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ORIGINAL ARTICLE

Annotating Multiple Prebiotic Synthesizing Capabilities Through Whole Genome Sequencing of *Fusarium* Strain HFK-74

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Abstract

In the present study, seven fungal isolates from effluent treatment plants were screened for the production of prebiotic fructooligosaccharide synthesizing enzymes with the highest activity of fructofuranosidase (17.52 U/mL) and fructosyl transferase (18.92 U/mL) in strain HKF-74. Mining of genome sequence of strain revealed the annotation of genes providing multiple carbohydrate metabolizing capacities, such as amylases (*AMY1*), beta-galactosidase (*BGAL*), beta-xylosidase (*Xyl*), β-fructofuranosidase (*ScrB*), fructosyltransferase (*FTF*), and maltose hydrolases (*malH*). The annotated genes were further supported by β-galactosidase (15.90 U/mL), xylanase (17.91 U/mL), and α-amylase (14.05 U/mL) activities for synthesis of galactooligosaccharides, xylooligosaccarides, and maltooligosaccharides, respectively. In addition to genes encoding prebiotic synthesizing enzymes, four biosynthetic gene clusters (BGCs) including Type I polyketide synthase (PKS), non-ribosomal peptide synthetase (NRPS), NRPS-like, and terpene were also predicted in strain HKF-74. This was significant considering their potential role in pharmaceutical and therapeutic applications as well as in virulence. Accurate taxonomic assignment of strain HKF-74 by in silico genomic comparison indicated its closest identity to type strains *Fusarium verticillioides* NRRL 20984, and 7600. The average nucleotide identity (ANI) of strain HKF-74 with these strains was 92.5% which was close to the species threshold cut-off value (95–96%) while the DNA–DNA hybridization (DDH) value was 83–84% which was greater than both, species delineating (79–80%), and also sub-species delineating (70%) boundaries. Our findings provide a foundation for further research into the use of *Fusarium* strains and their prebiotic synthesizing enzymes for the development of novel prebiotic supplements.

Keywords *Fusarium* · Whole genome sequencing · Prebiotics · Carbohydrate-active enzymes

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Introduction

Prebiotics are a type of food ingredient that have gained considerable attention in recent years owing to their ability to support the growth and activity of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, while inhibiting the growth of harmful bacteria in the gut [1–3]. Though, prebiotics cannot be digested by the host enzymes, their fermentation into short-chain fatty acids (SCFA) by colonic bacteria decreases the pH of colon and thus provides many health benefits to the host such as diarrhea prevention, constipation alleviation, colorectal cancer protection, and anti-inflammatory, immune response activation [4–6].

Prebiotics are naturally present in various dietary fibers such as in banana, chicory, wheat, onion, asparagus, and more [7, 8]. However, these natural plant sources of prebiotics often have limited prebiotic content which makes the extraction process labor intensive, while the synthetic prebiotics are expensive to produce due to which there has been an increase in interest in identifying new sources of prebiotics [9]. Microbial sources including several fungi possess potential to secrete certain enzymes that can catalyze the synthesis of prebiotics more efficiently [10]. Recent studies have focused on screening fungal isolates to identify strains that are efficient in using sugars as a carbon source and generating enzymes such as fructofuranosidase and galactosidase [11, 12]. Fungi, especially *Aureobasidium pullulans, Aspergillus* sp., *Fusarium solani,* and *Penicillium* sp., are some of the most investigated microorganisms for prebiotic synthesis [12–14].

Research highlights the potential of the genus *Fusarium* to produce valuable enzymes and secondary metabolites with industrial and medicinal applications, expanding its utility in bioprocessing, biofuels, and pharmaceuticals [15, 16]. Notably, several species of *Fusarium* have shown promise in producing prebiotic oligosaccharides through its carbohydrate metabolism enzymes, converting complex carbohydrates into gut-friendly compounds as in the case of fructooligosaccharides (FOS) reported in *Fusarium* sp. [17], *F. oxysporum* [18], *F. solani* [13], and xylooligosaccharides (XOS) [19]. The pathogenicity of members of *Fusarium* genus to crops can have significant implications during their use for prebiotic production, which may require implementation of rigorous safety measures and strategies for managing/inhibiting mycotoxin production [20].

In recent years, advancements in genomic technologies have revolutionized the way microorganisms can be exploited for novel functionalities. Whole genome sequencing of microorganisms has allowed for a deeper understanding of the genetic basis of their metabolic and functional capabilities [21–23]. Genome mining is a popular method for scanning genome databases in search of novel enzymes, and the gene functions can be annotated through various databases [24–26]. In the case of fungi, genomic analysis has enabled the identification of genes responsible for the synthesis of various bioactive compounds, including prebiotics [11, 15, 27].

The focus of this research was to screen fungal isolates and investigate their ability to use sugars as a carbon source especially focussing on the generation of enzymes fructofuranosidase (FFase), fructosyltransferase (FTase), β-galactosidase, xylanase, and α-amylase for the production of respective prebiotic oligosaccharides. Further, we aimed to identify the genetic basis of the prebiotic synthesizing capabilities in the potent fungal strain through whole genome sequencing followed by functional annotation for different genes encoding the enzymes for the potential prebiotic oligosaccharide synthesis, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS),

xylooligosaccharides (XOS), and maltooligosaccharides (MOS). Our findings could ultimately lead to the development of novel prebiotic supplements that promote gut health and prevent disease.

Material and Methods

Chemicals

All chemicals, such as potato dextrose agar (PDA), sucrose, lactose, maltose, xylose, yeast extract, $NaNO_3$, NaH_2PO_4, Na_2HPO_4, $MgSO_4.7H2O$, K_2HPO_4, KH_2PO_4, NH_4Cl, NaCl, were procured from of Himedia (Mumbai, India).

Inoculum Development and Fermentation Medium

The methodology involved the transfer of a 0.5-cm-diameter disc of mycelial growth from a 5-day-old PDA plate to 100 mL conical flasks containing 20 mL inoculum medium [28]. This medium was supplemented with 1% carbon source (sucrose/xylan/lactose/maltose) depending on the targeted prebiotic synthesizing enzyme to be produced, and 0.2% yeast extract as an organic nitrogen source. The initial pH of the inoculum development medium was set at 5.5, and the flasks were incubated for 24 h in a shaker at 120 rpm and 28 ± 2 °C. For submerged fermentation, in a 250 mL conical flask, 80 mL fermentation medium [28] containing 20 % sucrose, 0.2 % yeast extract, 2 % $NaNO_3$, 0.03 % $MgSO_4.7H2O$, 0.4 % K_2HPO_4, 0.9 % KH_2PO_4, 0.5 % NH_4Cl, and 0.25 % NaCl was taken and the initial pH was set at 5.5. Twenty milliliter of the 24-h grown inoculum was transferred to the 80 mL fermentation medium and the flasks were placed in a shaking incubator at 28 ± 2 °C and 120 rpm. The experiment was performed in triplicate.

Submerged Fermentation of Prebiotic Synthesizing Enzymes

Seven fungal cultures, viz., HKF-5, HKF-14, HKF-15, HKF-60, HKF-67, HKF-72, HKF-74, isolated from effluent treatment plants (ETPs) in a previous study by Deshmukh et al. [29] were revived from glycerol freezer stocks stored in laboratory culture collection. The revived isolates were initially screened for submerged fermentation of prebiotic FOS synthesizing enzymes FFase and FTase, in the aforesaid fermentation medium containing 20% sucrose as substrate. The isolate with the highest FFase and FTase activities, was further subjected to the production of other carbohydrate metabolizing enzymes β-galactosidase, xylanase, and α-amylase catalyzing the synthesis of prebiotic oligosaccharides GOS, XOS, and MOS, respectively. Fermentation was carried out under similar experimental conditions as in FFase and FTAse production, except that the carbon source sucrose was replaced with lactose (20%), xylan (1%), and maltose (20%), respectively.

