the immediate degradation of organic matter produced by autotrophic bacteria like *cyanobacteria* (Jones and Grant, 1999; Sorokin et al., 2014). Thus, we anticipate that *Halomonas* would generate a variety of hydrolytic enzymes, such as those necessary for the hydrolysis of complex polysaccharides, including cellulose, hemicellulose, pectin, and starch, which has also been indicated in a previous study (Tahrioui et al., 2013).

This study identified novel halo-alkaline and thermostable carbohydrate-degrading enzymes with potential applications in lignocellulosic biomass degradation. In order to use these enzymes in biofuel industrial applications, additional extensive molecular cloning, purification, and characterization studies are needed.

Conclusion

Enzymes from halophiles and alkaliphiles are the most promising for biofuel generation and other industrial processes due to their inherent salt tolerance and thermal and alkaline stability. Soda lakes, in this aspect, are a one-of-a-kind source of extremophiles capable of harboring enzymes (extremozymes) that are active at both high pH and salinity. However, there is a scarcity of reports on searching CAZymes using culture-independent approaches from soda lakes. The present study has revealed the potential of functional metagenomics for exploiting the abundant genetic resources in uncultured microorganisms from extreme environments. Moreover, this study identified multiple families of GHs, indicating that the Ethiopian soda lakes constitute a unique biological niche for identifying novel CAZymes for applications in complete lignocellulose biomass degradations. Furthermore, many reported GH enzymes originated from mesophilic microorganisms where optimal activity and stability were around neutral pH and in the absence of salt. However, the biochemical characterization of the amylase, pectinase, and cellulase enzymes in this work shows that these enzymes are halo-alkaline and thermally stable. These properties strongly indicate the enzymes' potential for use in various industrial processes, particularly biorefinery for lignocellulose biomass conversion.

Data availability statement

The data presented in the study are deposited in the NCBI repository, accession number PRJNA799030. This data can be found at: <https://www.ncbi.nlm.nih.gov/sra/PRJNA799030>.

Author contributions

OJ, AS, EA, EJ, and AG: designed the experiment and contributed to the study's conception and design. OJ: data collection and analysis, wrote the first draft of the manuscript. All

authors contributed to the article and approved the submitted version.

Funding

This study was financed by the Swedish International Development Cooperation Agency (SIDA) through the research and training grant awarded to Addis Ababa University and the Swedish University of Agricultural Sciences (AAU-SLU Biotech; <https://sida.aau.edu.et/index.php/biotechnology-phdprogram>).

Acknowledgments

The authors thank the Ethiopian Wildlife Conservation Authority for providing an access permit to sample from the three soda lakes. We would also like to thank the Ethiopian Biodiversity Institute and National Soil Testing Center, Ministry of Agriculture of Ethiopia, for granting permission for genetic material transfer. We also thank Ganapathi Varma Saripella for cooperating in the bioinformatics analysis and designing the workflow.

