Mushroom Biology and Mushroom Products, Royse (ed.) © 1996 Penn State Univ. ISBN 1-883956-01-3

Ligninolytic Enzyme Production and Secretion in Edible Mushroom Fungi

John A. Buswell, Yi Jin Cai and Shu-ting Chang
Department of Biology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong E–mail address: b202768@mailserv.cuhk.hk

ABSTRACT: The cultivation of edible mushrooms is a prime example of the bioconversion of many types of low-value lignocellulosic wastes, primarily from agricultural practices, into a higher-value commodity. *Lentinula edodes, Volvariella volvacea* and *Pleurotus sajor-caju* are three important commercially cultivated mushrooms which exhibit varying abilities to utilise different lignocellulosics as a growth substrate. Examination of the lignocellulolytic enzyme profiles of the three species reveals this diversity to be reflected in qualitative variations in the major enzymic determinants (i.e. cellulases, ligninases) required for substrate bioconversion. For example, *L. edodes*, which is cultivated on highly lignified substrates such as wood or sawdust, produces two extracellular enzymes which have been associated with lignin depolymerisation in other fungi, viz., manganese peroxidase and laccase. Conversely, *V. volvacea*, which prefers high cellulose-, low lignin-containing substrates, produces a multi-component enzyme system for the conversion of cellulose to glucose consisting of endo-1,4-ß-glucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and ß-glucosidase (β-D-glucosidic glucohydrolase; EC 3.2.1.21), but apparently none of the recognised lignin-degrading enzymes.

1 INTRODUCTION

There is considerable pressure nowadays to develop processes for the rational treatment and/or disposal of the vast quantities of waste lignocellulosic materials generated annually through activities of the agricultural, forestry and food processing industries. Of the various approaches adopted, one of the most significant in terms of producing a higher value prod-

uct from the waste is the cultivation of edible mushrooms by solid state fermentation (Chang and Miles 1991). Mushrooms provide a highly nutritious source of food (Buswell and Chang 1993) and, more recently, attention has focused on a second area of exploitation following the discovery that many of these fungi produce a range of metabolites of intense interest to the pharmaceutical (e.g. anti-tumour, immunomodulation agents and hypocholesterolaemic agents) (Mizuno *et al.* 1995) and food (e.g. flavour compounds) industries (Jong and Birmingham 1992).

Edible mushrooms are adapted to grow on a wide variety of lignocellulosic wastes including such diverse materials as cereal straws, bagasse, sawdust, cotton wastes from textile factories, banana leaves and coffee grounds. The utilisation of insoluble lignocellulosic substrates by edible mushrooms is dependent upon the production and secretion by these fungi of enzymes (cellulases, hemicellulases, ligninases) that bring about hydrolysis/oxidation of the macromolecular cellulose, hemicellulose and lignin components, respectively, thereby liberating low molecular growth nutrients (Buswell *et al.* 1993). Production of these enzymes by the fungal mycelium is a crucial part of the colonisation process and an important determinant of mushroom yields.

Over the past four years, research in this laboratory has been directed at a better understanding of lignocellululolytic enzyme production and secretion by selected edible fungi. Most attention has been given to *Volvariella volvacea, Lentinula edodes* and *Pleurotus* spp. which represent three of the five major cultivated mushrooms. Production of these mushrooms has increased dramatically over the last decade and the trend is set to continue. The three species vary considerably in substrate preference. The natural substrate for *V. volvacea* is paddy straw but higher fruit body yields are achieved under commercial cultivation conditions using cotton waste as a growth substrate (Chang 1974). Conversely, the natural substrate for *L. edodes* is wood and the mushroom is traditionally grown on wood logs although this procedure has been largely replaced by the use of artificial logs composed of sawdust and bran. *P. sajor-caju* is the most adaptable of the three species and will grow on a wide range of agricultural wastes of differing composition in terms of polysaccharide/lignin ratio. This paper summarises some of the data relating to lignocellulolytic enzyme production by these mushrooms which should facilitate the development of strategies for more efficient fungal colonisation/bioconversion of substrate and increased mushroom yields.

