



Identification of carbohydrate active enzymes from whole genome sequence of *Tilletia indica* and sporulation analysis

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ABSTRACT

Karnal bunt of wheat is an internationally quarantined disease. One of the major constraints in exporting wheat is prevalence of Karnal bunt (KB). It is caused by *Tilletia indica* which is a heterothallic fungus and requires fusion of different mating types for causing infection. The growth efficiency and aggressiveness of plant pathogens are often associated with their carbohydrate active enzymes (CAZymes). The present study was undertaken to identify the CAZymes in whole genome sequence of *Tilletia indica*, and conduct sporulation analysis. Total 315 predicted secretory proteins were annotated with the CAZyme database (dbCAN). The secreted carbohydrates active enzymes were identified as 105 glycosylhydrolases (GH), 85 glycosyltransferases (GT), 83 carbohydrate esterases (CE), 8 carbohydrate binding modules (CBM), 30 auxiliary activities (AA) and 4 polysaccharide lyases (PL). Based on sporulation analysis of *T. indica* isolates, KB1 and KB2 isolates were most sporulative among all the isolates of *T. indica*. These carbohydrate active enzymes can help to understand the pathogenesis mechanism(s) of Karnal bunt using functional genomics approach.

Key words: Carbohydrate active enzymes, Karnal bunt, Sporulation, *Tilletia indica*, Wheat

Wheat is the most important cereal crop grown and consumed worldwide. Wheat production has increased tremendously after the green revolution. Currently, India is self-sufficient in wheat production with surplus stock and has potential to produce more wheat. In India, wheat production was 98.40 mt with cultivated area of 31.78 mha in (Anonymous 2017-18). The occurrence of the Karnal bunt (KB) disease in North-western plain zone of India is a major constraint to wheat grain export. The disease is re-emerging in North-western plains of India. It affects grain quality and quantity of wheat (Gurjar *et al.* 2016). Total 77 countries imposed limitations on import of wheat from the areas where the Karnal bunt disease occurs (Bonde *et al.* 2004), took strict quarantine measures and insisted zero tolerance limit (Singh and Gogoi 2011).

Karnal bunt of wheat caused by the heterothallic fungus *Tilletia indica* was first reported from Karnal (Haryana) (Mitra 1931). *T. indica* is a hemibiotrophic fungus belonging

to order Ustilaginales and family ustilaginaceae (Nagarajan *et al.* 1997). *T. indica* requires fusion of different mating types for causing infection which results in genetic variation in pathogen (Krishna and Singh 1983, Aggarwal *et al.* 2010). It is a seed and soil-borne pathogen and also has an air-borne sporidial stage. The pathogen enters the grain through the germinal end and partially converts the kernels into sori filled with mass of teliospores (Aggarwal *et al.* 1999). It survives in the form of diploid teliospores in or on the seed and in agricultural soil. Teliospores of *T. indica* germinate to produce primary sporidia or the macro (filiform) sporidia, these are splash dispersed and in turn produce a large quantity of secondary or micro or allantoid spores. These allantoid spores are the only form that infects the wheat earhead (Dhaliwal and Singh 1988). In this study, we assessed the allantoid spores in different isolates of *T. indica*.

Whole genome of a virulent isolate *T. indica* has been sequenced and generated in Fungal Molecular Biology Laboratory, Division of Plant Pathology, ICAR-IARI, New Delhi (NCBI database with ID: RAKB_UP_1; accession numbers MBSW000000000) (Gurjar and Aggarwal 2018). A diagnostic marker has been developed to detect *T. indica* (Gurjar *et al.* 2017). Enzymes required for degrading plant cell walls is a crucial factor for pathogen invasion. The growth efficiency and aggressiveness of plant pathogens are often associated with their carbohydrate active enzymes

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(CAZymes). Keeping this in view, present study was undertaken to identify the CAZymes in whole genome sequence of *T. indica*.

MATERIALS AND METHODS

Pathogen and sporulation analysis: Twenty collected and well established isolates of *T. indica* from various locations in North-Western Plains Zone, were cultured and maintained at $16\pm 2^\circ\text{C}$ in incubator at Fungal Molecular Biology Laboratory, ICAR-IARI, New Delhi.

