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FOOD MICROBIOLOGY - RESEARCH PAPER

De novo genome sequencing of mycoparasite *Mycogone perniciosa* strain MgR1 sheds new light on its biological complexity

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Abstract

Mycogone perniciosa is a mycoparasite causing Wet Bubble Diseases (WBD) of *Agaricus bisporus*. In the present study, the whole genome of *M. perniciosa* strain MgR1 was sequenced using Illumina NextSeq500 platform. This sequencing generated 8.03 Gb of high-quality data and a draft genome of 39 Mb was obtained through a de novo assembly of the high-quality reads. The draft genome resulted into prediction of 9276 genes from the 1597 scaffolds. NCBI-based homology analysis revealed the identification of 8660 genes. Notably, non-redundant protein database analysis of the *M. perniciosa* strain MgR1 revealed its close relation with the *Trichoderma arundinaceum.* Moreover, ITS-based phylogenetic analysis showed the highest similarity of *M. perniciosa* strain MgR1 with *Hypomyces perniciosus* strain CBS 322.22 and *Mycogone perniciosa* strain PPRI 5784. Annotation of the 3917 genes of *M. perniciosa* strain MgR1 grouped in three major categories viz. biological process (2583 genes), cellular component (2013 genes), and molecular function (2919 genes). UniGene analysis identified 2967 unique genes in *M. perniciosa* strain MgR1. In addition, prediction of the secretory and pathogenicity-related genes based on the fungal database indicates that 1512 genes (16% of predicted genes) encode for secretory proteins. Moreover, out of 9276 genes, 1296 genes were identified as pathogenesis-related proteins matching with 51 fungal and bacterial genera. Overall, the key pathogenic genes such as lysine M protein domain genes, G protein, hydrophobins, and cytochrome P450 were also observed. The draft genome of MgR1 provides an understanding of pathogenesis of WBD in *A. bisporus* and could be utilized to develop novel management strategies.

Keywords Genome sequencing · Pathogenic genes · Mycoparasitism · *Mycogone perniciosa* · Wet Bubble Disease · *Agaricus bisporus*

Introduction

Agaricus bisporus is one of the most popular edible macrofungi and is commonly referred to as white button mushroom. Additionally, it is accounted for maximum share (73%) in total mushroom production of India [1]. However, the yield of the *A. bisporus* is adversely affected by various biotic and abiotic stresses. Among biotic factors, fungal pathogens like *Mycogone perniciosa* Magn. (Telomorph; *Hypomyces perniciosus*) are well known to cause economic losses to the growers across the globe [2–6]. *M. perniciosa* causes wet bubble disease (WBD) of *A. bisporus*. It is a contagious disease which frequently contaminates the *A. bisporus* growing facilities. *M. perniciosa* belongs to the division Eumycota, sub-division Deuteromycotina, class Hypomycetes, order Moniliales, and family Moniliaceae. While the perfect stage of *M. perniciosa*, Hypomyces belongs to division Eumycota, sub-division Ascomycotina, class Pyrenomycetes, order Sphaeriales, and family Hypomycetaceae [7]. This fungus is not observed during spawn run stage of the crop, but causes huge losses during primordial formation and other crop developmental stages [8]. WBD disease was first reported from Paris, France in 1888 [9]. The disease has also been reported to assume serious proportions in other major mushroom growing countries of the world such as Serbia [10], the United Kingdom [11], Netherlands [12], South Africa [13], Brazil [14], Spain [15], Poland [16], and China [8, 17, 18]. In India, this disease was reported for the first time in 1978 in the Union Territory of Jammu and

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Kashmir [7, 19]. Seasonal cultivation without environmental controlled facilities for pasteurization of compost, casing, and crop management are considered the plausible causes of heavy inoculum build-up and spread of *M. perniciosa* [7]. Later, the disease was also reported from other states of India such as Himachal Pradesh, Haryana, and Maharashtra [2–4, 6].

M. perniciosa and *M. rosae* are the two major species of the genus *Mycogon*e which infect mushroom crops. Notably, *M. perniciosa* is the most common and globally important species [20]. It enters into fruit bodies at the time of primordial formation and completely deformed the fruit body into sclerodermoid masses, i.e., primordial is not differentiated into stipe and pileus. The infected mushrooms are rendered unfit for market use [21–23]. On the other hand, *M. rosae* is rarely found in the mushroom growing facilities [22, 24].

M. perniciosa also infect other mushroom species including *A. campestris*, *Pleurotus eryngii*, *P. citrinopileatus*, *P. nebrodensis*, *P. ostreatus*, and *Volvarieila volvacea* [2, 3, 6]. Mycelium of *M. perniciosa* is initially white but brownish when the colony ages due to the colorful conidia generation [8]. Hyphae are hyaline produced one-celled conidia and large two-celled chlamydospore [3].

In the mushroom growing facilities, casing soil which is the top layer of compost of *A. bisporus* cultivation is considered the major source of inoculum for WBD [3]. This is due to the entry of the pathogen into mushrooms at the time of pinhead formation [23]. The pinhead formation of *A. bisporus* requires casing. Therefore, physiochemical and micro-flora quality of casing soil play key role in fruit body formation in *A. bisporus* [25, 26].

Moreover, inoculum of *M. perniciosa* could be easily carried from one place to another by the wind, water, contaminated clothing, or equipment or mechanically by mites and flies [7, 12, 15, 27]. WBD is very difficult to manage because none of the commercial available Indian strains of *A. bisporus* possessed complete resistance against *M. perniciosa*. Hygienic conditions and good management practices (GAP) are the most suggested remedies for the management of this disease [3]. Fungicides such as benzimidazoles, chlorothalonil, and prochloraz-Mn are the most widely used for WBD management [16–18, 28–30]. Despite the effectiveness of these fungicides in controlling WBD, the yield of the *A. bisporus* is declined due to infection [28]. On the other hand, botanical and microbial pesticides were also found effective against WBD, mostly through their protective applications [31–34]. Besides this, WBD management is complex and tedious due to the genetic variability of the *M. perniciosa* strains [8, 10, 11, 35, 36]. Considering the genetic diversity and the substantial economic losses pertaining to *M. perniciosa* [37], scanty information is available in literature on its genetics and pathogenicity aspects. Recently, Li et al. [38] reported genetic mechanism of pathogenesis and adaptation of *H. perniciosus* (*M. perniciosa*) under diverse range of environments. An array of the genes viz. transporters, protein kinases, CAZymes (GH 18), peptidases, cytochrome P450, and SMs of *H. perniciosus* plays an important role during the parasitic relationship with *A. bisporus*.