References

- Abdel-Hamid, A. M., Solbiati, J. O., and Cann, I. K. O. (2013). Insights into lignin degradation and its potential industrial applications. *Adv. Appl. Microbiol.* 82, 1–28. doi: 10.1016/B978-0-12-407679-2.00001-6
- Agirre, J., Ariza, A., Offen, W. A., Turkenburg, J. P., Roberts, S. M., McNicholas, S., et al. (2016). Three-dimensional structures of two heavily N-glycosylated *Aspergillus* sp. family GH3 β -D-glucosidases. *Acta Crystallogr. Sect. D Struct. Biol.* 72, 254–265. doi: 10.1107/S2059798315024237
- Andlar, M., Rezić, T., Mardetko, N., Kracher, D., Ludwig, R., and Šantek, B. (2018). Lignocellulose degradation: an overview of fungi and fungal enzymes involved in lignocellulose degradation. *Eng. Life Sci.* 18, 768–778. doi: 10.1002/elsc.201800039
- Andrews, S. (2010). FastQC-A quality control tool for high throughput sequence data. Available at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>. BabrahamBioinforma (Accessed November 2021)
- Antony, C. P., Kumaresan, D., Hunger, S., Drake, H. L., Murrell, J. C., and Shouche, Y. S. (2013). Microbiology of Lonar Lake and other soda lakes. *ISME J.* 7, 468–476. doi: 10.1038/ismej.2012.137
- Anwar, Z., Gulfranz, M., and Irshad, M. (2014). Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. *J. Radiat. Res. Appl. Sci.* 7, 163–173. doi: 10.1016/j.jrras.2014.02.003
- Ariaeenejad, S., Abdollahzadeh Mamaghani, A. S., Maleki, M., Kavousi, K., Foroozandeh Shahraki, M., and Hosseini Salekdeh, G. (2020). A novel high performance in-silico screened metagenome-derived alkali-thermostable endo- β -1,4-glucanase for lignocellulosic biomass hydrolysis in the harsh conditions. *BMC Biotechnol.* 20:56. doi: 10.1186/s12896-020-00647-6
- Asao, M., Pinkart, C. H., and Madigan, M. T. (2011). Diversity of extremophilic purple phototrophic bacteria in soap Lake, a Central Washington (USA) soda Lake. *Environ. Microbiol.* 13, 2146–2157. doi: 10.1111/j.1462-2920.2011.02449.x
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19, 455–477. doi: 10.1089/cmb.2012.0021
- Baruah, J., Nath, B. K., Sharma, R., Kumar, S., Deka, R. C., Baruah, D. C., et al. (2018). Recent trends in the pretreatment of lignocellulosic biomass for value-added products. *Front. Energy Res.* 6, 1–19. doi: 10.3389/fenrg.2018.00141
- Ben Hmad, I., and Gargouri, A. (2017). Neutral and alkaline cellulases: production, engineering, and applications. *J. Basic Microbiol.* 57, 653–658. doi: 10.1002/jobm.201700111
- Berini, F., Casciello, C., Marcone, G. L., and Marinelli, F. (2017a). Metagenomics: novel enzymes from non-culturable microbes. *FEMS Microbiol. Lett.* 364, 1–19. doi: 10.1093/femsle/fnx211
- Berini, F., Presti, I., Beltrametti, F., Pedroli, M., Vårum, K. M., Pollegioni, L., et al. (2017b). Production and characterization of a novel antifungal chitinase identified by functional screening of a suppressive-soil metagenome. *Microb. Cell Factories* 16:16. doi: 10.1186/s12934-017-0634-8
- Berlemont, R., and Martiny, A. C. (2015). Genomic potential for polysaccharide deconstruction in bacteria. *Appl. Environ. Microbiol.* 81, 1513–1519. doi: 10.1128/AEM.03718-14
- Bredon, M., Dittmer, J., Noël, C., Moumen, B., and Bouchon, D. (2018). Lignocellulose degradation at the holobiont level: teamwork in a keystone soil invertebrate 06 biological sciences 0605 microbiology. *Microbiome* 6, 1–19. doi: 10.1186/s40168-018-0536-y
- Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., et al. (2015). RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci. Rep.* 5, 1–6. doi: 10.1038/srep08365
- Cabrera, M. Á., and Blamey, J. M. (2018). Biotechnological applications of archaeal enzymes from extreme environments. *Biol. Res.* 51:37. doi: 10.1186/s40659-018-0186-3
- Cantarel, B. I., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., and Henrissat, B. (2009). The carbohydrate-active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* 37, D233–D238. doi: 10.1093/nar/gkn663
- Chakraborty, S., Khopade, A., Biao, R., Jian, W., Liu, X. Y., Mahadik, K., et al. (2011). Characterization and stability studies on surfactant, detergent and oxidant stable α -amylase from marine haloalkaliphilic *Saccharopolyspora* sp. A9. *J. Mol. Catal. B Enzym.* 68, 52–58. doi: 10.1016/j.molcatb.2010.09.009
- Collins, T., Gerday, C., and Feller, G. (2005). Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiol. Rev.* 29, 3–23. doi: 10.1016/j.femsre.2004.06.005
- Coughlan, L. M., Cotter, P. D., Hill, C., and Alvarez-Ordóñez, A. (2015). Biotechnological applications of functional metagenomics in the food and pharmaceutical industries. *Front. Microbiol.* 6:672. doi: 10.3389/fmicb.2015.00672
- Culligan, E. P., Sleator, R. D., Marchesi, J. R., and Hill, C. (2014). Metagenomics and novel gene discovery: promise and potential for novel therapeutics. *Virulence* 5, 399–412. doi: 10.4161/viru.27208
- Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., et al. (2021). Twelve years of SAMtools and BCFtools. *Gigascience* 10, 1–4. doi: 10.1093/gigascience/giab008
- DasSarma, S., and DasSarma, P. (2015). Halophiles and their enzymes: negativity put to good use. *Curr. Opin. Microbiol.* 25, 120–126. doi: 10.1016/j.mib.2015.05.009

