Characterization of a Novel Manganese Peroxidase-Lignin Peroxidase Hybrid Isozyme Produced by Bjerkandera Species Strain BOS55 in the Absence of Manganese*

Tünde Mester‡ and Jim A. Field

From the Division of Industrial Microbiology, Department of Food Technology and Nutrition Sciences, Wageningen Agricultural University, P. O. Box 8129, 6700 EV Wageningen, The Netherlands

A novel manganese-dependent peroxidase (MnP) isozyme produced in manganese-free cultures of Bjerkandera sp. strain BOS55 was purified and characterized. The production of the enzyme was greatly stimulated by the exogenous addition of various physiological organic acids such as glycolate, glyoxylate, and oxalate. The properties of the enzyme are similar to those of MnP isozymes from other white rot fungi (1). Furthermore, the isozyme was able to oxidize various substrates in the absence of manganese, such as 2,6-dimethoxyphenol, guaiacol, ABTS, 3-hydroxyanthranilic acid, and o- and p-anisidine. An interesting characteristic of the isozyme was its ability to oxidize nonphenolic substrates, veratryl alcohol and 1,4-dimethoxybenzene, without manganese addition. The affinity for veratryl alcohol ($K_m = 116 \mu M$) and its turnover number ($2.8 s^{-1}$) are comparable to those of lignin peroxidase (LiP) isozymes from other white rot fungi. The enzyme works efficiently in the oxidation of MnP (6, 9, 10). Mn(III) is stripped from the enzyme by organic acids, and the formed Mn(III)-organic acid complex acts as a diffusible mediator in the oxidation of lignin by MnP (6, 9). Mn(III) can also oxidize organic acids, yielding radicals (11). There are two possible sources of organic acids during lignin degradation. The fungi are able to produce de novo aliphatic organic acids, mainly oxalate (12). Moreover, organic acids are formed from the degradation of lignin (13, 14).

The ligninolytic enzymes are produced during the secondary metabolism triggered by nutrient limitation (1, 5). Manganese is an absolute requirement for expression of mnp genes in several white rot fungi (16, 17). Consistent with manganese requirement, putative metal response elements were found in the promoter regions (15, 18). Furthermore, enhanced MnP production is associated with increased manganese concentrations in many white rot fungi (17, 19, 20). Bjerkandera sp. strain BOS55 is a good MnP producer (20). Previous studies have shown that this fungus is nitrogen-unregulated, and MnP production is greatly increased by nutrient nitrogen sufficiency and excess (21). MnP production is also enhanced by supplementing the culture medium with simple organic acids (20). Following the general trend of most white rot fungi, manganese is stimulatory for MnP in this fungus (20). However, Bjerkandera sp. strain BOS55 is atypical since it produces considerable MnP in manganese-free media (20, 22). In this study, we characterized a MnP isozyme produced in the absence of manganese. Furthermore, the manganese dependence for the oxidation of various substrates was tested.

MATERIALS AND METHODS

Microorganism—Bjerkandera sp. strain BOS55 (ATCC 90940) was maintained in glucose/peptone/yeast extract slants as described earlier (23). Prior to the experiment, the fungus was transferred to glucose/malt extract plates (20), which were incubated for 4–6 days at 30 °C. As inocula, 6-mm diameter agar plugs from the leading mycelial edge were used in the experiments.

Media—The standard basal medium used contained 2.2 mM nitrogen as diammonium tartrate, 56 mM glucose, 2 mg/liter thiamin, and manganese-free BIII mineral medium (pH 4.5), and the standard buffer (2,2-dimethyl succinate) was omitted (24). All glassware was previously cleaned with diammonium tartrate, 56 mM glucose, 2 mg/liter thiamin, and manganese-free BIII mineral medium (pH 4.5), and the standard buffer (2,2-dimethyl succinate) was omitted (24). All glassware was previously
washed with 5 m HNO₃ to remove contaminating manganese. The measured concentration in the manganese-free medium was always <0.01 μM. In some experiments, the basal medium was also supplemented with manganese nutrients, providing a final concentration of 0.053 mM. Glycolic acid, glyoxylic acid, and oxalic acids (5 mM) were individually added neutralized to pH 4.5 to the cultures at the time of incubation in order to study their stimulatory effect on MnP production.