Knowledge of the pathogens at molecular level is required to provide a new insight of mechanism of disease establishment in the host [39]. As per the current knowledge of mushroom pathology, genomes of only few mycoparasites have been sequenced such as *Lecanicillium fungicola* 150–1[40], *Cladobotryum protrusum* [41], *Cladobotryum dendroides* [42], and *Trichoderma virens* [43]. Therefore, the present study was conducted to explore the genomic features of *M. perniciosa* MgR1 including its relatedness, pathogenesis-related genes, and secretory proteins. The predicted candidate genes of *M. perniciosa* will be helpful for understanding their role in pathogenesis and disease-associated mechanism (s) and ultimately provide a novel strategy to fight WBD.

Materials and methods

Strain and culture conditions

The WBD infected fruit bodies of *A. bisporus* were collected from Haryana state of India. The associated fungal pathogen was isolated on PDA media by placing small bits from the junction of healthy and diseased portion of infected fruit body. Inoculated plates were incubated at 25 °C for 10 days. Culture of the pathogen was further purified by transferring the actively growing hyphae into fresh media plates. The pathogenicity test was performed under mushroom house condition and symptomatology of the WBD was studied. Casing soil was inoculated on the same day of its application with the standardized aleuriospore suspension (5×10^4 spore/ml) of *M. perniciosa* [15]. The pathogen was identified using morphological characters of spores and ITS (internal transcribed spacer) markers. To isolate genomic DNA from mycelia, 250 ml flask containing 100 ml liquid medium (2.5% malt extract and 0.5% peptone) was inoculated with fresh mycelial plugs of 5 mm diameter (3 plugs/flask) and incubated at 25 ± 1 °C for 12 days with rotation. The fungal mat was removed from liquid media, washed with sterilized distilled water, and kept in deep freezer (− 80 °C) to utilize it further for DNA extraction.

Genome sequencing, assembly, and annotation

Genomic DNA was extracted from fungal mycelium using cetyl-trimethylammonium bromide (CTAB) method. In brief, the 0.1 g of lyophilized mycelia was grounded with the help of pestle-mortar. Furthermore, 1 ml of extraction buffer (50 mM Tris; 100 mM EDTA;

150 mM NaCl; pH 8.0) and 100 µl (10% SDS) were added and incubated at 37 °C for 1 h. After incubation, 125 µl of CTAB solution (10% CTAB; 0.7 M NACl) added and again incubated at 65 °C for 20 min, followed by addition of the equal volume of chloroform and isoamyl alcohol (24:1). The centrifugation was performed at 12,000 rpm for 12 min. Furthermore, supernatant was collected in a fresh tube followed by addition of 0.6 volume of isopropanol and 0.1 volume sodium acetate and kept at − 20 °C overnight. After centrifugation (12,000 rpm for 12 min), supernatant was discarded and pellet dissolved in 50 µl TE buffer (10 mM Tris and 1 mM EDTA). The quantification and qualification of the DNA were done with the 4200 Tape Station System. Paired-end libraries were constructed using TruSeq Nano DNA Library Prep Kit and reads were generated by sequencing libraries on NextSeq500 using 2 × 150 bp chemistry (Illumina Inc., San Diego, CA, USA). The sequenced raw data was processed to obtain highquality clean reads using Trimmomatic v0.38 to remove adapter sequences, ambiguous reads (reads with unknown nucleotides "N" larger than 5%), and low-quality sequences (reads with more than 10% quality threshold (QV) < 20 phred score) [44]. High-quality paired-end reads of Illumina Trueseq were then assembled using hybrid approach by SPAdes assembler v-3.13.0 [45]. Sequences were run on default k-mer and the sequence coverage was × 240. Protein coding genes were predicted in assembled scaffolds of *M. perniciosa* strain MgR1 using AUGUSTUS-3.2.1 gene prediction program [46]. *Aspergillus fumigatus* was used as model organism to predict genes from the assembled scaffolds [47]. To predict the functions of predicted genes, the genes were compared using DIAMOND program [48], which is a BLAST-compatible local aligner for mapping translated DNA query sequences against a protein reference database. DIAMOND (BLASTX alignment mode) finds the homologous sequences for the genes against NR (non-redundant protein database) from NCBI [49]. Gene ontology (GO) analysis of the genes predicted for *M. perniciosa* strain MgR1 was carried out using Blast2GO program [50]. The e-value of 1×10^{-5} selected for the BLAST analysis. The pathway annotation of the predicted genes was carried out against the curated KEGG GENES database using KAAS bi-directional best hit method (KEGG Automatic Annotation Server- http://www.genome.jp/kegg/ko.html) [51]. The KEGG Orthology "Fungi" database was used as the reference for pathway mapping. The genome completeness was assessed with gVolante platform [52] using the Core Eukaryotic Genes Mapping Approach (CEGMA) [53] and Benchmarking Universal Single-Copy Orthologs (BUSCO v2/v3) analysis [54].

Orthovenn and phylogenetic analysis

The protein content of MgR1 was compared against protein sequences of closely related organisms *Trichoderma arundinaceum* (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/003/012/105/GCA_003012105.1_Trichoderma_arundinaceum_IBT40837_contigs/GCA_003012105.1_Trichoderma_arundinaceum_IBT40837_contigs_protein.faa.gz) *and Trichoderma reesei* QM6A ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/167/675/GCF_000167675.1_v2.0/GCF_000167675.1_v2.0_protein.faa.gz. The Orthologs in MgR1 sample were identified using Orthovenn2 tool (55). Phylogenetic analysis at genome level was analyzed for *M. perniciosa* strain MgR1 against seven selected organisms, namely, *Trichoderma viride* strain Tv-1511 (NCBI ID. PRJNA543939), *T. virens* strain Gv29-8 (NCBI ID. PRJNA264113), *T. reesei* strain QM6a (NCBI Id. PRJNA325840), *T. longibrachiatum* strain ATCC 18,648 (NCBI ID. PRJNA207876), *T. harzianum* strain CBS (NCBI ID. PRJNA453596), *T. citrinoviride* strain TUCIM (NCBI ID. PRJNA453598), and *T. asperellum* strain CBS (NCBI ID. PRJNA453597) using neighbor-joining (NJ) algorithm of the AAF phylogeny tool (alignment and assembly free tool: version 2,016,104.1) [56]. The phylogeny plots were generated using iTOL [57]. Moreover, ITS region of *M. perniciosa* strain MgR1 was analyzed using the molecular evolutionary genetic analysis (MEGA v6) [58] with neighbor-joining (NJ) cluster method.