2 MATERIALS AND METHODS

2.1 *Fungal strains*

Lentinula edodes, (Berk.) Pegler, strain L54, *P. sajor-caju*, strain Pl-27, and *V. volvacea* (Bull ex Fr.) Sing., strain V14, were obtained from the culture collection of this department and maintained at 4°C on potato dextrose agar (PDA) slants with periodic transfer.

2.2 *Culture conditions*

To determine ligninolytic enzyme production by *L. edodes* and *P. sajor-caju,* the fungi were cultivated in stationary 250-ml Erlenmeyer flasks containing 50ml of a defined medium containing (g/l): KH_2PO_4, 0.2; $MgSO_4.7H_2O$, 0.05; $CaCl_2.2H_2O$, 0.013; glucose,10; 2,2'- dimethylsuccinate, 1.46; vitamins (Ohta *et al.* 1990); and 10ml of a trace element solution (Kirk *et al.* 1978). In culture medium for *V. volvacea,* the vitamin solution was replaced by 0.1g/litre yeast extract. Nitrogen was added as NH_4NO_3 and L-asparagine at concentrations of 2.6mM-N and 26mM-N for low nitrogen (LN) and high nitrogen (HN) media, respectively (Buswell *et al.* 1984). For cellulolytic enzyme production, fungi were grown in shake culture in a medium containing (g/l): KH_2PO_4, 1.0; K_2HPO_4, 0.4; $MgSO_4.7H_2O$, 0.5; $CaCl_2.2H_2O$, 0.013; yeast extract (Difco), 0.1; L-asparagine, 1.5; NH_4NO_3, 0.5; thiamine.HCl, 0.0025; and 1 ml of a trace element solution (Buswell *et al.* 1984). Cellulose (1%) (Sigmacell, Sigma Chemicals) or carboxymethylcellulose (1%) served as carbon source as indicated. Media were adjusted to pH 5.0 for *L. edodes*, and pH 6.0 for *P. sajor-caju* and *V. volvacea*. Growth temperatures were 25°C for *L. edodes,* 28°C for *P. sajor-caju*, and 32°C for *V. volvacea*. An aliquot (1 ml) of a hyphal suspension, prepared by blending mycelium from actively growing cultures of the three species grown on potato dextrose broth (Difco) was used as inoculum.

2.3 *Enzyme assays*

Endoglucanase (carboxymethylcellulase, CMCase) and exoglucanase (Avicelase) activity were determined by measuring the amount of reducing sugar (as glucose) released from carboxymethylcellulose and Avicel, respectively (Cai *et al.* 1994), using the Somogyi-Nelson method (Somogyi 1952). ß-Glucosidase was determined by measuring the amount of *p*-nitrophenol released from *p*-nitrophenyl-ß-D-glucopyranoside (Cai *et al.* 1994). Laccase activity was determined using 2,2'-azino-*bis*-ethylben-

thiazoline (ABTS) (Buswell *et al.* 1995). Manganese peroxidase (Mn-P) was assayed according to Glenn *et al.* (1986). Lignin peroxidase (LiP) was determined by measuring the oxidation of veratryl alcohol to veratraldehyde (Tien and Kirk 1984).

3 RESULTS AND DISCUSSION

3.1 *Volvariella volvacea*

Like many other cellulose-degrading fungi, *V. volvacea* produces a multicomponent enzyme system for the conversion of cellulose to glucose consisting of endo-1,4-ß-glucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and ß-glucosidase (ß-D-glucosidic glucohydrolase; EC 3.2.1.21) (Cai *et al*, 1994). Each enzyme exists as a number of separate isozymes. So far, five endoglucanases,five cellobiohydrolases, two intracellular and two extracellular ß-glucosidases have been identified. Detection of intracellular ß-glucosidase activity in *V. volvacea* conflicts with an earlier report (Chang and Steinkraus 1982) in which the enzyme was found to be exclusively extracellular. In some cases, individual isozymes have been purified and partially characterised, and all appear to be glycosylated. It is not yet clear to what extent these represent separate gene products or simply post-translational modification. However, genes encoding for individual components of the mushroom's cellulase complex have now been isolated using the heterologous probes *cbh-1* and *cbh-2* from *Phanerochaete chrysosporium* (Jia, J., Buswell, J.A. & Peberdy, J.F., to be published). Fluorescent antibody combined with confocal laser microscopy confirm both the intra- and extracellular location of ß-glucosidase, whereas endoglucanase appears to be 'assembled' in the periplasmic space prior to secretion.