Enzyme Assay

Samples were withdrawn from the fermentation flasks after every 24 h, centrifuged (4 °C, 10,000 rpm) in a cooling centrifuge (Remi C-30, India), and the supernatant was

separated and stored at −20 °C. This cell-free fermented broth was used as a source of crude mixture for assay of multiple carbohydrate metabolizing enzymes and analysis of residual sugar.

β-Fructofuranosidase (FFase) and Fructosyl Transferase (FTase) Assay

FFase (hydrolytic activity) assay was performed by incubating 1.5 mL of 0.5% (w/v) sucrose in 0.1 M citrate buffer (pH 5.5) with 0.5 mL crude enzyme in a water bath at 55 ± 1 °C for 1 h. The reaction was terminated by placing the tubes in a boiling water bath for 15 min and reducing sugar (glucose) released by action of FFase on sucrose was determined by the dinitrosalicylic acid reagent (DNSA) using a standard calibration curve of glucose [30]. One unit of FFase activity is defined as the amount of enzyme required to release 1 µmol of glucose per min from 0.5% sucrose at 55 °C and pH 5.5 [31].

FTase (transfructosylation activity) assay was performed by a method similar to the FFase assay, except that the sucrose concentration taken was 55% (w/v) in 0.1 M citrate buffer (pH 5.5). The amount of reducing sugar (glucose) released was determined by DNSA using a standard calibration curve of glucose [30]. One unit of FTase activity is defined as the amount of enzyme required to release 1 µmol of glucose per min from 55% sucrose at 55 °C and pH 5.5 [31].

β-Galactosidase Assay The galactosidase enzyme activity was determined by a spectrophotometric assay [32] in which a reaction mixture of 0.91 mL of lactose (10 mM final concentration, prepared in 50 mM sodium phosphate buffer, pH 6.8), 0.01 mL of $MgCl_2$ (50 mM) was incubated with 0.08 mL of the crude enzyme at 40 °C for 10 min. The reaction was stopped by adding 0.1 mL of 1 M Na_2CO_3, and the mixture was centrifuged at 10,000 rpm for 5 min. The amount of reducing sugar (glucose) released by action of galactosidase on lactose was estimated by DNSA reagent using a standard calibration curve of glucose [30]. One unit of β-galactosidase activity is defined as the amount of enzyme required to release 1 µmol of glucose per min under the specified assay conditions.

Xylanase Assay The enzymatic activity of xylanase was measured using the method described by Jiang et al. [33]. A reaction mixture containing 0.9 mL of 1% (w/v) beechwood xylan in citrate-phosphate buffer (pH 6.0) was incubated with 0.1 mL of a crude enzyme at 50 °C for 10 min in 0.05 M citrate-phosphate buffer (pH 6.0). The amount of reducing sugar (xylose) released by the action of xylanase on xylan was estimated by DNSA reagent using a standard calibration curve of xylose [30]. One unit of xylanase activity is defined as the amount of enzyme required to release 1 µmol of xylose per min under the specified assay conditions.

α-Amylase Assay α-amylase activity was estimated using the method described by Bernfeld et al. [34]. A reaction mixture of 0.5 mL crude enzyme and 0.5 mL of 1% starch solution was incubated at 45 °C for 30 min following which the reaction was stopped by adding 1 mL of DNSA reagent. The amount of reducing sugar (glucose) released by the action of amylase on starch was estimated by DNSA reagent using a standard calibration curve of glucose [30]. One unit of amylase activity is defined as the amount of enzyme required to release 1 µmol of glucose per min under the specified assay conditions.

Genome Sequencing and Phylogenetic Analysis

Genomic DNA was extracted from 3-day-old mycelial culture grown in potato dextrose broth (PDB) using FastDNA SPIN kit for soil (MP Biomedicals, USA), and DNA quality was checked using a Nano Drop spectrophotometer (ND-1000, Thermo Scientific, USA). Paired-end and mate-pair sequencing libraries were created from genomic DNA sample using the Library Preparation Kit (Nextera XT DNA Library Prep) after which, whole-genome sequencing was performed on the Illumina HiSeq X platform. The sequence data was subjected to de novo assembly and filtering of reads by Metaspades (v3.11.1) [35]. The functional gene annotation was performed using GeneMark-ES [36], Basic Local Alignment Search Tool (BLAST) [37], CAZyme [38], and other databases. Additionally, 18S rRNA sequence retrieved from the whole genome sequence of strain HKF-74 was used for comparative genomic analysis by constructing a phylogenetic tree with 18S rRNA sequences of other fungal strains which shared close homology as revealed by BLAST analysis [39].

Comparative Genomics

Genome-based methods were used for resolving the ambiguity in species level delineation. The web-based tool Genome-to-Genome Distance Calculator (GGDC 3.0) which determines the digital DNA-DNA hybridization (dDDH) values for bacterial, archaeal, and fungal genomes [40] was used for comparing the complete genome sequence of strain HKF-74 with reference strains to calculate their genetic relatedness (http://ggdc.dsmz.de/ggdc.php). ANI calculator was employed for estimating the average nucleotide identity between two genomic datasets using both one-way ANI and two-way ANI (http://enve-omics.ce.gatech.edu/ani/). The calculations are based on the approach using a BLAST algorithm for comparing homologous regions of the two genomes [41, 42].

To assess the conservation and divergence of the prebiotic synthesizing enzyme genes in strain HKF-74, the DNA sequences were submitted to the NCBI using the BLAST to identify their potential homologs. Phylogenetic trees were constructed for assessing functional relationships, using Molecular Evolutionary Genetics Analysis software (MEGA; version 11.0) with 1000 bootstrap replicates and subjected to multiple alignments using the Clustal Omega program [43]. The identified potential homologous gene sequences were translated into amino acid sequences using the NCBI ORF Finder tool (https://www.ncbi.nlm.nih.gov/orffinder/). The translated amino acid sequences were analyzed to determine the presence of conserved domains or motifs associated with the genes for respective enzymes [44]. The identified ORFs were then compared with known gene sequences in public databases using BLASTp to confirm their homology [45]. This methodology was employed for the identification of ORFs in genes encoding all the enzymes viz., fructosyltransferase, β-galactosidase, xylanase, and α-amylase.

Annotation of Genes for Multiple Carbohydrate Metabolizing Enzymes

GeneMark-ES was used for genome annotation and identification of nucleotide and protein coding-genes [46]. The fasta file of protein coding-genes was then used for protein annotation by aligning it against various protein databases including the CAZyme (http://www.cazy.org/) and Swiss-Prot databases (http://www.expasy.org/sprot/ Open;

http://www.ebi.ac.uk/swissprot/). The CAZyme database was used to classify enzymes involved in carbohydrate synthesis, metabolism, and identification, whereas the Swiss-Prot database was manually downloaded from NCBI and used for a blast run against the protein sequence file generated from Genemark-ES [11, 47].

To predict the genes involved, five widely used protein databases, namely Cluster of Orthologous Group of proteins (COG), gene ontology (GO), Swiss-Prot (with an *E*-value threshold of 1e-05), Non-Redundant Protein Database (NR), and Kyoto Encyclopedia of Genes and Genomes (KEGG), were used for annotation. Carbohydrate-utilizing enzymes were identified and studied by conducting a BLASTp search against the Carbohydrate-Active Enzyme (CAZy) database at an *E*-value cut-off of 1e-5 [48].

Prediction of Biosynthetic Gene Clusters (BGCs) for Secondary Metabolite Production

Fungal growth and the production of secondary metabolites can occur under favorable environmental conditions, such as when temperature and moisture levels are ideal. These products that are encoded by genes forming a part of the biosynthetic gene clusters (BGCs) do not contribute in any biochemical way to their own growth and development. Based on the phylogenetic analysis and considering that the members of identified genus were known for their phytopathogenic potential, AntiSMASH [24, 49] was used for the prediction of BGCs imparting secondary metabolite production and virulence traits in the genome of strain HKF-74.