Functional annotation of pathogenicity-related genes and MgR1 secretome

Pathogenicity-related genes were identified using PHI database [59]. Secretome annotation was performed for 9276 genes predicted. The predicted gene sequences were searched against the secretory proteins of Fungal Secretome KnowledgeBase (FunSecKB, http://bioinformatics.ysu.edu/secretomes/fungi.php) using Diamond (BlastX mode) at *e* value threshold 1e-5 [60].

Results

De novo genome sequencing of M. perniciosa strain MgR1

Formation of scleradermoid masses, thickened stipe, and droplets of dark brown color were recorded as the characteristic symptoms of WBD under pathogenicity test and as well as under field conditions (Fig. 1). The causal pathogen was identified as *M. perniciosa* Magn. using ITS markers and the sequence was submitted in NCBI GenBank (MN031246). The microscopic analysis of *M. perniciosa* revealed white,

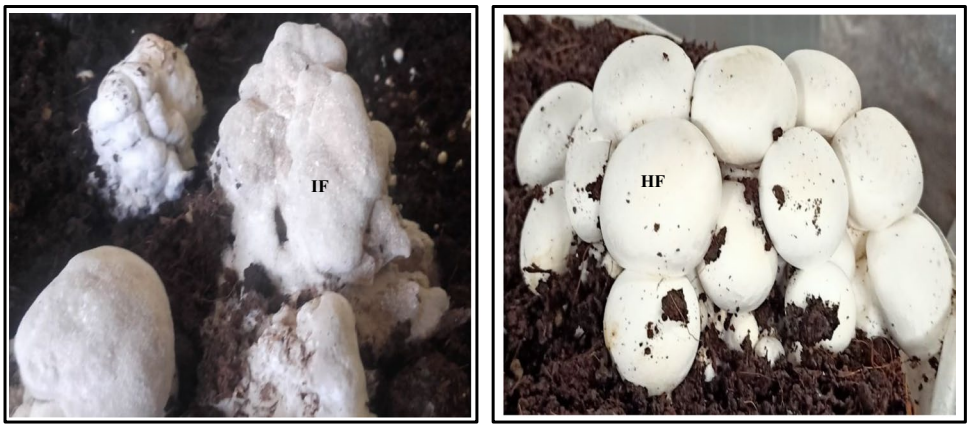

Fig. 1 Symptomatic fruit-body of white button mushroom (*Agaricus bisporus*). **a** IF, infected fruit-body; **b** HF, healthy fruit-body

compact, and fluffy mycelium. Hyphae were branched interwoven, septate, and thin-walled. Bicellular conidia were produced by *M. perniciosa* which are commonly referred to as either aleuriospores or chlamydospores. The *M. perniciosa* was isolated on PDA media and the purified culture was deposited in culture bank of ICAR- Directorate of Mushroom Research, Chambaghat, Solan (HP) with accession number DMRM04. The genome of *M. perniciosa* MgR1 was sequenced using Illumina NextSeq500 system which generated 27,285,801 reads. In total 8.03 Gb genomic data was generated and we obtained genome sequence of *M. perniciosa* after de novo assembly with size of 39 Mb which was accessioned by NCBI (PRJNA543984). The de novo assembled genome of MgR1 contained a total of 1579 scaffolds with the N50 scaffolds of 239,975 bp as well as a 44.40% GC content (Table 1). Genome completeness was evaluated with CEGMA and BUSCO. The analysis showed 98.79% and 99.66% for CEGMA and BUSCO, respectively, indicating high quality of MgR1 genome. A total number of 1512 protein-coding genes were predicted. In *M. perniciosa* genome, in total 9,276 genes were predicted with average gene length of 1625 bp. Maximum gene length of 52878 bp and minimum of 201 bp was recorded (Table 1). Majority of the BLAST hits (*e* value of 1×10^{-5}) were found to be against *Trichoderma arundinaceum* which belongs to the same family Hypocreaceae as that of *M. perniciosa*. *T. virens* which is closely related to *M. perniciosa* was found to be the third top blast hit (Fig. 2). Out of total 9276 genes, 8660 genes showed blast hits with the available genomic data of NCBI database, whereas, 616 genes remained without blast hits.

Gene annotation

Out of total 9276 predicted genes of *M. perniciosa* strain MgR1, only 3917 GO terms (representing 42.2% of the genes) were found to be associated with a GO category. Of these, 2583 were assigned to Biological Process, 2013 were assigned to Cellular Component, and the highest 6139 were assigned to molecular function. In the biological process domain, the majority of the genes belong to the organic substance metabolic processes (19%) followed by primary and cellular metabolic processes (18%) and nitrogen compound metabolic processes (16%) (Fig. 3a), while in the cellular process domain, the majority of the genes belong to the intracellular and intrinsic component of the membrane (19%) (Fig. 3b). Additionally, the molecular function domain includes the genes of the organic cyclic and heterocyclic compound binding (17%) and ion binding (16%) followed by hydrolase activity genes (11%) (Fig. 3c). Moreover, KEGG pathway analysis grouped 9276 genes in five major categories: metabolism, genetic information processing, environmental information processing, cellular

Table 1 Genome features of *Mycogone perniciosa* strain MgR1 using Illumina NextSeq500 platform