The activities of three recognized lignin-modifying extracellular oxido-reductases, namely lignin peroxidase (LiP) (Tien and Kirk 1983, Glenn *et al.* 1983), manganese-dependent peroxidase (MnP) (Kuwahara *et al.* 1984, Glenn and Gold 1985) and laccase (Reinhammar 1984), were examined in this study. No production of any of these enzymes was observed when *V. volvacea,* strain V14, was grown under a wide range of physiological and nutritional conditions reported to support ligninolytic enzyme production in other fungi (Buswell *et al.* 1993, Cai *et al.* 1994). It is not clear if this is characteristic of all strains of *V. volvacea* although no ligninolytic enzyme activity was detected in another strain (strain V34) examined as part of this investigation.

3.2 *Lentinula edodes*

Lentinula edodes, L54, grows very poorly in agitated submerged culture on crystalline cellulose (Avicel). This poor growth is reflected by the absence of detectable cellulolytic activity (endoglucanase, exoglucanase and ß-glucosidase) in culture supernatants. However, endoglucanase activity is induced in other strains of *L. edodes* during growth on this substrate (Yie, H.L., Wang, C.H. and Buswell, to be published). Mishra and Leatham (1990) reported endoglucanase, exoglucanase and ß-glucosidase activity in *L. edodes* grown in solid-state cultures with red oak wood (*Quercus rubra*) as substrate.

Lentinula edodes, strain L54, produces manganese-dependent peroxidase (MnP) and laccase, but not lignin peroxidase, when grown on a defined medium with glucose as sole carbon source. MnP production is suppressed by nitrogen whereas highest levels of laccase were observed when the fungus was grown under high nitrogen (26mM) conditions.The ligninolytic systems of several wood-rotting basidiomycetes, including the widely studied white-rot fungus *Phanerochaete chrysosporium*, are expressed only during the secondary metabolic phase of growth and are triggered by nutrient nitrogen depletion (Keyser *et al.* 1978). Given the poor nitrogen content of the woody tissues which serve as the natural growth substrate for *L. edodes* (Cowling and Merrill 1966), it is perhaps not surprising for low nitrogen levels to trigger lignin degradation in the same way as observed for *P. chrysosporium* (Keyser *et al.* 1978).

Both the titre and time of appearance of MnP were affected by the concentration of Mn in the culture medium with highest enzyme levels recorded in cultures supplemented with 1.1 ppm Mn. In the white-rot fungus *P. chrysosporium,* Mn regulates the expression of MnP by activating the transcription of the *mnp* gene via a growth-stage-specific and concentration- dependent mechanism (Brown *et al.* 1991). Purified MnP from *L. edodes* L54 has an apparent M_r of 59kDa and a p*I* of 5.6, and differs in several respects from a MnP isolated from *L. edodes* grown on a commercial wood substrate.

Unlike the multiple forms of MnP (Leisola *et al.* 1987, Niku-Paavola *et al.* 1988, Ruttimann-Johnson *et al.* 1994) and laccase (Morohoshi *et al.* 1987) reported in some other ligninolytic fungi, native polyacrylamide gel electrophoresis (PAGE) of crude *L. edodes* culture supernatants combined with activity staining with ABTS in the presence and absence of H_2O_2 revealed only one MnP protein and one laccase protein, respectively. Activity staining with ABTS in the presence of H_2O_2 revealed a single band with MnP activity only in gels of LN culture supernatants (Buswell *et al.* 1995). Forrester *et al.* (1988) also detected

only one major MnP by *L. edodes* when the fungus was grown on a commercial oak-wood substrate

No LiP activity was detected in the extracellular medium of *L. edodes* L54 grown under the range of growth conditions used in this study. Leatham (1986) was also unable to detect extracellular ligninase capable of oxidising veratryl alcohol to veratraldehyde in cultures of another strain of *L. edodes* although only HN conditions were employed. However, the fungus has been shown to produce LiP when grown on a synthetic medium containing low concentrations of Mn^{2+} (Bonnarme and Jeffries 1990) although the extent of lignin degradation (^{14}C-lignin → $^{14}CO_2$) was only 1.5% compared to 25% with Mn^{2+} supplementation (Leatham 1986). Thus, *L. edodes* resembles those ligninolytic fungi such as *Dichomitus squalens* (Bonnarme and Jeffries 1990) and *Rigidoporus lignosus* (Perie and Gold 1991) in apparently utilising MnP and laccase for lignin degradation.

