REPORT

Biochemical and molecular characterization of catalase enzyme in the saprobic fungus: *Sordaria fimicola*

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Abstract: Fungi have been used in modern scientific research due to their high potential for different enzymes production based on genomic features. The great proportion of soil mycoflora represented by saprobic fungi plays an important role in decomposition, thus contribute to the global carbon cycle. *Sordaria fimicola* strains (n= 61) collected from different environments were evaluated for catalase enzyme activity at first stage. Among all 61 isolates of *S. fimicola*, five strains viz. S1, S2, N7, N6 and SF13 were found to be most efficient in catalase enzyme activity. The complete catalase gene including exons and introns was amplified and sequenced from the most efficient strains of *S. fimicola* and then submitted in the NCBI data base under accession numbers KM282183, KM282184, KM282186, KM282185 and KM282182 for strains S1, S2, N7, N6 and SF13 respectively. The significant differences in the genes sequences and theoretically translated proteins were observed for all five strains of *S. fimicola.* As regards catalase enzyme activity, *S. fimicola* strains were found comparable to the *Aspergillus niger* strains, therefore being a saprophytic fungus with short life cycle *S. fimicola* can become a fungus of choice to produce catalase enzyme at large scale.

Keywords: *Sordaria fimicola*, catalase assay, ribotyping, gene sequencing.

INTRODUCTION

Filamentous fungi secrete different enzymes in the growth medium and most of these enzymes are hydrolytic in nature and employed in different industrial processes (Shakuntala et *al.,* 2009). Catalases (EC 1.11.1.6) are present in all those organisms which are exposed to oxygen and they catalyze the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen (Chelikani *et al.,* 2004). According to Montavon *et al.,* (2007) the conversion of H_2O_2 into water and oxygen is necessary in living systems as H_2O_2 is toxic to cells. Fungi are good producer of catalases as the fungal growth take place in intimate contact with environment; therefore catalases are continuously exposed and affected by physical and chemical stress factors (Kurakov *et al.,* 2001). Fungal catalases may have specialized functions, in case of *A. niger* extra cellular catalase prevents cells from hydrogen peroxide (H_2O_2) toxicity (Witteveen *et al.,* 1992). In fungi as a result of metabolic activities reactive oxygen species are formed and their production increases due to different stress factors like starvation, mechanical damage, light and interaction with other living organisms (Aguirre *et al.,* 2005).

In textile industry catalase is used to remove H_2O_2 from the fabrics (Goodsell, 2004) and in food industry this enzyme is used to get rid of hydrogen peroxide from food products (Chu *et al.,* 1975). Commercially, catalases are isolated from mammalian liver and *A. niger* (Frost and Moss, 1987). It has been revealed from genomic analysis that, two catalase peroxidases namely Kat-G1 and Kat-G2 are encoded by two genes in fungi (Zamocky *et al.,* 2012).

Sordaria fimicola has filamentous mycelium growing as extending branched tubular cells (hyphae) that generally grow radially with symmetric colony (Kavak, 2012). Colonies of *S. fimicola* grow rapidly on Potato Dextrose Agar (PDA) medium in 7 days at 18ºC reaching 9 cm in diameter, from brown to dark brown mycelium with homothallic perithecia (Jeamjitt, 2007). Being a saprophytic fungus, *S. fimicola* may have ability to produce relatively high levels of catalases, therefore, there is need to check its enzyme production potential and to get information about the unique gene sequences encoding the catalase enzymes.

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MATERIALS AND METHODS

In the current research, *S. fimicola* (Roberge ex Desm.) Ces. & De Not., strains belonging to sub-division Ascomycotina were used throughout to study different biochemical and molecular aspects of catalase. These isolates were initially isolated by Professor Nevo's colleagues (1995), from the soil samples of Evolution Canyon (EC) located at the Mount Carmel, Israel. Whereas, Evolution Canyon has two opposite slopes, South facing slope (SFS) which is hash with terrestrial climate and North facing slope (NFS), which is moist with temperate climate. These slopes are 200 to 500 meters away from each other and are dramatically different in flora and fauna. The SFS has 35° angle and 120 meters long with assigned stations 1 and 2 at the elevations of 120, 90 meters above the sea level respectively. The NFS has 25° angle and 180 meters long with stations 6 and 7 at 90 and 120 meters heights from sea level respectively (Nevo, 2001) and from each station 15 strains of *S. fimicola* included in the study. One *S. fimicola* strain (SF13) collected from the premises of University of Illinois at Urbana-Champaign (UIUC) USA., was also used in study. Potato Dextrose Agar (PDA) medium was used for reviving and sub culturing *S. fimicola* strains. Mycelia were transferred on the PDA media and incubated at 18°C for seven days to get good mycelium mat for DNA extraction. While, for biochemical analysis fungal mycelium was sub cultured in the PD broth media and incubated at 18˚C in a shaker for seven days.

