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Research Article

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Original Research Article

Genome analysis and CAZy repertoire of a novel fungus *Aspergillus sydowii* C6d with lignocellulolytic ability isolated from camel rumen

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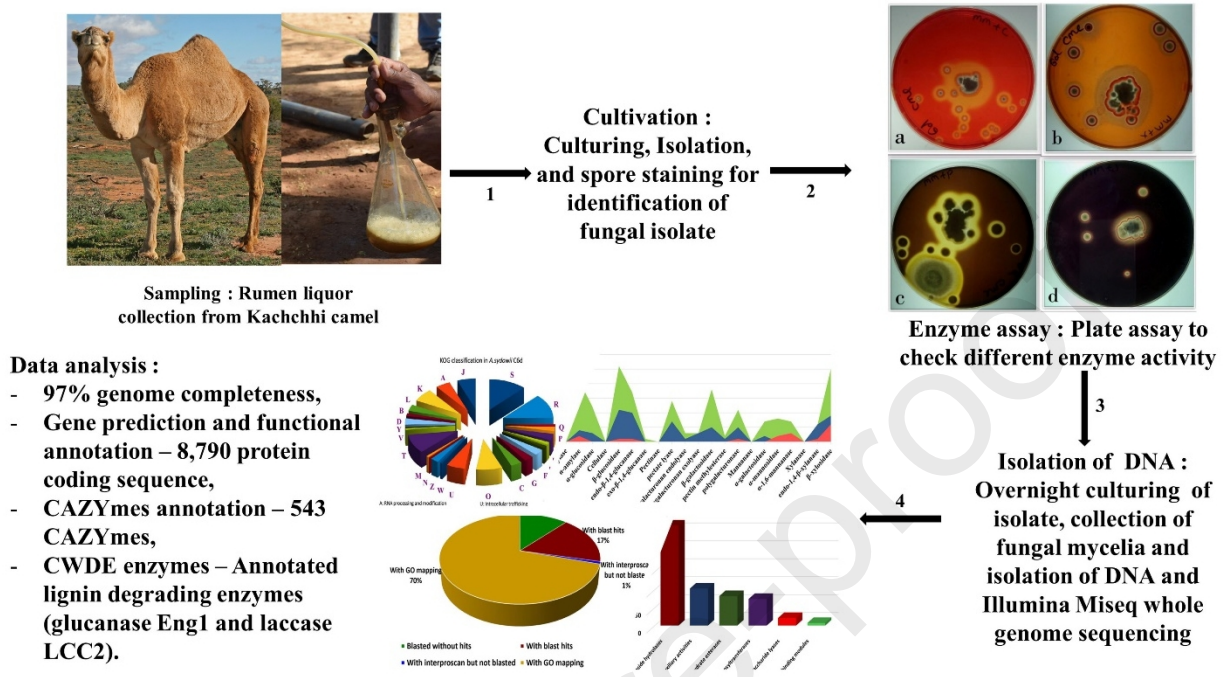
Abstract

Background: Camels are known for their survival under harsh and nutrient deficient climates. Camel rumen ecosystem presents a unique opportunity to study the ruminal microbes helping the camel in this task. The genus *Aspergillus* is extensively studied filamentous fungus due to its ability to secret industrially important enzymes. The present study was aimed to isolate and characterize microbes with lignocellulolytic capacity from camel rumen.
Results: The fungal isolate *Aspergillus sydowii* C6d, isolated from camel rumen was sequenced and analysed for its CAZyme content responsible for lignocellulose degradation. The C6d isolate was evaluated for its capacity to produce cellulase, pectinase, xylanase, and amylase with their respective assays and further evaluated for their optimum pH. The genome sequencing and assembly resulted in 32.27 Mb of genome size with a GC % of 50.59. The CAZyme analysis revealed that the C6d produced 543 polysaccharide degrading CAZymes amongst which, 148 CAZyme were potentially involved in lignocellulose degradation. The genomic comparison of the C6d with 30 commonly used lignocellulolytic fungi (white rot, brown rot, and soft rot fungus), showed enrichment of cellulolytic and pectinolytic CAZymes in C6d genome as compared to others. The saccharification of lignocellulosic substrate wheat straw resulted in release of 50.85% of reducing sugars.
Conclusions: The study provides important insights into the CAZymes responsible for lignocellulolytic ability in the novel fungus *Aspergillus sydowii* C6d isolated from camel rumen and presents a valuable source of CAZymes to be further evaluated for potential biotechnological applications.

Graphical abstract



Keywords: *Aspergillus sydowii;* Camel rumen; CAZymes; Filamentous fungus; Genome analysis; Genome sequencing; Glycoside hydrolase; Lignocellulolytic ability; Novel fungus; Ruminal microbes; Saccharification.

1. Introduction

The ruminant gastrointestinal tract harbours several symbiotic organisms, which helps the host animal by converting non-digestible feedstuff into readily absorbable nutrients. The camels are adapted to live in harsh and dangerous ecosystem like desert, due to their excellent water retaining capacity and ability to almost eat and digest any plant material. Unlike ruminants, the digestive system of the camel (pseudo-ruminant) consists of only three chambers with no omasum [1]. Amongst the three chambers, the forestomach of camel acts as a fermentation vat with complex microbial community consisting of bacteria, archaea, protozoa, and fungi [2]. Very few studies have reported the characterization of the camel rumen microbiome and profiling of carbohydrate-active enzymes (CAZymes) of the camel rumen [3,4,5,6]. The camel can digest variety of woody shrubs and low nutrient plants not favoured by majority of ruminants, with the help of ruminal microbes. Further, it has been reported that particle retention time is longer in *Camelus dromedarius* than other ruminants [7]. Consequently, this may put the camels at an advantage as compared to the other ruminants with respect to degradation of lignocellulosic biomass.

The plant skeleton mainly contains cellulose and hemicellulose, which constitutes the two largest regenerative organic resources on earth. Lignocellulose biomass typically consists of cellulose and hemicellulose varying in composition [8]. Now-a-days, use of single

microorganism with ability to produce cocktail of enzymes for the production of ethanol from lignocelluloses is considered a promising approach for sustainable biofuels production [9,10]. The utilization of lignocellulosic by-products or agro-industrial wastes for the production of ethanol with help of microbes is very attractive scenario [9,11]. Degradation of agricultural wastes such as wheat straw requires the combined action of several enzymes. Moreover, several industries like, bio hydrogen production industry, detergent industry, animal feed industry etc., requires mixtures of enzymes rather than a single enzyme [12,13]. This requires use of multiple microbes producing several CAZymes or single microbe with producing cocktail/mixture of lignocellulolytic enzymes.