**Culture Conditions**—Filter-sterilized 25-ml aliquots were placed in 250-ml cylindrical flasks, which were previously autoclaved with a drop of demineralized water (22). Each flask was inoculated with three agar plugs. Cultures were incubated statically under an air atmosphere at 27 °C.

**Enzyme Purification with Fast Protein Liquid Chromatography (FPLC)**—The collected supernatant was separated from the mycelial mat by centrifugation (20,000 × g). The extracellular fluid was washed with 10 mM sodium acetate (pH 6.0) and concentrated by ultrafiltration with a PM-10 membrane (10 kDa; Amicon, Rotterdam, The Netherlands). The concentrated supernatants were loaded in 10 mM sodium acetate onto a MonoQ anion-exchange column (Pharmacia, Uppsala, Sweden), and heme-containing proteins were monitored with a wavelength of 405 nm. Proteins were eluted with a linear gradient of sodium acetate up to 450 mM (pH 6.0) in 45 min, and 1-ml fractions were collected (1 ml/min) for 45 min. The fractions, 33–35, that contained the most MnP activity were collected and then washed and concentrated by ultrafiltration. The concentrate was eluted by creating a decreasing pH gradient in the pH range of 4.5–2.7 on a MonoP chromatofocusing column (Pharmacia) as described earlier (25), and 1-ml fractions were collected (1 ml/min) for 40 min. The MnP-containing fraction was washed with demineralized water and concentrated by ultrafiltration. The concentrate was used for enzyme characterization, kinetic, and substrate oxidation studies.

The MonoP enzyme concentrate was brought through two additional FPLC steps to confirm its purity. First, gel filtration was carried out using a Superdex 75 HR 10/30 column (10 × 300 mm, volume of 24 ml; Pharmacia). The enzyme was eluted in 10 mM sodium acetate buffer (pH 6.0) with a flow rate of 0.5 ml/min for 50 min, and 0.5-ml fractions were collected. A single symmetrical peak was observed, and the enzyme-containing fractions, 22 and 23, were pooled and concentrated by ultrafiltration. The enzyme was washed with 10 mM potassium phosphate buffer (pH 6.0). Diammonium sulfate was added to the protein, reaching a final concentration of 1 M, and loaded onto a 1-ml hydrophobic interaction chromatography column (phenyl-Sepharose HP, Pharmacia). Proteins were eluted in a decreasing linear gradient of 1 M diammonium sulfate in 10 mM potassium phosphate buffer (pH 6.0) in 45 min (1 ml/min). Fractions (1 ml) were also collected every minute. Again, a single symmetrical peak was observed eluting from 19 to 22 min.

**Enzyme Characterization**—Protein concentration was determined according to Bradford (26) using bovine serum albumin as a standard. The concentration of purified MnP was also measured at a wavelength of 406 nm and calculated using ε₂₀₀₀ nm = 129 mM⁻¹ cm⁻¹ (6), which gave approximately 12 mg/ml MnP. The molecular mass of the enzyme was determined by SDSPolyacrylamide gel electrophoresis (12% polyacrylamide gel) in a PhastSystem (Pharmacia) using marker proteins (low molecular mass calibration kit from Pharmacia) as standards. The isoelectric point was estimated by chromatofocusing by measuring the pH of the collected fraction containing MnP isozyme. The enzyme absorbance spectrum was determined with 86 mg/ml MnP in the presence of 50 mM malonate buffer (pH 4.5) by scanning the absorbance of the enzyme at a wavelength range of 350–700 nm using a Perkin-Elmer Lambda1 UV-visible spectrophotometer. The oxidized enzyme was scanned after addition of 0.2 mM H₂O₂.