Catalase assay

Bailey and Scott (1994) method was adopted for checking the presence of catalase in *S. fimicola* and *A. niger* strains were used as control. Briefly, 0.2g fungal colony of each strain of *S. fimicola* was dipped carefully with sterilized loop under aseptic conditions in test tubes having 5 ml 3% hydrogen peroxide solution and was looked for vigorous bubbling occurring within 30s. Rapid and sustained production of gas bubbles indicated the positive test, while few and somewhat sustained production of gas bubbles, showed low production of catalase and no bubbling indicated the absence of catalase.

DNA extraction and ribotyping

The manual DNA isolation was carried out by 1% CTAB method (Saghai-Maroof *et al.,* 1984). DNA quality was checked by performing 1% agarose gel electrophoresis and DNA concentration was measured by using *NanoDrop* ND-1000 (SPECTRAmax Plus). *Sordaria fimicola* strains found efficient in catalase enzyme production were subjected to ribotyping by amplification of 431 base long V4 region (Hyper variable) of 18S rRNA gene by employing the Machouart-Dubach *et al.* (2001) procedure with slight modifications.

Molecular analysis of catalase gene

Catalase gene from biochemically efficient strains of *S. fimicola* was amplified with self-designed primers (table 1) by using reference sequence of *Neurospora crassa* (Accession# AY027545.1). The conditions for catalase gene amplification were optimized by adjusting different melting temperatures (50-60˚C) and $MgCl_2$ concentration (1.5 to 3.0mM). The standard reagents were supplied by Promega, Madison, WI, USA. The 50µL reaction mixture contained 10µL Go *Taq* flexi® Buffer (5x), 3.0-6.0µL $MgCl_2$ (1.5-3.00mM), 1.0µL dNTPs (10mM each), 0.5µL Go*Taq*® DNA Polymerase (5U/µL), 0.2µL of each upstream and downstream Primers (25pmole/µL) and Template DNA (25ng/µL) was prepared and PCR tubes were placed in the PCR machine (Applied Biosystems, 2720 Thermocycler, USA) and temperature cycling conditions were adjusted as; initial denaturation at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30s, annealing at 50, 55 and 60˚C in independent reactions for 30 s, primer extension at 72˚C for 1 min and final extension at 72˚C for 7 min. The reaction was terminated at 4˚C. The amplified products were checked by 1% agarose gel electrophoresis and amplified DNA bands were purified from the gel using standard reagents supplied by Promega. Gel purified PCR products were sequenced in both directions from the Core sequencing facility, UIUC., USA. Chromas for peak correction, ClustalW and different protein translation tools were used to draw useful conclusions. Available sequences of catalase genes in Gene Bank database were extracted and aligned by using clastalW program. Molecular Evolutionary Genetics Analysis (MEGA 6.0.5) software was used for phylogenetic analysis and phylogeny was tested by bootstrap value of 100 replicates (Tamura *et al.,* 2013).

RESULTS

Catalase activity

In the current research work, 61 strains of *S. fimicola* collected from different environments were evaluated for catalase enzyme activity. Fifteen *S. fimicola* strains from each station viz. 1, 2, 6 and 7 located at EC were screened for catalase enzyme levels. As regards catalase enzyme activity from station 1, nine strains viz. AR25.4, S1, AR32.4, AR8.7, AR55.5, AR66.2, AR28.8, AR15.7 and AR21.3 showed maximum catalase enzyme activity (Tab. 2). From the station 2, five strains viz. IQ3.2, S2, IQ4.5, IQ17.8 and IQ21.4 were found to have highest levels of catalase enzyme potential (Tab. 2). In station 6, six strains viz. N6, MQ1.8, MQ5.4, MQ11.2, MQ8.1 and MQ52.7 showed highest levels of catalase activity (table 2) and as regard station 7; eight strains viz. HD70.5, HD1.3, HD56.7, HD68.8, N7, HD65.4, HD49.6 and HD8.8 were found to have maximum catalase enzyme potential. While *S. fimicola* strain named SF13 from UIUC, also showed maximum catalase enzyme activity (table 2).