3.3 *Pleurotus sajor-caju*

Extracellular endoglucanase and exoglucanase activities were readily detectable when *P. sajor-caju* was grown in submerged culture with either crystalline cellulose (Avicel) or CMC as carbon source. Enzyme titres were considerably higher compared with *V. volvacea*. Extracellular ß-glucosidase was also observed in culture supernatants of *P. sajor-caju* grown on either substrate although most of the enzyme activity appears to reside in extracts of fungal hyphae. Rai and Saxena (1990) also investigated cellulase production by *P. sajor-caju* during growth on rice straw. These authors reported no activity towards filter paper but very low levels of endoglucanase and ß-glucosidase appeared late in the cultivations.

Compared with *L. edodes*, relatively low levels of Mn-P were produced by the faster growing *P. sajor-caju* grown in stationary culture on a defined medium with glucose as the sole carbon source and under LN conditions. Enzyme activity was also detectable in HN cultures of *P. sajor-caju* although specific activities (U/mg mycelium) were much lower. The mushroom also produces copious quantities of laccase and at least five proteins exhibiting laccase activity can be separated by PAGE from culture supernatants of *P. sajor-caju* (Lo 1993).

No LiP activity was detected in the extracellular medium following growth of *P. sajor-caju* under the growth conditions used in this study. However, two veratryl alcohol oxidases have been reported in *P. sajor-caju* which may play a role in lignin degradation by this fungus (Bourbonnais and Paice 1988).

3.4 *Concluding remarks*

Lentinula edodes, Pleurotus sajor-caju and *Volvariella volvacea*, exhibit quite different growth responses to different lignocellulosic wastes. *Lentinula edodes* grows well on woody substrates and is traditionally cultivated on logs of Fagaceae species although this procedure has, to a considerable extent, been replaced by the "bag" system using supplemented sawdust media. The natural substrate for *V. volvacea* is paddy straw which has a relatively low lignin content (Dale 1987). The preference of this mushroom for 'less-lignified' substrates is exemplified by the increased production yields obtained when *V. volvacea* is cultivated on cotton wastes Chang 1974). *Pleurotus sajor-caju* is the most adaptable of the three species and can be grown on a wide variety of agricultural waste materials of differing composition in terms of polysaccharide/lignin ratio. Although the cellulolytic capacity of *V. volvacea* is clearly evident, the mushroom's aversion for highly lignified substrates is undoubtably reflected in the apparent inability of the fungus to synthesize any of the recognized lignin-transforming enzymes. The cellulose and hemicellulose components of the plant cell wall are intimately associated with the lignin moiety which presents a barrier to the hydrolytic enzymes catalyzing the degradation of the polysaccharides. Since *V. volvacea* appears to lack a ligninolytic or lignin transforming system, this will restrict fungal access to the polysaccharide components and will reduce the capacity of the fungus to grow and fruit in lignified substrates.

An alternative, or supplementary, explanation for the preference of *V. volvacea* for less-lignified substrates might also be found in a higher sensitivity to phenolic monomers and tannins, and in a more pronounced effect of these compounds on the production and/or activity of the straw mushroom's cellulases and hemicellulases (Martin and Akin 1988, Cai *et al.* 1993). This higher sensitivity relates perhaps to the absence of laccase in this fungus. Laccase generates phenoxy radicals from phenols which then spontaneously polymerise, and the enzyme may play a role in the detoxification of inhibitory phenolic compounds which are often present in lignocellulosic materials. Conferring to this fungus an ability to produce extracellular enzymes able to degrade lignin and/or to detoxify inhibitory phenolics may lead to increased substrate colonisation and improved fruit body yields during commercial cultivation.