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2022.1059061/full#supplementary-material>

- Dimitriu, P. A., Pinkart, H. C., Peyton, B. M., and Mormile, M. R. (2008). Spatial and temporal patterns in the microbial diversity of a meromictic soda Lake in Washington state †. *Appl. Environ. Microbiol.* 74, 4877–4888. doi: 10.1128/AEM.00455-08
- Eberhart, B., Cross, D. F., and Chase, L. R. (1964). β -Glucosidase system of *Neurospora crassa*. I. BETA-Glucosidase and cellulase activities of mutant and wild-type strains. *J. Bacteriol.* 87, 761–770. doi: 10.1128/jb.87.4.761-770.1964
- Ellilä, S., Bromann, P., Nyyssönen, M., Itävaara, M., Koivula, A., Paulin, L., et al. (2019). Cloning of novel bacterial xylanases from lignocellulose-enriched compost metagenomic libraries. *AMB Express* 9:124. doi: 10.1186/s13568-019-0847-9
- Ewels, P., Magnusson, M., Lundin, S., and Käller, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 32, 3047–3048. doi: 10.1093/bioinformatics/btw354
- Faris, G. (2017). Threats, use and management interventions for restoration of Lake Chitu west Arsi. *Ethiopia. Am. J. Biol. Environ. Stat.* 3:1. doi: 10.11648/j.ajbes.20170301.11
- Ferrer, M., Ghazi, A., Beloqui, A., Vieites, J. M., López-Cortés, N., Marin-Navarro, J., et al. (2012). Functional metagenomics unveils a multifunctional glycosyl hydrolase from the family 43 catalysing the breakdown of plant polymers in the calf rumen. *PLoS One* 7:e38134. doi: 10.1371/journal.pone.0038134
- Fongaro, G., Maia, G. A., Rogovski, P., Cadamuro, R. D., Lopes, J. C., Moreira, R. S., et al. (2020). Extremophile microbial communities and enzymes for multifunctional application based on multi-Omics tools. *Curr. Genomics* 21, 240–252. doi: 10.2174/1389202921999200601144137
- Garg, R., Srivastava, R., Brahma, V., Verma, L., Karthikeyan, S., and Sahni, G. (2016). Biochemical and structural characterization of a novel halotolerant cellulase from soil metagenome. *Sci. Rep.* 6, 1–15. doi: 10.1038/srep39634
- Gessesse, A., and Mamo, G. (1998). Purification and characterization of an alkaline xylanase from alkaliphilic micrococcus sp AR-135. *J. Ind. Microbiol. Biotechnol.* 20, 210–214. doi: 10.1038/sj.jim.2900503
- Grant, W. D., and Jones, B. E. (2016). “Bacteria, Archaea and viruses of Soda Lakes,” in *Soda Lakes of East Africa*. ed. Schagerl, M. (Cham: Springer International Publishing), 97–147. doi: 10.1007/978-3-319-28622-8_5
- Grant, W. D., and Sorokin, D. Y. (2011). “Distribution and diversity of soda Lake Alkaliphiles,” in *Extremophiles Handbook* ed. Horikoshi, K. (Tokyo: Springer)
- Horn, S. J., Vaaje-Kolstad, G., Westereng, B., and Eijsink, V. G. H. (2012). Novel enzymes for the degradation of cellulose. *Biotechnol. Biofuels* 5, 1–12. doi: 10.1186/1754-6834-5-45
- Houfani, A. A., Anders, N., Loogen, J., Heyman, B., Azzouz, Z., Bettache, A., et al. (2021). Lignocellulosic biomass degradation enzymes and characterization of cellulase and xylanase from *Bosea* sp. FBZP-16. *Biomass. Convers. Biorefinery*. 11, 1–19. doi: 10.1007/s13399-021-02044-1
- Humayoun, S. B., Bano, N., and Hollibaugh, J. T. (2003). Depth distribution of microbial diversity in mono Lake, a Meromictic soda Lake in California. *Appl. Environ. Microbiol.* 69, 1030–1042. doi: 10.1128/AEM.69.2.1030-1042.2003
- Jeilu, O., Gessesse, A., Simachew, A., Johansson, E., and Alexandersson, E. (2022). Prokaryotic and eukaryotic microbial diversity from three soda lakes in the east African Rift Valley determined by amplicon metagenomics. *Front. Microbiol.* 13, 1–14. doi: 10.3389/fmicb.2022.999876