Table 1: List of the primers used to amplify catalase gene in *S. fimicola*

Sr. No.	Name	Sequence (5'-3')	Expected PCR product (bp)
1	CATU-F	CTA GAT CCT GCA GCA GAA C	803
2	CATU-R	AAC GGG GAA TGG CGC GAT	
3	CATM-F	ATC GCG CCA TTC CCC GTT	811
4	CATM-R	CAG TGG TCG AGC TCG AAG	
5	CATL-F	GCC AGG CCC AGC TCT TCT	874
6	CATL-R	TAA ACA TTA GGT ATC ATA ACT ATC	

Table 2: Efficient strains of regarding catalase enzyme activity

Sr. No.	*S. fimicola* Strains	+++	++	+	-	Sr. No.	*S. fimicola* Strains	+++	++	+	-
	Station 1					3	IQ5.4	✓			
1	AR25.4	✓				4	IQ11.2	✓			
2	S1	✓				5	IQ8.1	✓			
3	AR32.4	✓				6	IQ52.7	✓			
4	AR8.7	✓					Station 7				
5	AR55.5	✓				1	HD70.5	✓			
6	AR66.2	✓				2	HD1.3	✓			
7	AR28.8	✓				3	HD56.7	✓			
8	AR15.7	✓				4	HD68.8	✓			
9	AR23.1	✓				5	N7	✓			
	Station 2					6	HD65.4	✓			
1	IQ3.2	✓				7	HD49.6	✓			
2	S2	✓				8	HD8.8	✓			
3	IQ4.5	✓					UIUC strain of *S. fimicola*				
4	IQ17.8	✓				1	SF13	✓			
5	IQ21.4	✓					Total	29 strains of *S.fimicola*			
	Station 6						*A. niger* strains				
1	N6	✓				1	AN744	✓			
2	MQ1.8	✓				2	An840	✓			

Key: +++: Spontaneous bubble formation; ++: Start slowly but gradually speed up; +: Very slow; -: Nil

Table 3: Analysis of catalase proteins derived from different strains of *S. fimicola* by ExPASy ProtParam tool

Strains	No. of Amino acid.	Mol. Wt. of Protein	PI. of Protein	Gene length (Exons) (bp)	Dominated Amino acid (%)
RefE	736	82267.9	6.21	2208	Ala (A) 10.2
CAT1-S1	736	82340.9	5.95	2208	Ala (A) 10.1
CAT 1-S2	736	82340.9	5.95	2208	Ala (A) 10.1
CAT 1-N6	736	82388.9	5.95	2208	Ala (A) 9.9
CAT 1-N7	736	82359.9	5.85	2208	Ala (A) 9.9
CAT 1-SF13	736	82388.0	6.0	2208	Ala (A) 9.9

Key: Catalase from Strain S1: CAT1-S1; Catalase from Strain S2: CAT1-S2; Catalase from Strain N6: CAT1-N6; Catalase from Strain N7: CAT1-N7 and Catalase from Strain SF13: CAT1-SF13. RefE: Catalase complete reference gene protein (Accession # AY027545.1) of *N. crassa*. PI: Isoelectric point of protein.

Catalase gene

Five strains of *S. fimicola* with maximum catalase enzyme activity, one from each different environment viz. S1, S2, N6, N7 and SF13, were short listed for catalase gene amplification. In 1% gel electrophoresis, a DNA band of ~15 kb was observed and DNA concentration was observed in the range of 25ng/µL. After ribotyping, the sequences of V4 domain of *S. fimicola* strains S1, S2, N6, N7 and SF13 were submitted to NCBI data base under accession numbers KF487278, KF487279, KF487281, KF487282 and LM654514, respectively (Ishfaq *et al.,* 2017).

After ribotyping, complete catalase gene including introns and exons was amplified and sequenced form all five strains of *S. fimicola* and on comparison with reference gene of *N. crassa* (Accession # AY027545.1) point mutations were observed in the exonic regions. As a result of 224 point mutations in exonic regions of catalase genes, 56 amino acid changes were detected in all strains of *S. fimicola* (fig. 1).

Key: Catalase reference sequence (Accession # AY027545.1): RefE; Catalase from Strain S1: CAT1-S1; Catalase from Strain S2: CAT1-S2; Catalase from Strain N6: CAT1-N6; Catalase from Strain N7: CAT1-N7 and Catalase from Strain SF13: CAT1-SF13.