Results and Discussion

Submerged Fermentation of Prebiotic Synthesizing Enzymes

The initial screening of fungal isolates was performed for submerged fermentation of enzymes FFase (hydrolysis of sucrose) and FTase (transfructosylation), which have been reported for synthesis of FOS [50]. Strain HKF-74 exhibited the highest activities of 17.52 U/mL (FFase) and 18.924 U/mL (FTase) at the end of 120 h and 96 h, respectively, after which a decline in activity was noted (Fig. 1a, b). The FFase and FTase activities for the remaining strains ranged between 8.15 and 15.28 U/mL, and 11.49 and 14.46 U/mL, respectively, with lowest enzyme production in the case of strains HKF-60 (lowest FFase activity) and HKF-14 (lowest FTase activity). Overall, these findings suggest that the strain HKF-74 has significant capacity for producing various extracellular enzymes, which could have potential for industrial application. Our results corroborated the FOS production capacity in *F. oxysporum,* reported in one of the earliest studies on invertase and FTase production in fungal genera, with highest specific activities of 0.6 to 0.8 and 0.1 to 0.57 U/mg, respectively, in different strains of *F. oxysporum* [18]. A study by Neera et al. [17] optimized the fermentation conditions for another similar enzyme, i.e., inulinase production by *Fusarium* sp., which catalyzed the production of FOS by hydrolysis of inulin, resulting in an enzyme activity of 131.6 U/mL. Another recent study reported the production of FOS by hydrolyzing inulin in the presence of inulinase secreted by *F. solani*, which reached an activity of 13.9 and 15.9 U/mg protein in the case of exo- and endo-inulinases, respectively [13]. These studies indicated the importance of optimization of fermentation conditions for

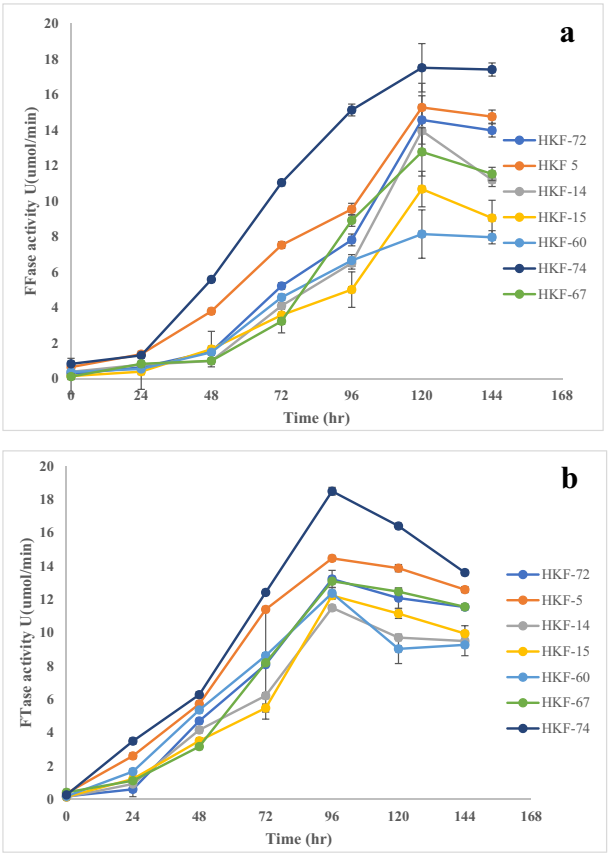

Fig. 1 Submerged fermentation of fungal isolates for production of prebiotic FOS synthesizing enzymes **a** Fructofuranosidase (FFase) and **b** Fructosyl transferase (FTase)

enhanced enzyme production and purification for realizing the full potential of this strain in industrial applications.

Strain HKF-74 with the highest FFase and FTAse production was further taken up for evaluating its capacity to produce other carbohydrate metabolizing enzymes. Results exhibited the significant capacity of strain HKF-74 for producing the highest galactosidase activity of 15.9 U/mL in the presence of lactose, while the highest xylanase and amylase activities were found to be 17.917 U/mL and 14.058 U/mL in 72 h in the presence of xylan and maltose, respectively (Fig. 2). The three enzymes galactosidase, xylanase, and α-amylase have been known for catalyzing the production of prebiotics GOS, XOS, and MOS, respectively [51–53]. Though, the multiple carbohydrate-metabolizing enzyme production in members of genus *Fusarium* has been demonstrated by many researchers, reports on the application of these fungal strains for synthesis of prebiotic oligosaccharides are scarce. The role of genus *Fusarium* in secreting the enzyme galactosidase was demonstrated by Gajdhane et al. [54]. The authors observed that the basal enzyme activity by *F. moniliforme* in the presence of wheat bran was slightly lower than the present study (13.17 U/g) which was enhanced by applying response surface methodology to 207.33 U/g. The basal xylanase activity in our study was observed to be nearly half that reported recently by Marinho et al. [55], who employed a strain of *Fusarium* sp. EA 1.3.1 for hydrolysis of different agricultural residues. By optimizing the fermentation conditions, the authors

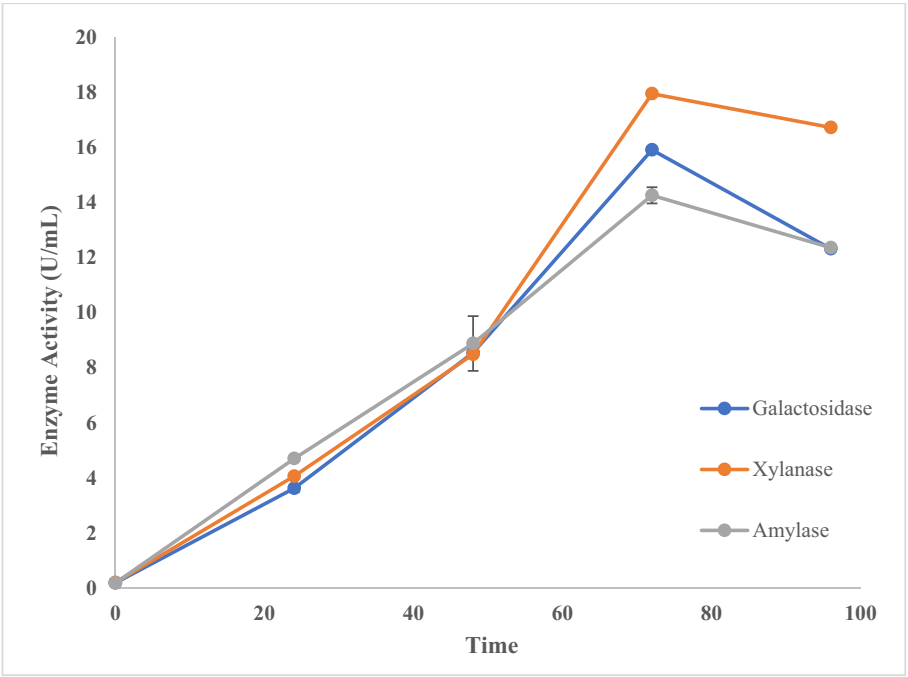

Fig. 2 Submerged fermentation of isolate HKF-74 for production of multiple carbohydrate metabolizing enzymes β-galactosidase, xylanase, and α-amylase

achieved a 1.2-fold improvement in xylanase activity to 35.5 U/mL in the presence of wheat bran. Similar application of a strain of *F. solani* NAIMCC-F-02956 for alpha-amylase production in the presence of mango kernel resulted in a basal enzyme activity of 0.889 U/g which could be enhanced further by optimization of fermentation conditions [56]. Our previous studies have also highlighted the significance of optimizing the fermentation conditions for achieving enhanced enzyme activity as demonstrated in case of other industrially important hydrolytic enzymes [57]. The present study exhibited the broad substrate specificity of strain HKF-74 owing to the diverse enzymatic activities induced by the specific substrates. However, considering that the enzyme activities were lower than reported values, future studies were necessary for enhancing the enzyme production for large-scale application in prebiotic oligosaccharide production.