**Kinetic Study**—Kinetic constants of MnP activities for H₂O₂ and Mn(II) were calculated by the formation of Mn(III) malonate complex at 24 °C, which gave approximately 12 mg/ml MnP. The molecular mass of the enzyme was determined by SDSPolyacrylamide gel electrophoresis (12% polyacrylamide gel) in a PhastSystem (Pharmacia) using marker proteins (low molecular mass calibration kit from Pharmacia) as standards. The isoelectric point was estimated by chromatofocusing by measuring the pH of the collected fraction containing MnP isozyme. The enzyme absorbance spectrum was determined with 86 mg/ml MnP in the presence of 50 mM malonate buffer (pH 4.5) by scanning the absorbance of the enzyme at a wavelength range of 350–700 nm using a Perkin-Elmer Lambda1 UV-visible spectrophotometer. The oxidized enzyme was scanned after addition of 0.2 mM H₂O₂.

**H₂O₂ Inactivation—MnP (4 μg) was incubated in 0.2 ml of 50 mM succinate buffer at 25 °C with or without 0.4 mM H₂O₂ to study the effect of either 0 or 0.1 mM veratryl alcohol on the inactivation rate.**

**Determination of Substrate Oxidation by High Performance Liquid Chromatography (HPLC)**—To test the oxidation of benzyl alcohol, veratryl alcohol, 1,4-dimethoxybenzene, and 2-chloro-1,4-dimethoxybenzeno, Bjerkandera MnP was incubated with 0.3 mM compound in 50 mM succinate buffer (pH 3.0). The reaction was initiated with 0.1 mM H₂O₂, H₂O₂ was added once every hour, reaching a final concentration of 0.4 mM. After 4 h of incubation, the reaction was stopped with 1 eq volume of acetoniitrite. Compound oxidation was detected by HPLC. All incubations were carried out in triplicate. 50-μl samples were analyzed by a Pascal series HPLC ChemStation (Hewlett-Packard, Waldbronn, Germany) equipped with an HP1040 series diode array detector. The column (200 × 3 mm) was filled with ChromoSpher C-18-PAH (5-μm particles; Chrompack, Middelburg, The Netherlands). The following gradient (0.4 ml/min, 30 °C) was used: 50:10, 0:100, and 0:100 ratios of H₂O₂ to CH₃CN at 0, 15, and 20 min, respectively. Compound identifications were based on matching retention times, and UV spectra with those of standards.

**Manganese Inhibition of Veratryl Alcohol Oxidation—**In some experiments, the effect of Mn(II) on veratryl alcohol oxidation was elucidated. The oxidation of 0.1 mM veratryl alcohol was tested during a 10-min incubation period with 0.4 mM H₂O₂ in 50 mM sodium succinate buffer (pH 3.8) with 2 mM sodium pyrophosphate and varying Mn(II) concentrations. For comparison, a similar experiment was conducted with purified LiP-5 isozyme from Bjerkandera sp. strain BOS55 (28) supplied at the same units in terms of veratryl alcohol oxidation. Formation of veratraldehyde was analyzed by HPLC.

**Determination of Background Manganese Content in Culture Media—**Manganese content of the culture media was measured by an inductivity-coupled plasma mass spectrometer by the Department of Soil Science and Plant Nutrition, Wageningen Agricultural University (Wageningen, The Netherlands). The detection limit for manganese is 0.002 μM.

**Terminal Sequence Determination—**The T-terminus amino acid sequence determination was carried out at the Rijks Universiteit Leiden, Faculteit der Geneeskunde, Vakgroep Medische Biochemie (Leiden, The Netherlands).

**Chemicals—**All chemicals were commercially available and used without further purification.

**RESULTS**

**MnP Production in the Cultures of Bjerkandera sp. Strain BOS55 and Enzyme Purification—**Bjerkandera sp. strain BOS55 was cultivated in manganese-free nitrogren-limited glucose medium in the presence and absence of simple organic acids. The FPLC studies using the MonoQ column demonstrated the increased production of homeprotein with MnP activity as a result of organic acid addition to the cultures. As an example, the FPLC profiles and enzyme activities measured in the collected fractions of extracellular fluid from a 7-day-old culture grown in the absence or presence of 5 mM glycolate are shown in Fig. 1. Similar results were obtained with oxalate and...
glyoxylate. In the cultures without the organic acids, very little hemoprotein and limited MnP activities were detected. The addition of glycolate stimulated the production of several heme peaks. However, most of the MnP activity was attributed to one peak (fractions 33–35). In other studies with glycolate and other organic acids (data not shown), the MnP activity was sometimes distributed between two major peaks: fractions 33–35 and 38–39, respectively. Since MnP activity was consistently present in fractions 33–35, this isozyme was isolated and used for further characterization. The purification is summarized in Table I.