Fig. 1: Multiple sequences alignment of catalase proteins isolated from different strains of *S. fimicola* along reference catalase protein (Accession # AY027545.1) with exons only.

The catalase proteins derived from all strains of *S. fimicola* and reference control of *N. crassa* showed 736 amino acids (table 3). *Neurospora crassa* reference protein (Accession # AY027545.1) showed different molecular weight (82267.9 Da) as compare to others (table 3). This difference in molecular weight of reference and catalase proteins derived from remaining fives isolates of *S. fimicola* is attributed due to substitutions of amino acids at total 56 different positions in all five strains as compare to reference control protein. The catalase proteins of isolates S1, S2 showed same molecular weight of 82340.9 Da, while N6 and SF13 showed almost same molecular weight of 82388.9 and 82388.0 Da respectively (table 3). However, isolate N7 showed unique molecular weight of 82359.9 Da which was not observed in any other strain of *S. fimicola* (table. 3). The catalase protein derived from strain N7 showed 92 Da difference with reference protein and 19 Da difference from strain S1, S2 proteins and 29 Da difference from strain N6, while difference with SF13 catalase proteins was 28.1 Da (table. 3).

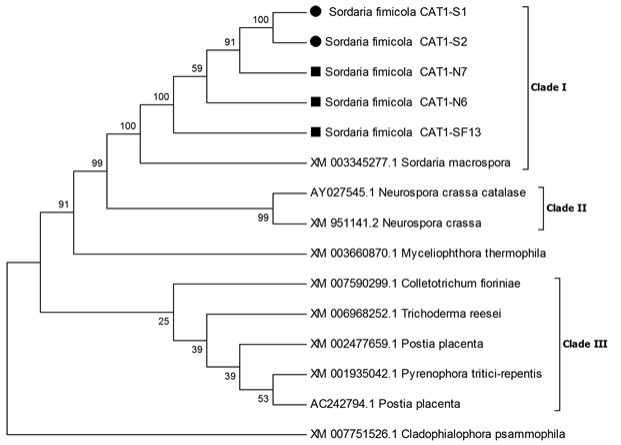

Fig. 2: Phylogenetic relationship of *S. fimicola* based on the catalase gene using Maximum likelihood method. The analysis involved 15 nucleotide sequences

The substitutions of amino acids in all five strains resulted in different isoelectric points (pI) of catalase proteins as compare to reference control of *N. crassa* (Accession # AY027545.1). Catalase proteins from *S. fimicola* strains S1, S2 and N6 showed same isoelectric point 5.95 (table 3), while catalase from strain SF13 and N7 showed 6.0 and 5.85 isoelectric points respectively and this change in isoelectric points of different catalase proteins was due to changes in amino acids in catalase proteins derived from different strains of *S. fimicola.*.

The phylogenetic analysis was performed based on catalase gene sequences of five strains of *S. fimicola* isolated from different environment including reported sequences of related species from Gene bank (fig. 2). The phylogenetic analysis included 15 nucleotide sequences and it clustered into three major clades. S1, S2, N7, N6 and SF13 were clustered in clade-I and were close to each other, while reference sequence (*N. crassa*, AY027545.1) was present in clade-II (fig. 2). In phylogenetic analysis *S. fimicola* has close relationship with *Sordaria macrospora* as both species separated under significant bootstrap value (100).

DISCUSSION

In the present study, out of 61 strains of *S. fimicola*, 29 were found most efficient in catalase enzyme production and catalase enzyme activity of *S. fimicola* was found almost equal to the *A. niger* strains AN744 and AN840 (Ishfaq *et al.,* 2014). Catalases (EC1.11.1.6) are important enzymes that catalyze the decomposition of H_2O_2 to oxygen and water as well as provide protection to the host organism from the oxidative damage of proteins and nucleic acids (Levy *et al.,* 1992). Fungi are reported to be high producer of catalases and different types of catalase genes have been isolated and characterized at molecular level from different fungi (Cordi *et al.,* 2007). According to Gerd *et al.,* (2006) there is needed to characterize fungal strains at molecular level for their efficient enzyme production.

The ribotyping results revealed that 431 bases long V4 region of 18S rRNA gene was 100% similar among all short listed strains as well with previously reported sequence of *S. fimicola* (Accession # AY545724.1), which confirmed that *S. fimicola* strains were pure cultures, without any contamination. The 18S rRNA gene sequencing was used earlier for species identification in different studies (Meyer *et al.,* 2010). Kappe *et al.,* (1996) described that hyper variable V4 region of the 18S rRNA can be used to find polymorphism prevailing among different fungal species.

