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SHORT REPORTS

Draft genome sequence of *Penicillium chrysogenum* strain HKF2, a fungus with potential for production of prebiotic synthesizing enzymes

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Abstract

In this study, we have characterized a novel set of extracellular enzymes produced by *Penicillium chrysogenum* strain HKF2. A draft genome data of 31.5 Mbp was generated and annotation suggested a total of 11,243 protein-coding genes out of which 609 were CAZymes, majority of which were found to have homology with *Penicillium rubens, Penicillium chrysogenum* followed by *Penicillium expansum* and *Penicillium roqueforti*. The prominent CAZyme genes identified in the draft genome encoded for enzymes involved in the production of prebiotics such as inulo-oligosaccharides and fructo-oligosaccharides. Corresponding enzyme assay indicated that the isolate possessed the potential to produce 11.8 and 3.8 U/mL of β-fructofuranosidase and inulinase, respectively. This study highlights the significance of Effluent Treatment Plants as novel and under-explored niche for isolation of fungi having the potential for production of prebiotics synthesizing enzymes.

Keywords Prebiotic · *Penicillium* · CAZymes · Draft genome

Prebiotics are non-digestible food ingredients which selectively promote the growth and activity of beneficial bacteria in the colon, thereby positively improving the health status of the host (Patel and Goyal 2012; Gujar et al. 2018). Popular prebiotics which include inulo-oligosaccharides (IOS), fructo-oligosaccharides (FOS), chito-oligosaccharides (COS), isomalto-oligosaccharides (IMO), and galacto-oligosaccharides (GOS) have gained much attention in pharmaceutical and food sector (Patel and Goyal 2011). *Penicillium* sp. is known to produce range of various carbohydrate active enzymes (CAZymes), e.g., exoinulinase (EC 3.2.1.80), β-fructofuranosidase (EC 3.2.1.26), endo-chitinase (EC 3.2.1.14), α-glucosidase (EC 3.2.1.20) and β-galactosidase (EC 3.2.1.23) which are used for industrial production of IOS, FOS, COS, IMO and GOS, respectively (Gyo et al. 2009; Park and Oh 2010; Prata et al. 2010; Patel and Goyal 2011; Singh and Shukla 2012; Schafhauser et al. 2015; Saqib et al. 2017).

Penicillium chrysogenum strain HKF2 was isolated from activated sludge samples from effluent treatment plant (ETP) located at Ankaleshwar in Gujarat State of India (Deshmukh et al. 2014). The details of the isolation have been provided in Table S1. In the present study, the strain was screened for production of extracellular β-fructofuranosidase and inulinase enzymes. Submerged fermentation was carried out for production of the two enzymes (Prata et al. 2010; Trivedi et al. 2012). Samples taken at a regular interval of 24 h were used as the source of crude enzyme, while the enzyme activities were determined as per the protocols developed by Sangeetha et al. (2004) and Trivedi et al. (2012). One unit of FFase activity was defined as the amount of enzyme required to produce 1 µmol of glucose per minute at 55 °C with 55% sucrose at pH 5.5 (Sangeetha et al. 2004). One unit of inulinase activity was defined as the amount of enzyme necessary to release one micromole of fructose per minute at 60 °C with 1% inulin at pH 5 (Trivedi et al. 2012). Production of FFase and inulinase by the fungal isolate is shown in Fig. S1. Increase in FFase activity was observed which reached a peak in 72 h (11.8 U/mL) with further incubation

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resulting in decrease in enzyme activity. The corresponding inulinase activity was also found to increase gradually to 1 U/mL in 48 h followed by a rapid increase to maximum level of 3.8 U/mL in 96 h.

For understanding the genetic potential of *P. chrysogenum* strain HKF2, whole-genome sequencing approach was used for mining of genes of various prebiotics synthesizing enzymes present in the genome. Genomic DNA was isolated with FastDNA SPIN Kit 116540600 (MP Biomedicals, USA) followed by preparation of paired-end and mate-pair sequencing libraries with mean sizes 639 and 679 bp, respectively. The libraries were sequenced (2 × 150 bp) on Illumina HiSeq 2500 platform. High-quality reads generated were used for de novo assembly using Soapdenovo2 assembler (Luo et al. 2012; He et al. 2017) resulting in a draft genome of 31.5 Mbp containing 296 contigs and 94 scaffolds (Table 1). Genome–genome comparison between *P. chrysogenum* strain HKF2 and closest matching *P. chrysogenum* strain P2niaD18 performed using MAUVE v.2.4.0 software (Darling et al. 2004) is shown in Fig. S2.