Genome Sequencing and Phylogenetic Analysis

Genome sequencing and annotation are pivotal techniques in deciphering the genomic potential and unexplored capacities inherent in any microbial strain [3, 22, 50]. In this study, de novo assembly generated 82,342,140 base pairs (bp). The draft genome size of *Fusarium* sp. strain HKF-74 was determined to be 43.05 Mb with a GC content of 48% (NCBI Accession number JAJFAS000000000.1). The whole genome of HKF-74 contained a total of 31,648 genes (Table 1). Annotation by the SWISS PROT protein sequence database provided information on the structure, function, and modifications of these proteins. The SwissProt analysis performed using the Linux operating system revealed that the total

Table 1 Genome features of strain HKF-74

Genome features	Strain HKF-74
Assembled genome size (Mb)	43.05 Mb
Number of scaffolds	8082
Total no. of contigs	222
Contig N50 (bp)	76188
Scaffold N50 (bp)	81047
N50	432218
N75	264244
L50	33
L75	65
K-mer	1522
Total genes	31648
Total reads	82342140
GC%	48%

functional proteins in the genome were comprised of 7983 coding sequences (CDs). These results provide valuable insights into the genetic potential of strain HKF-74 for the production of various enzymes including multiple prebiotic synthesizing enzymes and can be used as a basis for further investigation into the genetic mechanisms that govern enzyme production in fungi. Several studies have investigated the genetic potential of *Fusarium* strains for the production of various enzymes. For instance, a study by Li et al. [58] analyzed the genome of *F. graminearum* and identified a total of 15,327 protein-coding genes. *F. graminearum* has the potential to produce a variety of carbohydrate-active enzymes, including glycoside hydrolases, polysaccharide lyases, and carbohydrate esterases. The

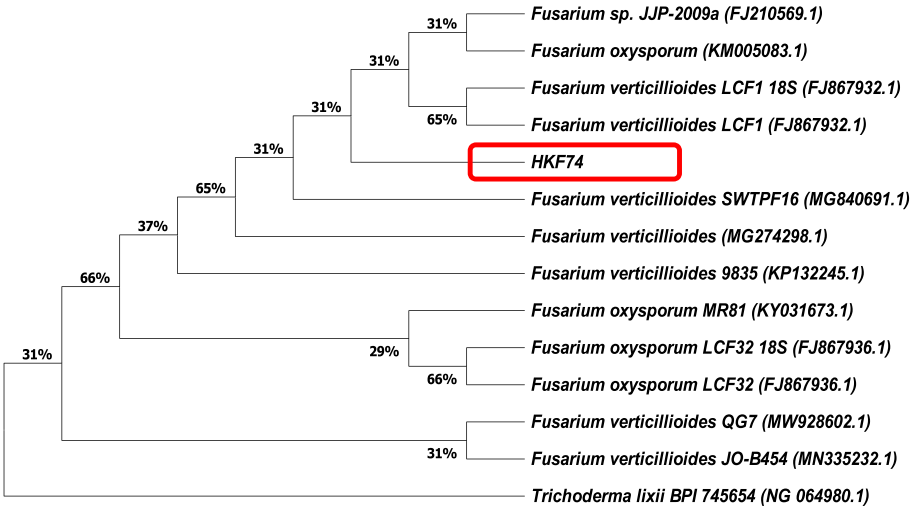

Fig. 3 18S rRNA phylogenetic tree representing genetic relation of strain HKF-74 with other strains of *Fusarium verticillioides* available on NCBI. The numbers on the branches indicate the bootstrap support achieved from 1000 replications

Table 2 Whole genome sequence-based comparison of strain HKF-74 with genomes of reference strains of genus *Fusarium*

Query genome	Reference genome name with accession number	Two-way ANI	dDDH	Model C.I.	Prob. DDH ≥ 70%	G + C difference
HKF-74	*Fusarium verticillioides* BRIP53263 (ASM331701v2)	92.55%	83.2	[79.3–86.4%]	93.74	0.46
	Fusarium verticillioides NRRL 20984 (ASM1375927v1)	92.55%	84	[80.2–87.2%]	94.32	0.89
	Fusarium verticillioides 7600 (ASM14955v1)	92.56%	83.9	[80.2–87.1%]	94.29	0.77
	Fusarium oxysporum f. sp. *lycopersici* 4287 (ASM14995v2)	88.86%	43.6	[40.3–47.1%]	3.81	0.48
	Fusarium graminearum PH-1 (ASM24013v3)	79.32%	15.9	[13–19.4%]	0	0.41
	Fusarium oxysporum Fo47 (ASM1308505v1)	88.84%	54.4	[50.9–57.9%]	23.09	0.2
	Fusarium musae F31 (ASM1991524v1)	92.52%	81	[77.1–84.4%]	91.97	0.68
	Fusarium fujikuroi IMI 58289 (IMI58289_V2)	88.95%	66.1	[62.3–69.7%]	63.27	0.45

Neighbor-Joining tree constructed using the 18S rRNA sequences showed that strain HKF-74 shared the closest homology (100%) with *Fusarium verticilloides* LCF1 (FJ867932.1) based on which the name *Fusarium verticilloides* strain HKF-74 was proposed for the isolate (Fig. 3).

The study further aimed to evaluate the genetic relatedness of the strain HKF-74 with reference strains of the *Fusarium* by analyzing their genomic features including genome size, GC content difference, ANI, and dDDH. Table 2 shows that the two-way ANI similarity between HKF-74 and eight reference genomes retrieved from NCBI varied in the range of 79.3–92.5%. The highest ANI similarity of 92.5% was shared with *Fusarium verticillioides* strains BRIP53263 (ASM331701v2), NRRL 20984 (ASM1375927v1), and 7600 (ASM14955v1), which, though not equal or greater, was closer to the species threshold cut-off of 95%. The slightly lower ANI similarity could be associated with the difference in genome size of strain HKF-74 which was slightly larger (43 Mb) than the aforementioned strains (around 42 Mb). Similarly, the strain HKF-74 also shared ANI similarity of 92.5% with *F. musae* strain F31 (ASM1991524v1), which, though differing in species name, had high similarity as it was reported to be a sister species of *F. verticillioides* [59]. The results were in agreement with dDDH values which were computed by determining the quantity of identical HSPs (high-scoring segment pairs) shared by the compared genomes. The dDDH values of strain HKF-74 were the highest and varied between 83 and 84% with *F. verticillioides* strains BRIP53263, NRRL 20984, and 7600, while it was computed to be slightly lower (81%) with *F. musae* strain F31. These values were not only greater than species delineating boundary of 70% but they were also greater than the sub-species delineating threshold of 79–80%. Another genome parameter which confirmed the assignment of the strain HKF-74 to *F. verticillioides* was the G + C content difference that did not exceed the threshold cut-off of 1%. Strain HKF-74 was deposited in the microbial culture collection at the National Centre for Microbial Resource (NCMR), National Centre for Cell Sciences, Pune, India, and has been assigned the accession number MCC 9904.

Phylogenetic trees were also constructed for assessing functional relationships between the genes encoding the various prebiotic producing enzymes from strain HKF-74 with the potential homologs available in NCBI database. The fructosyltransferase gene from HKF-74 shared the closest homology with glycosyl hydrolase family 32 superfamily gene from *F. tjaetaba* (FTJAE_7966), gene for uncharacterized protein from *F. musae* (J7337_013740), and gene for hypothetical protein from *F. verticillioides* 7600 (FVEG_12976) (Fig. 4a). A multiple sequence alignment was done with related sequences from reference strains to assess the conservation and divergence of the fructosyltransferase gene of HKF-74 which showed the highest evolutionary divergence from closely related strains (Fig. 4b). In the case of the xylanase gene of HKF-74, the highest homology was seen with related sequences from *F. verticillioides* 7600 (glycosyl hydrolase family 10; FVEG_12502), *F. musae* (uncharacterized protein; J7337_007833), and *F. proliferatum* ET1 (Endo 14-beta-xylanase A; FPRO_07211) (Supplementary Fig. 1a). The α-amylase gene from HKF-74 shared closed homology with related sequences from *F. tjaetaba* (α-amylase; FTJAE_7989), *F. musae* (uncharacterized protein; J7337_013719), and *F. verticillioides* 7600 (α-amylase; FVEG_12957) (Supplementary Fig. 1b). The phylogenetic tree prepared with gene sequences of the enzyme β-galactosidase highlighted the homology of this gene from HKF-74 with β-galactosidase from *F. tjaetaba* (FTJAE_4575), β-galactosidase from *F. verticillioides* 7600 (FVEG_08557), and uncharacterized protein from *F. musae* (J7337_012789) (Supplementary Fig. 1c). The multiple sequence alignment of xylanase, α-amylase, and β-galactosidase genes revealed a high level of conservation among the four strains with minor variations in specific regions (Supplementary Fig. 2a-c). The ORF