A mass of mycelium was put facing downward at the apical part of the PDA slant and the tubes were incubated at $16\pm 2^\circ\text{C}$. After 7–8 days creamy white growth of fungus appeared showering sporidia downward from the disc which covered the entire slant within a few days. Spore suspension for all isolates after 15 days old cultures were used for allantoid spore count through haemocytometer. Crescent shape allantoid spores are infective. The sporulation of all the isolates of *T. indica* was observed with three replications and statistically analyzed.

Identification of carbohydrate active enzymes (CAZymes): dbCAN is a database and web server for automated annotation of carbohydrate active enzyme. It provides a subfamily classification of the existing CAZymes families based on sequence similarities. *T. indica* genome sequence corresponding to all families belonging to the CE, GH, GT, AA, CBM and PL families were retrieved from the CAZymes database (Yin *et al.* 2012). Homologous sequences in *T. indica* genomes were obtained by screening the CAZymes sequences using BLASTP. The dbCAN (dbCAN HMMs 5.0) was used to detect carbohydrate active enzymes (CAZymes) based on the CAZymes database in

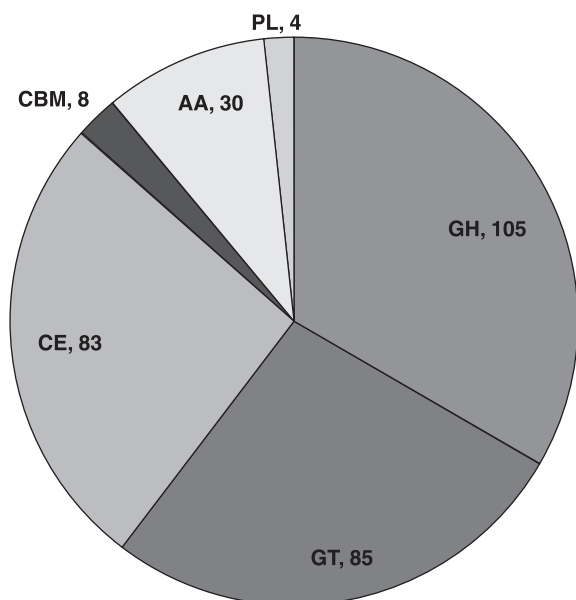


Fig 1 CAZymes identified in whole genome sequence of *Tilletia indica* using functional annotation. CAZymes consisted of Glycosyl Hydrolases (GH), Glycosyl Transferases (GT), Carbohydrate Esterases (CE), Carbohydrate Binding Modules (CBM), Auxiliary activities (AA) and Polysaccharide Lyases (PL).

Table 1 Sporulation of *Tilletia indica* isolates from North-Western Plains Zone

Isolates	Location	Average (10^4 spores/ml)
KB1	Aligarh, UP	32.00
KB2	New Delhi	30.33
KB3	Tarari	3.67
KB4	Pipli	2.78
KB5	Nilokheri	7.56
KB6	Sonipat, Haryana	6.56
KB7	Jind	1.66
KB8	Panipat, Haryana	6.89
KB9	Bareilly, UP	14.00
KB10	Bhiwani, Haryana	18.78
KB11	Pundri	21.67
KB12	Ambala	16.22
KB13	Pantnagar, Uttarakhand	0.22
KB14	Sultanpur	0.22
KB15	Allahabad, UP	2.11
KB16	Bulandshahr, UP	16.78
KB17	Saharanpur, UP	17.11
KB18	Mujaffar Nagar, UP	2.33
KB19	Patudi, Haryana	2.89
KB20	Shamli, Haryana	2.04
CD		0.12
SE(m)		0.04
SE(d)		0.06
CV		4.56

the *T. indica* secretome (Yin *et al.* 2012).

RESULTS AND DISCUSSION

Isolates of *T. indica* and sporulation: Among 20 isolates, KB1 (32×10^4 spores/ml) and KB2 (30.33×10^4 spores/ml) were highly sporulative, with no significant difference between them (Table 1). KB13 and KB14 isolates were least sporulative (0.22×10^4 spores/ml) among all the studied isolates. Allantoid spores are only infective spores that cause infection (Singh and Gogoi 2011). KB1 isolate showing highest sporulation count can be used for further expression studies and virulence analysis.

