Rhizosphere-derived Glycoside Hydrolases: A Biotechnological Treasure Trove from Arid Plant Ecosystems

Khulood F. Alabbosh [a] and Rewaa S. Jalal [b,*]

Wild plant-associated rhizospheric microbiomes represent largely unexplored reservoirs of carbohydrate-active enzymes (CAZymes) that have significant biotechnological potential. This metagenomic investigation examined glycoside hydrolase (GH) family distribution within rhizospheric microbial assemblages of two native Saudi Arabian plants: *Moringa oleifera* and *Abutilon fruticosum*. High-throughput shotgun sequencing revealed pronounced plant species-specific CAZyme specialization. *Moringa oleifera* rhizospheres exhibited exclusive enrichment in five GH families (GH105, GH106, GH25, GH28, and GH38), while *A. fruticosum* supported three distinct families (GH17, GH32, and GH33). Taxonomic analysis revealed differential microbial composition: *M. oleifera* communities were dominated by Actinobacteria (*Streptomyces*, *Micromonospora*) with significant eukaryotic representation, whereas *A. fruticosum* microbiomes showed bacterial predominance, primarily Proteobacteria (*Pseudomonas*, *Bradyrhizobium*). CAZyme-encoding sequences frequently exceeded 120 per GH family, indicating extensive catalytic potential. These specialized enzymes offer multifaceted applications across pharmaceutical glycoprotein synthesis, lignocellulosic biomass degradation for biofuels, food preservation systems, and biomaterial fabrication for tissue regeneration. The rhizosphere-specific enrichment of highly specialized CAZyme consortia positions these microbial communities as scalable biocatalytic platforms, providing eco-sustainable alternatives to conventional industrial methodologies across pharmaceutical, energy, food, and environmental sectors.

DOI: 10.15376/biores.21.1.706-749

Keywords: Metagenomics; Pectin depolymerization; Carbohydrate metabolism; Plant cell wall degradation; Rhamnogalacturonan hydrolysis; Lignocellulosic biomass conversion; Oligosaccharide production; Viral and bacterial sialidase

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INTRODUCTION

The two wild plant species *Moringa oleifera* and *Abutilon fruticosum* represent phytological entities indigenous to the septentrional territories of Saudi Arabia, manifesting diverse utilitarian applications spanning numerous sectorial domains (Al-Eisawi and Al-Ruzayza (2015). *Moringa oleifera*, colloquially designated the "miracle tree" or "tree of life," has garnered extensive global recognition owing to its exceptional

nutritional and medicinal attributes, encompassing anti-inflammatory, antimicrobial, antioxidant, anticancer, antihypertensive, hepatoprotective, and cardioprotective properties mediated by bioactive phytochemicals including flavonoids (quercetin, kaempferol, rutin), phenolic acids (gallic acid, ferulic acid, caffeic acid), glucosinolates (glucomoringin), and phytosterols (β-sitosterol) (Abdull Razis *et al*. 2014; Alegbeleye 2018; Islam *et al*. 2021; Pareek *et al*. 2023). The therapeutic applications extend to treating malnutrition, diabetes, cardiovascular disorders, inflammatory conditions, bacterial and fungal infections, wound healing, and liver diseases, while its leaves, seeds, roots, bark, and flowers yield distinct bioactive compounds facilitating diverse pharmacological interventions (Abdull Razis *et al*. 2014; Milla *et al*. 2021). Conversely, *A. fruticosum*, or sweet Indian mallow, demonstrates an ecological resilience and drought tolerance, serving pivotal roles in rangeland restoration, wildlife habitat provisioning, soil stabilization, and water quality enhancement in arid environments (Al-Quwaie and Alamoudi 2022; Gouda *et al*. 2022, 2023). This xerophytic perennial shrub exhibits important medicinal properties, with various plant parts employed in traditional remedies for inflammatory conditions, ulcers, rheumatism, and bladder ailments, while simultaneously providing critical ecosystem services including erosion control, carbon sequestration, and biodiversity conservation through its capacity to support diverse avian and ungulate populations.

Glycoside hydrolases (GHs) within rhizospheric microbial communities of wild plants can play critical ecological roles in the degradation of complex plant-derived polysaccharides—including cellulose, hemicellulose (xylan, arabinoxylan), pectin (homogalacturonan, rhamnogalacturonan), chitin, and β-glucans—thereby facilitating carbon cycling, nutrient mobilization, and plant-microbe symbiotic interactions in soil ecosystems. These enzymes catalyze the hydrolysis of glycosidic bonds with remarkable substrate specificity and catalytic efficiency, enabling microorganisms to access energy-rich carbohydrates that would otherwise remain recalcitrant in terrestrial environments (Berlemont and Martiny 2016; Wardman and Withers 2024; Tanimura *et al*. 2025).

Contemporary scholarly investigations into the rhizospheric microbiome gene catalogs affiliated with the two indigenous botanical taxa posit that these microbial consortia constitute reservoirs of high potential for ameliorating multidimensional tribulations confronting agricultural productivity, pharmacological innovation, environmental restoration, and biotechnological applications (Al-Quwaie and Alamoudi 2022; Cheng *et al*. 2023). The rhizospheric microbiomes associated with these botanical entities constitute several repositories of genomic and metabolic diversification, presenting opportunities for biotechnological and industrial innovations encompassing the development of novel biocatalysts, bioactive metabolites, antimicrobial compounds, stress-tolerance mechanisms, and ecosystem restoration strategies through microbiome engineering and synthetic biology approaches (Abdull Razis *et al*. 2014; Al-Quwaie and Alamoudi 2022; Alegbeleye 2018; Cheng *et al*. 2023; Hanif *et al*. 2024; Islam *et al*. 2021; Pareek *et al*. 2023; Sarsaiya *et al*. 2025; Sarsaiya *et al*. 2024; Sultan *et al*. 2025). These microbial communities exhibit high genetic diversity. They harbor specialized enzymatic machinery for carbohydrate processing, secondary metabolite biosynthesis, nutrient solubilization, stress adaptation, and plant-microbe signaling that can be harnessed for sustainable agriculture, pharmaceutical bioprospecting, environmental remediation, and biotechnological advancement through precision microbiome manipulation and

rhizospheric engineering strategies (Bowya and Balachandar 2020; Hanif *et al*. 2024; Sarsaiya *et al*. 2024; Sultan *et al*. 2025).

Leveraging metagenomic interrogation *via* next-generation sequencing (NGS) technologies, researchers have dissected the functional architecture of the complex microbial ecosystems of the two wild plants, unearthing a reservoir of genetic determinants encapsulated within comprehensive catalogs including CAZymes (Carbohydrate-Active Enzymes) (Igiehon and Babalola 2018). These repositories elucidate the metabolic and adaptive stratagems of rhizospheric communities, thereby furnishing a scaffold for translating microbial genomic diversity into industrial scalability. These enzymes play critical roles in carbohydrate modification, degradation, and biosynthesis, mainly supporting energy metabolism, cell structure maintenance (Cardoso *et al*. 2022; Thurimella *et al*. 2023; Wardman and Withers 2024). They also exhibit catalytic versatility, positioning them as pivotal candidates for advancing lignocellulosic biofuel synthesis, precision food enzymology, and carbohydrate-based industrial biomanufacturing (Alshareef 2024; Igiehon and Babalola 2018; Sonbol and Jalal 2025; Tashkandi and Baz 2023). Prevailing investigations demonstrate that glycoside hydrolases (GHs) within the CAZyme assemblage mediate highly discriminating structural reconfiguration of glycosidic linkages across diverse polysaccharidic substrates, catalyzing pioneering advancements in both biotechnology and industrial applications (Payne *et al*. 2015; Amin *et al*. 2021; Sethupathy *et al*. 2021).

The industrial and biotechnological significance of GH enzymes is substantial and multifaceted. In biofuel and biorefinery applications, cellulases (GH families 5, 6, 7) and hemicellulases (GH families 10, 11) enhance lignocellulosic biomass saccharification, increasing fermentable sugar yields for second-generation bioethanol production by 15 to 25%. In the pharmaceutical sector, GH enzymes enable glycoprotein remodeling for therapeutic antibody engineering, facilitate drug delivery through oligosaccharide excipient production, and support the synthesis of bioactive compounds with improved pharmacological properties. Food and beverage industries utilize pectinases (GH28, GH105, GH106) for fruit juice clarification and wine processing, achieving up to 20% improvements in yield and clarity, while invertases and fructanases (GH32) produce high-value prebiotics such as fructooligosaccharides with demonstrated gut health benefits. Additionally, GH enzymes contribute to textile bioprocessing through improved fiber treatment and reduced chemical consumption, and to biomaterial development *via* production of functional oligosaccharides for tissue engineering scaffolds. The thermostability and catalytic versatility of GH enzymes from extremophilic and stress-adapted microbiomes—such as those inhabiting arid plant rhizospheres—position them as particularly promising candidates for industrial-scale applications requiring robust performance under harsh processing conditions (Contesini *et al*. 2021; Samanta 2022; Ashcroft and Munoz-Munoz 2024; Wardman and Withers 2024; Ferrarotti and Costa 2026).

While prior investigations have documented the CAZyme repertoires of rhizospheric microbiomes associated with individual plant species or have characterized specific glycoside hydrolase families in isolation, this is the first comprehensive metagenomic study to systematically compare plant-specific glycoside hydrolase specialization between two distinct arid-adapted native species—*Moringa oleifera* and *Abutilon fruticosum*—and to directly correlate the plant species-specific enrichment of

distinct GH families with their taxon-specific microbial harbors and integrated biotechnological applications across pharmaceutical, biofuel, food processing, and biomaterial development sectors. Furthermore, the present analysis uniquely integrates functional genomic profiling with quantitative substrate specificity and mechanistic enzyme characterization to establish actionable biotechnological pipelines for next-generation bioprocessing platforms. This integrated, multimodal approach represents a significant advancement in linking rhizospheric microbiome composition, enzymatic capacity, and real-world industrial application potential for sustainable biotechnology innovation (Contesini *et al.* 2021; Samanta 2022; Ashcroft and Munoz-Munoz 2024; Wardman and Withers 2024; Ferrarotti and Costa 2026).

In the present investigation, designed as a primary research article with an extended literature review, there is an effort to delineate the distinctive enzymatic repertoires characteristic of predominant CAZy families within the glycoside hydrolases (GHs) classification across the rhizospheric microbiomes associated with the indigenous botanical taxa *M. oleifera* and *A. fruticosum*. By integrating both empirical analysis and comprehensive review of current literature, the present study reveals circumscribed CAZyme profiles that manifest unique taxonomic signatures specific to each microbiome assemblage. This pronounced enzymatic divergence exemplifies the ecological specialization and niche-specific adaptations inherent to sophisticated plant-microbe mutualistic partnerships, while simultaneously illuminating the high potential of lineage-restricted GH biocatalysts for industrial, pharmaceutical, medical, and biotechnological applications. This integrative approach underscores the translational promise of these enzymes in next-generation bioproduction, bioprocessing, and therapeutic innovations.

EXPERIMENTAL

The current study utilized six distinct rhizospheric soil samples (50 g each) collected from native endemic *M. oleifera* and *A. fruticosum* plant communities strategically positioned within the northwestern sector of the Mecca biogeographical region, Kingdom of Saudi Arabia, positioned in proximate adjacency to the Red Sea littoral zone at precisely delineated geographic coordinates 21.209430/39.530866 and 21.352751/39.578932, respectively (Al-Eisawi and Al-Ruzayza 2015). Systematic sampling procedures transpired after an extended period of protracted environmental aridity exceeding three consecutive months, with carefully selected solitary botanical specimens exhibiting uniformly consistent morphotypic characteristics and phenological developmental stages to ensure sampling homogeneity and experimental reproducibility. Rhizospheric microbiomes were systematically procured through precise excision of lateral root systems at standardized pedological depths ranging from 10 to 30 cm below the soil surface, followed by meticulous collection of non-adherent rhizospheric soil fractions maintained within a precisely defined 1.0 cm radius from exposed root surfaces, while corresponding bulk soil specimens serving as environmental controls were concurrently acquired at identical pedological depths positioned 10 meters distant from the sampled phytological entities to minimize potential contamination and ensure comparative analytical validity (Abulfaraj *et al*. 2024). All collected specimens underwent immediate cryopreservation at −20 °C to maintain genomic integrity and prevent nucleic acid

degradation, with subsequent total genomic DNA isolation performed *via* established CTAB/SDS (cetyltrimethylammonium bromide/ sodium dodecyl sulfate) extraction methodology and DNA concentrations systematically normalized to 10 ng/μL per rigorously validated protocols (Tashkandi *et al*. 2022).

A precisely measured 30 μL aliquot of each purified genomic extract underwent comprehensive high-throughput whole-metagenome shotgun sequencing utilizing state-of-the-art sequencing platforms at Novogene Co. (Helios, Singapore), with all generated raw sequencing data systematically deposited in the internationally recognized European Nucleotide Archive (ENA) under specifically assigned accession codes ERR10100770–72 (rhizosphere) and ERR10100773–74/ERR10100781 (bulk soil) for *M. oleifera*, and ERS15580318–20 (bulk) and ERS15580321–23 (rhizosphere) for *A. fruticosum* to ensure data accessibility and experimental reproducibility. Stringent quality control measures systematically excluded low-quality sequencing reads containing bases with quality scores ($Q \leq 38$) exceeding 40 bp in length and sequences containing ≥10 ambiguous nucleotides (Ns) to maintain data integrity and analytical precision. Following comprehensive post-filtering quality assessment, library construction and subsequent sequencing procedures adhered to previously established and validated methodologies (Tashkandi *et al*. 2022), with all assembled genomic sequences subjected to rigorous chimeric sequence elimination utilizing computationally validated bioinformatics pipelines (Oh *et al*. 2014), and comprehensive downstream phylogenetic and functional analyses conducted according to internationally standardized analytical frameworks (Mende *et al*. 2012; Huson *et al*. 2016). Sophisticated bioinformatics workflows systematically deployed MEGAHIT v1.2.9 for comprehensive *de novo* genomic assembly (Mende *et al*. 2012), SOAP v2.21 for precise read alignment (https://GitHub.com/ShujiaHuang/SOAPaligner, 5/2024), MetaGeneMark-2 for accurate high-abundance gene prediction , CD-HIT v4.8.1 for systematic non-redundant gene clustering and sequence deduplication, and MEGAN v6 for comprehensive reference-based taxonomic binning and functional annotation (Huerta-Cepas *et al*. 2017). Detailed protein-level functional mapping and enzymatic characterization utilized the advanced DIAMOND v2.1.8 algorithm (Buchfink *et al*. 2015) against the comprehensive CAZy database (*version* 2014.11.25) to systematically identify and classify carbohydrate-active enzymes (CAZymes), with subsequent hierarchical classification into distinct CAZy classes and families based on structural and functional homology (Lombard *et al*. 2014). Moreover, the workflow facilitated the inference of microbial lineages with the latent genomic potential to encode the identified CAZyme cohorts, thereby enhancing the precision of taxon-function linkage within complex environmental consortia.