Physical Properties and N-terminal Amino Acid Sequence—As the first step in the enzyme characterization, the molecular mass was measured by the SDS-polyacrylamide gel electrophoresis method and was found to be 43,000 Da (Fig. 2). This gel electrophoresis resulted in only one band, indicating a successful purification. The pl was 3.88 as estimated by the chromatofocusing method. The spectrum of the enzyme showed a typical peak for native enzyme at 407 nm with a molar extinction coefficient of 24,800 M$^{-1}$ cm$^{-1}$, and the addition of excess hydrogen peroxide resulted in a shift to 420 nm (Fig. 3). The N-terminal amino acid sequence of the protein was also determined up to 25 amino acids: VACPDGVNTATNAACCALF-

Catalytic Properties and Manganese Dependence of Substrate Oxidation—Different substrates were used to evaluate the manganese dependence of their oxidation at pH 4.5 in 50 mM malonate buffer. The results are summarized in Table II. The substrate 2,6-dimethoxyphenol (DMP) was oxidized in the presence and absence of manganese. The oxidation rate of DMP without manganese was 18% of that in the presence of manganese. This trend was observed in the case of other substrates such as guaiacol, ABTS, 3-hydroxyanthranilate, o-anisidine, and p-anisidine. The enzyme was also able to directly oxidize veratryl alcohol at pH 4.5 without any manganese (Table III).

The kinetics of the oxidizing substrate (H$_2$O$_2$) and of various direct reducing substrates of the enzyme were compared in terms of $K_m$ and turnover number (Table III). At a physiological pH of 4.5, Mn(II) was clearly the best reducing substrate, with a turnover of 59 s$^{-1}$. Nonetheless, veratryl alcohol, DMP, and ABTS were directly oxidized by the enzyme at this pH, with a turnover of 2.3 to 1.3 s$^{-1}$ in the absence of Mn(II). The enzyme had a high affinity for Mn(II), DMP, and ABTS, whereas the affinity for veratryl alcohol at pH 4.5 was an order of magnitude less.

The pH dependence of the oxidation of several substrates was also studied. Fig. 4 shows the pH dependence of Mn(II) and DMP oxidation. The highest rate of Mn(II) oxidation was observed at pH 4.5. At pH 3.0, the Mn(II) oxidizing activity was negligible. The direct oxidation of DMP was the highest at pH 3.0, and at this pH, the rate was comparable in the presence and absence of manganese. Likewise, the oxidation of ABTS and veratryl alcohol in the absence of manganese was 2–3-fold higher at pH 3.0 compared with pH 4.5. At pH 3.0, the affinity

**TABLE I**

| Step         | Protein | Activity$^a$ | Purification factor |
|--------------|---------|-------------|---------------------|
|              | mg      | units       | units/mg            |                      |
| EF           | 97      | 97          | 1.0                 | 1.0                 |
| Concentrated EF | 85     | 128         | 1.5                 | 1.5                 |
| MonoQ        | 1.6     | 85          | 53                  | 53                  |
| MonoP        | 0.87    | 70          | 80                  | 80                  |

$^a$ MnP activity was determined by monitoring Mn(III) malonate formation as described under “Materials and Methods.”

$^b$ EF, extracellular fluid.
of the Bjerkandera MnP for veratryl alcohol was ~5-fold greater than at pH 4.5 (Table III).

To verify that the manganese and veratryl alcohol oxidation activities were due to one isozyme, the enzyme was purified further by two additional FPLC steps: gel filtration and hydrophobic interaction. After each step, only one symmetrical peak was observed, and the molar ratio of 0.1 mM Mn(II) oxidation rate to 0.1 mM veratryl alcohol oxidation rate at pH 4.0 remained constant at 23 (data not shown).