Characteristics	*Mycogone perniciosa* strain MgR1
Size (Mb)	39 Mb
Protein-coding genes	1512
Average size of scaffolds (bp)	24,922
Scafold N50 (bp)	239,975
Max Scaffold size (bp)	1,327,753
Min Scaffold size (bp)	200
Number of genes	9276
Secretary proteins (no.)	1512
Pathogen-host interaction genes (no.)	1296
Average gene length (bp)	1625
Maximum gene length (bp)	52,878
Minimum gene length (bp)	201
GC-content (%)	44.40%
CEGMA	98.79%
BUSCO	99.66%

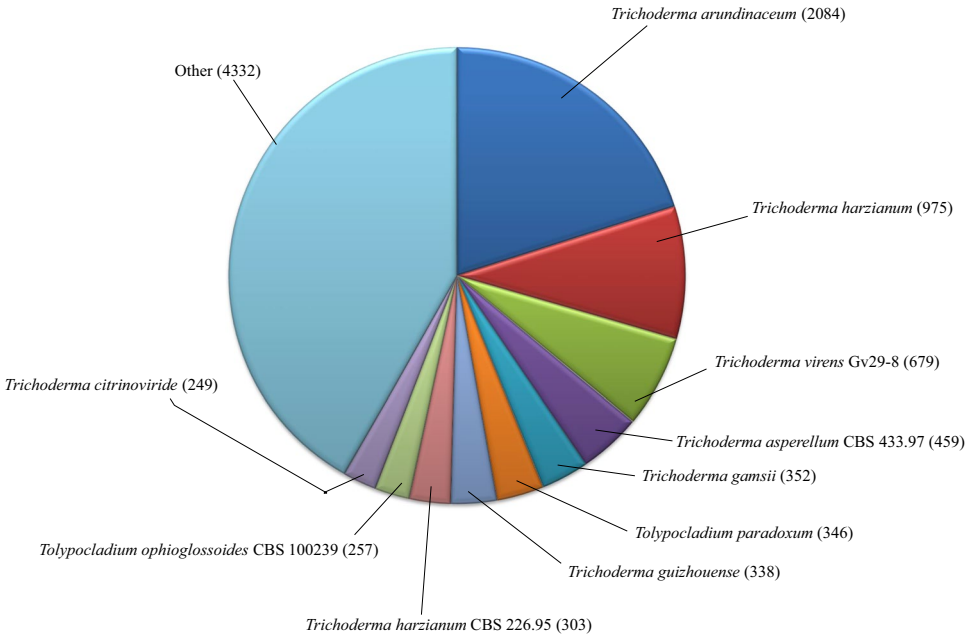

Fig. 2 Results of species distribution of sequence homology search against NCBI NR database for *M. perniciosa* strain MgR1. BLAST hits (*e* value of 1×10^{-5}) demonstrated by the different numbers viz. higher number revealed the higher hit. For instance, *M. perniciosa* strain MgR1 was found similar to *Trichoderma arundinaceium* (2084) followed by *T. harzianum* (975) and *T. virens* (679)

processes, and organismal systems. The output of KEGG analysis includes KEGG Orthology (KO) assignments and corresponding Enzyme Commission (EC) numbers and metabolic pathways of predicted genes using KEGG automated annotation server, KAAS. In addition, UniGene analysis identified the 2967 genes belongs to the twenty three pathways (Table 2). Most of the pathways were found into the categories of metabolic pathways, genetic information processing, environmental information processing, and cellular processes. Signaling molecules and interaction pathway were represented by a single gene, whereas translation pathway was represented by the highest numbers of genes.

Orthology and phylogenetic analysis

Orthovenn analysis of *M. perniciosa* strain MgR1, *Trichoderma arundinaceum*, and *Trichoderma reesei QM6A* revealed that 6532 clusters were found to be common between all the three organisms (Fig. 4). Notably, out of total 9276 proteins of MgR1, 7881 proteins formed 7394 orthologous clusters with the two reference organisms. Among three organisms, the highest orthologous clusters were obtained in *T. arundinaceum* (8263) with 1902 singletons. The phylogenetic tree obtained by whole genome sequence analysis of *M. perniciosa* MgR1 and seven species of the genus *Trichoderma* is presented in Fig. 5a. A neighbor-joining analysis of the whole genome-sequences of all the selected organisms demonstrated three distinct phylogenetic clades. Clade A comprised only *M. perniciosa* strain MgR1. Clade B represented three genomes of *T. citrinoviride* strain TUCIM (NCBI ID. PRJNA453598), *T. reesei* strain QM6a (NCBI Id. PRJNA325840), and *T. longibrachiatum* strain ATCC 18,648 (NCBI ID. PRJNA207876). Clade C represented four genome of *T. asperellum* strain CBS (NCBI ID. PRJNA453597), *T. harzianum* strain CBS (NCBI ID. PRJNA453596), *Trichoderma viride* strain Tv-1511 (NCBI ID. PRJNA543939), and *T.* virens strain Gv29-8 (NCBI ID. PRJNA264113). Moreover, the phylogenetic tree was also constructed based on the comparison of the internal transcribed spacer (ITS) region sequences of *M. perniciosa* strain MgR1 along with other closely related organisms, e.g., *Hypomyces* spp. and *Trichoderma* spp. The phylogenetic analysis grouped in three clades. The Clade A clustered all the *Trichoderma* spp., i.e., *T. harzianum*, *T. aggressivum*, *T. arundinaceum*, *T. longibrachiatum*, *T. citrinoviride*, and *T. viride*. On the other hand, Clade B grouped the *M. pernicosa* strain MgR1 with *Hypomyces perniciosus*, *M. rosea*, *M. perniciosa* strain PPRI5784 and CBS322.52. The clade C included only the *Cladobotryum protrusum* (Fig. 5b).