The illustrations were produced through a combination of BioRender software and manual sketching, while Perplexity was engaged exclusively as an AI-facilitated instrument to augment the research process, functioning in a complementary and supportive role.

RESULTS

CAZy Class Distribution in Plant Rhizosphere Microbiomes: Dominance of Glycoside Hydrolase Families

A comprehensive annotation of the glycoside hydrolase (GH) gene repertoire—meticulously curated from the CAZy database—across two distinct edaphic microbiome contexts, *e.g.*, bulk (S) and rhizospheric (R) soils, respectively, associated with the wild plants *M. oleifera* and *A. fruticosum* is presented in Tables S1 and S2. Each Query ID in these tables corresponds to a discrete gene or gene fragment whose nucleotide sequence exhibits homologous alignment with a specific CAZyme-encoding allele (subject ID) in the gene bank, while the Novo_MIX ID denotes an aggregated set of low-abundance, rigorously quality-filtered sequencing reads that have been consolidated, reassembled, and algorithmically mapped across all six microbiome datasets of each plant species, often appearing in multiple environmental compartments. This high-throughput metagenomic annotation pipeline leverages advanced computational assembly and mapping protocols to comprehensively profile carbohydrate-active enzymatic determinants across diverse soil niches.

The results in Fig. S1 depict the percentage distribution of gene queries encoding the glycoside hydrolase (GH) CAZy families in relation to other CAZy classes within the soil microbiomes of the wild plant species *M. oleifera* (MO) and *A. fruticosum* (AF). The analysis encompasses alternative CAZy categories, including auxiliary activities (AA), carbohydrate-binding modules (CBMs), carbohydrate esterases (CE), glycosyltransferases (GT), and polysaccharide lyases (PL). The figure expectedly demonstrates that the GH class constitutes as high as ~40% of all CAZy-encoded gene queries across both plant species, underscoring its predominance and central role in the carbohydrate-active enzymatic repertoire regardless of soil type. Detailed characterizations of each CAZy class are provided in Table S3.

Plant Species - Specific Glycoside Hydrolase Family Specialization: Abundance Patterns and Rhizosphere Enrichment

The results in Fig. 1 present a comparative matrix profiling gene query encoding the most abundant glycoside hydrolase (GH) CAZy families (threshold ≥120 gene queries) within the rhizospheric microbiomes of MO and AF, deliberately excluding less abundant GH families to focus on the dominant carbohydrate-active enzyme repertoires that drive plant-microbe interactions.

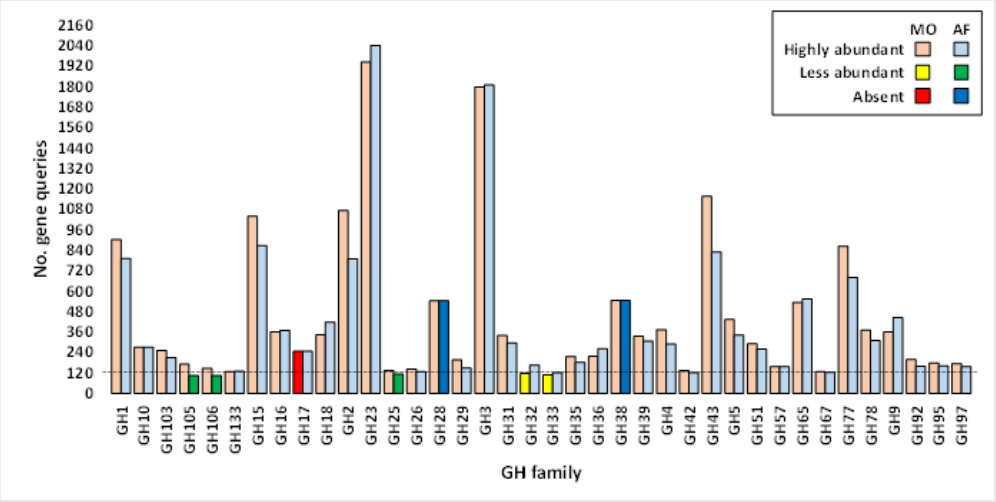

Fig. 1. Comparative profiling of gene queries encoding predominant GH-CAZy families (threshold: ≥120 gene queries) within the rhizosphere microbiomes of MO and AF. Rose and light blue boxes denote GH-CAZy families highly abundant and predominantly associated with MO and AF rhizospheres, respectively; yellow and green boxes represent less abundant families in MO and AF; while red and dark blue highlight GH families entirely absent from the MO and AF microbiomes, respectively. The term "Absent" denotes absence in one plant's microbiome relative to the other, not global absence from both systems.

The visualization in Fig. 1 distinctly reveals lineage-specific and differentially enriched CAZy family repertoires between the two plant species (MO and AF) and facilitates rapid identification of unique or shared CAZy capabilities within their respective rhizospheric communities of the two plant species, with further information cataloged in Tables S4 and S5, respectively. The visualization employs a strategic color-coding system where rose-colored boxes denote GH-CAZy families that are highly abundant and predominantly associated with MO microbiome, while light blue boxes highlight families that are highly enriched within AF microbiome, thereby revealing plant species-specific enzymatic preferences and metabolic specialization.

Yellow and green boxes represent GH families of less abundance in MO and AF, respectively, indicating shared but less dominant carbohydrate-processing capabilities, while red boxes signify GH families that are entirely absent from MO microbiomes and dark blue boxes represent families completely absent from AF microbiomes, thus delineating exclusive enzymatic niches. Among the selected highly abundant families, the analysis specifically targets those that exist exclusively in one of the two plant species' microbiomes to elucidate the differential biochemical interactions and metabolic dependencies between each plant and its associated soil microbial consortium, providing insights into how plant-specific root exudates, secondary metabolites, and rhizodeposition patterns selectively enrich distinct carbohydrate-degrading microbial populations. Further supplementary details are cataloged in Tables S4 and S5.

Across both species MO and AF, gene abundances for these GH families are consistently elevated in rhizospheric soils compared to bulk soils, underscoring enhanced microbial activity and carbohydrate metabolism in root-associated niches. This analysis elucidates plant species-specific and niche-specific enrichment patterns among dominant

GH family repertoires within the respective soil microbial communities. Further details for these GH families for both soil types are cataloged in Tables S6 and S7, respectively.

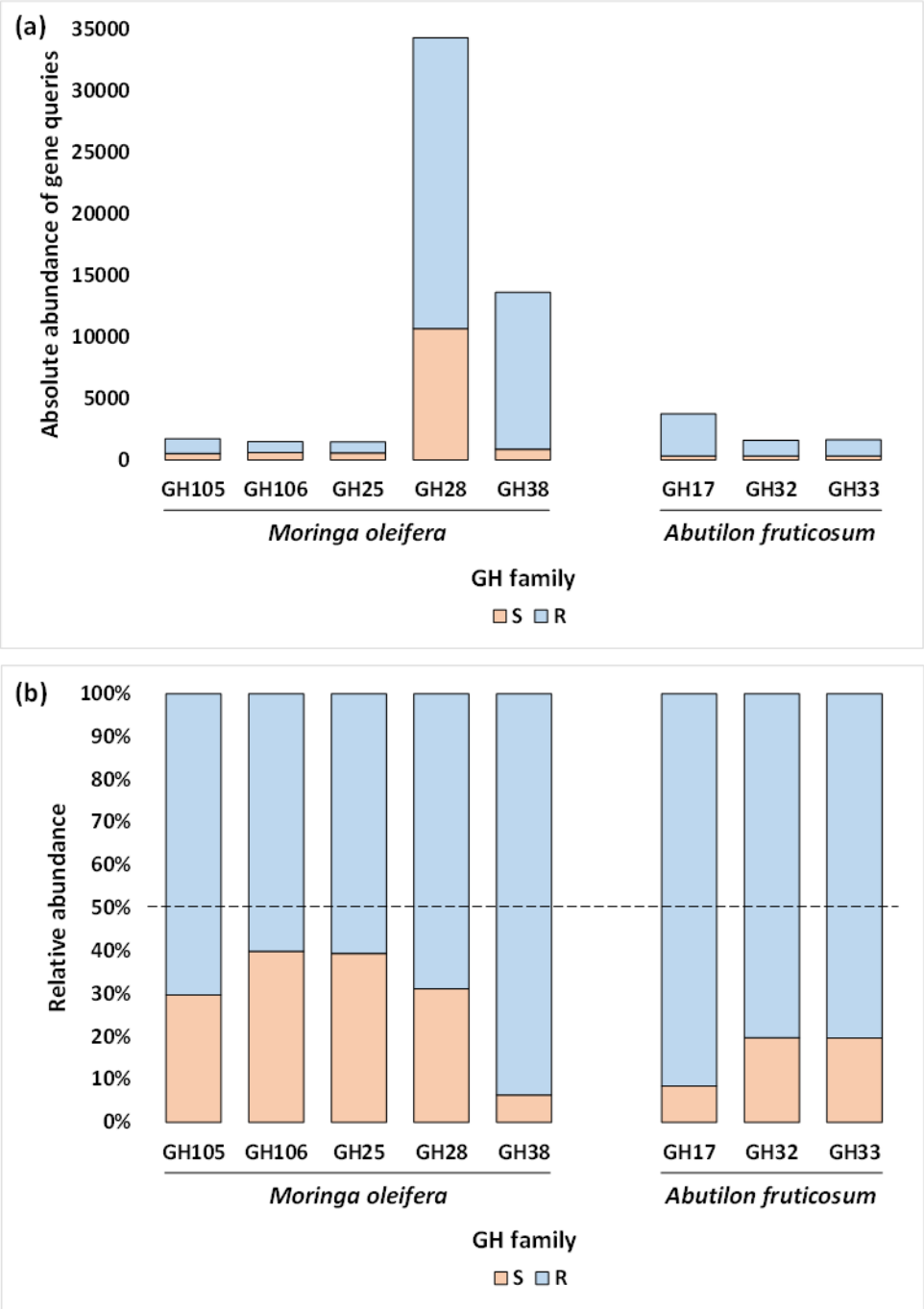

Fig. 2. Absolute (a) and relative abundance (b) of genes encoding dominant GH-CAZy family members (threshold: ≥120 gene queries) uniquely associated with the soil microbiomes of MO or AF. GH families GH105, GH106, GH25, GH28, and GH38 are predominantly linked to MO, whereas GH17, GH32, and GH33 are principally associated with AF microbiomes.

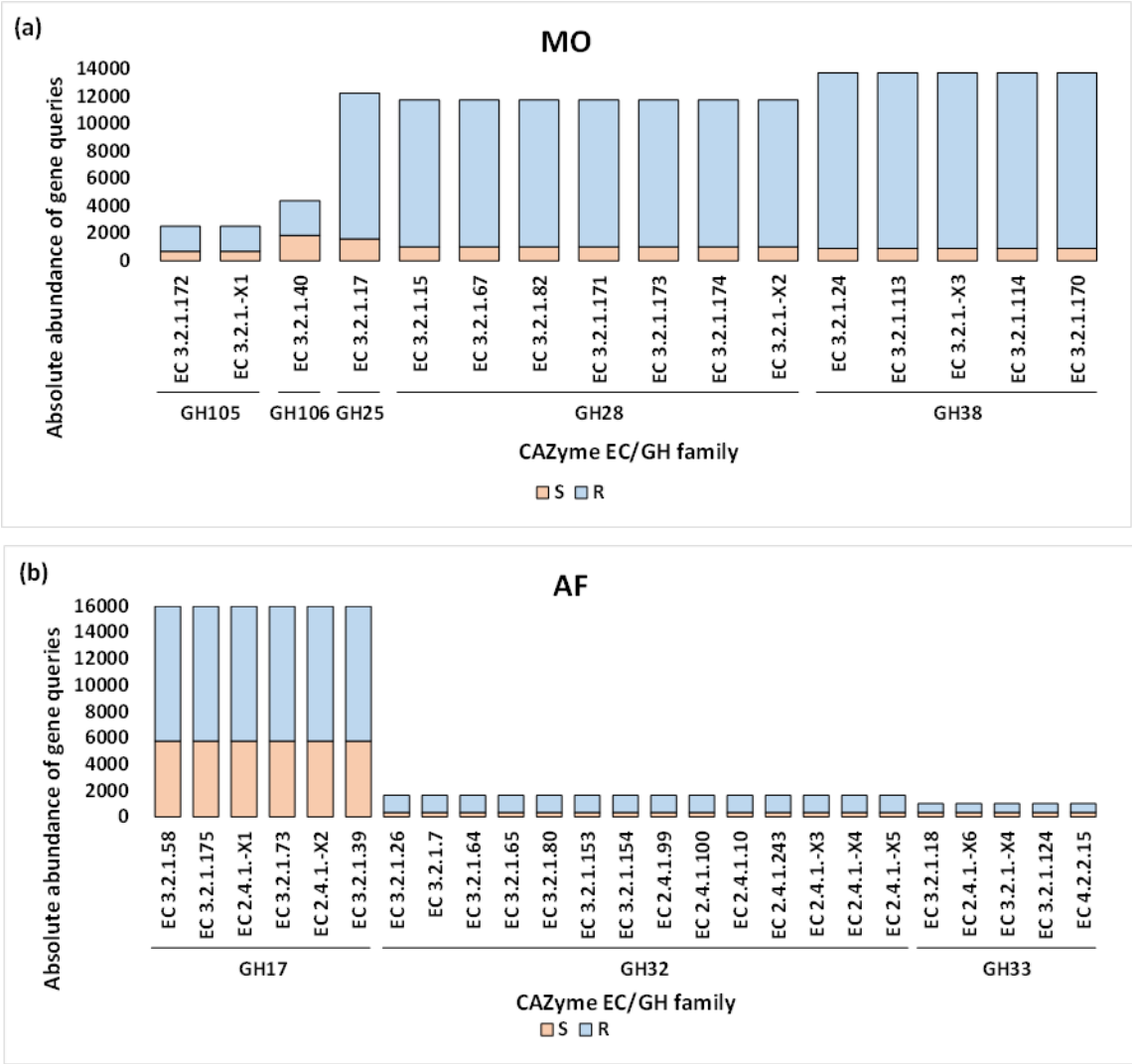

Fig. 3. Absolute abundance of gene queries encoding CAZymes from dominant GH families (threshold: ≥120 gene queries) identified as exclusively prevalent within the soil microbiomes of MO (panel a) and AF (panel b). GH families GH105, GH106, GH25, GH28, and GH38 exhibit pronounced enrichment in MO, while GH17, GH32, and GH33 are distinctly affiliated with AF

Comprehensive Abundance Profiling of Plant-Exclusive CAZyme-Encoding Genes from Dominant GH Families

The results in Figs. S2, 3, and 4 and Tables S6-S11 collectively present a comprehensive analysis of gene queries encoding CAZymes from the highly abundant glycoside hydrolase (GH) families that are exclusively associated with each plant species, examining both absolute and relative abundance patterns of these specialized carbohydrate-active enzymes. Figure S2 displays the number of gene queries encoding CAZymes from the dominant GH families (threshold ≥120 gene queries) that are exclusively present in the soil microbiomes of MO and AF, specifically highlighting the five MO-exclusive families (GH105, GH106, GH25, GH28, and GH38) and the three AF-exclusive families (GH17, GH32, and GH33).