Identification of CAZymes (Carbohydrate active enzymes) in *T. indica* genome: Enzymes required for degrading plant cell walls is a crucial factor for pathogen invasion. The growth efficiency and aggressiveness of plant pathogens are often associated with their carbohydrate active enzymes. Therefore, CAZymes were also identified in genome of *T. indica*. Total 315 predicted secretory proteins were annotated with the CAZyme database. The secreted carbohydrates enzymes consisted of 105 glycosyl hydrolases, 85 glycosyl transferases, 83 carbohydrate esterases, 8 carbohydrate binding modules, 30 auxiliary

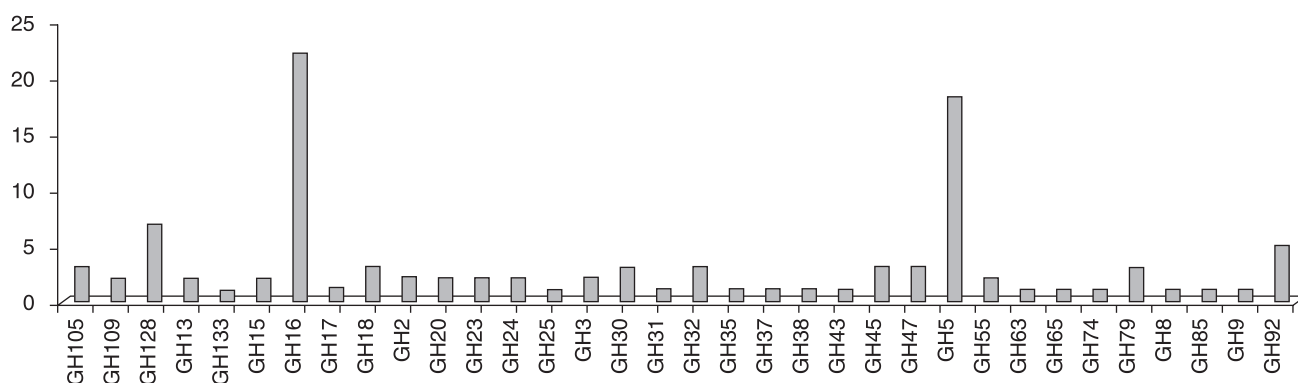


Fig 2 Glycosyl hydrolase enzymes grouped into different GH families.

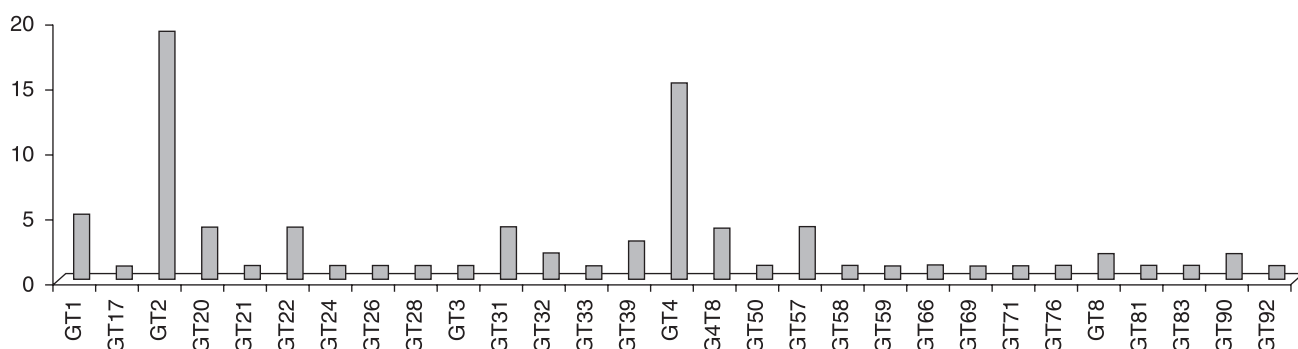


Fig 3 Glycosyl transferase enzymes grouped into different GT families.

activities (AA) and 4 polysaccharide lyases (PL) (Fig 1).