Pathogenic genes and secretory proteins

The predicted 9276 genes were also searched through BlastX against PHI-base proteins and 1296 genes were aligned to PHI-base proteins (Table 1). Figure 6 depicts the classification of genes of *M. perniciosa* strain MgR1 based on the PHI-base, with the numbers and proportions of genes indicated. Based on the homology of pathogenicity proteins, 451 genes were related to reduced virulence, 21 genes to increased virulence, 133 genes to loss of pathogenicity, 89 genes to lethal, and 588 genes were related to others. The highest number of PHI genes of MgR1 was obtained in phytopathogens like *Fusarium* spp. (650) followed by *Magnaporthe* spp. (243), *Aspergillus* spp. (43), *Colletotrichum* spp. (31), *Parastagonospora* spp. (25), *Botrytis* spp. (14), *Alternaria* spp. (12), *Cochliobolus* spp. (12), *Ustilago* spp. (11), *Zymoseptoria* spp. (8), *Verticillium* spp. (7), *Cercospora* spp. (6). *Leptosphaeria* spp. (6), *Claviceps* spp. (5), *Gaeumannomyces* spp. (5), etc. Sufficient numbers of gene of MgR1 were also found in animal pathogenic fungi like

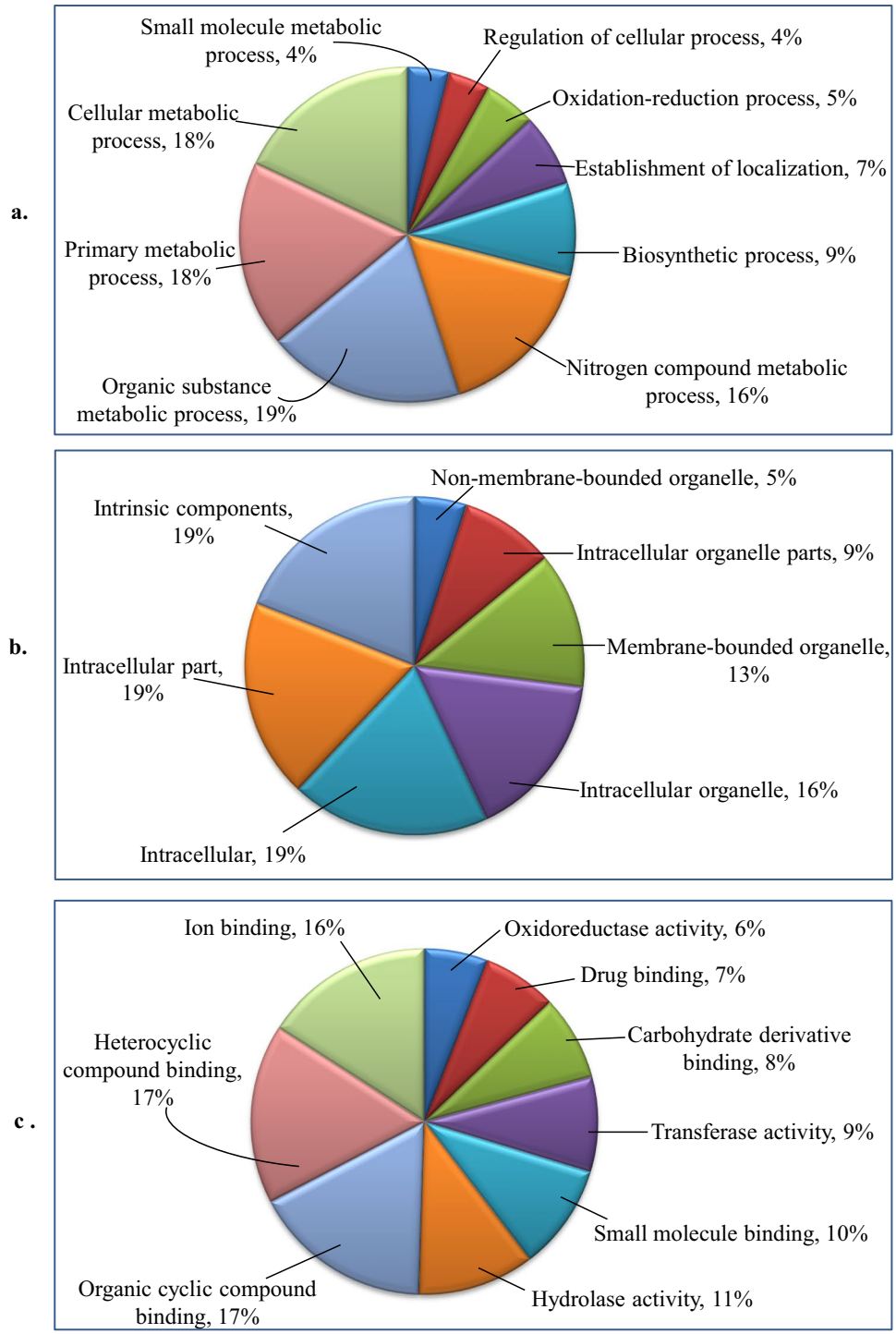

Fig. 3 GO-based functional annotation of genes of *M. perniciosa* strain MgR1 genome. The pie chart shows the top significant enrichment terms represented by **a** biological process, **b** cellular component, and **c** molecular function. The percent value indicates the percentage of number of genes associated with the corresponding GO term represented by each sector of the pie chart. Corresponding number of genes represented in parentheses

Candida spp. (53) and *Cryptococcus* spp. (38) and human pathogenic fungi such as *Histoplasma* spp. (1), *Legionella* spp. (1), and *Trichophyton* spp. (1). MgR1 genes were also found in protozoan parasites of human and animals like *Toxoplasma* spp. (1) and *Leishmania* spp. (2). No doubt the highest similarity of MgR1 was recorded with *Trichoderma* spp.; however, its only 7 pathogenesis-related genes of MgR1 were obtained in *Trichoderma* spp. Large numbers of genes were also obtained in entomophagous fungi like *Beauveria* spp. (29) and *Metarhizium* spp. (17). Surprisingly, many genes were also found in bacterial pathogens of human, animals, plants, and fungi such as *Acinetobacter* spp. (1), *Actinobacillus* spp. (1), *Burkholderia* spp. (3), *Edwardsiella* spp. (3), *Erwinia* spp. (1), *Francisella* spp. (2), *Pseudomonas* spp. (2), *Ralstonia* spp. (2), *Salmonella*