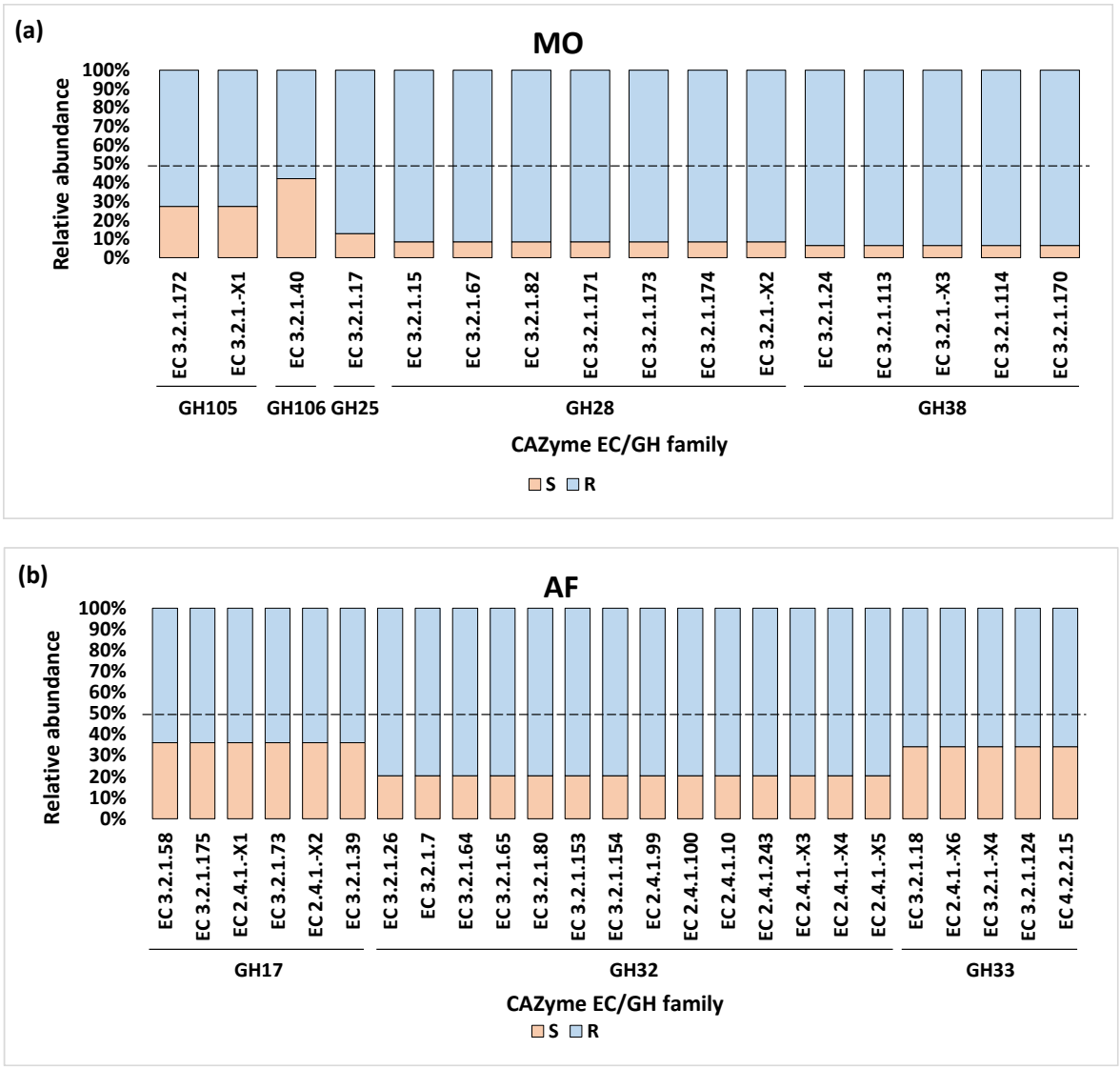

Fig. 4. Relative abundance of genes encoding CAZymes from dominant GH families—defined as those surpassing the threshold of 120 gene queries—that are exclusively prevalent within the soil microbiomes of MO and AF. Specifically, families GH105, GH106, GH25, GH28, and GH38 demonstrate pronounced enrichment in MO, whereas GH17, GH32, and GH33 are uniquely associated with AF.

Figure 3 extends this analysis by presenting the absolute abundance profiles of genes encoding CAZymes from these same exclusive GH families, demonstrating the pronounced enrichment of the specific families in MO microbiomes and AF-specific families in soil environments, with consistently elevated gene abundances observed in rhizospheric soils compared to bulk soils for both plant species. Figure 4 complements these findings by illustrating the relative abundance of genes encoding CAZymes from these dominant, plant-exclusive GH families, providing proportional perspectives that reveal the specialized carbohydrate-active enzyme repertoires characteristic of each plant species' associated microbiome, thereby elucidating the distinct metabolic capabilities and plant-microbe interaction patterns that define the functional specialization of rhizospheric microbial communities in these two wild plant ecosystems. Absolute abundance of genes

encoding CAZymes from the specified GH families across both wild plant taxa is comprehensively consolidated in Table S12.

The gene abundance profiles for these families reveal significant elevation within rhizospheric (R) *versus* bulk soil (S) microbiomes for both species reflecting intensified microbial metabolic dynamics in the root-associated niche. Exhaustive distributional data for all GH-CAZy families across disparate soil compartments of the two plant species are catalogued in Tables S10 and S11, respectively. This analysis delineates the plant species-specific specialization of carbohydrate-active enzyme repertoires, and comprehensive distributions across soil compartments of the two plant species are detailed in Tables S10 and S11, respectively.

Taxonomic Hierarchy Analysis of Plant-Exclusive GH-CAZyme Distribution: From Kingdom to Species-Level Microbial Community Specialization

Figures S3, S4, 5, and 6 and Tables S13 and S14 collectively present a comprehensive taxonomic hierarchy analysis examining the number of gene queries encoding CAZymes from the selected GH-families across kingdom, phylum, and genus/species levels in the soil microbiomes of both *Moringa oleifera* and *Abutilon fruticosum*. Figure S3 displays the gene query number at the kingdom taxonomic level, demonstrating the predominance of bacterial kingdoms in harboring these specialized GH CAZy families, with bacterial representatives exhibiting relatively uniform gene query abundances across the plant-exclusive GH families associated with MO (GH105, GH106, GH25, GH28, and GH38), whereas eukaryotic microorganisms display elevated gene query numbers for the AF-associated families (GH17, GH32, and GH33). Figure S4 provides a more refined phylum-level analysis, demonstrating that Actinobacteria, Proteobacteria, and Bacteroidetes represent the predominant bacterial phyla harboring these exclusive GH CAZymes, with Actinobacteria particularly enriched in MO-microbiomes and Proteobacteria showing substantial representation in AF-environments, while the eukaryotic phylum Streptophyta contributes notably to certain GH families, reflecting plant-associated microorganisms within the soil matrix. Figures 5 and 6 reveal striking plant species-specific microbial assemblages harboring unique glycoside hydrolase (GH) CAZy families, with MO and AF each selecting for distinct carbohydrate-degrading communities. MO-associated microbiomes are dominated by five exclusive GH families (GH105, GH106, GH25, GH28, GH38) distributed across 17 microbial taxa, while AF microbiomes harbor three unique GH families (GH17, GH32, GH33) represented by 13 taxa.

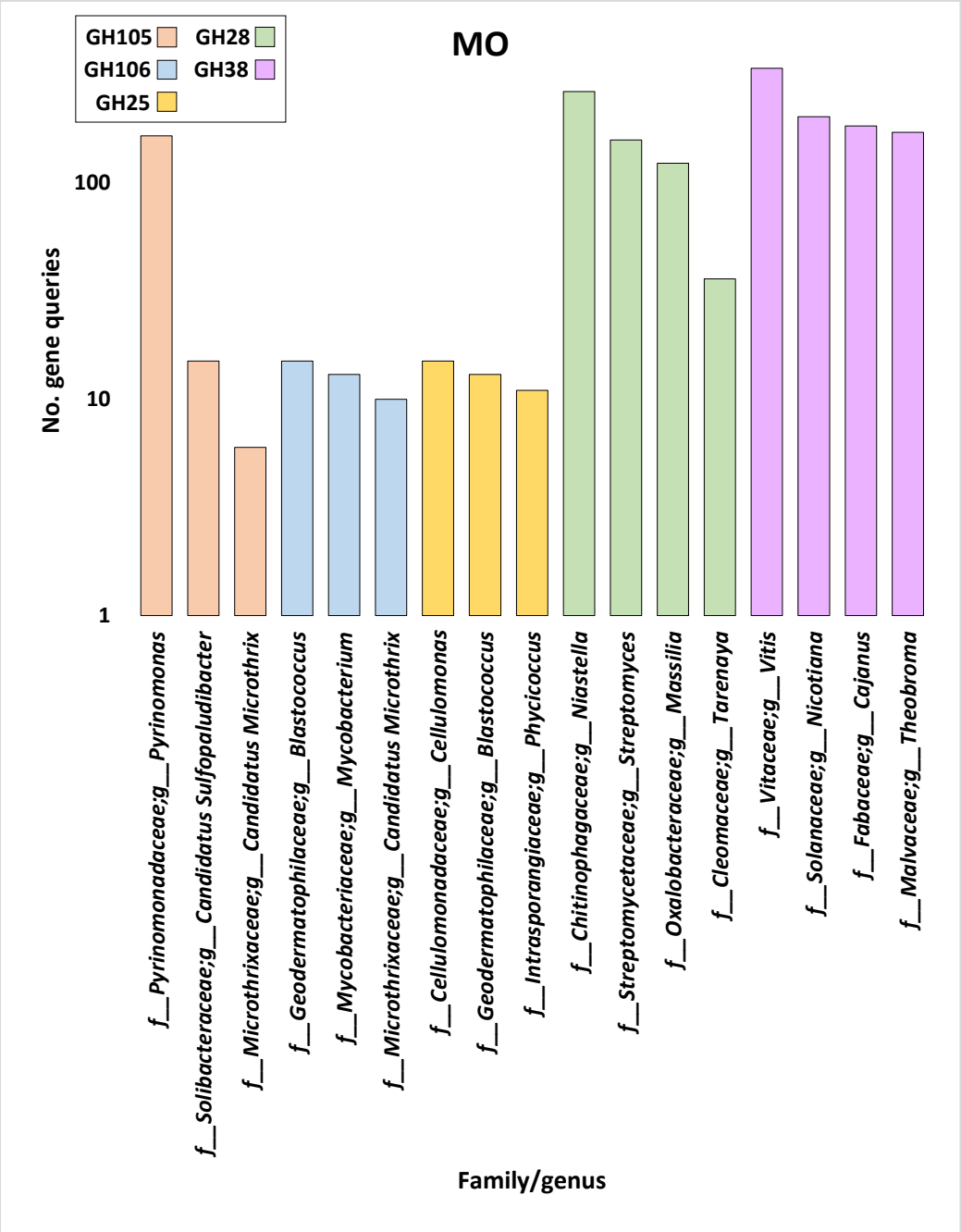

Fig. 5. Number of gene queries attributed to the most prevalent microbial families and descending genera—including both bacterial and eukaryotic lineages—that harbor GH-CAZy families uniquely associated with the soil microbiomes of MO

Figure 5 provides a family- and genus-level perspective on niche-adapted carbohydrate-active enzymatic capacities underpinning the plant-specific microbial communities. Exhaustive taxonomic and functional breakdowns of CAZyme-bearing microbial taxa, alongside the distributions of their respective GH-CAZy families, relevant to these two wild plant species, are systematically cataloged in Tables S13 and S14, respectively.