The repeat sequences were masked using RepeatMasker and the RepBase library, and a total of 588,673 bp (1.87%) were masked, and the repeats masked assembly was used for coding gene prediction. Coding sequences (CDS) in the genome were predicted by GeneMarkES (Borodovsky and Lomsadze 2011) which resulted in a total of 11,243 protein-coding genes. For the predicted CDS, a similarity search was done against NCBI's non-redundant (nr) database using the BLASTP algorithm. Top-hit distribution proteins revealed that majority of the hits were found to have homology with *P. rubens, P. chrysogenum* followed by *P. expansum* and *P. roqueforti*. All proteins were also searched for similarity against Swiss-Prot, KOG and Pfam databases using BLASTP, rpsblast and Hmmscan algorithms (via webMGA). Gene Ontology (GO) annotation was obtained for nr database annotated proteins using Blast2GO Pro and those with similar functions were allotted to the same GO functional groups (Götz et al. 2008). Analysis of GO sequence distributions revealed the presence of all three GO domains, i.e., Biological Processes (3980), Molecular Function (3349) and Cellular Components (3946). KEGG automatic annotation server (KAAS) was used for assigning orthologs and mapping of proteins to the biological pathways which were then compared against the KEGG database using BLASTP. CAZymes analysis was carried out through dbCAN and all data generated in dbCAN were based on the family classification from CAZy database (Yin et al. 2012).

Gene annotation revealed the presence of genes coding for various prebiotic synthesizing enzymes in *P. chrysogenum* strain HKF2 genome, which were labeled with locus id/tag numbers viz., gene 7365 (exoinulinase), gene 7089 (β-fructofuranosidase), gene 629 (endochitinase), gene 1498 (α-glucosidase) and gene 1128 (β-galactosidase). The amino acid sequence of predicted genes was searched for similarity against reference proteins (refseq_proteins) database using BLASTP (version 2.7.1), and top 10 hits were chosen to study phylogeny of respective enzymes. Sequences were aligned using ClustalW interface in MEGA 7 based on the neighbor joining method, and bootstrap values were based on 1000 replicates (Fig. 1). The nearest neighbor analysis of exoinulinase, β-fructofuranosidase, endochitinase, α-glucosidase and β-galactosidase showed the diversification associated with strain which allowed the survival in such extreme environments as existing in ETPs. Exoinulinase (gene 7365) exhibited 95% similarity with *P. rubens* strain wisconsin 54-1255 exoinulinase. β-fructofuranosidase (gene 7089) displayed 86% similarity with β-fructofuranosidase of *P. expansum* isolated from infected apple (Accession Number XP_016592914.1) (Ballester et al. 2015). The genes, gene 629 (endochitinase), gene 1498 (α-glucosidase), and gene 1128 (β-galactosidase) showed 87, 93 and 86% identities, respectively, with corresponding enzymes of *P. digitatum* Pd1 which was isolated from infected grapefruit (Accession Numbers XP_014534878.1, XP_014538379.1, XP_014530847.1) (Marcet-Houben et al. 2012). Comparative genome analysis was carried out to determine the novelty of extracellular CAZymes secreted by this strain needs further exploration for identifying their role in utilization of complex polysaccharides.