**Product Identification of Nonphenolic Compound Oxidation**—Several nonphenolic compounds were incubated in the absence of manganese for 4 hours with the Bjerkandera MnP at pH 3.0 in 50 mM succinate buffer. The products of the oxidation were identified by HPLC diode array detector. The results are summarized in Table IV. The main product of veratryl alcohol oxidation was veratraldehyde, recovered with a molar yield of 46%. 1,4-Benzoquinone was also identified as a product of 1,4-dimethoxybenzene oxidation, with a molar yield of 41%. Even 2-chloro-1,4-dimethoxybenzene was oxidized to a small extent by the isozyme. Benzyl alcohol was not significantly oxidized by this enzyme.

**Effect of Manganese on Veratryl Alcohol Oxidation**—The veratryl alcohol oxidation to veratraldehyde by Bjerkandera MnP and LiP was compared in the presence of manganese (Table V). LiP was unaffected by the presence of either 0.3 mM Mn(II) or Mn(III). However, Bjerkandera MnP behaved differently. Mn(II) in the concentration range of 0.1–1 mM inhibited the oxidation of veratryl alcohol. Interestingly, Mn(II) at a low concentration (0.033 mM) stimulated the veratryl alcohol oxidation to veratraldehyde.

**DISCUSSION**

Bjerkandera sp. strain BOS55 produces MnP in high quantities under different culture conditions. An interesting characteristic of this fungus is the ability to produce MnP in manganese-free media (20, 22). Recently, the production of MnP under manganese deficiency was reported for two Pleurotus species, *P. eryngii* and *P. ostreatus*, and the isozymes were characterized (29, 30). In the *Pleurotus* strains, MnP production was inhibited by even trace amounts of manganese. In contrast, Bjerkandera sp. strain BOS55 MnP production is stimulated by manganese nutrients. The same MnP isozymes produced under manganese deficiency were also detected in manganese-deficient cultures (data not shown). A previous study showed that the addition of certain organic acids enhanced MnP production under manganese-excess conditions (20). In this study, glycolate, glyoxylate, and oxalate highly stimulated MnP production under manganese-deficient conditions. These acids are physiological metabolites of white rot fungi (6, 12, 14, 31). The role of the organic acids in MnP production still requires further studies.

The physical characteristics in terms of molecular mass, isoelectric point, and heme absorbance of the purified MnP do not differ from those of other MnP isozymes described from other white rot fungi (32–35). The turnover number of MnP from Bjerkandera sp. strain BOS55 shows that this isozyme is efficient and comparable to other MnP isozymes described in the literature (28, 29, 36–38). However, the Bjerkandera MnP appears to be unique since it has a broader substrate specificity compared with most MnP isozymes reported. This isozyme is able to directly oxidize various aromatic amines as well as guaiacol and DMP in the absence of manganese. Although the oxidation rate was lower in the absence of manganese than in its presence at pH 4.5, the oxidation of these compounds was completely independent of manganese at pH 3.0.

Remarkable was the observation that Bjerkandera MnP was even able to oxidize veratryl alcohol and 1,4-dimethoxybenzene, which are traditional LiP substrates, in the absence of manganese. Since veratryl alcohol was oxidized, but not benzyl alcohol, the oxidation of veratryl alcohol most likely proceeds via a cationic radical mechanism rather than a benzylic radical mechanism. The observed oxidation of 1,4-dimethoxybenzene to 1,4-benzoquinone also supports a cation radical mechanism, which is characteristic of this fungus is the ability to produce MnP in manganese-free media (20, 22). Recently, the production of MnP under manganese deficiency was reported for two Pleurotus species, *P. eryngii* and *P. ostreatus*, and the isozymes were characterized (29, 30). In the *Pleurotus* strains, MnP production was inhibited by even trace amounts of manganese. In contrast, Bjerkandera sp. strain BOS55 MnP production is stimulated by manganese nutrients. The same MnP isozymes produced under manganese deficiency were also detected in manganese-deficient cultures (data not shown). A previous study showed that the addition of certain organic acids enhanced MnP production under manganese-excess conditions (20). In this study, glycolate, glyoxylate, and oxalate highly stimulated MnP production under manganese-deficient conditions. These acids are physiological metabolites of white rot fungi (6, 12, 14, 31). The role of the organic acids in MnP production still requires further studies.