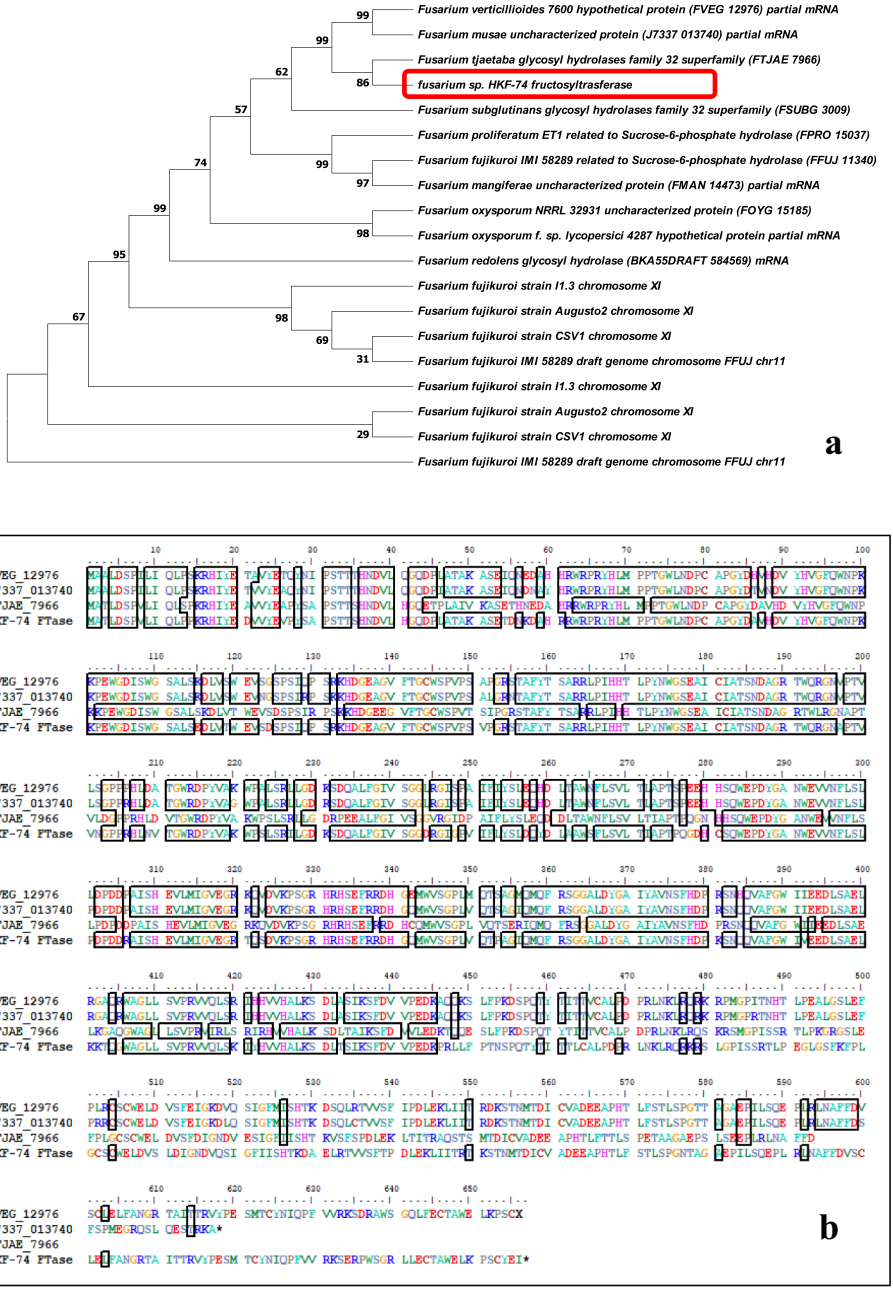

Fig. 4 **a** Phylogenetic analysis. **b** Multiple sequence alignment for confirming the homology of gene of prebiotic synthesizing enzyme fructosyltransferase from strain HKF-74 with known gene sequences from closely related strains

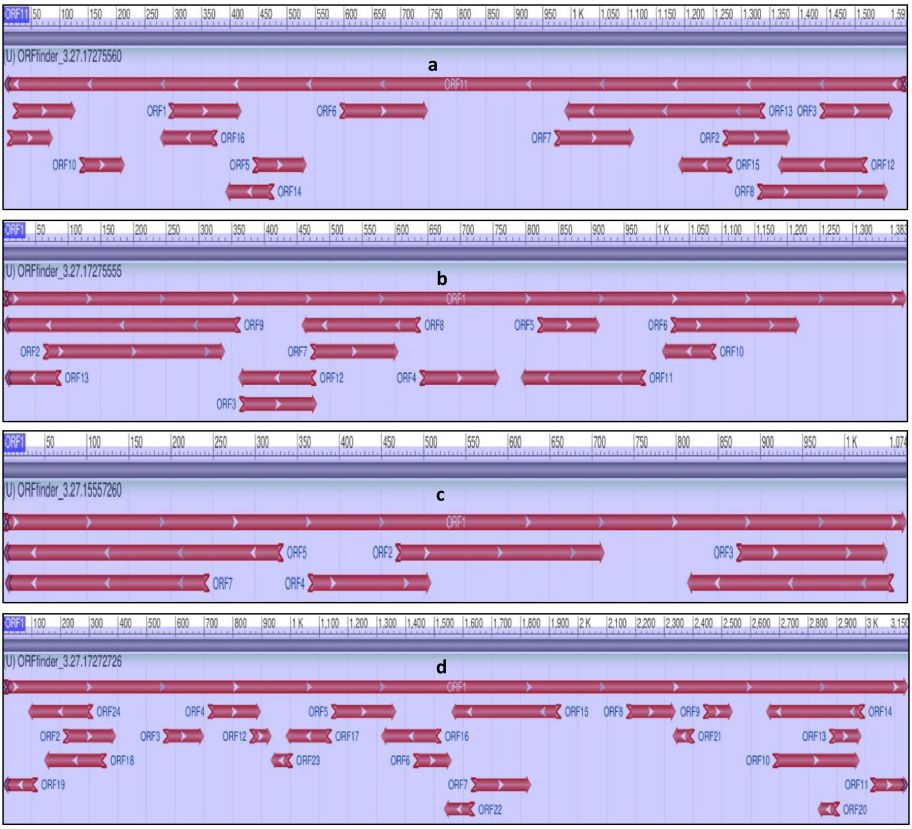

Fig. 5 Prediction of ORFs for confirming the homology of genes from strain HKF-74 with known gene sequences of **a** fructosyltrnsferase, **b** xylanase, **c** α-amylase, and **d** β-galactosidase

finder tool was able to predict a total of 16 ORFs in the sequence of fructosyltransferase enzyme while 13, 7, and 24 ORFs were predicted in genes encoding the enzymes xylanase, α-amylase, and β-galactosidase, respectively (Fig. 5).