Table 2 Summary of KEGG pathway analysis

Category	Pathway	Gene count
Metabolism	Carbohydrate metabolism	280
	Energy metabolism	150
	Lipid metabolism	156
	Nucleotide metabolism	72
	Amino acid metabolism	247
	Metabolism of other amino acids	83
	Glycan biosynthesis and metabolism	77
	Metabolism of cofactors and vitamins	137
	Metabolism of terpenoids and polyketides	34
	Biosynthesis of other secondary metabolites	42
	Xenobiotics biodegradation and metabolism	77
Genetic information processing	Transcription	137
	Translation	308
	Folding, sorting and degradation	237
	Replication and repair	98
Environmental information processing	Membrane transport	12
	Signal transduction	236
	Signaling molecules and interaction	1
Cellular processes	Transport and catabolism	265
	Cell growth and death	163
	Cellular community—eukaryotes	38
	Cell motility	22
Organismal systems	Environmental adaptation	95

spp. (7), *Staphylococcus* spp. (1), *Xanthomonas* spp. (2), and *Yersinia* spp. Fungal Secretome KnowledgeBase based annotation of secretory revealed that *M. perniciosa* MgR1 genome encodes 1512 secretory proteins (Table 1). The highest number of secretory proteins genes in *M. perniciosa* strain MgR1 was found in *Hypocrea* spp. (345) followed by *Aspergillus* spp. (120), *Fusarium*

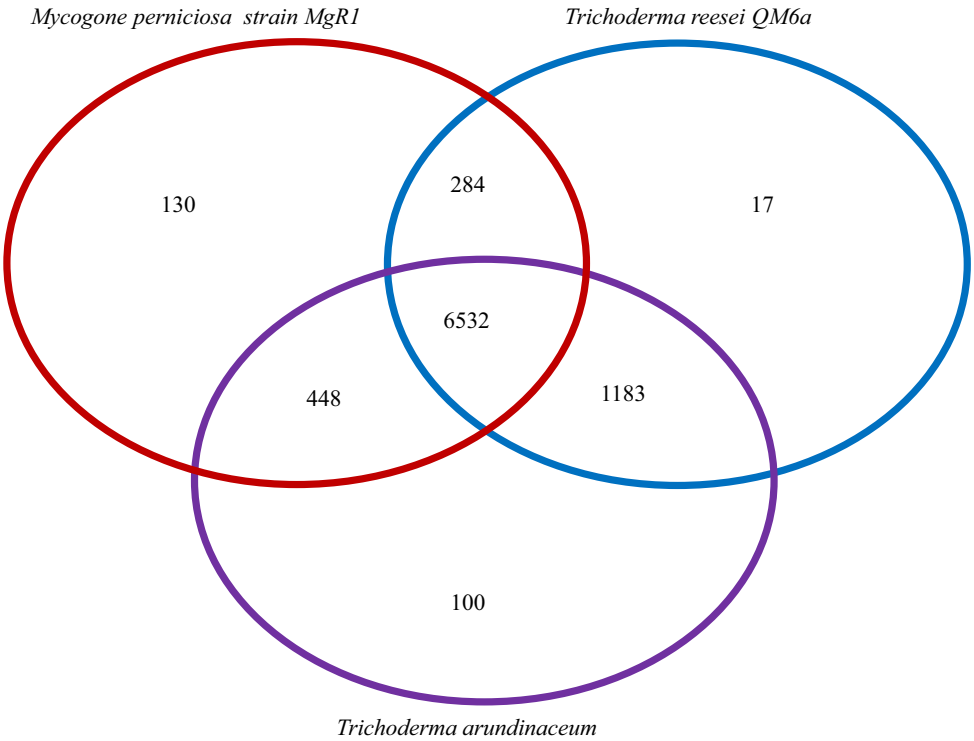

Fig. 4 The Venn diagram shows shared and unique orthologous gene clusters of *M. perniciosa* strain MgR1, with *Trichoderma reesei* QM6A and *Trichoderma arundinaceum*

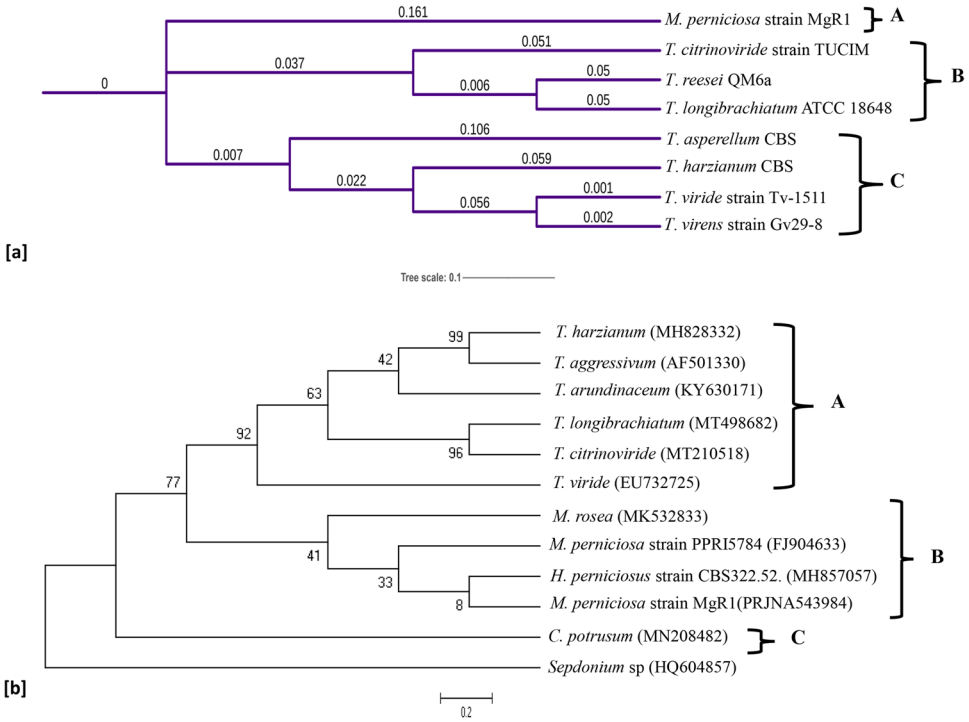

Fig. 5 Phylogenetic analysis. **a** Whole genome sequences. The number above the branches shows length and scale bar represents phylogenetic tree distance of 10%. **b** Internal transcribed spacer (ITS) sequences of *M. perniciosa* strain MgR1 and other closely related organisms. Bootstrap support values from 1000 replicates are shown at the nodes and scale bar represents 0.2 changes. *Sepedonium* sp. Gene bank accession: HQ604857 was used as an outgroup. Corresponding NCBI accession numbers represented in parentheses. Phylogenetic tree constructed by the neighbor-joining method and A, B, and C in both the phylogenetic trees indicates three distinct clades