- Jones, B. E., and Grant, W. D. (1999). “Microbial diversity and ecology of the Soda Lakes of East Africa,” in *Proceedings of the 8th International Symposium on Microbial Ecology*.
- Jönsson, L. J., and Martín, C. (2016). Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol.* 199, 103–112. doi: 10.1016/j.biortech.2015.10.009
- Kabeyi, M. J. B., and Olanrewaju, O. A. (2022). Sustainable energy transition for renewable and low carbon grid electricity generation and supply. *Front. Energy Res.* 9, 1–45. doi: 10.3389/fenrg.2021.743114
- Kern, M., McGeehan, J. E., Streeter, S. D., Martin, R. N. A., Besser, K., Elias, L., et al. (2013). Structural characterization of a unique marine animal family 7 cellobiohydrolase suggests a mechanism of cellulase salt tolerance. *Proc. Natl. Acad. Sci. U. S. A.* 110, 10189–10194. doi: 10.1073/pnas.1301502110
- Khatri, B. P., Bhattarai, T., Shrestha, S., and Maharjan, J. (2015). Alkaline thermostable pectinase enzyme from *Aspergillus niger* strain MCAS2 isolated from Manaslu conservation area, Gorkha, Nepal. *Springerplus* 4, 1–8. doi: 10.1186/s40064-015-1286-y
- Lakhundi, S., Siddiqui, R., and Khan, N. A. (2015). Cellulose degradation: A therapeutic strategy in the improved treatment of *Acanthamoeba* infections. *Parasit. Vectors* 8:23. doi: 10.1186/s13071-015-0642-7
- Lam, K. N., Cheng, J., Engel, K., Neufeld, J. D., and Charles, T. C. (2015). Current and future resources for functional metagenomics. *Front. Microbiol.* 6, 1–8. doi: 10.3389/fmicb.2015.01196
- Langmead, B., and Salzberg, S. (2013). Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9, 357–359. doi: 10.1038/nmeth.1923.
- Lanzén, A., Simachew, A., Gessesse, A., Chmolewska, D., Jonassen, I., and Øvreås, L. (2013). Surprising prokaryotic and eukaryotic diversity, community structure and biogeography of Ethiopian soda lakes. *PLoS One* 8:e72577. doi: 10.1371/journal.pone.0072577
- Liew, K. J., Liang, C. H., Lau, Y. T., Yaakop, A. S., Chan, K. G., Shahar, S., et al. (2022). Thermophiles and carbohydrate-active enzymes (CAZymes) in biofilm microbial consortia that decompose lignocellulosic plant litters at high temperatures. *Sci. Rep.* 12:2850. doi: 10.1038/s41598-022-06943-9
- Limsakul, P., Phitsuan, P., Waeonukul, R., Pason, P., Tachaapaikoon, C., Poomputsa, K., et al. (2021). A novel multifunctional arabinofuranosidase/endoxylanase/ β -xylosidase gh43 enzyme from *paenibacillus curdianolyticus* b-6 and its synergistic action to produce arabinose and xylose from cereal arabinoxylan. *Appl. Environ. Microbiol.* 87:e0173021. doi: 10.1128/AEM.01730-21
- Liu, Z., Zhao, C., Deng, Y., Huang, Y., and Liu, B. (2015). Characterization of a thermostable recombinant β -galactosidase from a thermophilic anaerobic bacterial consortium YTY-70. *Biotechnol. Biotechnol. Equip.* 29, 547–554. doi: 10.1080/13102818.2015.1015244
- López-Mondéjar, R., Tláškal, V., Větrovský, T., Šturová, M., Toscan, R., Nunes da Rocha, U., et al. (2020). Metagenomics and stable isotope probing reveal the complementary contribution of fungal and bacterial communities in the recycling of dead biomass in forest soil. *Soil Biol. Biochem.* 148:107875. doi: 10.1016/j.soilbio.2020.107875
- Lu, Y., He, Q., Fan, G., Cheng, Q., and Song, G. (2021). Extraction and modification of hemicellulose from lignocellulosic biomass: a review. *Green Process. Synth.* 10, 779–804. doi: 10.1515/gps-2021-0065