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### Table IV

| Compound                  | Identified product | % of oxidation | Molar product yield |
|---------------------------|--------------------|----------------|---------------------|
| Benzyl alcohol            | None               |                | NA*                 |
| Veratryl alcohol          | Veratraldehyde     | 34 ± 5         | 46 ± 4              |
| 1,4-Dimethoxybenzene      | 1,4-Benzoquinone   | 35 ± 7         | 41 ± 8              |
| 2-Chloro-1,4-dimethoxybenzene | 2-Chloro-1,4-benzoquinone | 10 ± 1     | 23 ± 3              |

*% of oxidation = 100 × (|substrate|_{initial} – |substrate|_{final})/|substrate|_{initial}.

*Molar product yield = 100 × (|product|/|substrate|_{initial} – |substrate|_{final}).

*NA, not applicable.

*Results are the means ± S.D. of three parallel incubations.
TABLE V
Effect of manganese on the oxidation of veratryl alcohol to veratraldehyde by the purified Bjerkandera MnP in comparison with Bjerkandera LiP-5 isozyme

The reaction mixture contained 10 mg/liter MnP, 0.1 mM veratryl alcohol, and 0.4 mM H$_2$O$_2$ in 50 mM succinate buffer with 2 mM pyrophosphate (pH 3.8). The incubation time was 10 min. The LiP-5 isozyme was added at equivalent veratryl alcohol-oxidizing units as MnP.

| Mn conc     | MnP | LiP  |
|-------------|-----|------|
| 0.000 mM Mn(II) | 38.6 ± 0.4 | 54.5 ± 3.3 |
| 0.033 mM Mn(II) | 63.6 ± 1.7 | NT$^a$ |
| 0.100 mM Mn(II) | 24.2 ± 0.5 | NT  |
| 0.300 mM Mn(II) | 8.6 ± 0.3  | 51.4 ± 7.8  |
| 1.000 mM Mn(II) | 4.4 ± 0.3  | NT  |
| 0.300 mM Mn(III) | NT  | 57.6 ± 6.1  |

$^a$ Values are presented as the means ± S.D. of three parallel incubations.

were found to severely inhibit the oxidation of veratryl alcohol by the *Bjerkandera* MnP. This observation would be expected since both compounds as substrates of the enzyme would have to compete for the same oxidized heme group. The fact that a low rate-limiting (noncompetitive) concentration (0.033 mM) of Mn(II) stimulated veratryl alcohol oxidation indicates that Mn(II) helped to protect the enzyme from H$_2$O$_2$ inactivation, probably by completing the catalytic cycle. It was also observed that veratryl alcohol (0.1 mM) incubated with the enzyme without Mn(II) could delay the H$_2$O$_2$ inactivation of the enzyme as measured by Mn(II)-oxidizing activity (data not shown). These phenomena can only be explained by a single enzyme utilizing both compounds as a substrate.

Veratryl alcohol is a *de novo* secondary metabolite produced by many white rot fungi, including *Bjerkandera* sp. strain BOS55 (3). Concentrations ranging from 0.01 to 0.80 mM occur in liquid cultures as well as during solid-state fermentation of wood (23, 43). The oxidation of veratryl alcohol has generally been attributed to LiP as opposed to MnP due to the high ionization potential of the compound, which is not directly oxidized by Mn(III). Veratryl alcohol has been implicated in several roles in the catalysis of LiP. This metabolite mediates the oxidation of aromatic compounds with lower redox potential than that of veratryl alcohol cation radical (44, 45). As the ionization potential of the compound, which is not directly oxidized by MnP, 63.6

In conclusion, the MnP from *Bjerkandera* sp. strain BOS55 is a unique enzyme that can best be described as a hybrid between manganese peroxidase and lignin peroxidase. The catalytic efficiencies in Mn(II) and veratryl alcohol oxidation by this single isozyme are comparable to those of MnP and LiP, respectively, from other white rot fungi.