Microbial enzymes are more economical, easier, and faster in production than the enzymes of plant and animal origin. The fungi are generally more preferred over other microorganisms, due to better yield and production of diverse set of GH enzymes [14]. Industrially, fungal enzymes have been widely used for production of biofuel, paper and pulp, detergents, textiles [15], beverages, wine and in food and feed industries [16,17]. Filamentous fungi are most efficient in yielding cocktail of various enzymes when encountered with lignocellulose biomass [18]. *Aspergillus* is the most widely studied filamentous fungi due to its extensive set of enzymatic cocktail for degradation of lignocellulosic biomass [19]. Several studies have reported fungi involved in lignocellulolytic degradation [20,21,22], however, very few studies have attempted isolation of lignocellulolytic fungi from rumen of Indian camel. In the present study, culture-dependent method was applied to isolate a novel fungal strain C6d from the camel rumen liquor, followed by its genome sequencing and CAZyme analysis for the presence of lignocellulolytic enzymes. Further, fungal culture was used to evaluate degradation of lignocellulosic biomass into fermentable sugars.

2. Materials and methods

2.1 Sampling and cultivation

Kachchhi camels were maintained on a specific diet to enrich cellulolytic microbes at the National Research Centre on Camel, Bikaner, Rajasthan. Rumen liquor was collected aseptically using probang from camel under mild sedative conditions, as described elsewhere [7]. Collected sample was serially diluted and 10^{-1} dilution was inoculated on plates containing minimal medium with 50 µg/mL of ampicillin and 1% (w/v) of substrates (starch, carboxymethyl cellulose, xylan birchwood, tributyrin, pectin, and Azure B) as a sole carbon source. Plates were incubated at 30°C for 4-5 d to obtain fungal isolates. The isolates were further screened for their cellulolytic, amylolytic, pectinolytic and xylanolytic activities [23,24]. Further, the isolates were identified based on colony morphology as well as microscopic characteristics of spore shape and spore stalk. The fungal isolate with good enzymatic activity on the plates was selected for further study.

2.2 Enzyme assay

Fresh conidiospores of the selected fungal isolate (hereafter denoted as C6d) were inoculated into 200 mL flasks containing optimized medium (ammonium sulphate 1.4 g/L, KH_2PO_4 2 g/L, $MgSO_4 \cdot 7H_2O$ 0.3 g/L, $CaCl_2$ 0.3 g/L, ammonium nitrate 2.3 mg/L) and

incubated at 30°C and 120 rpm. The supernatant was harvested on 1, 2, 3, 4, 5, and 7 days after inoculation to measure specific enzymatic activities. The enzyme assays for Cellulase, Xylanase, Amylase and Pectinase enzymes were performed by the DNS (3,5-dinitrosalicylic acid) assay to estimate the amount of reducing sugars liberated after hydrolysis [25]. The reaction mixtures containing 50 µl of 1% substrate solution (CMC, xylan, starch, and galacturonic acid), 50 µl of supernatant and 100 µl buffer (pH 4, 4.5, 5, 5.5 to 8.0) were incubated at 30°C for 30 min. Subsequently, DNS was added to stop the reaction and samples were boiled for ten minutes, cooled and the optical density was measured at 540 nm. One unit of enzymatic activity (U) was defined as amount of enzyme that catalyses the transformation of one micromole of substrate per minute under specified condition [26].

2.3 Saccharification of wheat straw

Lignocellulosic substrate wheat straw (100 gm) was saccharified in an incubator shaker at 120 rpm and 30ºC for 48 h. Pre mashed substrate with suitable particle size (1–2 mm) was autoclaved at 121ºC for 15 min, washed with distilled water until the pH was neutral and dried in an oven. Substrate was suspended in 50 mM sodium citrate buffer (pH 5), supplemented with a with 5 ml spore suspension (10^7 spores) and kept for incubation. The supernatant was used to estimate reducing sugars using the DNS reagent. Degree of saccharification (DoS) was calculated using **Equation 1** [27]:

D_OS = cvf1/mf2 X 100

Equation 1

where c is the sugar concentration in the hydrolysate estimated as total reducing sugars, in mg/ml; v is the liquid volume of the hydrolysates, in ml; f1 is the factor (0.90 for hexoses) used to convert monosaccharide to polysaccharide due to water uptake during hydrolysis; m is the amount of initial substrate dry weight, in mg; and f2 is the factor for the carbohydrate content of the substrate (total carbohydrate, mg/total substrate, mg).

2.4 Whole genome sequencing

Approximately, 10^7 of fungal spores were inoculated in 200-ml flasks containing potato dextrose broth (PDB) and incubated for 5 d at 30°C and 150 rpm. The genomic DNA was extracted from harvested fungal mycelia by standard phenol-chloroform DNA extraction procedure. The genomic DNA was quantified and normalized to 0.2 ng/µl, using a Quant-iT™ dsDNA assay (Thermo Fisher Scientific). The DNA was further processed for the library preparation using the Illumina's Nextera XT index kit (Illumina, SD) following the manufacturer's instructions. After confirmation of library size with an Agilent 2100 Bioanalyzer high Sensitivity Kit (Agilent, USA), sequencing was carried out on Illumina MiSeq desktop sequencer using 2 × 250 bp chemistry.

2.5 Data pre-processing and Genome assembly

Raw reads were evaluated using FastQC and further curated using Prinseq [28] to remove sequencing adapters and bases having phred score (Q) less than 25. The high quality

reads were retained and assembled using SPAdes v3.1.1 with default parameters [29]. The integrity of the genome assembly was evaluated using FGMP (Fungal Genome Mapping Project; https://github.com/stajichlab/FGMP) [30].

2.6 Phylogenetic analysis

For the phylogenetic analysis, the ITS database of fungi was retrieved from NCBI and a local blast of assembled contigs was done. Further, ITS sequences of selected species of *Aspergillus* genera were downloaded, and multiple sequence alignment was done with ITS of isolated fungus. A phylogenetic tree was constructed with MEGAX using the Maximum Likelihood method and Tamura-Nei model [31].