Both *L. edodes* and *P. sajor-caju* degrade lignin, and produce both manganese peroxidase and laccase. The former enzyme is generally recognised as an important component of fungal ligninolytic systems and the recognition of the role played by nitrogen in the regulation of Mn-P

may has far-reaching implications for more efficient substrate colonisation and utilisation, and hence for improved fruit body yields, by these important commercially cultivated mushrooms.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Hong Kong Research Grants Council (Grant No. CUHK 18/92M) and a Strategic Research Grant from The Chinese University of Hong Kong.

REFERENCES

Bonnarme, P., and T. W. Jeffries. 1990. Mn(II) regulation of lignin peroxidases and manganese-dependent peroxidases from lignin-degrading white-rot fungi. Appl. Environ. Microbiol. 56:210-217.

Bourbonnais, R., and M. G. Paice. 1988. Veratryl alcohol oxidase from the lignin-degrading basidiomycete *Pleurotus sajor-caju.* Biochemical J. 255:445-450.

Brown, J. A., M. Alic, and M. H. Gold. 1991. Manganese peroxidase gene transcription in *Phanerochaete chrysosporium*: activation by manganese. J.Bacteriol. 173:4101-4106.

Buswell, J. A., and S. T. Chang. 1993. Edible mushrooms: attributes and applications. In Genetics and breeding of edible mushrooms, eds S.T. Chang, J.A. Buswell, and P.G. Miles. pp. 297-324. Philadelphia: Gordon and Breach Scientific Publishers.

Buswell, J. A.,Y. J. Cai, and S. T. Chang. 1993. Fungal- and substrate-associated factors affecting the ability of individual mushroom species to utilise different lignocellulosic growth substrates. In: Mushroom biology and mushroom products eds. S.T. Chang, J. A. Buswell and S. W. Chiu. pp.141-150 Hong Kong: Chinese University Press.

Buswell, J. A., Y .J. Cai, and S. T. Chang. 1995. Effect of nutrient nitrogen on manganese peroxidase and laccase production by *Lentinula (Lentinus) edodes*. FEMS Microbiol. Lett. 128:81-88.

Buswell, J.A., B. Mollet, B. and E. Odier. 1984. Ligninolytic enzyme production by *Phanerochaete chrysosporium* under conditions of nitrogen sufficiency. FEMS Microbiol. Lett. 25:295-299.

Cai, Y-J., J. A. Buswell, and S. T. Chang. 1993. Effect of lignin-related phenols and tannic acid derivatives on the growth of edible mushrooms. World J. Microbiol. Biotechnol. 9:503-507.

Cai, Y .J., J. A. Buswell, and S. T. Chang. 1994. Cellulases and hemicellulases of *Volvariella volvacea* and the effect of Tween 80 on enzyme production. Mycol. Res. 98:1019-1024.

Chang, S. T. 1974. Production of the straw mushroom (*Volvariella volvacea*) from cotton wastes. Mushroom J. 21:348-354.

Chang. S. C., and K. H. Steinkraus. 1982. Lignocellulolytic enzymes produced by *Volvariella volvacea*, the edible straw mushroom. Appl. Environ. Microbiol. 43:440-446.

Chang, S. T., and P. G. Miles. 1991. Recent trends in world production of cultivated mushrooms. Mushroom J. 503:15-18.

Cowling, E. B., and W. Merrill. 1966. Nitrogen in wood and its role in wood deterioration. Can. J. Bot. 44:1539-1554.

Dale, B. E. 1987. Lignocellulose conversion and future fermentation technology. Trends Biotechnol. 5:287-291.

Forrester, I. T., C. G. Grabski, R. R. Burgess, and G. F. Leatham. 1988. Manganese, Mn-dependent peroxidases, and the biodegradation of lignin. Biochem.Biophys. Res. Commun. 157:922-929.

Glenn, J.K., and M.H. Gold. 1985. Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete, *Phanerochaete chrysosporium.* Arch. Biochem. Biophys. 242:329-341.

Glenn, J. K., L. Akileswarean, and M. H. Gold. 1986. Mn(II) oxidation is the principle function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium.* Arch. Biochem. Biophys. 251:688-696.