- Mamo, G. (2020). “Alkaline active Hemicellulases,” in *Advances in Biochemical Engineering/Biotechnology* in, eds. G. Mamo and B. Mattiasson (Cham: Springer International Publishing), 245–291. doi: 10.1007/10_2019_101
- Martin, M., and Vandenbol, M. (2016). The hunt for original microbial enzymes: an initiatory review on the construction and functional screening of (meta)genomic libraries. *BASE*, 20, 523–532. doi: 10.25518/1780-4507.13201
- Martins, R. F., Davids, W., Abu Al-Soud, W., Levander, F., Rådström, P., and Hatti-Kaul, R. (2001). Starch-hydrolyzing bacteria from Ethiopian soda lakes. *Extremophiles* 5, 135–144. doi: 10.1007/s007920100183
- Maruthamuthu, M., Jiménez, D. J., Stevens, P., and van Elsas, J. D. (2016). A multi-substrate approach for functional metagenomics-based screening for (hemi)cellulases in two wheat straw-degrading microbial consortia unveils novel thermoalkaliphilic enzymes. *BMC Genom.* 17:86. doi: 10.1186/s12864-016-2404-0
- Masasa, M., Kushmaro, A., Chernova, H., Shashar, N., and Guttman, L. (2022). Carbohydrate-Active Enzymes of a Novel Halotolerant Alkalihalobacillus Species for Hydrolysis of Starch and Other Algal Polysaccharides. *Microbiol. Spectr.* 10:e0107822. doi: 10.1128/spectrum.01078-22
- Miller, G. L. (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31, 426–428. doi: 10.1021/ac60147a030
- Minig, M., Walker, D., Ledesma, P., Martínez, M. A., and Breccia, J. D. (2009). Bacterial isolates from ethiopian soda lake producers of alkaline-active β -glucanases resistant to chelating and surfactant compounds. *Res. J. Microbiol.* 4, 194–201. doi: 10.3923/jm.2009.194.201
- Mohammadi, S., Tarrahimofrad, H., Arjmand, S., Zamani, J., Haghbeen, K., and Aminzadeh, S. (2022). Expression, characterization, and activity optimization of a novel cellulase from the thermophilic bacteria *Cohnella* sp. A01. *Sci Rep* 12:10301. doi: 10.1038/s41598-022-14651-7
- Möllers, K. B., Cannella, D., Jørgensen, H., and Frigaard, N. U. (2014). Cyanobacterial biomass as carbohydrate and nutrient feedstock for bioethanol production by yeast fermentation. *Biotechnol. Biofuels* 7, 1–11. doi: 10.1186/1754-6834-7-64
- Montella, S., Ventorino, V., Lombard, V., Henrissat, B., Pepe, O., and Faraco, V. (2017). Discovery of genes coding for carbohydrate-active enzyme by metagenomic analysis of lignocellulosic biomasses. *Sci. Rep.* 7, 1–15. doi: 10.1038/srep42623
- Moshfegh, M., Shahverdi, A. R., Zarrini, G., and Faramarzi, M. A. (2013). Biochemical characterization of an extracellular polyextremophilic α -amylase from the halophilic archaeon *Halorubrum xinjiangense*. *Extremophiles* 17, 677–687. doi: 10.1007/s00792-013-0551-7
- Ngara, T. R., and Zhang, H. (2018). Recent advances in function-based metagenomic screening. *Genom. Proteom. Bioinform.* 16, 405–415. doi: 10.1016/j.gpb.2018.01.002
- Nguyen, N. H., Maruset, L., Uengwetwanit, T., Mhuantong, W., Harnpicharnchai, P., Champreda, V., et al. (2012). Identification and characterization of a cellulase-encoding gene from the buffalo rumen metagenomic library. *Biosci. Biotechnol. Biochem.* 76, 1075–1084. doi: 10.1271/bbb.110786
- Ondov, B. D., Bergman, N. H., and Phillip, A. M. (2011). Interactive metagenomic visualization in a web browser. *BMC Bioinform.* 12, 1–9. doi: 10.1186/1471-2105-12-385
- Oriez, V., Peydecastaing, J., and Pontalier, P. Y. (2019). Lignocellulosic biomass fractionation by mineral acids and resulting extract purification processes: conditions, yields, and purities. *Molecules* 24, 1–21. doi: 10.3390/molecules24234273