2.7 Gene prediction and functional annotation

The gene prediction was performed using AUGUSTUS software v3.2.2 [32] with default parameters in gene find parameter. Sequence similarity search was performed for de novo assembled contigs against National Centre for Biotechnology Information (NCBI) non-redundant protein (NR) database with an E value cut off of 1e−6 and blast hits up to 10 sequences. The functional assignment of coding sequences was performed using Blast2GO Pro Ver. 4.0.7 [33] and InterproScan v5.25–64.0 [34]. Further, predicted protein sequences were used for searching and determining the motifs and protein domains with InterProScan v5.25–64.0 against InterPro databases. In addition, predicted genes were assigned to cluster of Orthologous Groups of proteins (COG) and Eukaryotic orthologous group (KOG) of proteins using online WebMGA server [35].

2.8 CAZyme annotation and comparison

The CAZyme annotation was done using dbCAN server [36]. The CAZymes were classified according to the type of reaction: glycoside hydrolases (GH), glycosyl transferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE) and carbohydrate-binding modules (CBM) [37]. Further, CAZymes were analysed for presence of signal peptide cleavage sites and transmembrane helices, using SignalP v.4.1 [38] and the webserver TMHMM v.2.0 [39], respectively. For the identification of cell wall degrading enzymes (CWDE), a local blastp analysis was executed against PhiBase 4.5 database (http://www.phi-base.org).

Comparison of CAZys of the C6d with widely used fungi from three different group of lignocellulolytic was performed. For this, annotated genomes of (a) 10 popular white rot fungal strains – *Ceriporiopsis subvermispora* B, *Heterobasidion annosum* v2.0, *Fomitiporia mediterranea* v1.0, *Phanerochaete carnosa* HHB- 10118**,** *Pycnoporus cinnabarinus* BRFM 137**,** *Phanerochaete chrysosporium* R78 v2.2**,** *Dichomitus squalens* LYAD-421 SS1, *Trametes versicolor* v1.0**,** *Punctularia strigosozonata* v1.0 and *Phlebia brevispora* HHB-7030 SS6; (b) 10 popular brown rot fungal strains – *Postia placenta* MAD 698-R v1.0, *Fibroporia radiculosa* TFFH 294, *Wolfiporia cocos* MD-104 SS10 v1.0, *Dacryopinax primogenitus* DJM 731 SSP1 v1.0, *Daedalea quercina* v1.0, *Laetiporus sulphureus* var v1.0, *Postia placenta* MAD-698-R-SB12 v1.0, *Neolentinus lepideus* v1.0, *Serpula lacrymans* S7.9 v2.0, *Calocera cornea* v1.0 and (c) 10 popular soft rot fungal

strains – *Trichoderma reesei* v 2.0, *Rhizopus oryzae* 99-880, *Aspergillus wentii* v1.0**,** *Penicillium chrysogenum* Wisconsin 54-1255, *Daldinia eschscholzii* EC12 v1.0, *Hypoxylon* sp. CI-4A v1.0, *Aspergillus niger* ATCC 1015 v4.0, *Hypoxylon* sp. EC38 v3.0, *Hypoxylon* sp. CO27-5 v1.0, *Neurospora crassa* OR74A v2.0, were retrieved from the JGI (Joint Genome Institute) MycoCosm database. Genome-level annotations of the selected fungal strains especially CAZy were retrieved from the JGI-MycoCosm database (https://mycocosm.jgi.doe.gov/mycocosm/).

3 Results

3.1 Cultural isolation and identification

Five carbohydrate degrading fungal isolates (C4E, P7E-6, P7E, C6d, and X1F-2) were isolated from camel rumen liquor. To obtain isolates with multi substrate activities, the isolates were incubated in liquid medium supplemented with the respective substrates at 30°C for 7 d followed by DNS assay. Amongst all isolates, C6d exhibited higher multi-substrate activity on the plate as well as DNS assay **(Fig. S1, Table 1).** Accordingly, the isolate C6d was selected for further analysis using genome sequencing. Based on colony morphology, microscopic characteristics of the spores and ITS analysis, C6d fungal isolate was identified as *Aspergillus sydowii* **(Fig. S2a and Fig.S2b).** Maximum Likelihood tree inferring the relationship of the ITS of C6d with other lignocellulolytic fungi, showed closest species as *A. sydowii* **(Fig. S3).**

3.2 Genomic features of the C6d isolate

Whole genome sequencing generated 731,483 raw reads with an average read length of 227 bp corresponding to 375.4 Mbp of data. Quality filtering with Prinseq retained 340 Mbp of data, further subjected to de novo assembly using SPAdes v3.1.1. The assembly resulted into genome size of 32.27 Mbp with largest contig of 43,950 bp and an N50 of 6905 bp. The GC content of the *A. sydowii* C6d genome was 49.39% for predicted protein-coding genes and 50.59% of the assembled whole genome. The assessment of genome completeness by FGMP showed 97% genome completeness with 93% conserved DNA segments **(Table 2).**

3.3 Genome annotation

Ab initio gene prediction using AUGUSTUS software v3.2.2 revealed a total of 8,790 protein-coding sequences in the C6d genome. To get an insight into the biological processes in C6d isolate, predicted genes were subjected to gene ontology (GO) analysis using Blast2GO Pro v4.0.7. As a result, 6,182 (70.32%) were mapped to the NCBI non redundant database with GO assignment, while 17.26 % and 11.5% of the sequences remained unassigned and unmatched, respectively **(Fig. S4)**. The majority of hits were observed from *Aspergillus* (98.21%), followed by few hits (1.77%) from other genera. Within the *Aspergillus* genus, the maximum number of hits (11,616, 87.22%) were observed against *A. sydowii* CBS 595.63 **(Fig. 1)**, further substantiating results of morphology and ITS analysis.