Glenn, J. K., M. A. Morgan, M. B. Mayfield, M. Kuwuhara, and M. H. Gold. 1983. An extracellular H_2O_2-requiring enzyme preparation involved in lignin degradation by the white rot basidiomycete *Phanerochaete chrysosporium.* Biochem. Biophys. Res. Commun. 114:1077-1083.

Jong, S. C., and J. M. Birmingham. 1992. Medicinal benefits of the mushroom *Ganoderma*. Adv. Appl. Microbiol. 37:101-134.

Keyser, P., T. K. Kirk, and J. G. Zeikus. 1978. Ligninolytic enzyme system of *Phanerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation. J. Bacteriol. 135:790-797.

Kuwuhara, M., J. K. Glenn, M. A. Morgan, and M. H. Gold. 1984. Separation and characterization of two H_2O_2-dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium.* FEBS Lett. 169:247-250.

Leatham, G. F. 1986. The ligninolytic activities of *Lentinus edodes* and *Phanerochaete chrysosporium.* Appl. Microbiol. Biotechnol. 24:51-58.

Leisola, M. S. A., B. Kozulic, F. Meussdoerfer, and A. Fiechter. 1987. Homology among ultiple extracellular peroxidases from *Phanerochaete chrysosporium.* J. Biol. Chem. 262:419-424.

Lo, S. C. 1993. M. Phil. Thesis. The Chinese University of Hong Kong.

Martin, S. A., and D. E. Akin. 1988. Effect of phenolic monomers on the growth and ß-glucosidase of *Bacteroides ruminocola* and on the carboxymethylcellulase, ß-glucosidase and xylanase from *Bacteroides succinogenes.* Appl. Environ. Microbiol. 54:3019-3022.

Mishra, C., and G. F. Leatham. 1990. Recovery and fractionation of the extracellular degradative enzymes from Lentinula edodes cultures cultivated on a solid lignocellulosic medium. J. Ferment. Eng. 69:8-15.

Mizuno, T., G. Wang, J. Zhang, H. Kawagishi, T. Nishitoba, and J. Li. 1995. Reishi, *Ganoderma lucidum* and *Ganoderma tsugae*: Bioactive substances and medicinal effects. Food Rev. Int. 11:151-166.

Morohoshi, N., N. Wariishi, C. Muraiso, T. Nagai, and T. Haraguchi. 1987. Degradation of lignin by the extracellular enzymes of *Corious versicolor.* IV. Properties of three laccase fractions fractionated from the extracellular enzymes. Mokuzai Gakkaishi. 33:218-225

Niku-Paavola, M.-L., E. Karhunen, P. Salola, and V. Raunio. 1988. Ligninolytic enzymes of the white rot fungus *Phlebia radiata.* Biochem. J. 254:877-884.

Ohta, A., M. Shimada, T. Hattori, T.Higuchi, and M.Takahashi. 1990. Production of secondary metabolites including a new metabolite *p*-methoxyphenylpropanol by the brown-rot fungus *Lentinus lepideus*. Mokuzai Gakkaishi 36:225-231

Perie, F. H., and M. H. Gold. 1991. Manganese regulation of manganese peroxidase expression and lignin degradation by the white rot fungus *Dichomitus squalens*. Appl. Environ. Microbiol. 57:2240-2245.

Rai, R. D., and S. Saxena. 1990. Extracellular enzymes and non-structural components during growth of *Pleurotus sajor-caju* on rice straw. Mush. J. Tropics. 10:69-73.

Reinhammer, B. 1984. Laccase. In: Copper proteins and copper enzymes, Vol 3. ed Lontie, L. pp.1-36 Boca Raton, Florida:CRC Press.

Ruttimann-Johnson, C., D. Cullen, and R. T. Lamar. 1994. Manganese peroxidases of the white rot fungus *Phanerochaete sordida*. Appl. Environ. Microbiol. 60:599-605

Somogyi, M. 1952. Notes on sugar determination. J. Biol. Chem. 195:19-23.

Tien, M. and T. K. Kirk. 1983 Lignin degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science 221:661-663.

Tien, M., and T. K. Kirk. 1984. Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a unique H_2O_2-requiring enzyme. Proc. Natl. Acad. Sci. 81:2280-2284.