In the current research, 56 amino acid changes were detected in the exonic regions of the catalase genes among all strains of *S. fimicola,* which suggested that environmental stresses may induce different kind of mutations in specific gene or on genome which lead to genetic diversity among organisms and their products as reported earlier (Moller and Mousseau, 2006). The fungal catalases (Cho *et al.,* 2000) are very diverse regarding their structures, functions and locations. On genomic analysis, two different catalase-per oxidases (Kat-G1 and Kat-G2) were reported in fungi by Zamocky *et al.,* (2013). Chary and Natvig (1989) carried out biochemical and molecular studies on three catalases encoded by three different structural genes in *N. crassa* and reported the specific response of these enzymes to heat shock, development and superoxide-mediated stress. Switala *et al.,* (1999) reported that Cat-1 of *Aspergilli* is more resistant to heat than that of *Escherichia coli*. Catalases (*Cat A* and *Cat B*) genes in *Aspergillus nidulans* code two true catalases of <84 and <79 kDa respectively (Kawasaki *et al.,* 1997). In phylogenetic analysis it was found that *S. fimicola* has close relationship with *S. macrospora* as both species separated under significant bootstrap value of 100. The molecular evolutionary relationship among 36 fungal and 13 bacterial KatG genes was reported earlier by using neighbor joining, maximum likelihood and maximum parsimony methods (Zamocky *et al*., 2009).

CONCLUSION

In the current research catalase gene sequence from *S. fimicola* were reported for the first time and submitted to NCBI data base under accession numbers KM282183, KM282184, KM282185, KM282186, and KM282182 for *S. fimicola* strains S1, S2, N6, N7 and SF13 respectively. In biochemical assay the catalase enzyme activity of *S. fimicola* was found almost compare able to the *A. niger*, therefore, *S. fimicola* can also be exploited further for industrial use.

REFERENCES

Aguirre J, Momberg MR, Hewitt D and Hansberg W (2005). Reactive oxygen species and development in microbial eukaryotes. *Trend Microbiology,* **13**: 111-118.

Bailey RW and Scott EG (1994). Diagnostic microbiology, 2nd edn. The C.V. Mosby Company publisher, Saint Louis.

Chary P and Natvig DO (1989). Evidence for three differentially regulated catalase genes in: effects of oxidative stress, heat shock and development. *J. Bacteriol.,* **171**: 2646-2652.

Chelikani P, Fita I and Loewen PC (2004). Diversity of structures and properties among catalases. *Cell Mol. Life Sci.,* **61**: 192-208.

Cho YH, Lee EJ and Roe JH (2000). A developmentally regulated catalase required for proper differentiation and osmoprotection of *Streptomyces coelicolor. Mol. Microbiol.,* **35**: 150-160.

Chu H, Leeder J and Gilbert S (1975). Immobilized Catalase Reactor for Use in Peroxide. Sterilization of Dairy Products. *J. Food Sci.,* **40**: 641-645.

Cordi L, Minussi RC, Freire RS and Dur´an N (2007). Fungal laccase: copper induction, semi-purification, immobilization, phenolic effluent treatment and electrochemical measurement. *Afr. J. Biotechnol.,* **6**: 1255-1259.

Frost MG and Moss DA (1987). Production of enzymes by fermentation. *Biotechnol.,* **7a**: 65-102.

Gerd JM, Huaming W, Elizabeth B, Jens W, Kay V, Claudia V, Caroline F, Abigail CL, Gethin A, Valerie H, Giselle G, Nigel DC, Marvin K, Hans GL, Thomas S, Claus B, Geoffrey T, Bernhard N and Reinhard F (2006). Use of laccase as a novel, versatile reporter system in filamentous fungi. *Appl. Environ. Microb.,* **72**: 5020-5026.

Goodsell DS (2004). Catalase Molecule of the Month. RCSB Protein Data Bank. Retrieved, (2007)-02-11.

Ishfaq M, Mahmood N, Nasir IA and Saleem M (2014). Molecular and Biochemical screening of local *Aspergillusniger* strains efficient in catalase and laccase enzyme production. *IJAB.,* **16**: 177-182.

Ishfaq M, Mahmood N, Nasir IA and Saleem M (2017). Biochemical and molecular analysis of superoxide dismutase in *Sordaria fimicola* and *Aspergillus niger*

collected from different environments. *Pol. J. Environ. Stud*., **26**(1): 115-125.