In terms of biological processes, most of the genes were involved in organic substance metabolic processes, primary metabolic process, and cellular metabolic processes. In the cellular component category, most annotated categories were membrane, intrinsic components of membrane, organelles, and intracellular organelle, while, categories of ion binding, organic cyclic compound binding, hydrolase binding, and DNA binding transcription factor activity were found to be enriched in molecular functions **(Fig. 2).** Further, to complement the GO annotation process, the protein-coding genes were annotated in terms of COG and KOG classifications. In total, 3816 and 5384 genes were assigned COG and KOG classifications out of 8790 protein-coding genes. In COG classification, the group of Unknown function (1347) formed the highest cluster, followed by General function prediction (702) and Amino acid transport and metabolism (270), while, Cytoskeleton, Nuclear structure, and Extracellular structure categories represented the smallest groups (**Fig. 3, Table S1**). Amongst 25 annotated KOG categories, most abundant class belonged to general function prediction (687), followed by function unknown (651) and Signal transduction modification (539), while the extracellular structures (40); Nuclear structure (33), and Cell motility (10) represented smallest groups (**Fig. S5, Table S2).**

3.4 CAZymes production potential of C6d

Using the CAZy database, 543 CAZymes were functionally annotated, including 255 glycoside hydrolase (GH), 103 auxiliary activities (AA), 74 glycosyltransferases (GT), 21 polysaccharide lyases (PL), 82 carbohydrate esterase (CE) and 8 carbohydrate binding module (CBM). The most representative families were CE10 (48), GH43 (24) and GT2 (13) (**Fig. S6**). A total of 255 CAZymes, specifically involved in degradation of cellulose, hemicellulose, pectin and starch, were analysed for presence of transmembrane helices and signal peptide, which revealed 24 (9.45%) and 95 (37.25%) CAZymes, respectively (**Fig. S7** and **Table S3)**.

Total eight GH families encoding cellulase were annotated from CAZy database in fungal genome. Amongst these, GH-5 encoding endocellulases, GH-6 and GH-7 encoding cellobiohydrolases as well as GH-1 and GH-3 encoding β-glucosidases were detected. Also, GH10, GH11 and GH30 families encoding for xylan degradation; GH2, GH5 and GH26 constituting β-mannanase and β-mannosidase and GH28, PL1, PL3, PL4 and CE8 for pectin degradation were detected. Additionally, α-glucosidase (GH31), α-amylase (GH13), α-glucoamylase (GH15) and N-terminal starch binding modules (CBM21) were also annotated (**Table S4**).

The annotation of auxiliary activity families in C6d revealed the presence of multicopper oxidase (AA1_2 and AA3_3), lignin-modifying peroxidases (AA2) including lignin peroxidases (LiP) cellobiose dehydrogenases containing an iron reductase domain (AA8-AA3_1), benzoquinone reductases (AA6), versatile peroxidases (VP), manganese peroxidases (MnP), galactose oxidase (AA5_2) and glucose-1 oxidase (AA3_2). Additionally, annotations of multicopper oxidases (AA1_3), aryl alcohol oxidases (AA3_2), glucooligosaccharide oxidases (GOO) (AA7) and copper dependent lytic polysaccharide monooxygenases (LPMOs) (AA9-AA11-AA13) were also reported **(Table S5).**

3.5 Comparison of lignocellulolytic CAZymes with other fungi

The C6d CAZymes were compared with ten white rot, ten brown-rot and soft rot fungi which are prominently involved in lignocellulose degradation. Overall, among thirty-one fungi analysed, the number of AA (103), PL (21) and CE (82) classes of CAZy observed in C6d were found to be in similar proportions as compared to others (**Fig. S8**). Further, we calculated the total number of genes involved in ligninolytic, cellulolytic, hemicellulolytic and pectinolytic abilities based on the annotations provided in JGI-MycoCosm database. The results revealed that, highest ligninolytic ability was observed in *Phanerochaete chrysosporium R78* and *Trametes versicolor* (white rot fungi), *Laetiporus sulphureus* (brown rot fungi) and *Hypoxylon* sp. EC38 (soft rot fungi) respectively. Similarly, the highest cellulolytic ability was observed in *Phanerochaete carnosa* (white rot fungi), *Neolentinus lepideus* (brown rot) and *Hypoxylon sp.* EC38 (soft rot fungi) respectively. Highest hemicellulolytic ability was observed in *Punctularia strigosozonata* (white rot fungi), *Dacryopinax primogenitus* (brown rot fungi) and *Hypoxylon sp.* EC38 and *Hypoxylon sp*. CO27-5 (soft rot fungi) respectively. Overall, highest pectinolytic ability was observed in camel rumen C6d amongst all fungi, while it was also higher in *Punctularia strigosozonata* (white rot fungi), *Fibroporia radiculosa* TFFH 294 (brown rot fungi) and *Aspergillus niger* ATCC 1015 v4.0 (soft rot fungi) respectively **(Fig. 4).**

3.6 Cell wall degrading enzymes (CWDE) from PHI database analysis of C6d

The predicted protein sequences were searched against the Pathogen-Host Interaction Database (PHI database) for the genes responsible in plant cell wall degradation. Annotated genes shared PHI homology with plant cell wall degrading enzyme specifically pectin-acting polygalacturonase (PG). These enzymes are secreted at early stages of contact between pathogenic fungi and plant cell walls. Also, homology was identified with plant polysaccharides and lignin degrading enzymes, particularly endo-1,4-beta-xylanase GH10, glucanase Eng1 and laccase LCC2. CWDEs, mainly include pectinase, glycosyl hydrolase and laccase which are primary weapons of fungal attacks causing the plant cell wall to become less compact **(Table S6).**

3.7 Saccharification of wheat straw

The crude enzymes from C6d efficiently saccharified lignocellulosic substrate wheat straw. It was showed that pre-treated substrates yielded more reducing sugar (50.85%, 48 h) than untreated substrates. The results indicated the suitability of the C6d in pre-treatment of lignocellulosic biomass to yield reducing sugar, which can be utilized in biofuel production.

4. Discussion

The camels were chosen as experimental animal in this study due to their ability to survive in harsh environmental condition with very limited food and water and comparatively more efficient fore-stomach digestion as compared to other ruminants. Complex fungi residing inside the gastrointestinal tract of ruminants, are gaining the attention of scientists across the globe for playing a key role in the degradation of plant biomass through diverse

enzymatic strategies. Fungal genome possesses multi-gene (mostly cellulase and hemi-cellulase) machinery to effectively degrade lignocellulose [40]. Physiologically, in association with other rumen bacteria, fungi colonize on the lignocellulose-rich plant cell walls, inside the rumen, and produce catalytic and non-catalytic proteins to degrade plant materials [41]. In this study, the fungus *Aspergillus sydowii* C6d isolated from camel rumen liquor was identified and screened for multiple enzyme activities. Further, whole-genome sequencing was done to gauge the ability of the C6d to produce CAZymes involved in lignocellulose degradation.