- Oumer, O. J., and Abate, D. (2017). Characterization of pectinase from *Bacillus subtilis* strain Btk 27 and its potential application in removal of mucilage from coffee beans. *Enzyme Res.* 2017, 1–7. doi: 10.1155/2017/7686904
- Övreås, L., Daae, F. L., Torsvik, V., and Rodríguez-Valera, F. (2003). Characterization of microbial diversity in hypersaline environments by melting profiles and reassociation kinetics in combination with terminal restriction fragment length polymorphism (T-RFLP). *Microb. Ecol.* 46, 291–301. doi: 10.1007/s00248-003-3006-3
- Pabbathi, N. P. P., Velidandi, A., Tavarna, T., Gupta, S., Raj, R. S., Gandam, P. K., et al. (2021). Role of metagenomics in prospecting novel endoglucanases, accentuating functional metagenomics approach in second-generation biofuel production: a review. *Biomass Convers. Biorefinery.*, 11, 1–28. doi: 10.1007/s13399-020-01186-y
- Patel, S., and Saraf, M. (2015). "Perspectives and application of halophilic enzymes," in *Halophiles: Biodiversity and Sustainable Exploitation*. vol 6. Springer, Cham. doi: 10.1007/978-3-319-14595-2_15
- Peng, X., Qiao, W., Mi, S., Jia, X., Su, H., and Han, Y. (2015). Characterization of hemicellulase and cellulase from the extremely thermophilic bacterium *Caldicellulosiruptor owensensis* and their potential application for bioconversion of lignocellulosic biomass without pretreatment. *Biotechnol. Biofuels* 8:131. doi: 10.1186/s13068-015-0313-0
- Privé, F., Newbold, C. J., Kaderbhai, N. N., Girdwood, S. G., Golyshina, O. V., Golyshin, P. N., et al. (2015). Isolation and characterization of novel lipases/esterases from a bovine rumen metagenome. *Appl. Microbiol. Biotechnol.* 99, 5475–5485. doi: 10.1007/s00253-014-6355-6
- Rastogi, M., and Shrivastava, S. (2020). "Glycosyl hydrolases and biofuel" in *Industrial Applications of Glycoside Hydrolases* eds. Shrivastava, S. (Singapore: Springer) (Singapore: Springer). doi: 10.1007/978-981-15-4767-6_6
- Reichart, N. J., Bowers, R. M., Woyke, T., and Hatzenpichler, R. (2021). High potential for biomass-degrading enzymes revealed by hot spring Metagenomics. *Front. Microbiol.* 12, 1–12. doi: 10.3389/fmicb.2021.668238
- Salam, L. B. (2018). Detection of carbohydrate-active enzymes and genes in a spent engine oil-perturbed agricultural soil. *Bull. Natl. Res. Cent.* 42, 1–18. doi: 10.1186/s42269-018-0013-6
- Schagerl, M. (2016). *Soda Lakes of East Africa*. New York, NY: Springer International Publishing. doi: 10.1007/978-3-319-28622-8
- Sharma, S., Khan, F. G., and Qazi, G. N. (2010). Molecular cloning and characterization of amylase from soil metagenomic library derived from northwestern Himalayas. *Appl. Microbiol. Biotechnol.* 86, 1821–1828. doi: 10.1007/s00253-009-2404-y
- Shen, J., Zheng, L., Chen, X., Han, X., Cao, Y., and Yao, J. (2020). Metagenomic analyses of microbial and carbohydrate-active enzymes in the rumen of dairy goats fed different rumen degradable starch. *Front. Microbiol.* 11. doi: 10.3389/fmicb.2020.01003
- Shrivastava, S. (2020). "Introduction to glycoside hydrolases: Classification, identification and occurrence" in *Industrial Applications of Glycoside Hydrolases* (Singapore: Springer). doi: 10.1007/978-981-15-4767-6_1
- Shuddhodana, S., Gupta, M. N., and Bisaria, V. S. (2018). Stable cellulolytic enzymes and their application in hydrolysis of Lignocellulosic biomass. *Biotechnol. J.* 13, 1–10. doi: 10.1002/biot.201700633