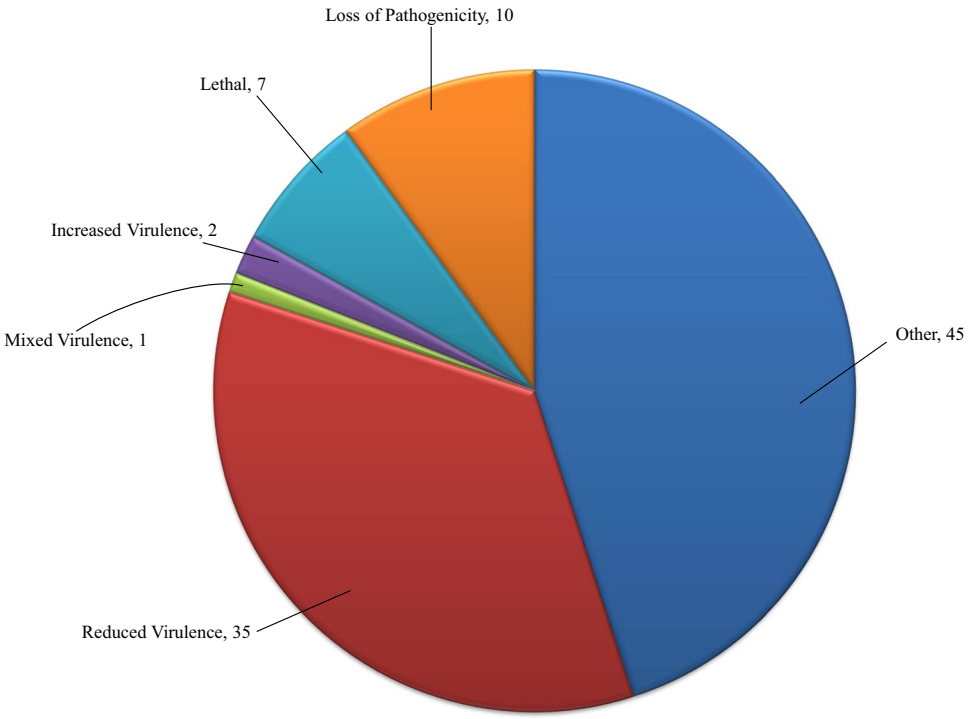

Fig. 6 Functional annotation of predicted genes using the PHI database in *M. perniciosa* strain MgR1

spp. (95), *Metarhizium* spp. (77), *Colletotrichum* spp. (67), and *Beauveria* spp. (29).

Discussion

Fungal diseases of cultivated mushrooms like WBD (*M. perniciosa*) are more devastating compared to viral and bacterial diseases of mushrooms. WBD causes severe economic losses to mushroom growers in all mushroom growing areas of the world. If the weather conditions are congenial for the disease development, it could cause complete crop failure [3, 18]. In pathogenic trial of *M. perniciosa*, WBD manifested in casing soil after 13 days of inoculation as deformed primordia of *A. bisporus*. Hyphae of *M. perniciosa* were found branched interwoven, septate, hyaline having verticillate branches bearing spores. *M. perniciosa* produced two types of spores which are small thin-walled phialoconidia or phialospores on *Verticillium*-like conidiophores with larger bicellular aleuriospores or chlamydospores. *M. perniciosa* can survive for up to 3 years in form of chlamydospores in and around *A. bisporus* growing facilities. Aleuriospores are probably responsible for secondary infection of WBD [3]. Chemical control of this disease through fungicides is still the most preferred practice in mushroom farming [28]. However, so far in India, none of the pesticides has registered to be used on mushroom crops.

Genome sequencing of mycoparasites is the key feature to understand host–pathogen interaction. Notably, genome sequencing of a mycoparasite of edible mushrooms explored the disease-associated mechanisms. For instance, genome sequence of the causal agent of dry bubble disease (*Lecanicillium fungicola* 150–1) [40]; WBD (*Hypomyces perniciosus*); [38]; Cobweb disease (*Cladobotryum dendroides*) [42]; and *C. protrusum* [41] dissected various pathogenesis-related genes. In this study, draft genome sequence data was generated using de novo approach to accomplish the genome sequence of *M. perniciosa* (WBD). Majority of the genes were found to be homologous with *Trichoderma arundinaceum*. Beside this, the protein coding genes in *M. perniciosa* MgR1 (9276) were low compared with closely related organism *T. arundinaceum* (10,473), *T. viride* (12,250), and *T. hamatum* (14,294) [61]. It may be attributed to high numbers of repeat elements in the MgR1 genome [38]. Moreover, higher orthologous clusters were observed in MgR1 compared with *Trichoderma* spp. Notably, the percentage of singletons was comparatively less in MgR1 (18.86%) than *T. arundinaceum* (23.01%). Our results are in concordance with the data reported on recently sequenced *H. perniciosus* strain HP10, which also shares the highest number of orthologous genes with *Trichoderma* spp. [38]. Phylogenetic analysis of whole genome sequences revealed close relatedness of *M. perniciosa* strain MgR1 sequences with earlier reported sequences of *T. citrinoviride* strain TUCIM (NCBI ID. PRJNA453598) and *T. reesei* strain QM6a (NCBI Id. PRJNA325840). In our findings, we found *M. perniciosa* strain MgR1 very similar to the *Trichoderma* spp., a well-known competitor mold under mushroom cultivation [62] and a potential bio-control agent against various diseases of crop plants [63].