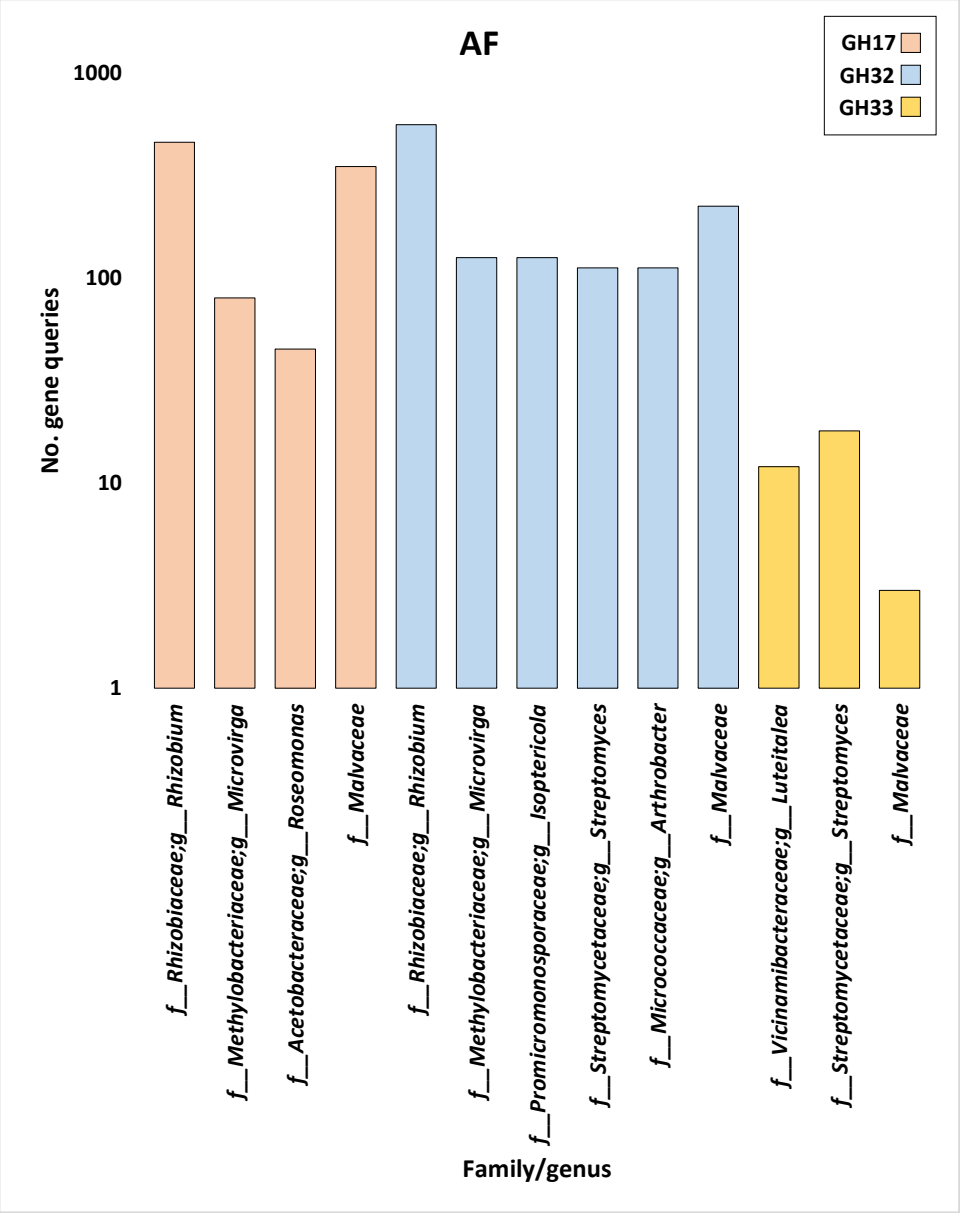

Fig. 6. Number of gene queries attributed to the most prevalent microbial families and descending genera—including both bacterial and eukaryotic lineages—that harbor GH-CAZy families uniquely associated with the soil microbiomes of AF

Figure 6 provides a family- and genus-level perspective on niche-adapted carbohydrate-active enzymatic capacities underpinning the plant-specific microbial communities. Exhaustive taxonomic and functional breakdowns of CAZyme-bearing microbial taxa, alongside the distributions of their respective GH CAZy families, relevant to these two wild plant species, are systematically cataloged in Tables S13 and S14, respectively.

The taxonomic composition reveals fundamental differences, where MO exhibits 53.5% eukaryotic dominance with major contributions from plant families including Vitaceae (genus *Vitis*), Solanaceae (genus *Nicotiana*), Fabaceae (genus *Cajanus*), and

Malvaceae (genus *Theobroma*), contrasted by 46.5% bacterial representation featuring Actinobacteria-rich lineages such as Chitinophagaceae (genus *Niastella*), Streptomycetaceae (genus *Streptomyces*), and Pyrinomonadaceae (genus *Pyrinomonas*) (Fig. 5). Conversely, AF demonstrates pronounced bacterial dominance (74.1%) *versus* limited eukaryotic participation (25.9%), with Proteobacteria-dominated assemblages led by Rhizobiaceae (genus *Rhizobium*, achieving high numbers of gene queries across GH32 and GH17), Methylobacteriaceae (genus *Microvirga*), and Acetobacteraceae (genus *Roseomonas*), while eukaryotic contributions are restricted primarily to Malvaceae family members (Fig. 6). The bacterial community compositions are entirely distinct between the two plant species, with MO uniquely enriched in nine bacterial families (Cellulomonadaceae, Geodermatophilaceae, Chitinophagaceae, Pyrinomonadaceae, Oxalobacteraceae, among others) *versus* AF's six exclusive families (Rhizobiaceae, Methylobacteriaceae, Acetobacteraceae, Micrococcaceae, Promicromonosporaceae, Vicinamibacteraceae), sharing only Streptomyce-taceae as a common bacterial lineage. This differential microbial recruitment underscores plant species-specific rhizospheric engineering, where MO selects for diverse eukaryotic contributors alongside specialized Actinobacteria, while AF preferentially enriches Proteobacteria with minimal eukaryotic involvement, reflecting distinct plant-microbe interaction strategies and niche-adapted carbohydrate metabolism pathways within their respective soil ecosystems.

Functional and Industrial Insights into CAZyme-Enriched Rhizospheric Microbiomes of Wild Plants

Figures 7 to 12 and S5 to S8 highlight the substrate specificities, catalytic proficiencies, and commercial relevance of CAZymes that are uniquely enriched in the rhizospheric microbiomes of *Moringa oleifera* (GH105, GH106, GH25, GH28, GH38) and *Abutilon fruticosum* (GH17, Gh32, Gh33). Figure 7 presents a comparative overview showing that the MO-microbiome is characterized by enzymes such as GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172), GH106 α-L-rhamnosidase (EC 3.2.1.40), GH25 lysozyme (EC 3.2.1.17), GH28 endo-polygalacturonase (EC 3.2.1.15), and GH38 α-mannosidase (EC 3.2.1.113). These enzymes act on complex substrates such as pectin, rhamnogalacturonan, peptidoglycan, and N-glycans. In contrast, the AF-microbiome is enriched in GH17 β-1,3-glucanase or glucan endo-beta-1,3-glucosidase (EC 3.2.1.39), GH32 invertase (EC 3.2.1.26), and GH33 sialidase (EC 3.2.1.18), which enable the decomposition of β-glucans, fructans, and sialic acid-containing compounds. Figure 8 illustrates the cooperative action of GH28 polygalacturonase (EC 3.2.1.15) and GH105 hydrolase (EC 3.2.1.172) in degrading plant cell wall pectin. Polygalacturonase targets α-(1→4)-link galacturonic acid residues to release oligomers, while GH105 cleaves rhamnogalacturonan disaccharides following pectin lyase pretreatment, resulting in the liberation of galacturonic acid and rhamnogalacturonide oligomers. Figure S5 further details the pectin depolymerization cascade in *M. oleifera*, beginning with PL1 pectate lyase (EC 4.2.2.2), which generates unsaturated oligosaccharides. These intermediates are subsequently processed by abundant GH105, GH106, and GH28 enzymes, which efficiently cleave and debranch the pectic network.

In MO, the unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.72) classified under GH105 orchestrates the depolymerization of pectin. Similarly, α-L-rhamnosidase (EC 3.2.1.174) from GH106 demonstrates selectivity for rhamnogalacturonan substrates,

while GH25's lysozyme/muramidase (EC 3.2.1.17) preferentially cleaves peptidoglycan. The endo-polygalacturonase hydrolase (EC 3.2.1.15) of GH28 exhibits pronounced activity on pectic polysaccharides, and α-mannosidase (EC 3.2.1.113) of GH38 targets N-glycan structures with marked specificity.

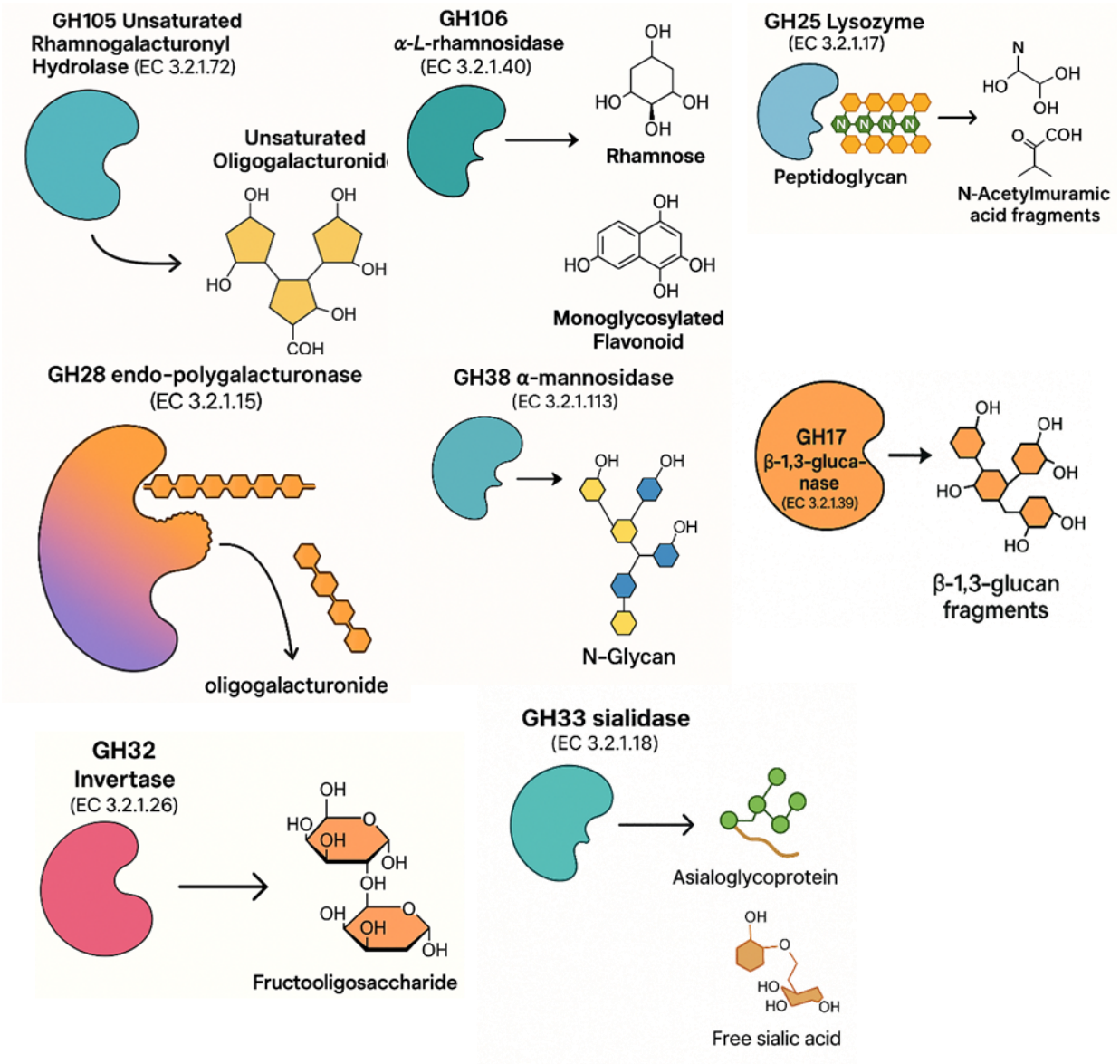

Fig. 7. Substrate specificities and catalytic proficiencies characteristic of premier carbohydrate-active enzymes (CAZymes) within GH families, each distinctively prevalent in the rhizospheric microbiota of MO or AF

Conversely, the AF microbiome manifests a unique enzymatic repertoire, where β-glucanase (EC 3.2.1.139) from GH17 hydrolyzes β-glucans, GH32's invertase (EC 3.2.1.26) catalyzes the decomposition of fructans, and GH33 sialidase (EC 3.2.1.18) expedites the release of sialic acid moieties. These discrete substrate–enzyme dynamics exemplify the highly tailored carbohydrate-processing capabilities inherent to each microbiome, underpinning specialized plant–microbe symbioses and illuminating potent

biotechnological prospects for precision-driven substrate valorization across industrial sectors.

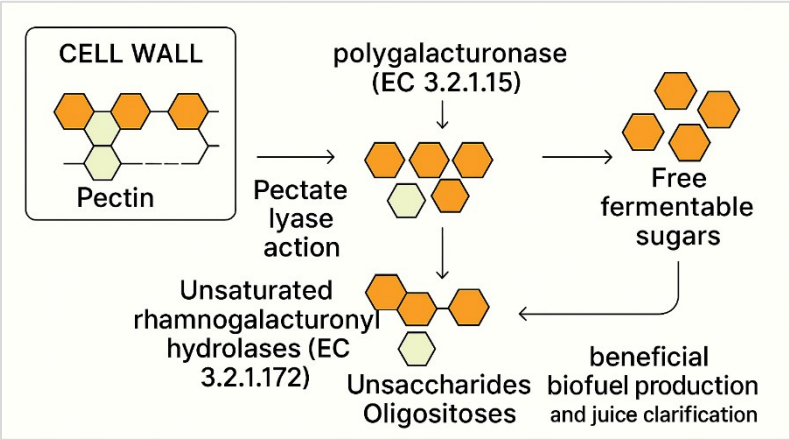

Fig. 8. Pectic polysaccharide cleavage by GH28 polygalacturonase (EC 3.2.1.15) and GH105 unsaturated rhamnogalacturonyl hydrolases (EC 3.2.1.172) in rhizospheric microbiome of *Moringa oleifera* and their industrial valorization

This schematic in Fig. 8 illustrates the depolymerization of homogalacturonan and rhamnogalacturonan I regions of plant cell-wall pectin by polygalacturonases and unsaturated rhamnogalacturonyl hydrolases, respectively. The figure shows hydrolytic cleavage of α-(1→4) galacturonic acid linkages, and the removal of unsaturated rhamnogalacturonan disaccharide units generated by pectin lyase. The arrows indicate released galacturonic acid oligomers and rhamno-galacturonides with defined degrees of polymerization. Industrial and biotechnological utilization of these two enzymes encompasses multifarious processes, notably the refinement of fruit juices and wines wherein their catalytic action markedly diminishes viscosity and turbidity, thereby yielding products of exceptional translucency and elevated organoleptic sophistication. In the realm of bioethanol and biorefinery processes, concerted enzymatic hydrolysis pathways precipitate augmented yields of fermentable monosaccharides from polysaccharide-rich biomass. Such innovations potentiate the conversion efficiency of biomass into renewable biofuels, concomitantly reducing energy expenditure and extending the applicability across a diversified array of feedstocks.