The camel rumen is anoxic environment mostly dominated by the anaerobic organisms. Still, several aerobic bacteria and fungi are present in the rumen which also aids in the rumen fibre degradation. There are several reports in the literature, where researchers have reported the presence of aerobic microbes in the rumen. Earlier study reported predominance of *Aspergillus spp*. and *Pichia kudriavzevii* fungi among isolates from the rumen of dairy cattle fed tropical forages [42]. Also, similar findings were reported where authors have concluded that rumen is initially dominated by the aerobic microbes which are gradually replaced by the anaerobic microorganisms, but still there are presence of several aerobic microbes amongst predominantly anaerobic microbes [43,44].

A. sydowii C6d was tested for its capacity to produce lignocellulolytic enzymes i.e. cellulase, pectinase, xylanase and amylase, using both plate as well as liquid assay to check its candidature for biofuel production [45,46]. The activities observed for C6d cellulase, pectinase, xylanase, and amylase were 0.357, 0.849, 0.695 and 0.386 U /ml, respectively. The observed activities were lower as compared to other such studies involving measurement of multi-enzymes (cellulase, pectinase, xylanase) produced by *A. niger* [22] or amylase from *A. terreus* [47]. However, the quantification of enzyme activities depends on final concentration of the enzyme as well as induction conditions on different substrates, which can be optimized further. The optimum temperature observed for various enzymes in C6d was 30°C, which is in accordance with the reported range of 25-30°C [48,49]. The pH plays an important role in the structural modifications of the active site of the enzyme, glycosylation of the enzymes and transport of molecules across the membrane. The optimal pH for enzymes produced by C6d was within the reported range of acidic pH (4.5 to 6.0) (**Fig. S9**) [14,50].

Saccharification of lignocellulosic biomass wheat straw by C6d showed that pre-treatment enhanced saccharification of wheat straw as compared to untreated substrates. Similar results were also reported in a recent study where pre-treatment with crude enzymes from five different fungi improved saccharification of grape stalks [51]. Pre-treatment of the lignocellulosic substrate is essential for efficient enzymatic hydrolysis because of the various physical and chemical barriers that greatly inhibit the accessibility of the polysaccharide to hydrolytic enzymes [52].

The Blast2GO analysis revealed 6.33%, 22.92%, 2.67% of genes responsible for DNA binding, ion binding, and protein binding, respectively in molecular function category (**Fig. 2**). These might be involved in gene transcription regulation and protein-folding transportation process [53]. According to COG database, 7.07% predicted genes were involved in amino acid transport and metabolism, 6.76% in energy production and

conversion, 6.42% in translation, 6.23% in replication, 6.05% in transcription and 6.02% in carbohydrate transport and metabolism as the gene-rich classes in the COG groupings. The enrichment of this categories in the C6d suggests presence of diverse protein and energy metabolism functions and in turn its potential in transformation of nutrients from substrates present in rumen environment [54].

Cellulases, hemi-cellulases, pectinases and LPMOs reported in the C6d genome might serve as candidate genes to use as components of industrial cocktail involved in biomass degradation [55]. The various CAZy families (CBM21, CE5, GH2, GH12, GH92, and GT34) were reported in C6d, which may play a role in breaking down the barrier of the plant cell-wall polysaccharides by using plant polysaccharides as a carbon source [56,57,58]. The presence of several important enzymes like GH5, β glucosidase, endoglucanase enzymes, endo-β-1,4-xylanase, β-1,4-D-xylosidase and cellobiohydrolase involved in lignocellulose degradation enables use of C6d for commercial applications [59,60,61,62]. The presence of GH 28 family comprising of exo and endo polygalacturonases, rhamnogalacturonases, and pectin lyase families 1, 3 and 4 in C6d indicated its role in the disruption of pectin backbone [63,64]. Furthermore, there were 8 genes annotated as cellulose-binding domain (CBM) in C6d, which facilitates the lignocellulose-degrading enzyme attachment to distinct regions on a polysaccharide substrate, such as cellulose fibres and starch granules, causing the substrate to loosen and become more exposed for efficient degradation of plant biomass [21,65].

Copper-dependent lytic polysaccharide monooxygenases (AA9, AA11 and AA13) were also identified in this study, which may augment the enzymatic decomposition of polysaccharides [66,67]. The C6d reported AA9 family of genes classified as GH61 glycoside hydrolases, which are believed to act directly on cellulose, rendering it more accessible to traditional CAZyme action [68]. The C6d also produced AA3_1 cellobiose dehydrogenases (CDH) and AA2 family of enzymes containing, class II lignin-modifying peroxidases with the ability to degrade and modify lignin polymers helping the fungus in oxidization of variety of phenolic compound [69,70,71]. Moreover, comparison of annotated CAZyme genes in C6d with 30 commonly used ligninolytic fungi, showed higher numbers of PL, CE, and AA families and second highest GH number in C6d, suggesting its promising role in catabolism of lignocellulose through hydrolysis of glycosidic linkages [37].

The identification of CWDEs by mapping against PHI database, indicated the mode of plant cell wall degradation in C6d is hemi biotrophic with the capacity to produce several enzymes which can easily digest plant polysaccharides. Several genes of C6d showed homology with PHI genes involved in lignin depolymerisation specifically polygalacturonases, endo-1,4-beta-xylanase, glucanase, and laccase, which cause the plant cell wall-less compact and enhances the degradation by cellulase and hemicellulase enzymes [72].

The camels are very efficient to survive in arid climates with very scarce nutrition. However, it is imperative to identify and characterize the rumen microbes helping them in this process to employ these microbes for further manipulation. The *Aspergillus* spp. are good producers of series of enzymes which can be employed in lignocellulose containing

plant biomass degradation. Here we have isolated *Aspergillus sydowii* C6d from camel rumen along with its genome sequencing for elucidation of CAZymes involved in lignocellulose degradation. The isolate C6d can produce enzymatic cocktail, which makes it suitable for its use in biofuel production. Additionally, the analysis revealed presence of a higher number of glycosyl hydrolase (GHs), carboxyl esterases (CEs) and auxiliary activity enzyme families (AAs), which assists in lignin decomposition and facilitates easy degradation of plant polysaccharides. Further, the isolate C6d is capable of saccharification of lignocellulose biomass, hence it can be used in production of reducing sugars to use in ethanol production. In conclusion, *A. sydowii* C6d serves as a valuable source, which can be exploited for CAZyme expression and manipulation to further study its applications in lignocellulose degradation and biofuel production.