Table 1 Features of draft genome sequence of *Penicillium chrysogenum* strain HKF2

Features	*Penicillium chrysogenum* strain HKF2 draft genome
No. of reads	42,170,766
Pair end read length	150 bp
Genome assembly size	31,484,772
Number of contigs	6194
Number of scaffolds	94
Contig N50	10,874
Gaps between scaffolds	0
Scaffold N50	3,449,782
No. of gene model	11,243
No. of exon per gene	3.15
Mean protein length	476.36
GC content	53.21%
No. of tRNA	188
NR annotated	11,172
SwissProt annotated	7580
KEGG annotated	2128
KOG annotated	6003
Pfam annotated	8174
CAZymes	609

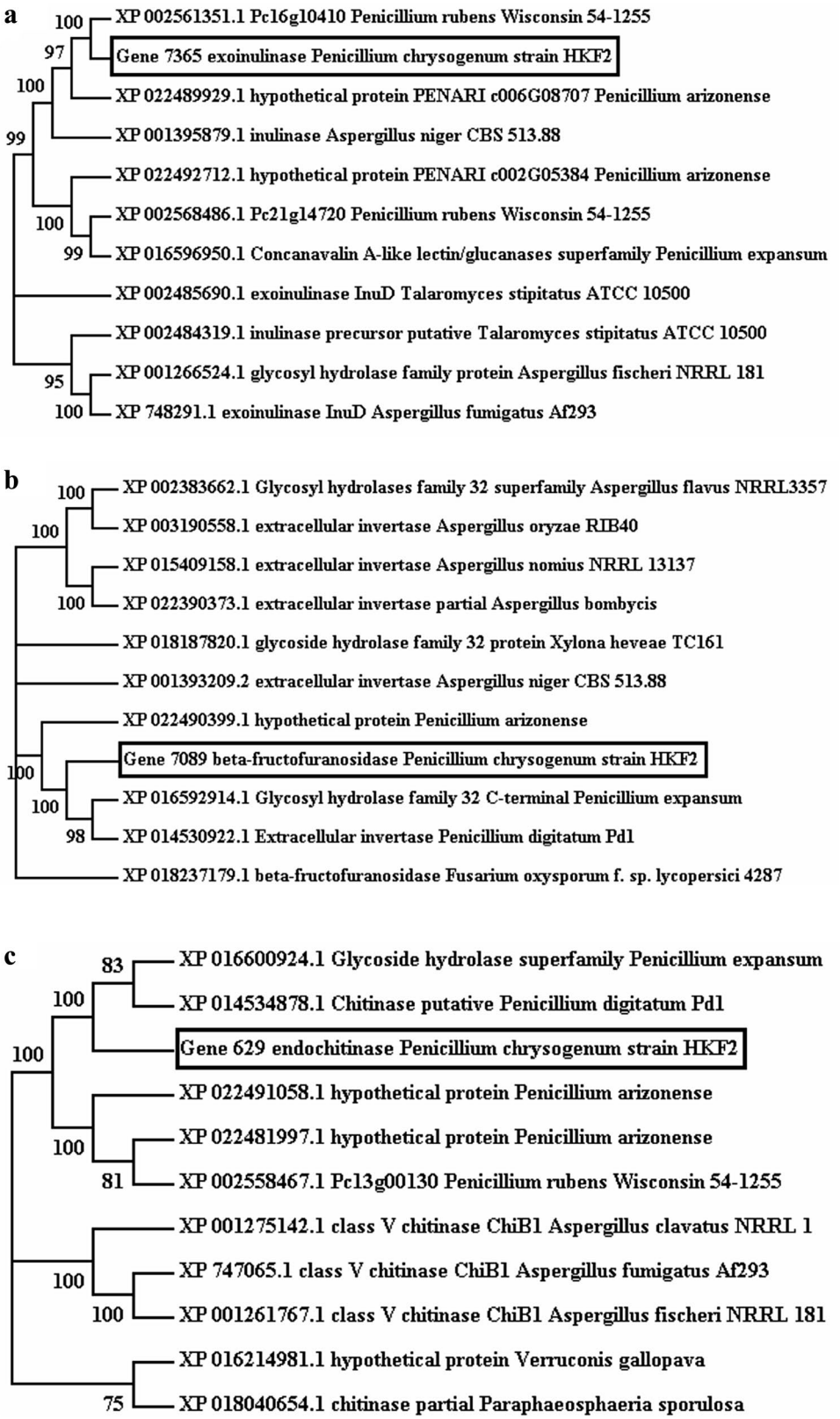
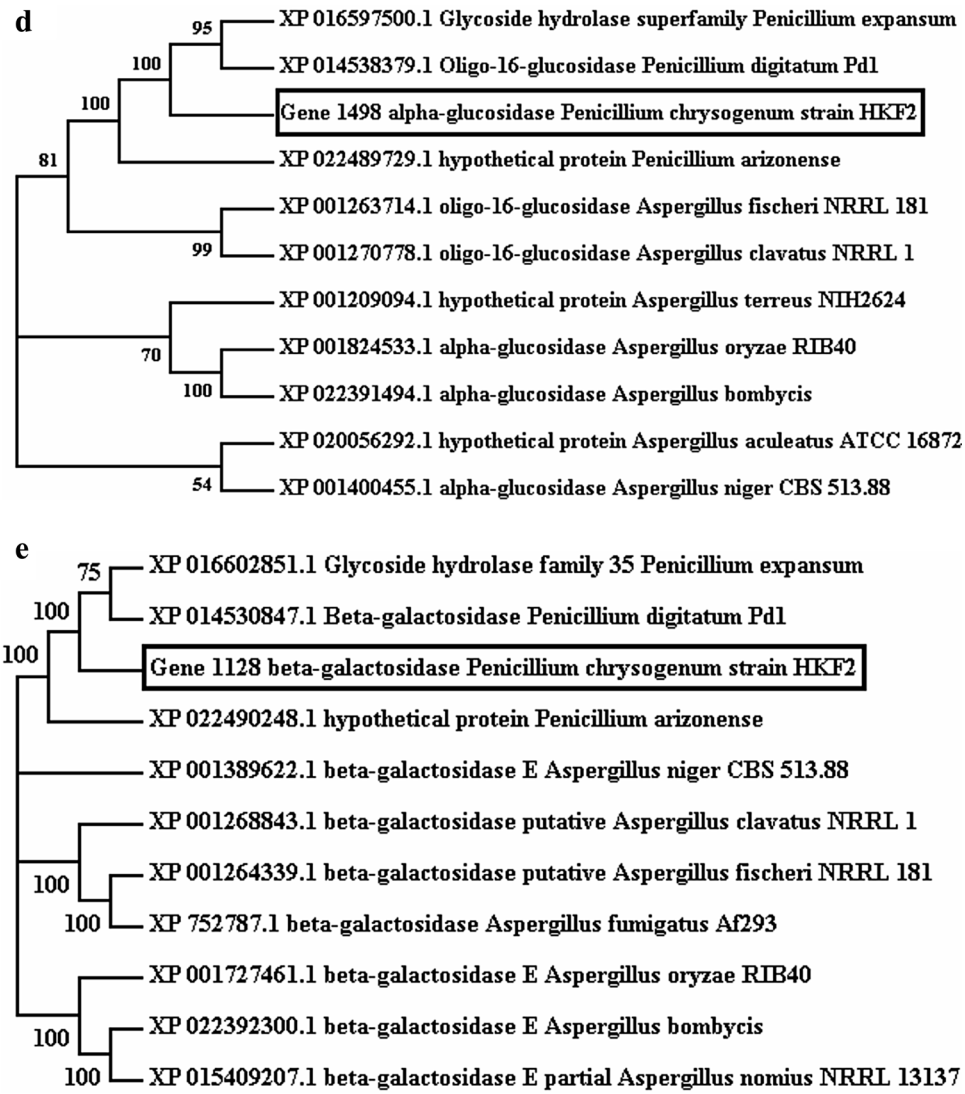


Fig. 1 Neighbor Joining Tree calculated by ClustalW for **a** Gene 7365 exoinulinase, **b** Gene 7089 β-fructofuranosidase, **c** Gene 629 endochitinase, **d** Gene 1498 α-glucosidase, **e** Gene 1128 β-galactosidase from *Penicillium chrysogenum strain* HKF2 constructed by MEGA 7. Numbers displayed on the branches are the bootstrap support obtained through 1000 replications

Fig. 1 (continued)



Nucleotide sequence accession numbers This Whole Genome Shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number MUXA00000000. The version described here is MUXA01000000.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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