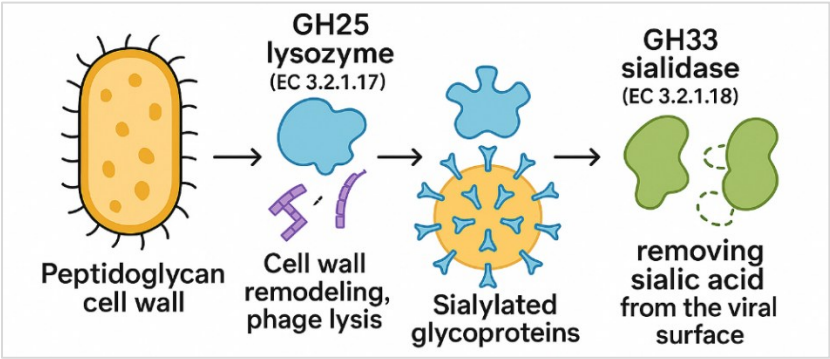

Fig. 9. Microbial interactions mediated by highly abundant GH25 and GH33 glycoside hydrolase enzymes within the rhizospheric microbiomes of the two wild plants

Figures 9, 10, S6, and S7 explore the ecological roles and industrial relevance of three highly abundant glycoside hydrolases—GH25, GH33, and GH38. Figures 9 and S6 highlight GH25 lysozyme (EC 3.2.1.17) and GH33 sialidase (EC 3.2.1.18) as central mediators of microbial interactions. GH25 lysozymes, known for their peptidoglycan-cleaving activity, play a key role in antagonistic intermicrobial dynamics. GH33 sialidase (Ec 3.2.1.18), which cleave terminal sialic acids from glycan chains, modulate microbial and host surface glycoconjugates. Its action influences nutrient cycling, microbial colonization, and communication by altering root exudate accessibility and microbial recognition pathways.

The schematics in Fig. 9 illustrates the functional roles of GH25 lysozyme (EC 3.2.1.17) and GH33 sialidase (EC 3.2.1.18), as revealed in rhizospheric microbiomes of MO and AF species. GH25 lysozyme catalyzes peptidoglycan cell wall remodeling and phage lysis, facilitating substrate turnover and microbial community dynamics. Meanwhile, GH33 sialidase acts on sialylated glycoproteins, removing sialic acid moieties from viral surfaces and modulating intermicrobial signaling and host–microbe interactions. These enzymatic mechanisms underscore the pivotal ecological functions of CAZyme-encoded gene repertoires in shaping niche-specific carbohydrate processing, microbial network architecture, and the metabolic capacity for complex glycan transformation within the rhizosphere environment.

Figures 10 and S7 focus on GH38 α-mannosidase (EC 3.2.1.24) that catalyzes the stepwise hydrolysis of terminal, non-reducing α-D-mannose residues from N-glycan chains on both plant and microbial glycoproteins. This activity facilitates glycoprotein maturation, turnover, and remodeling of surface glycoconjugates, which in turn regulate microbial colonization, nutrient exchange, and molecular signaling in the rhizospheric. GH38 enzyme role in modifying cell-surface glycans enhances host–microbe and microbe–microbe interactions, contributing to rhizospheric homeostasis.

Figure 11 details the substrate specificities and catalytic functions of glycoside hydrolase families GH17 and GH32, both of which are highly abundant in the rhizospheric microbiome of *Abutilon fruticosum*. GH17 enzymes, chiefly β-1,3-glucanases (including 1,3-β-D-glucan endohydrolases and exohydrolases), specialize in hydrolyzing β-1,3-glucans—major components of plant and fungal cell walls.

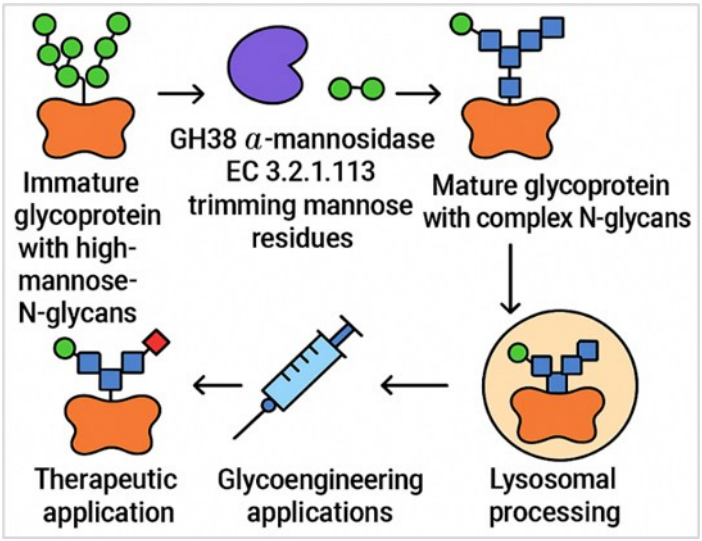

Fig. 10. Functional roles of glycoside hydrolase families GH38 (α-mannosidase; EC 3.2.1.113) and their impact on glycoprotein maturation and biotechnological applications in the rhizospheric microbiomes of *Moringa oleifera*

This schematic illustrates the enzymatic trimming of mannose residues from immature glycoproteins bearing high-mannose N-glycans by GH38 α-mannosidase (EC 3.2.1.113), culminating in the maturation of glycoproteins with complex N-glycans. Downstream processes depicted include lysosomal processing, glycoengineering applications, and therapeutic deployment of engineered glycoproteins. The figure underscores the metabolic and adaptive versatility of rhizospheric microbiomes in *Moringa oleifera* and *Abutilon fruticosum*, highlighting the ecological specialization and industrial relevance of GH-catalyzed glycan transformation for advanced bioprocessing, medical biotechnology, and precision carbohydrate engineering.

Their activity enables the breakdown of these glucans into oligosaccharides and glucose, facilitating nutrient recycling, microbial cell wall turnover, and promoting plant-microbe interactions within the rhizosphere. Meanwhile, GH32 enzymes encompass invertases and inulinases, which display distinct activity towards fructan substrates. GH32 invertases (β-fructofuranosidases or invertase, EC 3.2.1.26) cleave sucrose and related oligosaccharides by hydrolyzing terminal fructose units, thereby enabling efficient saccharification, energy release, and carbohydrate flux in the soil environment. GH32 endo-inulinase (EC 3.2.1.7) acts specifically on inulin-type fructans—performing internal cleavage of β-2,1-fructosidic bonds to generate fructo-oligosaccharides, which serve as key prebiotic molecules and energy sources for soil microbes.

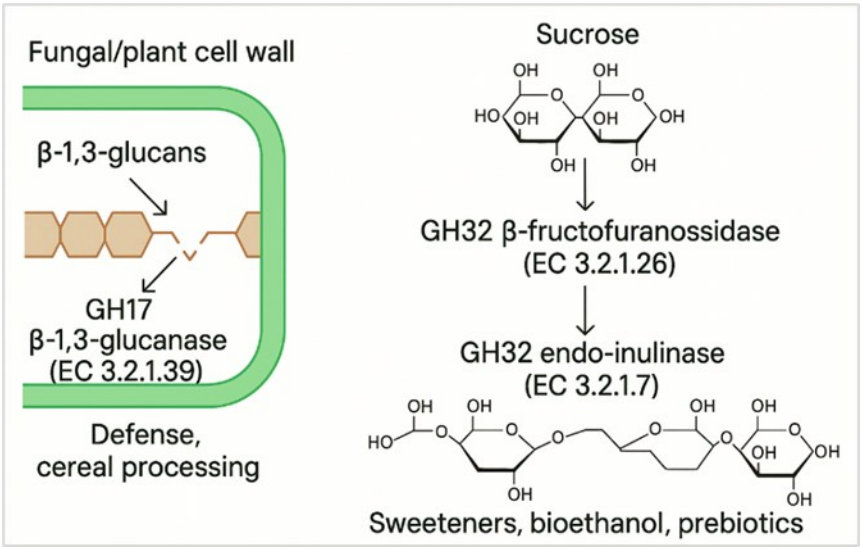

Fig. 11. Substrate specificity and catalytic activities of glycoside hydrolase families GH17 and GH32 in rhizospheric microbiomes of *Abutilon fruticosum*

The schematic in Fig. 1 depicts the enzymatic roles of GH17 β-1,3-glucanase (EC 3.2.1.39), which targets β-1,3-glucans in fungal and plant cell walls, and GH32 invertase (EC 3.2.1.26), which hydrolyzes sucrose and fructans, generating substrates for diverse industrial applications. Activity of GH17 enzymes is crucial for cellular defense and cereal processing, while GH32-mediated saccharification processes facilitate the biotechnological production of sweeteners, bioethanol, and prebiotics. The integrated depiction illustrates how these CAZyme families regulate substrate turnover and enable niche-specific plant–microbe interactions, with significant promise for bioprocess engineering, sustainable biofuel production, and food industry innovation.

This sequential action supports both primary carbon cycling and enhances soil health through the generation of functional oligosaccharides. Figure 12 depicts the enzymatic transformation pathway mediated by GH32 fructanase (exo-inulinase, EC 3.2.1.80) in the rhizospheric microbiome of *Abutilon fruticosum*. In this metabolic sequence, the GH32 enzyme specifically targets inulin-type fructan polymers, catalyzing the hydrolysis of terminal β-D-fructofuranosidic linkages to release fructose monomers. This initiates the depolymerization of inulin into shorter fructo-oligosaccharides, which serve as essential energy resources for soil microorganisms and contribute to carbon cycling in the rhizosphere. The figure highlights the dual role of GH32 exo-inulinase (EC 3.2.1.80) in promoting nutrient turnover and biotechnological innovation within the wild plant-associated microbiome.

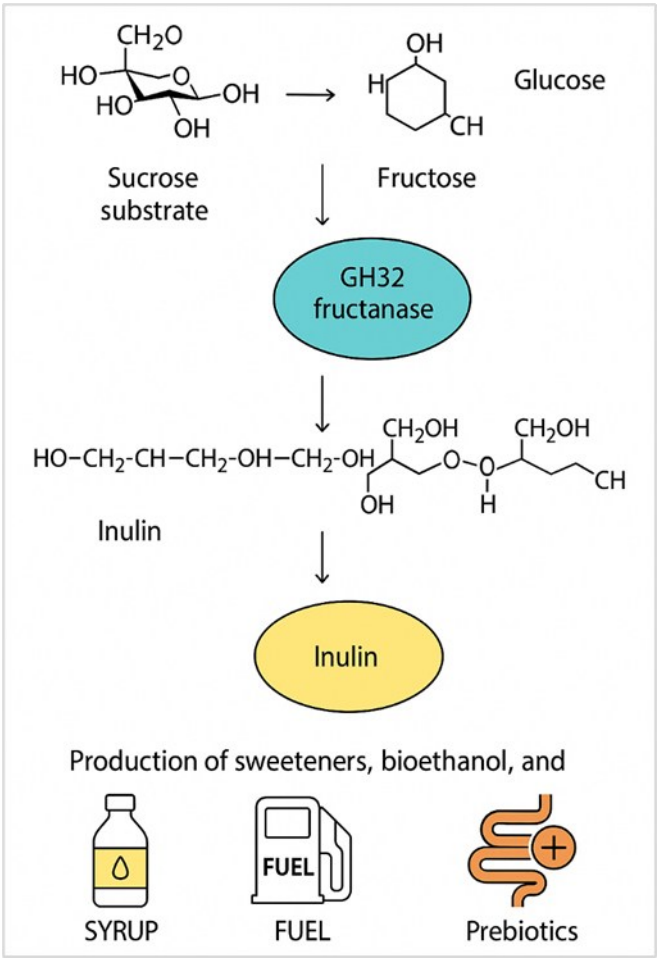

Fig. 12. Enzymatic transformation pathway mediated by GH32 fructanase (exo-inulinase, EC 3.2.1.80) in *Abutilon fruticosum* rhizospheric microbiomes

Sucrose derived from the plant rhizosphere is enzymatically cleaved to yield glucose and fructose. The highly specialized GH32 fructanase, prevalent in *A. fruticosum*-associated soil communities, converts fructose into inulin polymers. Inulin, as a functionally important bioproduct, underpins diverse industrial applications, including the sustainable manufacture of sweeteners, bioethanol, and prebiotic formulations, demonstrating the unique metabolic repertoire and biotechnological potential of lineage-restricted glycoside hydrolases in wild plant soil ecosystems.

Figure S8 illustrates the metabolic cascade mediated by two highly abundant CAZymes of the GH32 family—invertase (EC 3.2.1.26) and endo-inulinase (EC 3.2.1.7)—in the rhizospheric microbiome of *A. fruticosum*. This pathway begins with GH32 invertase catalyzing the hydrolysis of sucrose into monosaccharides, which supports the subsequent synthesis of inulin-type fructans in the soil microenvironment. Downstream, GH32 endo-inulinase targets these inulin polymers by cleaving internal β-2,1-fructosidic linkages, thereby producing shorter-chain fructooligo-saccharides. Through these sequential enzymatic actions, the rhizospheric microbiome efficiently converts plant-derived carbohydrates into high-value compounds. Figure S9 provides a comparative protein architecture analysis of glycoside hydrolase families enriched in wild plant rhizospheric

microbiomes. The figure highlights the structural diversity underpinning catalytic specialization, featuring the $(\alpha/\alpha)_6$-barrel motif of GH105 unsaturated rhamnogalacturonyl hydrolases, the β-propeller fold shared by GH32 and GH33 enzymes (such as invertase, endo-inulinase, and sialidase), and other prevalent motifs such as β-sandwich, immunoglobulin-like, and α/β domains. These structural frameworks reflect both evolutionary adaptation and mechanistic versatility, enabling the recognition and transformation of distinct carbohydrate substrates and supporting innovative applications in biotechnology, food processing, biofuel production, and therapeutic biomaterial development.