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Ethical approval

The experiment was performed under the approval of the institutional animal ethics committee of the National Research Centre on Camel, Bikaner, Rajasthan.

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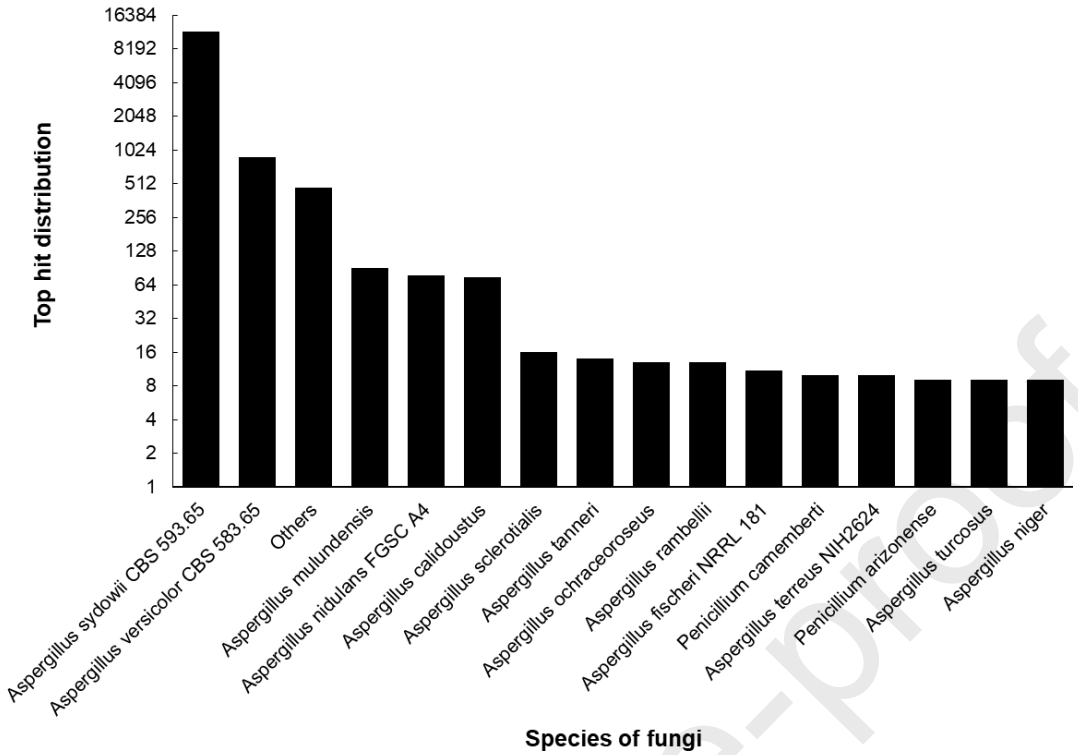


Fig. 1. Top hit distribution of C6D sequences against different fungal species. Distribution of sequences on y-axis have been transformed using the logarithm function.