Present investigation revealed that *T. indica* had 105 glycosyl hydrolase enzymes categorized into 34 GH families. The maximum number of glycosyl hydrolase enzymes belonged to GH16 and GH5 with 22 and 18 enzymes respectively (Fig 2). Glycosyl transferases enzymes (85) were grouped into 29 GT families. The maximum number of glycosyl transferases enzymes belonged to GT2 and GT4 (Fig 3). Total 83 carbohydrate esterases were assigned into 8 CE families. CE10 family was found most prevalent containing 33 CE enzymes (Fig 4). Thirty auxiliary activities enzymes were grouped into 7 AA families. Among seven families, AA7 and AA3 were most prevalent (Fig 5). Eight carbohydrate binding module enzymes were grouped into 6 CBM families. CBM50 was most prevalent family among them (Fig 6). Four polysaccharide lyases enzymes were grouped into three PL families. The PL4 (2 enzymes) family was most predominant (Fig 7). Glycosyl hydrolases, glycosyl transferases and carbohydrate esterases families were highly prevalent which are required for degradation of plant cell

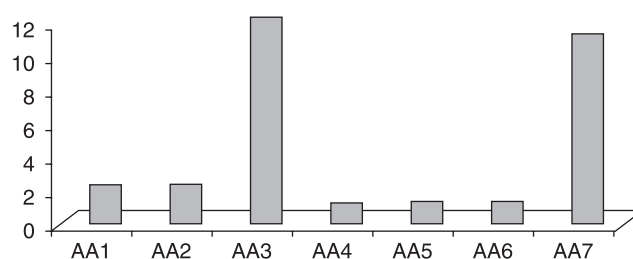


Fig 5 Auxiliary activities enzymes grouped into different AA families.

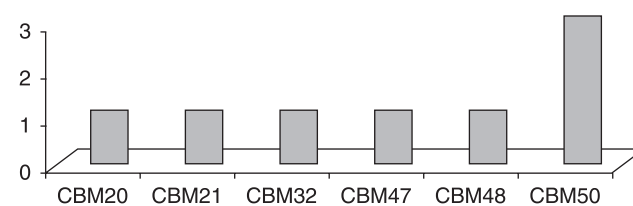


Fig 6 Carbohydrate binding modules grouped into different CBM families.

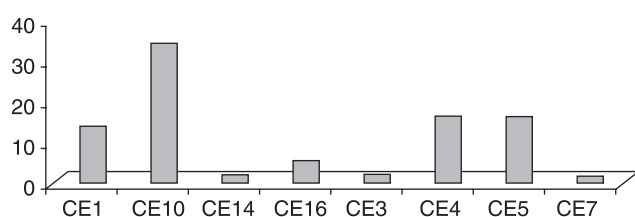


Fig 4 Carbohydrate esterases enzymes grouped into different CE families.

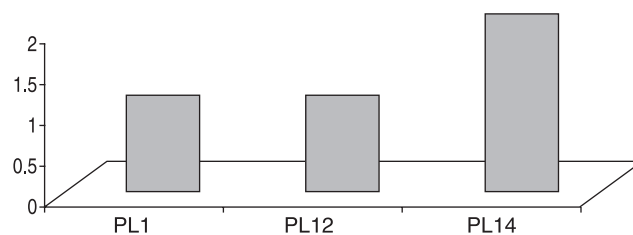


Fig 7 Polysaccharide lyase enzymes grouped into different PL families.

wall. Earlier studies revealed that carbohydrate active enzymes were identified in *Phytophthora* sp. The CE, GH, and PL superfamilies are involved in cell wall degrading enzymes (CWDE) (Ospina-Giraldo *et al.* 2010). The GH, PL, and CE enzymes may have a role in pathogenicity to cause diseases (Walton 1994, Ospina-Giraldo *et al.* 2003). The number of putative GT enzymes was considerably high as glycosidic bonds during biosynthesis of polysaccharides require the transfer of sugar moieties (Lairson *et al.* 2008).

The genome analyzed for CAZymes was found to be enriched with glycoside hydrolases f, glycosyltransferases, carbohydrate esterases (CE), auxiliary activities (AA), carbohydrate binding modules (CBM) and polysaccharide lyases (PL) f. Among these, glycosyl hydrolases, carbohydrate esterases and glycosyl transferases family genes were more prevalent. These are required for degradation of plant cell wall. These enzymes can be utilized for functional genomics approach to understand complex biology of Karnal bunt of wheat.

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