Annotation of Genes for Multiple Carbohydrate Metabolizing Enzymes

The KEGG Orthology (KO) database was utilized to annotate the molecular functions in the genome sequence of strain HKF-74. The annotations can be used to thoroughly investigate the metabolic pathways of gene products in cells and their functions. They also allow for the networked display of genes and their expression levels. The KO annotation showed that the HKF-74 genome sequence was comprised of different subsystems for metabolism (50%), followed by genetic information processing (25%), cellular processes (13%), human diseases (6%), environmental processing (5%), and organismal systems (1%) (Supplementary Fig. 3). More than half of the genes were associated with metabolism including carbohydrate metabolism (15%), amino acid and derivatives (14%), protein metabolism (12%), cofactor and vitamins (10%), RNA metabolism (8%), aromatics compounds (3%), respiration (2%), and miscellaneous (20%) subsystems. Among different subsystems,

carbohydrate metabolism was the most abundant which was further divided into fructose and mannose metabolism (13%), galactose metabolism (14%), starch and sucrose metabolism (7%), propanoate metabolism (14%), pyruvate metabolism (14%), pentose phosphate pathway (11%), and miscellaneous (27%). These results suggested that the energy metabolism of strain HKF-74 has unique characteristics. The distinctive nature of energy metabolism in strain HKF-74 is highlighted from the high prevalence of genes associated with fructose and mannose metabolism, galactose metabolism, starch and sucrose metabolism, propanoate metabolism, pyruvate metabolism, and pentose phosphate pathway.

The abundances of various genes annotated in the genome sequence of strain HKF-74, related to carbohydrate metabolism from the KEGG pathway [60] and SEED server are given in Supplementary Fig. 4. For CAZyme analysis, CAZyme (http://www.cazy.org/) annotation using dbCAN [61] was performed; all data in dbCAN was generated based on the family classification from CAZy database [62, 63]. Microbial xylanolytic enzyme systems typically consist of three major constituents, namely xylanase (endo-1,4-β-xylanase), β-xylosidase (β-D-xyloside xylohydrolase), and acetylxylan esterase. The endoxylanase (E.C 3.2.1.8) enzyme is classified into several glycoside hydrolase (GH) families based on its specific fold function, including GH7, GH10, GH11, GH30, GH43, GH51, GH98, and GH141. As shown in Supplementary Fig. 3, strain HKF-74 possessed the multiple number of genes for carbohydrate metabolism, viz., beta-glucosidase, beta-glucanase, beta-galactosidase, beta-xylosidase, beta-fructosidases, maltose hydrolases, and others. Each of these enzymes is involved in metabolizing specific type of carbohydrate. For example, beta-glucosidase breaks down beta-glucans, which are a type of complex carbohydrate found in the cell walls of many fungi, bacteria, and plants.

Hydrolysis plays a pivotal role in the enzymatic degradation of polysaccharides [27, 64–66]. Specifically, beta-xylosidase assumes a significant role in the catabolism of xylan, a structurally intricate carbohydrate component prominently found in plant cell walls. However, the presence β-xylosidase also suggested that the application of this strain would result in low yield of XOS owing to complete hydrolysis of XOS to xylose sugar. This issue has been demonstrated to be overcome by knocking out of the *β-xyl1* gene encoding β-xylosidase which not only resulted in a reduction in the activity of this enzyme but also a drastic decrease in the yield of xylose and a concomitant enhancement in XOS accumulation [67]. Owing to the presence of genes for multiple carbohydrate metabolizing enzymes, the strain HKF-74 was well-adapted for hydrolyzing a variety of complex carbohydrates. Our results are consistent with previous studies showing that *Fusarium* strains can produce a variety of carbohydrate-active enzymes [68]. This was an important trait that could benefit the fungus in its natural environment, where it may need to compete with other organisms for limited resources. It is well known that hydrolysis was the rate limiting step in various industrial applications such as VFA fermentation and anaerobic digestion of lignocellulosic biomass. The hydrolytic capabilities of strain HKF-74 had tremendous potential for exploitation as a cost-effective and eco-friendly pre-treatment agent in comparison to physical and chemical methods of pre-treatment for achieving high VFA and biogas yields [69, 70]

Prediction of Biosynthetic Gene Clusters (BGCs) for Secondary Metabolite Production

The significance of characterizing the BGCs in prebiotic production was suggested by Peng et al. [71] since the role of secondary metabolites was shown to enhance the functionality of

prebiotics by promoting the growth of beneficial gut bacteria, inhibiting the growth of harmful bacteria, and modulating the immune system. It is worth considering that prebiotics can sometimes yield secondary benefits by promoting the beneficial gut bacteria to produce metabolic compounds that inhibit the growth of enteric pathogens or mitigate their virulence [72]. The secondary metabolite biosynthetic potential of the strain HKF-74 was also investigated and the key features of its genome are listed in Supplementary Table 1. The antiSMASH analysis revealed the presence of four BGCs including Type I polyketide synthase (PKS; 9), non-ribosomal peptide synthetase (NRPS; 2), NRPS-like (2), and terpene (5).

Several of the BGCs showed homology with genes encoding compounds with potential pharmaceutical and therapeutic applications. For instance, squalestatin S1 is being investigated for its role in lipid metabolism and its potential use in cholesterol-lowering drugs [73]. Other compounds like oxyjavanicin, equisetin, bikaverin, and asperfuranone, which have been reported for their antibacterial, antifungal, nematicidal, and anti-cancer properties, can find applications in medicine or food preservation [74–77]. Compounds like gibberellin are naturally occurring plant hormones. While not directly toxic to humans, they have been used in agriculture to modify plant growth and may indirectly affect humans through their impact on crop yields and food production [78]. The remaining BGCs shared homology with genes encoding compounds for which limited information is available and since their effect on human and plant health are not well-documented, further research would be needed to determine any potential impacts.

Of particular concern in context of the present study are the BGCs listed to have similarity with genes encoding known mycotoxins. Though, the mycotoxins such as alternapyrone, ACT-Toxin II, fusaric acid, gliotoxin, equisetin, and neurosporin A and their derivatives exhibit toxicity in plants and animals including humans [20, 79], adoption of integrative approach combining enzymatic control of prebiotic oligosaccharides with genetic perturbation of pathogens could enable the application of strains of *Fusarium* in prebiotics. One promising approach in the production of specific prebiotic oligosaccharides involves purification and immobilization of enzymes on suitable matrices. By using pure fungal enzymes for specific carbohydrate metabolism, this approach would do away with the need for the utilization of whole fungal mycelium in fermentation and thus prevent the production of mycotoxins in the fermented broth. The choice of substrate is critical, as it dictates the composition of the prebiotic oligosaccharides to be produced. For instance, sucrose may be selected to generate fructooligosaccharides (FOS), consisting of fructose molecules linked together. This enzymatic conversion of substrates yields precise control over prebiotic oligosaccharide production while minimizing the risk of pathogenic contamination [80]. Immobilization process could also be applied for achieving enhanced enzyme stability, reusability, and facilitating control over enzymatic reactions. Furthermore, modern molecular biology tools such as recombinant DNA technology could also be applied for precise targeting and alteration of genes responsible for pathogenic virulence factors, leading to the attenuation or elimination of pathogenicity thus mitigating the harm caused by pathogens and reducing the risk of disease transmission. A reduction in the virulence potential of pathogens could also be achieved by modulating the expression of virulence-associated genes through cutting-edge methodologies such as RNA interference (RNAi).

Concluding Remarks

In conclusion, this study provides valuable insights into the potential of *Fusarium* sp. HKF-74 for secreting several carbohydrate metabolizing enzymes for the synthesis of prebiotic oligosaccharides. Not only has the broad substrate specificity been highlighted by submerged fermentation, but these capacities have been validated by the annotation of respective genes in the whole genome sequence. The results demonstrate the effectiveness of genomics and bioinformatics in analyzing the whole genome sequence data of the fungi, with a focus on identifying genes and enzymes involved in oligosaccharide synthesis. The draft whole-genome sequence and annotation of genes related to oligosaccharide synthesis have been deposited in NCBI, enabling further research on the genetic basis of prebiotic synthesis in this strain. Future research will be needed to evaluate the specific prebiotics that can be synthesized by *Fusarium* sp. HKF-74 and their effects on the gut microbiome, as well as the safety of using these strains and their enzymes as prebiotic supplements. Nonetheless, our study represents a promising step towards the development of more effective strategies for promoting gut health and preventing disease, highlighting the potential of whole genome sequencing as a tool for identifying novel sources of prebiotic-synthesizing enzymes.