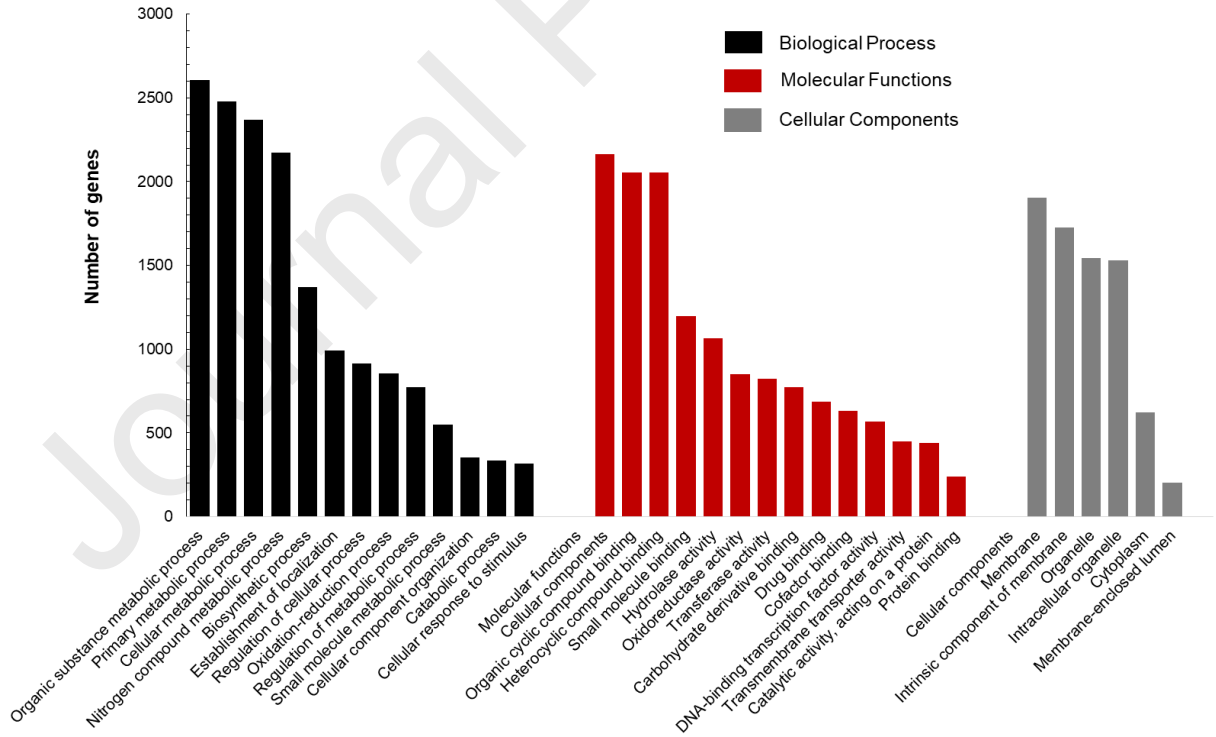
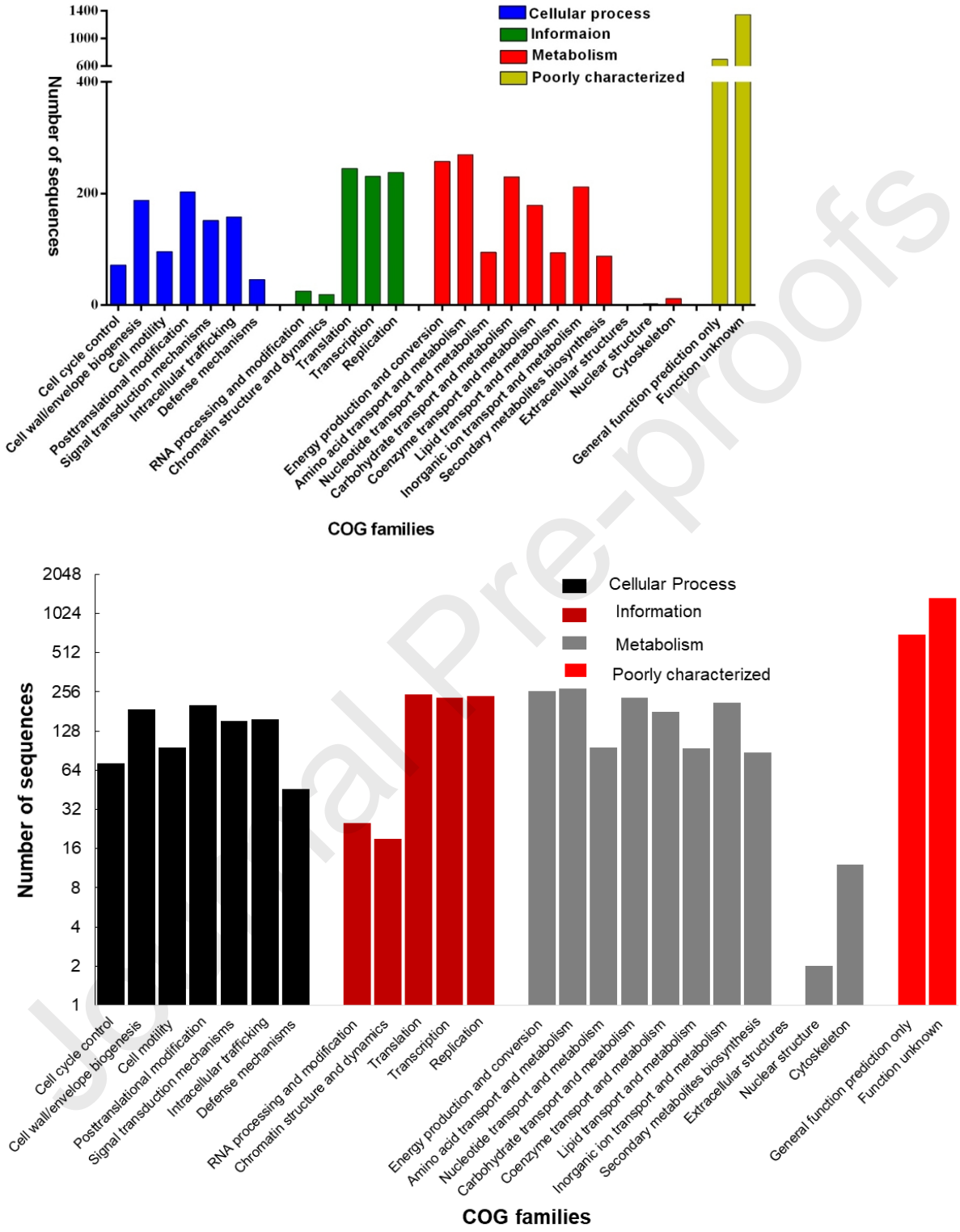


Fig. 2. GO classification of the C6d genome: The sequences were annotated by level 3 and grouped by three main functional categories. (GO terms with less than 200 annotated genes are not shown).



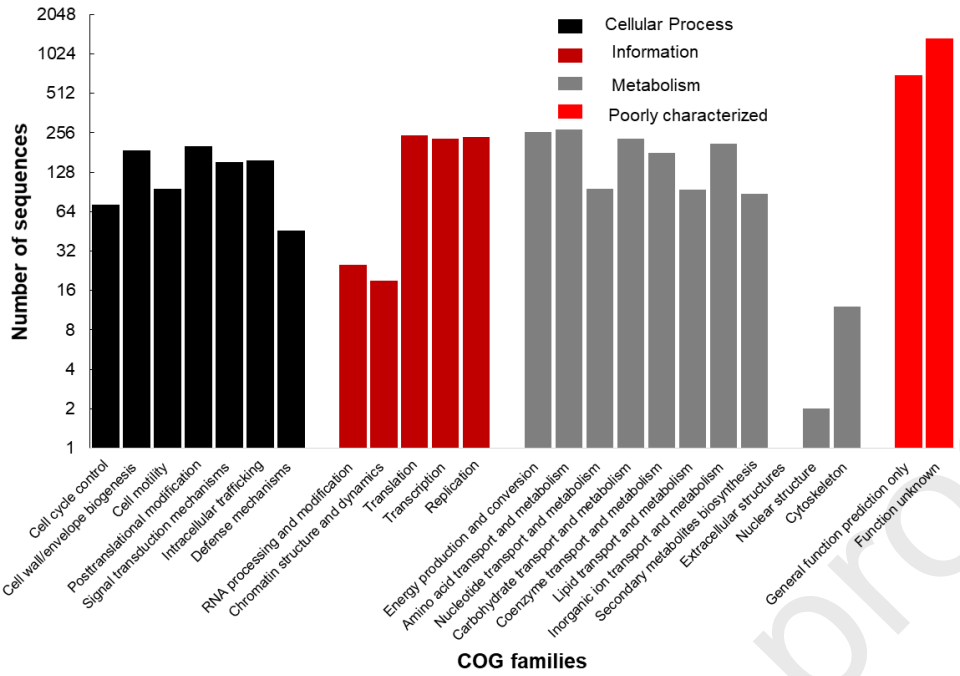


Fig. 3. The Clusters of Orthologous Groups classification in C6d isolate.