- Simon, C., and Daniel, R. (2011). Metagenomic analyses: past and future trends. *Appl. Environ. Microbiol.* 77, 1153–1161. doi: 10.1128/AEM.02345-10
- Sorokin, D. Y., Banci, H. L., and Muyzer, G. (2015). Functional microbiology of soda lakes. *Curr. Opin. Microbiol.* 25, 88–96. doi: 10.1016/j.mib.2015.05.004
- Sorokin, D. Y., Berben, T., Melton, E. D., Overmars, L., Vavourakis, C. D., and Muyzer, G. (2014). Microbial diversity and biogeochemical cycling in soda lakes. *Extremophiles* 18, 791–809. doi: 10.1007/s00792-014-0670-9
- Sorokin, D. Y., and Kuenen, J. G. (2005). Chemolithotrophic haloalkaliphiles from soda lakes. *FEMS Microbiol. Ecol.* 52, 287–295. doi: 10.1016/j.femsec.2005.02.012
- Stewart, R. D., Auffret, M. D., Warr, A., Wisner, A. H., Press, M. O., Langford, K. W., et al. (2018). Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen. *Nat. Commun.* 9:870. doi: 10.1038/s41467-018-03317-6
- Sun, S. F., Yang, J., Wang, D. W., Yang, H. Y., Sun, S. N., and Shi, Z. J. (2021). Enzymatic response of ryegrass cellulose and hemicellulose valorization introduced by sequential alkaline extractions. *Biotechnol. Biofuels* 14:72. doi: 10.1186/s13068-021-01921-1
- Sweeney, M. D., and Xu, F. (2012). Biomass converting enzymes as industrial biocatalysts for fuels and chemicals: recent developments. *Catalysts* 2, 244–263. doi: 10.3390/catal2020244
- Sysoev, M., Grötzing, S. W., Renn, D., Eppinger, J., Rueping, M., and Karan, R. (2021). Bioprospecting of novel Extremozymes from prokaryotes—the advent of culture-independent methods. *Front. Microbiol.* 12, 1–16. doi: 10.3389/fmicb.2021.630013
- Tahrioui, A., Schwab, M., Quesada, E., and Llamas, I. (2013). Quorum sensing in some representative species of Halomonadaceae. *Life* 3, 260–275. doi: 10.3390/life3010260
- Teather, R. M., and Wood, P. J. (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl. Environ. Microbiol.* 43, 777–780. doi: 10.1128/aem.43.4.777-780.1982
- Thakur, P., Singh, A. K., and Mukherjee, G. (2021). Isolation and characterization of alkaline pectinase productive *Bacillus tropicus* from fruit and vegetable waste dump soil. *Brazilian Arch. Biol. Technol.* 64, 1–14. doi: 10.1590/1678-4324-2021200319
- Tiwari, R., Kumar, K., Singh, S., Nain, L., and Shukla, P. (2016). Molecular detection and environment-specific diversity of glycosyl hydrolase family 1 β -glucosidase in different habitats. *Front. Microbiol.* 7, 1–12. doi: 10.3389/fmicb.2016.01597
- Tiwari, R., Singh, P. K., Singh, S., Nain, P. K. S., Nain, L., and Shukla, P. (2017). Bioprospecting of novel thermostable β -glucosidase from *Bacillus subtilis* RA10 and its application in biomass hydrolysis. *Biotechnol. Biofuels* 10:246. doi: 10.1186/s13068-017-0932-8
- Verma, D. (2021). Extremophilic Prokaryotic Endoxylanases: Diversity, Applicability, and Molecular Insights. *Front. Microbiol.* 12, 1–21. doi: 10.3389/fmicb.2021.728475
- Verma, D., and Satyanarayana, T. (2011). An improved protocol for DNA extraction from alkaline soil and sediment samples for constructing metagenomic libraries. *Appl. Biochem. Biotechnol.* 165, 454–464. doi: 10.1007/s12010-011-9264-5
- Verma, D., and Satyanarayana, T. (2020). Xylanolytic Extremozymes Retrieved From Environmental Metagenomes: Characteristics, Genetic Engineering, and Applications. *Front. Microbiol.* 11, 1–18. doi: 10.3389/fmicb.2020.551109