Taken together, these enzymatic functions demonstrate the metabolic ingenuity and practical importance of soil microbial communities associated with wild plants in promoting industrial and biotechnological advancements.

DISCUSSION

The Mecca region was deliberately prioritized and selected as the focal sampling locale owing to its unparalleled floristic biodiversity, particularly exemplified by its extraordinary pharmacobotanical heterogeneity and ethnomedicinal significance, while the taxonomically distinct selected botanical specimens, *e.g.*, *Moringa oleifera* and *Abutilon fruticosum*, were judiciously chosen based on their demonstrable ecological ubiquity, environmental adaptability, and multifaceted socioeconomic valorization potential across diverse sectorial domains (Khalik *et al*. 2013; Al-Eisawi and Al-Ruzayza 2015; Abdel Khalik *et al*. 2017; Al Masoudi 2024). Notably, the thermotolerant and xerotolerant CAZyme suite identified in the rhizospheric microbiomes of these plants potentially demonstrates optimal alignment with the demanding physicochemical exigencies and operational parameters characteristic of large-scale industrial bioreactor operations and advanced lignocellulosic biomass bioconversion systems requiring sustained enzymatic activity under harsh processing conditions, alongside numerous other commercially significant applications (Liew *et al*. 2022; Sallam *et al*. 2025). For instance, in biofuel and biorefinery contexts, cellulolytic biocatalysts from families GH5, GH6, and GH7, together with hemicellulases of families GH10, and GH11, orchestrate the depolymerization of lignocellulosic matrices and fungal or algal β-glucans into fermentable monosaccharides, thereby amplifying yields of second-generation ethanol, biobutanol, and allied bioproducts (Sethupathy *et al*. 2021; Wang *et al*. 2020).

Recent advances in metagenomic approaches have enabled detailed characterization of carbohydrate-active enzymes (CAZymes) associated with plant root systems across diverse ecological contexts, yet significant gaps persist in our understanding of plant species-specific CAZyme specialization. Martin and colleagues (2025) demonstrated that root-associated *Bacteroidota* species, particularly *Flavobacterium*, possess specialized Polysaccharide Utilization Loci (PULs) encoding glycoside hydrolases that efficiently degrade xyloglucan—a major hemicellulose component—thereby establishing competitive advantages in the rhizosphere, and revealed that the GH5_4 subfamily exhibits distinct evolutionary adaptations in soil microbiomes compared to engineered or gut systems (Martin *et al.* 2025). Liu and colleagues (2024) employed comparative metagenomics to examine rhizosphere microbiome assembly in Chinese

cabbage (*Brassica rapa*), revealing that CAZyme-mediated metabolic pathways play crucial roles in microbial-mediated disease resistance against the clubroot pathogen *Plasmodiophora brassicae* (Liu *et al*. 2024). In the context of *Moringa oleifera*, Tashkandi and colleagues (2023) investigated CAZymes encoded by highly abundant genes in rhizosphere soil using a gene-centric approach; however, this work focused exclusively on the most abundant CAZyme-encoding genes, leaving the broader taxonomic and functional diversity of GH families largely unexplored (Tashkandi and Baz 2023). Additional studies have characterized CAZyme repertoires in specialized niches, where Ferrer and colleague. (2012) identified multifunctional glycosyl hydrolases from cow rumen microbiomes with unprecedented substrate promiscuity (Ferrer *et al*. 2012), Montella and colleagues (2017) discovered lignocellulose-hydrolyzing CAZymes from energy crops including *Arundo donax*, *Eucalyptus*, and *Populus* (Montella *et al*. 2017), and Masasa and colleagues (2022) characterized CAZymes of halotolerant bacteria adapted to saline environments (Masasa *et al*. 2022). Despite these advances, comparative analyses of plant species-specific CAZyme specialization remain scarce, particularly for native arid-adapted plants where unique environmental stressors impose distinct selective pressures on rhizosphere microbial communities, and no prior study has systematically compared glycoside hydrolase family composition, taxonomic distribution, and industrial valorization potential between two distinct arid-adapted native plant species using high-resolution shotgun metagenomic sequencing combined with functional annotation and enzyme characterization. The present investigation addresses these gaps by providing the first comprehensive comparative metagenomic analysis of plant species-specific glycoside hydrolase specialization in the rhizospheres of *Moringa oleifera* and *Abutilon fruticosum*—two ecologically and taxonomically distinct native Saudi Arabian plants adapted to arid conditions—thereby advancing our mechanistic understanding of how plant identity shapes rhizosphere CAZyme assembly and establishing a translational framework for leveraging arid-adapted rhizosphere microbiomes in next-generation bioprocessing platforms.

The plant species-specific CAZyme specialization observed in this study provides strong evidence for synergistic mutualistic partnerships wherein plants secrete polysaccharide-rich root exudates that support microbial growth, while enriched bacteria and fungi produce CAZymes enabling nutrient mineralization that enhances plant nutrient uptake under nutrient-limited arid conditions (Lyu *et al*. 2021; Qu *et al*. 2022; Wu *et al*. 2024; Yang *et al*. 2024). The distinct taxonomic profiles—with *M. oleifera* harboring Actinobacteria-dominated communities including *Streptomyces* and *Micromonospora*, and *A. fruticosum* enriched in nitrogen-fixing Proteobacteria—reflect active plant-mediated recruitment of microbes with complementary enzymatic functions, while the substantial eukaryotic contribution (53.5%) to *M. oleifera* CAZyme diversity versus *A. fruticosum*'s bacterial-dominated (74.1%) profile indicates distinct coevolutionary strategies for nutrient acquisition reflecting specific adaptations to arid stress (Lyu *et al*. 2021; Shami *et al.* 2022; Ashy *et al*. 2023; Tashkandi and Baz 2023; Sefrji *et al*. 2025). These findings underscore that observed CAZyme specialization patterns represent outcomes of long-term plant-microbe coevolution, wherein each plant species selectively maintains distinct microbial consortia optimized to degrade specific root exudate polysaccharides and enhance plant fitness in nutrient-poor, water-limited ecosystems.

Industrial Applications of CAZymes: From Biotechnology Manufacturing to Biomedical Innovations

Biotechnology and manufacturing

In food and beverage applications, various glycoside hydrolases significantly enhance processing efficiency and product quality. GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172), in conjunction with pectin lyase pretreatment, facilitates the degradation of residual pectin in fruit juice and cider, thereby improving clarification, increasing yield, and preserving aromatic compounds (Chettri *et al*. 2022; Chettri *et al*. 2020). GH106 α-L-rhamnosidase (EC 3.2.1.174) is employed to mitigate bitterness in citrus juices and liberate aromatic monoterpenes during wine-making (Li *et al*. 2018; Pan *et al*. 2023). Enzymes such as GH28 endo-polygalacturonase (EC 3.2.1.15) and rhamnogalacturonan hydrolase (EC 3.2.1.67) target pectic polysaccharides to reduce viscosity and enhance clarity and yield—up to 15%—in juices and wines (Lu *et al*. 2022; Wang *et al*. 2015). Additionally, GH33 sialidases (EC 3.2.1.18) contribute to umami flavor enhancement and color development in fermented condiments like soy and fish sauces (Chen *et al*. 2025). In brewing and cereal processing, GH17 endo-1,3-β-glucanase (EC 3.2.1.39) and mixed-linkage lichenase (EC 3.2.1.73) decrease mash viscosity and improve filtration and extract yields, respectively, while maintaining foam stability (Srivastava *et al*. 2019). GH38 α-mannosidase (EC 3.2.1.113) markedly reduces turbidity by up to 60% during beverage aging and enhances sensory attributes such as mouthfeel and foam persistence (Suits *et al*. 2010). For antimicrobial preservation, GH25 lysozymes (EC 3.2.1.17) act as natural, non-antibiotic preservatives in dairy and alcoholic beverages, effectively inhibiting Gram-positive spoilage organisms and reducing reliance on chemical preservatives; notably, egg-white lysozyme at 200 mg/L lowers lactic acid bacteria counts by over 3 log CFU/mL in cheese brine (EFSA Panel on Food Contact Materials *et al*. 2023). In sweetener and prebiotic production, GH32 β-fructofuranosidase (EC 3.2.1.26) catalyzes the synthesis of fructooligosaccharides (FOS) from sucrose, yielding over 60 g/L FOS with high purity (>80%) and a degree of polymerization of 2–4 under optimized industrial conditions (55°C, pH 6.0), while GH32 endo-inulinase (EC 3.2.1.7) converts inulin from chicory and Jerusalem artichoke into high-purity FOS streams suitable for syrups and fiber-enriched formulations (Martins *et al*. 2019).

In the context of biofuel and biorefinery processes, several glycoside hydrolases have demonstrated significant utility in enhancing lignocellulosic biomass conversion and valorization. GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) facilitates the saccharification of pectin-rich biomass by removing rhamnogalacturonan I (RG-I) layers, thereby increasing fermentable sugar release by 15% and boosting ethanol production (Chettri *et al*. 2022; Chettri *et al*. 2020). GH106 α-L-rhamnosidase (EC 3.2.1.174) contributes to the debranching of RG-I, enhancing saccharification efficiency by 20% to 30% and enabling the liberation of L-rhamnose for downstream fermentation into bioethanol and 1,2-propanediol (MacCabe *et al*. 2020; Pardo and Orejas 2014). In biomass valorization, GH25 muramidases (EC 3.2.1.17) improve microbial cell wall degradation in feed applications, increasing digestibility by 12% and accelerating saccharification for single-cell protein generation (Moroz *et al*. 2021). GH28 endo-polygalacturonase (EC 3.2.1.15), alone or in combination with rhamnogalacturonan hydrolase (EC 3.2.1.67), releases galacturonic acid monomers and enhances sugar recovery by 20%, leading to a 10% rise in ethanol titers (Thakur *et al*. 2010). GH38 α-mannosidase

(EC 3.2.1.113) enhances glucose yields by 18% through the removal of mannose-rich side chains and provides mannose substrates suitable for ethanol and 2,3-butanediol biosynthesis (Mayer-Laigle *et al*. 2018). For improved saccharification, GH17 endo-1,3-β-glucanase (EC 3.2.1.39) augments the degradation of fungal and algal biomass, increasing glucose release by 18% and ethanol output by 12%, while the GH17 mixed-linkage lichenase (EC 3.2.1.73) accelerates cereal straw breakdown, enabling a 15% reduction in enzyme usage (Srivastava *et al*. 2019). GH32 endo-inulinase (EC 3.2.1.7) enhances ethanol yields by 25% through pretreatment of inulin-rich feedstocks, while β-fructofuranosidase (EC 3.2.1.26) enables the conversion of sucrose-rich molasses into fermentable sugars for co-production of bioethanol and biobutanol (Martins *et al*. 2019; Rawat *et al*. 2024a). Currently, GH33 sialidases have limited applicability in biorefinery settings due to the low abundance of suitable substrates.

In the pulp, paper, and textile industries, various glycoside hydrolases contribute to more sustainable and efficient processing through improved fiber treatment, reduced chemical usage, and enhanced material quality. GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) facilitates fiber separation and bleaching by targeting pectic substances, leading to a 20% reduction in chlorine dioxide consumption and increased pulp brightness; in textile applications, it enhances softness, dye affinity, and tensile strength by effectively removing pectin from plant fibers (Chettri *et al*. 2020, 2022). GH106 α-L-rhamnosidase (EC 3.2.1.174) degrades rhamnogalacturonan, decreasing chlorine dioxide use in pulp mills by 15% and improving textile whiteness by 1.2% during bioscouring (Gomes *et al*. 2016). GH25 lysozymes (EC 3.2.1.17) function as environmentally friendly antimicrobial agents in recycled pulp, reducing microbial load by 90% and chlorine dioxide demand by 15%, while also imparting antimicrobial properties to textiles without compromising tactile quality (EFSA Panel on Food Contact Materials *et al*. 2023; Moroz *et al*. 2021). GH28 endo-polygalacturonase (EC 3.2.1.15) and rhamnogalacturonan hydrolase (EC 3.2.1.67) act synergistically to lower kappa number by 12%, reduce chlorine dioxide usage by 18%, and simultaneously enhance dye uptake by 25% and decrease alkaline requirements by 30% (Lu *et al*. 2022; Wang *et al*. 2015). GH38 α-mannosidase (EC 3.2.1.113) aids in softwood pulp de-gumming, reducing hemicellulose content by 25%, chlorine dioxide use by 20%, and wastewater pollutant load by 30%, while improving fiber softness (Iacono *et al*. 2023). GH17 endo-1,3-β-glucanase (EC 3.2.1.39) significantly reduces slime accumulation by 60% in recycled pulp, whereas lichenase (EC 3.2.1.73) enhances fiber wettability and dye uptake by 20%, coupled with a 30% decrease in alkali consumption (Singh *et al*. 2016). GH32 and GH33 enzymes currently show limited relevance in these sectors due to substrate specificity constraints.

Healthcare and medicine

In pharmaceutical research and development, diverse glycoside hydrolases are being harnessed for their roles in drug formulation, therapeutic protein engineering, and diagnostics. GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) facilitates the generation of pectic oligosaccharides that function as pH-sensitive excipients, thereby enhancing the oral bioavailability of encapsulated drugs (Chung *et al*. 2017; Tang *et al*. 2025). GH106 α-L-rhamnosidase (EC 3.2.1.174) enables the synthesis of monoglycosylated flavonoids with superior bioavailability and catalyzes the conversion of chloropolysporin B into the more potent antibiotic chloropolysporin C (Guillotin *et al*.