Jeamjitt O (2007). Diversity of Coprophilous Fungi, Antagonism against Plant Pathogenic Fungi and Secondary Metabolites of *Ascodesmis macrospora* and *S. fimicola.* Kasetsart University, Thail., pp. 117-119.

Kappe R, Fauser C, Okeke N and Maiwald M (1996). Universal fungus specific primers systems and group specific hybridization oligonucleotides for 18S rDNA. *Mycoses,* **39**: 25-30.

Kavak H (2012). Some Biological Parameters in *Sordaria fimicola. Pakistan J. Bot.,* **44**: 1079-1082.

Kawasaki L, Wysong D, Diamond R and Aguirre J (1997). Two divergent catalase genes are differentially regulated during *Aspergillus nidulans* development and oxidative stress. *J. Bacteriol.,* **179**: 3284-3292.

Kurakov AV, Kupletskaya MB, Skrynnikova EV and Somova NG (2001). Search for micromycetes producing extracellular catalase and study of conditions of catalase synthesis. *Appl. Biochem. Micro.,* **37**: 59-64.

Levy E, Eyal Z and Hochmant A (1992). Purification and characterization of a catalase-peroxidase from the fungus *Septoria tritici*. *Arch. Biochem. Biophys.,* **296**: 321-327.

Machouart-Dubach CL, Martine FD, Chauvin I, Gall LE, Catherine G, Frederick L and Francis D (2001). Rapid discrimination among Dermatophytes, Scytalidium spp. and other fungi with a PCR-Restriction Fragment Length Polymorphism Ribotyping method. *J. Clin. Microbiol.,* **29**: 685-690.

Meyer A, Todt C, Mikkelsen NT and Lieb B (2010). Fast evolving 18S rRNA sequences from Solenogastres (Mollusca) resist standard PCR amplification and give new insights into mollusk substitution rate heterogeneity. *BMC Evol. Biol.,* **10**: 70.

Møller AP and Mousseau TA (2006). Biological consequences of Chernobyl: 20 years after the disaster. *Trends Ecol. Evol.,* **21**: 200-207.

Montavon P, Kukic KR and Bortlik K (2007). A simple method to measure effective catalase activities: optimization, validation and application in green coffee. *Annu. Rev. Biochem.,* **306**: 207-215.

Nevo E (1995). Asian, African and European biotas meet at Evolution Canyon Israel. Local tests of global biodiversity and genetic diversity patterns. *Proc. R. Soc. Lond.,* **262**: 149-155.

Nevo E (2001). Evolution of genome-phenome under environmental stress at Evolution Canyon, Israel. *Proc. Natl. Acad. Sci.,* **262**: 149-155.

Saghai-Maroof MA, Soliman KM, Jorgensen RA and Allard RW (1984). Fungal DNA isolation. *Proc. Natl. Acad. Sci.,* **81**: 8014-8018.

Shakuntala G, Samudra PB, Deepak V, Sudesshna C, Soumya M and Suman K (2009). Fungal biotechnology in food and feed processing. *Food Res. Int.,* **42**: 577-587.

Switala J, O'Neil JO and Loewen PC (1999). Catalase HPII from *Escherichia coli* exhibits enhanced resistance to denaturation. *Biochemistry,* **38**: 3895-3901.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.,* **30**: 2725-2729.

Witteveen CFB, Veenhuis M and Visser J (1992). Localization of glucose oxidase and catalase activities in *Aspergillus niger*. *Appl. Env. Microbiol.,* **58**: 1190-1194.

Zamocky M, Furtmüller PG and Obinger C 2009. Two distinct groups of catalase/peroxidases. *Bioch. Soc. Trans.*, **37**: 772-777.

Zamocky M, Droghetti E, Bellei M, Gasselhuber B, Pabst M, Furtmuller PG, Battistuzzi G, Smulevich G and Obinger C (2012). Eukaryotic extra cellular catalase-peroxidase from *Magnaporthe grisea*-Biophysical/ chemical characterization of the first representative from a novel phytopathogenic Kat G group. *Biochimie,* **94**: 673-83.

Zamocky M, Sekot G, Buckova J, Schaffer C, Farkasovsky M, Obinger C and Polek B (2013). Intracellular targeting of ascomycetous catalase-peroxidases (KatG1s). *Arch. Microbiol.,* **195**: 393-402.