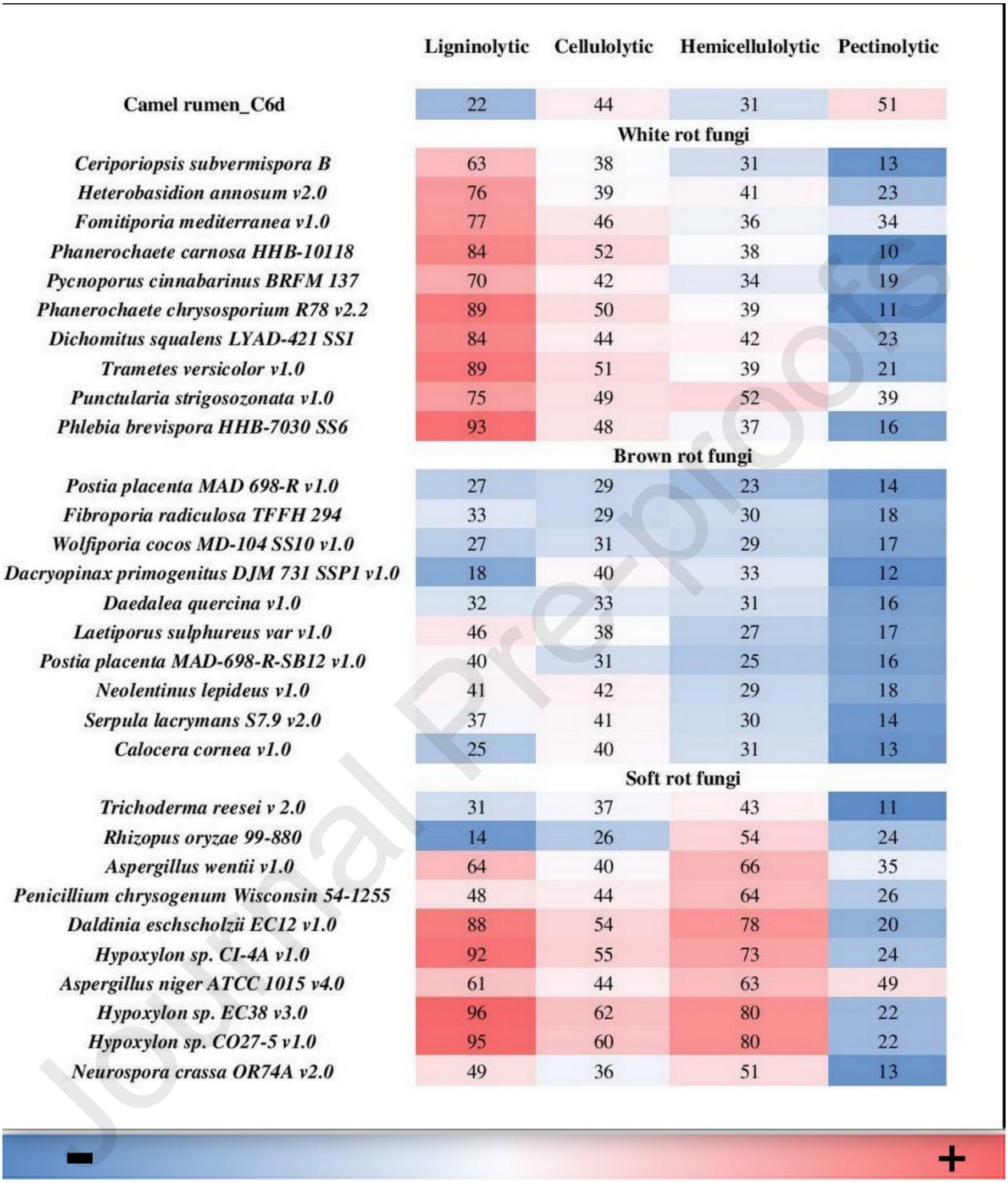


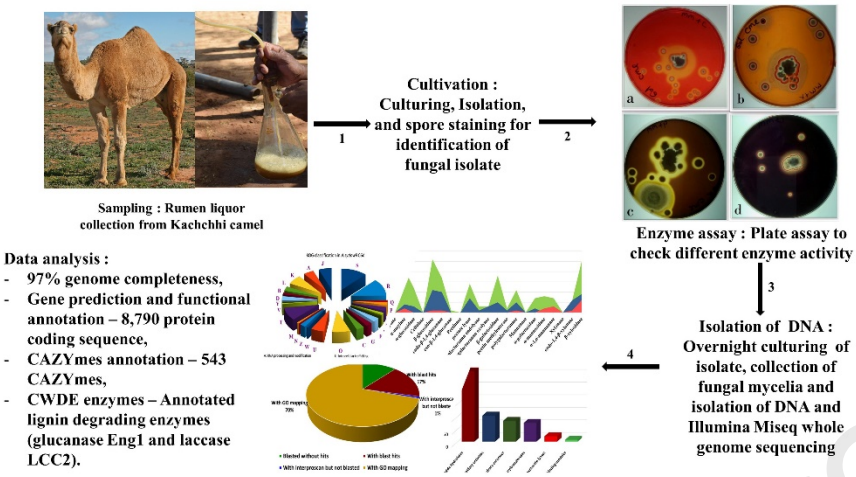
Fig. 4. Heatmap showing the genome-wide distribution of total ligninolytic, cellulolytic, hemicellulolytic and pectinolytic CAZymes in selected popular white rot, brown rot and soft rot and C6d fungi.

Table 1. Enzyme activities of five camel rumen fungal isolates on different substrates.

Fungal isolate	**Highest enzymatic activity reported (IU/L)**			
	Amylase	Cellulase	Pectinase	Xylanase
P3d2	No activity	73.21	86.94	No activity
P1d	No activity	91.08	82.71	No activity
P6d-3	87.36	68.94	75.31	No activity
C6d	71.40	70.45	98.19	88.39
P2dLE	No activity	No activity	71.36	No activity

Table 2. Genomic features of the *A. sydowii* C6d.

Attributes	**Numbers**
Raw reads	731,483
Clean reads	660,003
Number of bases (bp)	32,275,363
Number of contigs	7,181
Size of assembled genome (Mbp)	32.27
Average contig size (bp)	4,494
Largest contig size (bp)	43,950
N50 contig size (bp)	6,905
GC content of assembled genome	50.59%
Genome completeness	97%



Genome analysis and CAZy repertoire of a novel fungus *Aspergillus sydowii* C6d with lignocellulolytic ability isolated from camel rumen

Conflict of interest statement:

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the research paper.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process.

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