2019; Yu *et al*. 2022). GH25 lysozymes (EC 3.2.1.17) are employed as preservatives in ophthalmic and otic preparations to mitigate microbial contamination without promoting antibiotic resistance (EFSA Panel on Food Contact Materials *et al*. 2023; Moroz *et al*. 2021). For therapeutic protein modification, GH28 rhamnogalacturonan hydrolase (EC 3.2.1.67) generates rhamno-galacturonide structures that form ionic cross-links with cationic drugs, stabilizing pH-sensitive formulations (Chung *et al*. 2017; Lu *et al*. 2022). GH38 α-mannosidase (EC 3.2.1.113) trims high-mannose N-glycans on therapeutic glycoproteins, producing uniform antibody glycoforms with extended half-lives and diminished immunogenicity (Méndez-Yáñez *et al*. 2024; Suits *et al*. 2010). GH17 Endo-1,3-β-glucanase (EC 3.2.1.39) is supplied as high-purity recombinant enzyme for biochemical and *in vitro* diagnostic assays, where it specifically degrades fungal and cereal β-glucan contaminants to enable accurate quantification of residual glucans in biopharmaceutical preparations and cell-culture media, ensuring product safety and reproducibility (Kuge *et al*. 2015). GH32 β-fructofuranosidase or invertase (EC 3.2.1.26) generates fructooligosaccharide (FOS) excipients that improve solubility and stability of peptide-based drugs, while endo-inulinase (EC 3.2.1.7) produces high-purity inulo-oligosaccharides used as prebiotic biomarkers: their quantification in fecal samples by HPLC–MS after GH32 digestion correlates with gut microbiota composition and can serve as a diagnostic indicator of dysbiosis in irritable bowel syndrome and inflammatory bowel disease (Martins *et al*. 2019; Rawat *et al*. 2024a). GH33 sialidase (EC 3.2.1.18) produces asialoglycoproteins for targeted delivery and enhances monoclonal antibody pharmacokinetics (Lipničanová *et al*. 2020).

In diagnostic contexts, GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) activity levels have been associated with dysbiosis and colorectal cancer risk, offering promise as noninvasive biomarkers (Chung *et al*. 2017; Tang *et al*. 2025). GH106 alpha-L-rhamnosidase (EC 3.2.1.40) supports bacterial serotyping through depolymerization of O-antigen chains, and GH25-based kits detect bacterial load via peptidoglycan fragments (Guillotin *et al*. 2019; Yu *et al*. 2022). GH28 endo-polygalacturonase (EC 3.2.1.15) produces oligogalacturonides as potential biomarkers for gut permeability in inflammatory bowel disease (Martins *et al*. 2019; Rawat *et al*. 2024a). GH38 assays are applied to measure serum α-mannosidase as indicators of lysosomal storage disorders. GH17 endo-1,3-β-glucanase (EC 3.2.1.39) is critical for ensuring the safety of biopharmaceuticals by degrading β-glucan impurities (Kuge *et al*. 2015). GH32 endo-inulinase (EC 3.2.1.7) generates prebiotic compounds used as diagnostic markers for dysbiosis (Martins *et al*. 2019; Rawat *et al*. 2024a), while GH33 sialidase or neuraminidase (EC 3.2.1.18) activity assays quantify serum sialidase levels, providing insights into cancer and inflammatory conditions (Lipničanová *et al*. 2020).

In tissue engineering, glycoside hydrolases are increasingly recognized for their roles in enhancing scaffold functionality, promoting cell adhesion, and modulating immune responses. GH105-derived unsaturated pectic oligosaccharides function as reactive groups for collagen and gelatin crosslinking *via* Michael-type addition reactions, resulting in hydrogels with customizable mechanical characteristics suitable for cartilage and skin scaffold applications; additionally, they stimulate endothelial cell proliferation and angiogenesis through lectin receptor engagement (Chung *et al*. 2017; Munoz-Munoz *et al*. 2017). GH106 α-L-rhamnosidase (EC 3.2.1.174) produces rhamno-oligosaccharides that act as adhesive ligands, fostering epithelial and endothelial cell proliferation while

preserving tight-junction integrity in gut-on-chip and dermal tissue models (Maria-Ferreira *et al*. 2018). GH25 lysozyme (EC 3.2.1.17) coatings offer robust antimicrobial defense by inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms, while simultaneously supporting cell viability; controlled degradation of peptidoglycan by this enzyme also promotes macrophage M2 polarization and angiogenesis (Moroz *et al*. 2021). GH28 endo-polygalacturonase (EC 3.2.1.15) generates oligogalacturonides that contribute to the formation of microporous scaffold structures conducive to chondrocyte proliferation, whereas rhamno-galacturonan hydrolase (EC 3.2.1.67) enhances endothelial adhesion and capillary network formation (Wang *et al*. 2015; Lu *et al*. 2022). GH38 α-mannosidase (EC 3.2.1.113) produces mannose-depleted extracellular matrix scaffolds that support stem cell differentiation and reduce immunogenicity in grafts (Méndez-Yáñez *et al*. 2024; Suits *et al*. 2010). In immunomodulatory and vascularization contexts, GH17 lichenase (EC 3.2.1.73) yields β-glucan oligosaccharides that suppress dendritic cell-derived pro-inflammatory cytokines, fostering a tolerogenic immune environment for graft acceptance (Akkerman *et al*. 2020). GH32 endo-inulinase (EC 3.2.1.7) produces inulo-oligosaccharides that increase scaffold porosity and drive chondrogenic differentiation, while β-fructofuranosidase (EC 3.2.1.26) enhances endothelial adhesion and vascular formation via FOS grafting (Martins *et al*. 2019). GH33 sialidase (EC 3.2.1.18) removes terminal sialic acid residues, unveiling glycan binding sites that improve both stem and endothelial cell adhesion, thereby promoting neovascularization within engineered tissues (Yilmaz and Becer 2014).

In wound-healing applications, glycoside hydrolases play pivotal roles in modulating matrix remodeling, promoting epithelial regeneration, enhancing antimicrobial defense, and influencing immune and microbiome dynamics. GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) generates pectic fragments that enable controlled hydrogel degradation for sustained release of antimicrobials and growth factors, while promoting keratinocyte migration and collagen synthesis through upregulation of TGF-β1 and MMP-9 (Church *et al*. 2023; Tang *et al*. 2025). GH106 α-L-rhamnosidase (EC 3.2.1.174) yields oligosaccharides that significantly accelerate epithelial wound closure—up to 84% within 24 h—by enhancing fibroblast migration and re-epithelialization (Maria-Ferreira *et al*. 2018). Similarly, GH28 endo-polygalacturonase (EC 3.2.1.15) produces oligogalacturonides that improve re-epithelialization by 60%, while rhamnogalacturonan hydrolase (EC 3.2.1.67) generates antimicrobial rhamno-galacturonides that inhibit *S. aureus* biofilms and reduce inflammatory cytokine expression (Chung *et al*. 2017; Zhao and Jia 2024). GH25 lysozymes (EC 3.2.1.17) deliver sustained antimicrobial effects and support fibroblast proliferation, modulating systemic inflammation *via* gut-skin axis signaling and promoting epithelial repair through TGF-β1 activation (Zhang *et al*. 2025). GH38 α-mannosidase (EC 3.2.1.113) produces mannooligosaccharides that recruit macrophages and endothelial cells, thereby enhancing neovascularization and enabling a 45% increase in wound closure rates in diabetic models (Cobucci-Ponzano *et al*. 2010; Suits *et al*. 2010). GH17 endo-1,3-β-glucanase (EC 3.2.1.39) and lichenase (EC 3.2.1.73) generate β-glucan oligosaccharides that stimulate macrophage infiltration and growth factor secretion, leading to a two-fold acceleration in chronic wound healing (Grip *et al*. 2018; Majtan and Jesenak 2018; Seo *et al*. 2019). Additionally, GH32 β-fructofuranosidase (EC 3.2.1.26) produces FOS that suppress pathogenic *Staphylococcus* colonization and enhance antimicrobial peptide levels. GH32 endo-inulinase (EC 3.2.1.7) yields inulo-

oligosaccharides that promote fibroblast migration and re-epithelialization by inducing TGF-β1 and VEGF, resulting in a 30% acceleration in wound closure (Rawat *et al*. 2024a). Lastly, GH33 sialidase (EC 3.2.1.18) exposes galactose residues that activate keratinocyte migration and macrophage efferocytosis, facilitating rapid re-epithelialization and reduced scar formation (Lipničanová *et al*. 2020).

Health and nutrition

In the field of nutraceuticals, glycoside hydrolases are increasingly recognized for their capacity to generate bioactive oligosaccharides with profound prebiotic, immunomodulatory, metabolic, and neurodevelopmental effects. GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) produces low-degree polymerization oligomers that selectively stimulate *Faecalibacterium prausnitzii*, resulting in a two-fold increase in butyrate production and attenuation of inflammatory cytokine levels; these oligosaccharides also demonstrate antioxidant activity (IC_{50} = 150 μg/mL) and promote cholesterol efflux, contributing to cardiovascular health (Chung *et al*. 2017; Tang *et al*. 2025). GH106 α-L-rhamnosidase (EC 3.2.1.174) yields rhamno-oligosaccharides that enrich *Bifidobacterium adolescentis* and *Lactobacillus plantarum* by three-fold, increasing acetate and propionate production by 2 to 3 fold, while free L-rhamnose offers potential as a low-glycemic sweetener (GI = 32) (Pan *et al*. 2023). GH28 endo-polygalacturonase (EC 3.2.1.15) generates oligogalacturonides that enhance beneficial bacterial populations by 2.5 to 3 fold and butyrate levels by 1.8-fold, while exhibiting antioxidant properties (IC_{50} = 200 μg/mL) (Onumpai *et al*. 2011; Chung *et al*. 2017). For gut health and immune modulation, GH25 muramidase (EC 3.2.1.17) reduces pathogenic *Enterococcus* and *Clostridium* counts by 2 log CFU/g and increases *Lactobacillus* abundance by 1.5-fold, with released peptidoglycan fragments activating NOD2 receptors to induce defensins and anti-inflammatory cytokines, supporting mucosal immune equilibrium (Xiong *et al*. 2019; Zhou *et al*. 2019). GH38 α-mannosidase (EC 3.2.1.113) produces mannooligosaccharides that elevate *Lactobacillus rhamnosus* and *Bifidobacterium longum* by two-fold, enhancing short-chain fatty acids (SCFA) production by 1.5–2 fold; D-mannose further prevents *E. coli* adhesion to uroepithelial cells by 80% (Li *et al*. 2020; Pugliese *et al*. 2020). GH17 endo-1,3-β-glucanase (EC 3.2.1.39) and lichenase (EC 3.2.1.73) generate β-glucan oligosaccharides that double the growth of beneficial gut bacteria and modulate immune responses by increasing IL-10 levels by 40% and decreasing TNF-α by 30%. Metabolically, GH32 β-fructofuranosidase (EC 3.2.1.26) produces fructooligosaccharides (FOS) that lower fasting glucose and triglycerides and enhance gut barrier function by 2–3 fold, while GH32 endo-inulinase (EC 3.2.1.7) generates inulo-oligosaccharides (IOS) with potent bifidogenic effects, increasing *Faecalibacterium prausnitzii* by 2.5-fold and butyrate levels by 1.8-fold (Martins *et al*. 2019; Rawat *et al*. 2024a). Lastly, GH33 sialidase (EC 3.2.1.18) liberates sialic acid for use in infant formulas, promoting *Bifidobacterium longum* proliferation by 2.3-fold and enhancing cognitive development and synaptogenesis in juvenile models (Conroy *et al*. 2021).

In biomaterial development, glycoside hydrolases contribute significantly to scaffold crosslinking, mechanical tuning, and biofunctionalization of hydrogels and films. GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) produces pectic fragments capable of forming hydrogels *via* Michael-type addition with thiolated gelatin, yielding stiffness values between 0.5 and 5 kPa suitable for tissue fillers and wound healing

matrices; these can be integrated with chitosan scaffolds to create antimicrobial films with controlled growth factor release (Chung *et al*. 2017; Tang *et al*. 2025). GH106 α-L-rhamnosidase (EC 3.2.1.174) generates rhamno-oligosaccharides that interact with plant lectins within hyaluronic acid-based hydrogels, improving chondrocyte adhesion with stiffness tunability from 1 to 8 kPa, while L-rhamnose conjugation to poly(lactic-co-glycolic acid (PLGA) nanoparticles facilitates macrophage-targeted delivery in nutraceutical applications (Maria-Ferreira *et al*. 2018; Pan *et al*. 2023). GH25 muramidase (EC 3.2.1.17) yields peptidoglycan nanofibrils (~100 nm) that support M2 macrophage polarization and vascular endothelial growth factor (VEGF) release in collagen matrices, while also forming photocrosslinkable, antimicrobial hydrogels (Moroz *et al*. 2021). GH28 endo-polygalacturonase (EC 3.2.1.15) produces oligogalacturonides that act as calcium-mediated crosslinkers, enhancing scaffold compressive modulus from 0.8 to 3.2 kPa. In parallel, rhamnogalacturonan hydrolase (EC 3.2.1.67) generates oligosaccharides that double fibroblast adhesion and accelerate wound closure by 40% (Wang *et al*. 2015; Lu *et al*. 2022). GH38 α-mannosidase (EC 3.2.1.113) produces mannooligosaccharides that increase scaffold porosity and stimulate stem cell proliferation by 60%, concurrently enhancing chondrogenic marker expression; D-mannose-chitosan conjugates form films inhibiting *S. aureus* growth by over 90% (Li *et al*. 2020; Asbury and Saville 2025). GH17 endo-1,3-β-glucanase (EC 3.2.1.39) produces β-glucan-based crosslinkers that improve hydrogel compressive modulus from 0.8 to 2.5 kPa, while lichenase (EC 3.2.1.73) enhances fibroblast adhesion by 60% and promotes wound closure by 35% (Akkerman *et al*. 2020; Majtan and Jesenak 2018). GH32 β-fructofuranosidase (EC 3.2.1.26) generates fructooligosaccharides (FOS) that increase hydrogel porosity by 35% and water retention by 50%, supporting stem cell viability, while GH32 endo-inulinase (EC 3.2.1.7) produces inulo-oligosaccharides (IOS) that act as polymerization initiators in injectable hydrogels with stiffness ranging from 1.0 to 5 kPa, optimized for probiotic delivery (Martins *et al*. 2019). GH33 sialidase (EC 3.2.1.18) reveals surface binding ligands that enhance Caco-2 cell adhesion by 45% and suppress TNF-α secretion by 30% in bioactive surface coatings (Lipničanová *et al*. 2020).