Several gene families were identified in MgR1 such as hydrolases, peptidases, lipases, transporters (sugar, ion, phosphate, etc.), different types of ankyrin repeats, and several signaling pathway genes like kinases and transcription factors. MgR1 genome carries different type of genes belonging to important gene family which might play role in its aggressiveness against different strains of *A. bisporus*, its survival and adaptability to varied environmental conditions. Because of the wider adaptability of the pathogen [64], in India, none of the available commercial strains of button mushroom is found resistant against the pathogen. In addition, contraction in gene family of *M. perniciosa* has been reported compared to other members of Hypocreales [38]. Based on the similar observation it can hypothesized that reduction in size of gene family may certainly be the plausible cause to narrow down of its host range and confinement mainly to the *A. bisporus*. On the other hand, secretory proteins play a significant role during the establishment of infection in host [60]. The host–pathogen interactions majorly depend on the secretory proteins and pathogenicity-related genes [38, 65, 66]. Recently, researchers reported less number of secretory proteins than *C. protrusum* and *Trichoderma* spp. Moreover, comparative secretory protein analysis of this study revealed higher secretory proteins in *M. perniciosa* strain MgR1 compared with *Hypocrea, Aspergillus*, *Fusarium*, *Metarhizium*, *Colletotrichum*, and *Beauveria* [38].

Pathogen–host interaction database analysis suggested that 35% genes were related to reduced virulence, 2% genes to increased virulence, 10% genes to loss of pathogenicity, and 7% genes to lethal. PHI database statistics of *M. perniciosa* strain MgR1 showed higher PHI genes then other fifty one fungal genera. This might be due to the fact that MgR1 possess lysine M protein domain genes, G protein or guanine nucleotide-binding proteins, hydrophobins, and cytochrome P450. LysM helps the pathogens by not allowing the host to detect the chitin on the surface of the hyphae. This type of mechanism exists in those fungal pathogens which lack specialized food absorbing organs like appressoria. Apart from the LysM, the other genes help in breaking the physical barrier of the host and in establishing the host–pathogen relationship [67]. Cell wall is an important structural component of fungi and is composed of chitin, glucans, and glycoproteins [68]. It has been reported that hyphal walls of *Agaricus bisporus* are mainly composed of carbohydrates (78.3–79.2%), lipids

(9.9–10.1%), and proteins (8.7–10.2%) [69]. Among the various events of mycoparasitism, the degradation of cell wall of host fungus by chitinases, glucanases, and proteases is the most important one [70]. Genome analysis of *M. perniciosa* strain MgR1 revealed that the numbers of chitinase, glucanase, protease, and lipase-encoding genes are present. Like other mycoparasite such as *Trichoderma* spp. [71], *M. perniciosa* strain MgR1 contains chitinases having carbohydrate binding modules (CBMs) which support the enzyme to attach with insoluble substrates and its breakdown. Chitin is the major structural unit of the cell wall of mycoparasite which is filled with glucans. Besides chitinases, *M. perniciosa* strain MgR1 contains endo and exo-glucanases which play vital role in mycoparasitism in term of degradation of glucans. Role of glucanases in mycoparasitism has been well explained in *Trichoderma* species [72, 73]. Proteins and lipids are also the integral parts of cell wall structure [74]. *M. perniciosa* strain MgR1 genome also contained proteinase and lipase-encoding genes which probably help the mycoparasite in degrading proteins and lipid content and getting energy by utilizing the carbon source of cell wall of *A. bisporus*. Previously, role of proteinases of *Trichoderma* spp. in degrading the cell wall has also been established in *Rhizoctonia solani* [75].

Recently, [38] genome of *H. perniciosus* strain HP10 [Syn: *M. perniciosa*] was sequenced to predict pathogenesis-related genes and secretory proteins. They have reported its close similarity with *Cladobotryum protrusum* fungus. Contrary to this, results of NR database analysis demonstrated the highest similarity of *M. perniciosa* strain MgR1 with *T. arundinaceum* followed by *T. harizianum*, *T. virens*, *T. asperellum*, and *T. gamsii*. Additionally ITS-based phylogenetic analysis of *M. perniciosa* strain MgR1 revealed its highest similarity with *H. perniciosus*, *M. rosea*, *M. perniciosa* strain PPRI5784 and CBS322.52. Moreover, *C. protrusum* formed separate clade in the ITS-based phylogenetic tree, though low bootstrap value was observed in this clade, which might be due to the scant information in the ITS dataset of the *Mycogone* spp. Furthermore, the highest number of pathogenesis-related genes was obtained in *Fusarium* spp. followed by *Magnaporthe* spp. MgR1 strain found more complex compared to *H. perniciosus* strain HP10 because its pathogenic genes were obtained in 51 fungal genera whereas in the case of HP10, pathogenesis-related genes were found in 10 genera such as *Fusarium* spp., *Magnaporthe* spp., *Cochliobolus* spp., *Ustilago* spp., *Metarhizium* spp., *Beauveria* spp., *Botrytis* spp., *Trichoderma* spp., *Cryptococcus* spp., and *Candida* spp. However, it was noted that maximum number of pathogenic genes of MgR1 strain was matched with *Fusarium* spp. which is in concurrence with *H. perniciosus* strain HP10 [38].

Conclusions

De novo genome sequencing of *M. perniciosa* strain MgR1 unravel various facts about its relatedness, survival, pathogenicity, and narrow host range. MgR1 contains an array of gene families related to various cellular, biological, and molecular processes including genes of different families such as hydrolases, peptidases, and lipases. Genome of MgR1 is also rich in genes of biocontrol genera such as *Beauveria* spp. and *Metarhizium* spp. Besides this, it is rich in lysine M protein domain genes, G protein or guanine nucleotide-binding proteins, hydrophobins, and cytochrome P450. Furthermore, it can be also hypothesized that presence of bacterial genes in *M. perniciosa* enables it to produce brown droplets on the surface of infected sporophore of button mushroom. The generated genomic data of this study can be further utilized to explore bio-control like qualities of MgR1, to determine plausible cause of its aggressiveness in mushroom fungi and in devising suitable eco-friendly disease management strategies.

Author contribution Material preparation, data collection, and analysis and preparation of the first draft were performed by Anil Kumar. All the co-authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The whole genome shotgun project has been deposited at NCBI under the accession numbers PRJNA543984. The version described in this paper is version PRJNA543984. The NCBI accession number of bio-sample is SAMN11793478 and ID is 11793478.

Declarations

Competing interests The authors declare no competing interests.

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