- Vester, J. K., Glaring, M. A., and Stougaard, P. (2015). An exceptionally cold-adapted alpha-amylase from a metagenomic library of a cold and alkaline environment. *Appl. Microbiol. Biotechnol.* 99, 717–727. doi: 10.1007/s00253-014-5931-0
- Voget, S., Steele, H. L., and Streit, W. R. (2006). Characterization of a metagenome-derived halotolerant cellulase. *J. Biotechnol.* 126, 26–36. doi: 10.1016/j.jbiotec.2006.02.011
- Wang, L., Hatem, A., Catalyurek, U. V., Morrison, M., and Yu, Z. (2013). Metagenomic insights into the carbohydrate-active enzymes carried by the microorganisms adhering to solid digesta in the rumen of cows. *PLoS One* 8, 1–11. doi: 10.1371/journal.pone.0078507
- Wang, M., Lai, G. L., Nie, Y., Geng, S., Liu, L., Zhu, B., et al. (2015). Synergistic function of four novel thermostable glycoside hydrolases from a long-term enriched thermophilic methanogenic digester. *Front. Microbiol.* 6, 1–10. doi: 10.3389/fmicb.2015.00509
- Wang, L., Zhang, G., Xu, H., Xin, H., and Zhang, Y. (2019). Metagenomic analyses of microbial and carbohydrate-active enzymes in the rumen of Holstein cows fed different forage-to-concentrate ratios. *Front. Microbiol.* 10, 1–14. doi: 10.3389/fmicb.2019.00649
- Wood, R. B., Baxter, R. M., and Prosser, M. V. (1984). Seasonal and comparative aspects of chemical stratification in some tropical crater lakes. *Ethiopia. Freshw. Biol.* 14, 551–573. doi: 10.1111/j.1365-2427.1984.tb00176.x
- Wood, D. E., Lu, J., and Langmead, B. (2019). Improved metagenomic analysis with kraken 2. *Genome Biol.* 20:257. doi: 10.1186/s13059-019-1891-0
- Xiao, C., and Anderson, C. T. (2013). Roles of pectin in biomass yield and processing for biofuels. *Front. Plant Sci.* 4, 1–7. doi: 10.3389/fpls.2013.00067
- Yu, P., Zhang, Y., and Gu, D. (2017). Production optimization of a heat-tolerant alkaline pectinase from *Bacillus subtilis* ZGL14 and its purification and characterization. *Bioengineered* 8, 613–623. doi: 10.1080/21655979.2017.1292188
- Zhang, T., Datta, S., Eichler, J., Ivanova, N., Axen, S. D., Kerfeld, C. A., et al. (2011). Identification of a haloalkaliphilic and thermostable cellulase with improved ionic liquid tolerance. *Green Chem.* 13:2083. doi: 10.1039/c1gc15193b
- Zhang, L., Fu, Q., Li, W., Wang, B., Yin, X., Liu, S., et al. (2017). Identification and characterization of a novel β -glucosidase via metagenomic analysis of *Bursaphelenchus xylophilus* and its microbial flora. *Sci. Rep.* 7:14850. doi: 10.1038/s41598-017-14073-w
- Zhao, K., Guo, L. Z., and Lu, W. D. (2012). Extracellular production of novel halotolerant, thermostable, and alkali-stable carboxymethyl cellulase by marine bacterium *Marinimicrobium* sp. LS-A18. *Appl. Biochem. Biotechnol.* 168, 550–567. doi: 10.1007/s12010-012-9796-3
- Zhao, Z., Liu, H., Wang, C., and Xu, J. R. (2014). Correction to comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi [BMC genomics 14(2013) 274]. *BMC Genom.* 15, 1–15. doi: 10.1186/1471-2164-15-6
- Zhu, D., Adebisi, W. A., Ahmad, F., Sethupathy, S., Danso, B., and Sun, J. (2020). Recent development of extremophilic bacteria and their application in biorefinery. *Front. Bioeng. Biotechnol.* 8, 1–18. doi: 10.3389/fbioe.2020.00483
- Zoghiani, A., and Paës, G. (2019). Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis. *Front. Chem.* 7, 1–11. doi: 10.3389/fchem.2019.00874