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Author Contribution A.R. Chavan: investigation, formal analysis, visualization, data curation, writing—original draft; A. A. Khardenavis: supervision, formal analysis, writing—review and editing

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Declarations

Ethics Approval Not applicable

Consent to Participate Not applicable

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

1. Gujar, V. V., et al. (2017). Recent advances in bioprocess optimization of fructo-oligosaccharides. In V. Rai (Ed.), *Recent Advances in Biotechnology* (Vol. 3, pp. 215–238). Shree Publishers & Distributors India.

2. Mutanda, T., et al. (2014). Microbial enzymatic production and applications of short-chain fructooligosaccharides and inulooligosaccharides: Recent advances and current perspectives. *Journal of Industrial Microbiology and Biotechnology Advances, 41*(6), 893–906.
3. Qureshi, A., et al. (2014). Genome sequence of Lactobacillus plantarum EGD-AQ4, isolated from fermented product of Northeast India. *Genome Announcements, 2*(1). https://doi.org/10.1128/genomea.01122-13
4. Chavan, A. R., et al. (2023). Recent trends in the biotechnology of functional non-digestible oligosaccharides with prebiotic potential. *Biotechnology and Genetic Engineering Reviews*, (Epub ahead of print). https://doi.org/10.1080/02648725.2022.2152627
5. Hills, R. D., et al. (2019). Gut microbiome: Profound implications for diet and disease. *Nutrients, 11*(7), 1613.
6. Sabater-Molina, M., et al. (2009). Dietary fructooligosaccharides and potential benefits on health. *Journal of Physiology and Biochemistry, 65*, 315–328.
7. Joshi, D., Roy, S., & Banerjee, S. (2018). Prebiotics: A functional food in health and disease. In S. C. Mandal, V. Mandal, & T. Konishi (Eds.), *Natural Products and Drug Discovery* (pp. 507–523). Elsevier.
8. Roberfroid, M. (2002). Functional food concept and its application to prebiotics. *Digestive and Liver Disease, 34*, S105–S110.
9. Green, M., Arora, K., & Prakash, S. (2020). Microbial medicine: Prebiotic and probiotic functional foods to target obesity and metabolic syndrome. *International Journal of Molecular Sciences, 21*(8), 2890.
10. Silvério, S. C., et al. (2018). New β-galactosidase producers with potential for prebiotic synthesis. *Bioresource Technology, 250*, 131–139.
11. Gujar, V. V., et al. (2018). Draft genome sequence of Penicillium chrysogenum strain HKF2, a fungus with potential for production of prebiotic synthesizing enzymes. *3 Biotech, 8*, 106.
12. Nascimento, A., et al. (2016). Screening of fungi from the genus Penicillium for production of β-fructofuranosidase and enzymatic synthesis of fructooligosaccharides. *Journal of Molecular Catalysis B: Enzymatic, 134*, 70–78.
13. Galvão, D. F., et al. (2022). A comparative study between Fusarium solani and Neocosmospora vasinfecta revealed differential profile of fructooligosaccharide production. *Folia Microbiologica, 67*(6), 873–889.
14. Nobre, C., et al. (2018). Production of fructo-oligosaccharides by Aspergillus ibericus and their chemical characterization. *LWT, 89*, 58–64.
15. Deshmukh, R., Khardenavis, A. A., & Purohit, H. J. (2016). Diverse metabolic capacities of fungi for bioremediation. *Indian Journal of Microbiology, 56*, 247–264.
16. Ibrahim, S. R., et al. (2021). Bright side of Fusarium oxysporum: Secondary metabolites bioactivities and industrial relevance in biotechnology and nanotechnology. *Journal of Fungi, 7*(11), 943.
17. Neera, K., Ramana, N. G., & Sharma, R. (2018). Production of Inulinase by Fusarium sp. and its application for fructo-oligosaccharide production for use as prebiotics. *Defence Life Science Journal, 3*(1), 45–50.
18. Patel, V., Saunders, G., & Bucke, C. (1994). Production of fructooligosaccharides by Fusarium oxysporum. *Biotechnology Letters, 16*, 1139–1144.
19. Santibáñez, L., et al. (2021). Xylooligosaccharides from lignocellulosic biomass: A comprehensive review. *Carbohydrate Polymers, 251*, 117118.
20. Perincherry, L., Lalak-Kańczugowska, J., & Stępień, Ł. (2019). Fusarium-produced mycotoxins in plant-pathogen interactions. *Toxins, 11*(11), 664.
21. Gupta, R. K., et al. (2022). Role of heterotrophic nitrifiers and aerobic denitrifiers in simultaneous nitrification and denitrification process: A nonconventional nitrogen removal pathway in wastewater treatment. *Letters in Applied Microbiology, 74*(2), 159–184.
22. Paliwal, V., et al. (2014). Pseudomonas putida CSV86: A candidate genome for genetic bioaugmentation. *PloS one, 9*(1), e84000.
23. Singh, A. K., et al. (2022). Genomic characterization of denitrifying methylotrophic *Pseudomonas aeruginosa* strain AAK/M5 isolated from municipal solid waste landfill soil. *World Journal of Microbiology and Biotechnology, 38*, 140.
24. Blin, K., et al. (2021). antiSMASH 6.0: Improving cluster detection and comparison capabilities. *Nucleic Acids Research, 49*(W1), W29–W35.
25. Fuke, P., et al. (2018). In silico characterization of broad range proteases produced by Serratia marcescens EGD-HP20. *Journal of Basic Microbiology, 58*(6), 492–500.
26. Pal, R. R., Khardenavis, A. A., & Purohit, H. J. (2015). Identification and monitoring of nitrification and denitrification genes in Klebsiella pneumoniae EGD-HP19-C for its ability to perform