Neuroscience

In the context of neuroplasticity, glycoside hydrolases contribute significantly to gut-brain axis modulation and direct neural remodeling by influencing microbial metabolites, neurotrophic signaling, and synaptic architecture. GH105 unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) produces pectic oligosaccharides that enrich butyrogenic microbiota, resulting in a two-fold increase in serum acetate and propionate levels, which in turn elevate hippocampal BDNF expression by 1.8-fold and enhance dendritic spine density (Church *et al*. 2023). Similarly, GH106 α-L-rhamnosidase (EC 3.2.1.174) generates rhamno-oligosaccharides that boost cortical BDNF by 1.7-fold and promote long-term potentiation (LTP) *via* elevated propionate (Pan *et al*. 2023; Tautau *et al*. 2020). GH25 muramidase (EC 3.2.1.17) further supports hippocampal BDNF upregulation (2.1-fold) and cognitive enhancement, as demonstrated by improved Morris water maze performance, likely through dysbiosis correction and increased SCFAs (Xiong *et al*. 2019; Zhang *et al*. 2025). GH28 endo-polygalacturonase (EC 3.2.1.15) raises hippocampal BDNF by 1.9-fold and enhances memory consolidation *via* SCFA-mediated BDNF-TrkB signaling (Zhao and Jia 2024). Beyond microbiota-mediated effects, GH38

α-mannosidase (EC 3.2.1.113) facilitates synaptic glycoprotein remodeling, enhancing AMPA receptor mobility and PSA-NCAM turnover, thereby promoting neurite outgrowth and dendritic spine maturation; its inhibition reduces LTP magnitude by 45% (Cobucci-Ponzano *et al*. 2010). GH17 endo-1,3-β-glucanase (EC 3.2.1.39) and lichenase (EC 3.2.1.73) produce β-glucan-derived fragments that double SCFA levels, raise hippocampal BDNF 1.9-fold, and stimulate PSA-NCAM-driven neurite development, increasing dendritic branching and spine density by 45% and 30%, respectively (Majtan and Jesenak, 2018; Church *et al*. 2023). GH32 β-fructofuranosidase (EC 3.2.1.26) enhances butyrate production 2.5-fold, resulting in doubled BDNF expression and improved synaptic plasticity, while GH32 endo-inulinase (EC 3.2.1.7) promotes *Faecalibacterium prausnitzii* proliferation three-fold and upregulates synaptic proteins such as synapsin I and PSD-95 (Divyashri *et al*. 2021; Rawat *et al*. 2024a). Finally, GH33 sialidase (EC 3.2.1.18) increases PSA-NCAM cleavage by 60%, augmenting neurite length by 40% and spine density by 35% through enhanced BDNF-TrkB pathway activation (Conroy *et al*. 2021).

In the realm of neuroprotection, glycoside hydrolases exhibit multifaceted roles through anti-inflammatory, antioxidant, and metabolic mechanisms. GH105 unsaturated rhamno-galacturonyl hydrolase (EC 3.2.1.172) produces oligosaccharides that significantly attenuate hippocampal TNF-α and IL-1β levels (by 45%–50%) and enhance neuronal viability by 35% in LPS-induced neurotoxicity models (Divyashri *et al*. 2021). GH106 α-L-rhamnosidase (EC 3.2.1.174) generates rhamno-oligosaccharides that mitigate oxidative stress by reducing ROS accumulation by 55% and suppressing caspase-3 activation by 40% in H_2O_2-challenged cortical neurons (Pan *et al*. 2023). GH25 lysozyme (EC 3.2.1.17) exhibits neuroprotective effects by diminishing microglial cytokine release (TNF-α and IL-1β) by 50% and neuronal ROS by 45%, while also lowering hippocampal microglial activation by 40% and preventing CA1 synaptic loss following systemic administration (Moroz *et al*. 2021). GH28 rhamnogalacturonan hydrolase (EC 3.2.1.67) significantly reduces LDH release (by 45%) and caspase-3 activity (by 35%) in glutamate-induced excitotoxicity, alongside a >50% suppression of hippocampal inflammatory cytokines (Guo *et al*. 2023; Zhao and Jia 2024). GH38 α-mannosidase (EC 3.2.1.113) enhances ischemic resilience by reducing infarct volume by 35%, preserving neuronal ATP levels, and promoting glycoprotein aggregate clearance, resulting in a 50% reduction in tau accumulation and attenuated apoptotic signaling (Méndez-Yáñez *et al*. 2024). GH17 endo-1,3-β-glucanase (EC 3.2.1.39) and lichenase (EC 3.2.1.73) yield β-glucan fragments that lower hippocampal TNF-α and IL-1β levels by 50% to 55%, reduce ROS by 60%, and rescue neuronal viability by 40% through decreased caspase-3 activation (by 45%) (Majtan and Jesenak 2018; Akkerman *et al*. 2020). GH32 β-fructofuranosidase (EC 3.2.1.26) generates fructooligosaccharides (FOS) that suppress hippocampal TNF-α and IL-6 by over 40% and lower lipid peroxidation markers such as malondialdehyde by 50%, while GH32 endo-inulinase (EC 3.2.1.7) produces inulo-oligosaccharides (IOS) that reduce ROS by 55% and caspase-3 activity by 45% in excitotoxic models (Divyashri *et al*. 2021). Finally, GH33 sialidase (EC 3.2.1.18) downregulates microglial pro-inflammatory cytokines (TNF-α and IL-1β) by 45% to 50% and reduces infarct volume by 30% *via* diminished complement pathway activation following intracerebral administration (Lipničanová *et al*. 2020).

Complementary Roles of Highly Abundant Glycoside Hydrolase Family Genes

The glycoside hydrolase families GH105 and GH28 both play integral roles in the degradation of complex pectic polysaccharides, particularly rhamnogalacturonan I regions of plant cell walls (Figs. 8 and S5). GH105 enzymes act as unsaturated rhamnogalacturonyl hydrolases, cleaving oligosaccharides produced by pectate lyases, while GH106 α-L-rhamnosidases hydrolyze terminal α-L-rhamnose residues from a range of glycoconjugates including pectin and flavonoid glycosides (Fig. S5). Together, these enzymes facilitate comprehensive pectin saccharification, which is a crucial step in biomass conversion for biofuel production, and enhance industrial processes such as fruit juice clarification and extraction of bioactive compounds. Similarly, GH28 polygalacturonases contribute to pectin breakdown by hydrolyzing homogalacturonan backbones, aiding in fruit ripening and plant cell wall softening, which has broad applications in food, agriculture, and textile industries (Fig. 8). In the realm of microbial interactions and medical biotechnology, GH25 lysozymes specialize in degrading bacterial peptidoglycan, thereby playing vital roles in bacterial cell wall remodeling, phage-mediated lysis, and antibacterial defense strategies (Figs. 9 and S6). Complementing these functions, GH33 sialidases remove terminal sialic acids from glycoconjugates, modulating host-pathogen interactions, viral infectivity, and serving as key antiviral drug targets (Figs. 9 and S6). Both families exhibit distinct but crucial activities in microbial ecology and infection control. GH38 α-mannosidases hold a pivotal role in glycoprotein maturation and lysosomal degradation, with mutations linked to lysosomal storage disorders such as α-mannosidosis (Figs. 10 and S7). These enzymes are key in glycoengineering applications to produce therapeutic glycoproteins with optimized properties. Finally, GH17 β-1,3-glucanases target β-1,3-glucans in fungal and plant cell walls, contributing to plant defense mechanisms and modification of cereal-based foods (Fig. 11), while GH32 invertases and fructanases facilitate carbohydrate metabolism by hydrolyzing fructose-containing polysaccharides like inulin and sucrose, supporting industrial production of sweeteners, bioethanol, and prebiotics (Figs. 11, 12, and S8).

Collectively, these selected glycoside hydrolase family enzymes illustrate a complementary network of enzymatic activities essential for the degradation and modification of diverse polysaccharides in nature and industry. Their structural diversity—from (α/α)_6 barrels in GH105 to β-propeller folds in GH32 and GH33 (Fig. S9)—and distinct catalytic mechanisms underpin their specialized roles in biomass conversion, pathogen defense, and biotechnological innovations.

Industrial and Biotechnological Potential of CAZyme-Encoding Microbial Taxa in Wild Plant Rhizospheres

Leveraging the multifaceted taxonomic elucidations delineated within Figs. 5, 6, and S3—in conjunction with the expansive corpus of scholarly literature substantiating microbial CAZyme functionality—the heterogeneous phylogenetic stratification of CAZyme-encoding microbial assemblages inhabiting the rhizospheric niches of *Moringa oleifera* and *Abutilon fruticosum* epitomizes an extraordinary biotechnological repository replete with enzymatic capabilities for multitudinous industrial valorization (Lewin *et al*. 2016; Bano *et al*. 2021; Tashkandi and Baz, 2023; Deng *et al*. 2024).

In the soil around *M. oleifera* roots, Actinobacteria—mainly *Streptomyces* and *Micromonospora* species—are the most common bacteria and carry large sets of genes for

GH105, GH106, GH25, GH28, and GH38 enzymes (Figs. 5 and 6). These bacteria are becoming important sources for making enzymes used in biotechnology and medicine. *Streptomyces* bacteria show remarkable enzyme diversity, containing up to 86 different GH-families in their genomes, and they produce enzymes outside their cells just as well as the industrial standard *Trichoderma reesei*. The GH105 and GH106 enzymes from these bacteria are essential for making pectic oligosaccharides used in drug delivery systems and for activating flavonoid compounds, while GH38 α-mannosidases are key tools for engineering therapeutic proteins with better drug properties (Lewin *et al*. 2016; Lacombe-Harvey *et al*. 2018; Tashkandi and Baz 2023; Mufida *et al*. 2024; Rawat *et al*. 2024b). In contrast, the soil microbes around *A. fruticosum* are rich in Proteobacteria, especially *Pseudomonas* and *Bradyrhizobium* species, which contain GH17, GH32, and GH33 enzymes important for bioenergy and medical diagnostics. *Pseudomonas putida* bacteria have particularly useful genetic characteristics including high GC content (61% to 63%), flexible metabolism, and natural resistance to toxic compounds—features that make them excellent platforms for integrated bioprocessing. GH17 β-glucanases from these bacteria help break down plant biomass, GH32 invertases convert fructan sugars into fermentable compounds for bioethanol production, and GH33 sialidases help make antiviral drugs (Weimer *et al*. 2020; Chauhan *et al*. 2023; Zhou *et al*. 2024; Silva *et al*. 2025). Firmicutes bacteria, including *Bacillus* and *Clostridium* types, add more enzyme capabilities through their GH25 and GH28 activities. *Bacillus* species are recognized as safe organisms that control about half of the world's industrial enzyme market and are excellent at producing enzymes outside their cells, including heat-stable α-amylases and protein-digesting enzymes used in food processing, textile production, and antimicrobial products (Adrio and Demain 2014; Gavande *et al*. 2021; Harirchi *et al*. 2022). Fungal microorganisms—mainly from Ascomycota and Basidiomycota groups—provide additional enzyme functions. Ascomycete fungi specialize in enzymes that process sulfur and phosphorus compounds, while Basidiomycete fungi are known for powerful lignin-degrading enzyme systems. Scientists have identified 295 different "Function;Family" CAZyme patterns across fungal genomes, showing their versatility for applications in biofuel production, nutritional supplements, and drug manufacturing (Maciel and Ribeiro 2010; Barrett *et al*. 2020; Wan Mohtar *et al*. 2022; Manici *et al*. 2024).

The pronounced accumulation of CAZyme-encoding genetic elements within rhizospheric microbiomes reinforces the theoretical paradigm that native, plant-associated microbial consortia represent scalable, taxonomically diverse reservoirs for industrial and biotechnological utilization. Their inherent enzymatic complexity, ecological niche-adaptation, and phylogenetic plurality provide a resilient and multifaceted platform for the advancement of next-generation bioprocessing systems applicable to pharmaceutical synthesis, renewable energy generation, innovative food technologies, and ecological detoxification strategies (Levasseur *et al*. 2013; Lewin *et al*. 2016; Chettri *et al*. 2020; Weimer *et al*. 2020; Pantigoso *et al*. 2022; Cheng *et al*. 2023).

CONCLUSIONS

The comprehensive metagenomic analysis of rhizospheric microbiomes associated with *Moringa oleifera* and *Abutilon fruticosum* has revealed distinct CAZyme repertoires with extraordinary potential for industrial and biotechnological applications.

1. The plant species-specific enrichment of glycoside hydrolase families—with *M. oleifera* harboring GH105, GH106, GH25, GH28, and GH38, while *A. fruticosum* exclusively contains GH17, GH32, and GH33—represents a valuable biological resource for next-generation bioprocessing technologies. These enzymes demonstrate versatile applications across multiple sectors: pharmaceutical manufacturing through therapeutic glycoprotein engineering and drug delivery systems, sustainable biofuel production *via* enhanced lignocellulosic biomass conversion, food and beverage processing through natural preservation and clarification, and advanced biomaterial development for tissue engineering applications.
2. The taxonomic diversity of carbohydrate-active enzyme (CAZyme)-harboring micro-organisms, including specialized Actinobacteria, Proteobacteria, and fungal lineages, provides scalable platforms for enzyme production. This study has established wild plant rhizospheric microbiomes as untapped reservoirs for biotechnological innovation, offering sustainable alternatives to conventional industrial processes while supporting the development of precision bioprocessing technologies across pharmaceutical, energy, food, and environmental sectors.

SUPPLEMENTARY DATA CAN BE ACCESSED AT:

https://drive.google.com/drive/folders/1R93l5aLZhfOCJxQ-2qv-9nWqyKrNoMcE?usp= share_link

Data Availability Statement

All generated raw sequencing data was systematically deposited at the European Nucleotide Archive (ENA), an internationally recognized repository, under the following designated accession numbers: ERR10100770–72 (rhizosphere samples) and ERR10100773–74/ERR10100781 (bulk soil samples) for *M. oleifera*, and ERS15580318–20 (bulk soil samples) and ERS15580321–23 (rhizosphere samples) for *A. fruticosum*.

ACKNOWLEDGMENTS

The authors thank the University of Hail for its support. We wish to clarify that our use of artificial intelligence, specifically the Perplexity application, was exclusively for enhancing the precision and polish of the English language in our manuscript and not for content generation or for the development of scientific concepts. This distinction is especially significant given that, to the best of our knowledge, no prior published work in the literature has provided an integrative and comprehensive examination of all the enzyme

classes discussed in this study within a single manuscript. Consequently, our submission represents an original synthesis, and AI-assisted tools were employed to improve linguistic clarity for scientific communication.

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Article submitted: September 23, 2025; Peer review completed: October 25, 2025; Revised version received and accepted: November 8, 2025; Published: December 8, 2025.
DOI: 10.15376/biores.21.1.706-749