heterotrophic nitrification and aerobic denitrification. *Functional and Integrative Genomics, 15*(1), 63–76.
27. Deshmukh, R., & Purohit, H. J. (2014). siRNA mediated gene silencing in Fusarium sp. HKF15 for overproduction of bikaverin. *Bioresource Technology, 157*, 368–371.
28. Sangeetha, P., Ramesh, M., & Prapulla, S. (2004). Production of fructosyl transferase by Aspergillus oryzae CFR 202 in solid-state fermentation using agricultural by-products. *Applied Microbiology and Biotechnology Advances, 65*, 530–537.
29. Deshmukh, R., Mathew, A., & Purohit, H. J. (2014). Characterization of antibacterial activity of bikaverin from Fusarium sp. HKF15. *Journal of Bioscience and Bioengineering, 117*(4), 443–448.
30. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426–428.
31. Sangeetha, P., Ramesh, M., & Prapulla, S. (2004). Production of fructo-oligosaccharides by fructosyl transferase from Aspergillus oryzae CFR 202 and Aureobasidium pullulans CFR 77. *Process Biochemistry, 39*(6), 755–760.
32. Osman, A., et al. (2014). Synthesis of prebiotic galactooligosaccharides from lactose using bifidobacterial β-galactosidase (BbgIV) immobilised on DEAE-Cellulose, Q-Sepharose and amino-ethyl agarose. *Biochemical Engineering Journal, 82*, 188–199.
33. Jiang, Z., et al. (2005). Improvement of the breadmaking quality of wheat flour by the hyperthermophilic xylanase B from Thermotoga maritima. *Food Research International, 38*(1), 37–43.
34. Bernfeld, P. (1955). [17] Amylases, α and β. *Methods in Enzymology, 1*, 149–158.
35. Nurk, S., et al. (2017). metaSPAdes: a new versatile metagenomic assembler. *Genome Research, 27*(5), 824–834.
36. Ter-Hovhannisyan, V., et al. (2008). Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. *Genome Research, 18*(12), 1979–1990.
37. Johnson, M., et al. (2008). NCBI BLAST: a better web interface. *Nucleic Acids Research, 36*(suppl_2), W5–W9.
38. Drula, E., et al. (2022). The carbohydrate-active enzyme database: Functions and literature. *Nucleic Acids Research, 50*(D1), D571–D577.
39. Zhang, X., et al. (2022). In silico methods for identification of potential therapeutic targets. *Interdisciplinary Sciences: Computational Life Sciences, 14*, 285–310.
40. Meier-Kolthoff, J. P., et al. (2022). TYGS and LPSN: A database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Research, 50*(D1), D801–D807.
41. Goris, J., et al. (2007). DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *International Journal of Systematic and Evolutionary Microbiology, 57*(1), 81–91.
42. Luo, C., Rodriguez-r, L. M., & Konstantinidis, K. T. (2014). MyTaxa: An advanced taxonomic classifier for genomic and metagenomic sequences. *Nucleic Acids Research, 42*(8), e73–e73.
43. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022–3027.
44. Henikoff, S., & Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. *Proceedings of the National Academy of Sciences of the United States of America, 89*(22), 10915–10919.
45. Altschul, S. F., et al. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*(17), 3389–3402.
46. Hoff, K. J., et al. (2016). BRAKER1: Unsupervised RNA-Seq-based genome annotation with GeneMark-ET and AUGUSTUS. *Bioinformatics, 32*(5), 767–769.
47. Bairoch, A., & Apweiler, R. (1997). The SWISS-PROT protein sequence data bank and its supplement TrEMBL. *Nucleic Acids Research, 25*(1), 31–36.
48. Liang, X., et al. (2018). Pathogenic adaptations of Colletotrichum fungi revealed by genome wide gene family evolutionary analyses. *PloS One, 13*(4), e0196303.
49. Liu, Y., et al. (2022). Genome features and AntiSMASH analysis of an endophytic strain Fusarium sp. *R1. Metabolites, 12*(6), 521.
50. Gujar, V. V., et al. (2018). Annotation and de novo sequence characterization of extracellular β-fructofuranosidase from Penicillium chrysogenum strain HKF42. *Indian Journal of Microbiology, 58*, 227–233.
51. de Souza, P. M., & de Oliviera, M. P. (2010). Application of microbial α-amylase in industry-A review. *Brazilian Journal of Microbiology, 41*, 850–861.
52. Nordberg Karlsson, E., et al. (2018). Endo-xylanases as tools for production of substituted xylooligosaccharides with prebiotic properties. *Applied Microbiology, 102*, 9081–9088.
53. Liburdi, K., & Esti, M. (2022). Galacto-oligosaccharide (GOS) synthesis during enzymatic lactose-free milk production: state of the art and emerging opportunities. *Beverages, 8*(2), 21.

54. Gajdhane, S. B., Bhagwat, P. K., & Dandge, P. B. (2016). Response surface methodology-based optimization of production media and purification of α-galactosidase in solid-state fermentation by Fusarium moniliforme NCIM 1099. *3 Biotech, 6*, 1–14.
55. Marinho, G. O., et al. (2023). An environmentally safe production of xylanases by Fusarium sp. EA 1.3. 1 using agroindustrial residues: biochemical characterization and potential applications. *Asian Journal of Biochemistry, Genetics and Molecular Biology, 14*(4), 11–26.
56. Kumar, D., et al. (2013). Production and characterization of [alpha]-amylase from mango kernel by Fusarium solani NAIMCC-F-02956 using submerged fermentation. *Journal of Environmental Biology, 34*(6), 1053.
57. Fuke, P., Gujar, V. V., & Khardenavis, A. A. (2018). Genome annotation and validation of keratin-hydrolyzing proteolytic enzymes from Serratia marcescens EGD-HP20. *Applied Biochemistry and Biotechnology, 184*(3), 970–986.
58. Li, Y., et al. (2011). The HDF1 histone deacetylase gene is important for conidiation, sexual reproduction, and pathogenesis in Fusarium graminearum. *Molecular Plant-Microbe Interactions, 24*(4), 487–496.
59. Molnár, O., Bartók, T., & Szécsi, Á. (2015). Occurrence of Fusarium verticillioides and Fusarium musae on banana fruits marketed in Hungary. *Acta Microbiologica Et Immunologica Hungarica, 62*(2), 109–119.
60. Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research, 28*(1), 27–30.
61. Yin, Y., et al. (2012). dbCAN: A web resource for automated carbohydrate-active enzyme annotation. *Nucleic Acids Research, 40*(W1), W445–W451.
62. Cantarel, B. L., et al. (2009). The Carbohydrate-Active EnZymes database (CAZy): An expert resource for glycogenomics. *Nucleic Acids Research, 37*(suppl_1), D233–D238.
63. Prasanth, C. N., et al. (2022). Carbohydrate active enzymes (CAZy) regulate cellulolytic and pectinolytic enzymes in Colletotrichum falcatum causing red rot in sugarcane. *3 Biotech, 12*(2), 48.
64. Gulhane, M., et al. (2016). Biomethanation of vegetable market waste in an anaerobic baffled reactor: effect of effluent recirculation and carbon mass balance analysis. *Bioresource Technology, 215*, 100–109.
65. Bohra, V., Dafale, N. A., & Purohit, H. J. (2019). Understanding the alteration in rumen microbiome and CAZymes profile with diet and host through comparative metagenomic approach. *Archives of Microbiology, 201*, 1385–1397.
66. Poddar, B., et al. (2022). A comprehensive review on the pretreatment of lignocellulosic wastes for improved biogas production by anaerobic digestion. *International Journal of Environmental Science and Technology*, *19,* 3455–3456.
67. Long, C., et al. (2018). Improvement in xylooligosaccharides production by knockout of the β-xyl1 gene in Trichoderma orientalis EU7-22. *3 Biotech, 8*(1), 26.
68. Hao, Z., et al. (2021). Genome sequence analysis of the fungal pathogen Fusarium graminearum using Oxford Nanopore Technology. *Journal of Fungi, 7*(9), 699.
69. Nakhate, S.P., R,K. Gupta, B.J. Poddar, et al. (2022). Influence of lignin level of raw material on anaerobic digestion process in reorganization and performance of microbial community. *International Journal of Environmental Science and Technology*, *19,* 1819–1836.
70. Varghese, V. K., et al. (2022). A comprehensive review on current status and future perspectives of microbial volatile fatty acids production as platform chemicals. *Science of The Total Environment, 815*, 152500.
71. Peng, M., et al. (2020). Effectiveness of probiotics, prebiotics, and prebiotic-like components in common functional foods. *Comprehensive Reviews in Food Science and Food Safety, 19*(4), 1908–1933.
72. Lordan, C., et al. (2020). Potential for enriching next-generation health-promoting gut bacteria through prebiotics and other dietary components. *Gut Microbes, 11*(1), 1–20.
73. Gonçalves, M. F., et al. (2021). Genome and metabolome MS-based mining of a marine strain of Aspergillus affinis. *Journal of Fungi, 7*(12), 1091.
74. Bhavaniramya, S., et al. (2019). Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain & Oil Science and Technology, 2*(2), 49–55.
75. Kurobane, I., et al. (1980). Metabolites of Fusarium solani related to dihydrofusarubin. *The Journal of Antibiotics (Tokyo), 33*(11), 1376–1379.
76. Toghueo, R. M. K. (2020). Bioprospecting endophytic fungi from Fusarium genus as sources of bioactive metabolites. *Mycology, 11*(1), 1–21.
77. Wang, C. C., et al. (2010). Asperfuranone from Aspergillus nidulans inhibits proliferation of human non-small cell lung cancer A549 cells via blocking cell cycle progression and inducing apoptosis. *Basic and Clinical Pharmacology and Toxicology, 107*(1), 583–589.

78. Cen, Y.-K., et al. (2020). The gibberellin producer Fusarium fujikuroi: Methods and technologies in the current toolkit. *Frontiers in Bioengineering and Biotechnology, 8*, 232.
79. Ismaiel, A. A., & Papenbrock, J. (2015). Mycotoxins: Producing fungi and mechanisms of phytotoxicity. *Agriculture, 5*, 492–537.
80. Choukade, R., & Kango, N. (2021). Production, properties, and applications of fructosyltransferase: A current appraisal. *Critical Reviews in Biotechnology, 41*(8), 1178–1193.