REFERENCES
1. Kirk, T. K., and Farrell, R. L. (1987) *Annu. Rev. Microbiol.* 41, 465–505.
2. Hammel, K. E., Jensen, K. A., Jr., Mozuch, M. D., Landucci, L. L., Tien, M., and Pease, E. A. (1993) *J. Biol. Chem.* 268, 12274–12281.
3. de Jong, E., Field, J. A., and de Bent, J. A. M. (1994) *FEBS Microbiol. Rev.* 13, 153–188.
4. Glenn, J. K., Akleswaran, L., and Gold, M. H. (1986) *Arch. Biochem. Biophys.* 251, 688–696.
5. Warishii, H., Akleswaran, L., and Gold, M. H. (1988) *Biochemistry* 27, 5366–5370.
6. Warishii, H., Valli, K., and Gold, M. H. (1992) *J. Biol. Chem.* 267, 23688–23695.
7. Sundaramoorthy, M., Kishi, K., Gold, M. H., and Poulsus, T. L. (1994) *J. Biol. Chem.* 269, 32759–32767.
8. Sundaramoorthy, M., Kishi, K., Gold, M. H., and Poulsus, T. L. (1997) *J. Biol. Chem.* 272, 17574–17580.
9. Kuan, I. C., and Tien, M. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90, 1242–1246.
10. Shimada, M., Ma, D. B., Akamatsu, Y., and Hattori, T. (1994) *FEBS Microbiol. Rev.* 13, 285–296.
11. Khindaria, A., Grover, T. A., and Aust, S. D. (1994) *Arch. Biochem. Biophys.* 314, 301–306.
12. Dutton, M. V., and Evans, C. S. (1996) *Can. J. Microbiol.* 42, 881–895.
13. Hammel, K. E., Mozuch, M. D., Jensen, K. A., and Kersten, P. J. (1994) *Biochemistry* 33, 1338–1344.
14. Roy, B. P., and Archibald, F. (1993) *Appl. Environ. Microbiol.* 59, 1855–1863.
15. Gold, M. H., and Alic, M. (1993) *Microbiol. Rev.* 57, 605–622.
16. Alic, M., Akileswaran, L., and Gold, M. H. (1991) *J. Bacteriol.* 173, 4101–4106.
17. Périé, F., and Gold, M. H. (1991) *Appl. Environ. Microbiol.* 57, 2240–2245.
18. Alic, M., Akleswaran, L., and Gold, M. H. (1997) *Biochem. Biophys. Acta* 1338, 137–147.
19. Bonnarme, P., and Jeffries, T. W. (1990) *Appl. Environ. Microbiol.* 56, 210–217.
20. Mester, T., and Field, J. A. (1997) *FEBS Microbiol. Lett.* 155, 161–168.
21. Mester, T., Peña, M., and Field, J. A. (1996) *Appl. Microbiol. Biotechnol.* 44, 267–278.
22. Moreira, M. T., Feijoo, G., Sierra-Alvarez, R., Lema, J., and Field, J. A. (1997) *Appl. Environ. Microbiol.* 63, 1749–1755.
23. Mester, T., de Jong, E., and Field, J. A. (1995) *Appl. Environ. Microbiol.* 61, 1881–1887.
24. Tien, M., and Kirk, T. K. (1988) *Methods Enzymol.* 161B, 238–248.
25. Johansson, T., and Nyman, P. O. (1993) *Arch. Biochem. Biophys.* 306, 49–56.
26. Field, J. A. (1986) *Appl. Environ. Microbiol.* 52, 248–254.
27. Wolden, B. S., and Willson, R. L. (1982) *J. Chem. Soc. Perkin Trans. II* 805–812.
28. van Have, R., Hartmans, S., Teunissen, P. J. M., and Field, J. A. (1998) *FEBS Lett.* 422, 391–394.
29. Martínez, M. J., Ruiz-Duenas, F. J., Guillen, F., and Martínez, A. T. (1996) *Eur. J. Biochem.* 237, 424–432.
30. Sarkar, S., Martínez, A. T., and Martínez, M. J. (1997) *Biochim. Biophys. Acta* 1339, 23–30.
31. Kersten, P. J., and Kirk, T. K. (1987) *J. Bacteriol.* 169, 2185–2201.
32. Guha, J. K., and Gold, M. H. (1985) *Arch. Biochem. Biophys.* 242, 329–341.
33. Périé, H. F., Sheng, D., and Gold, M. H. (1996) *Biochim. Biophys. Acta* 1297, 139–148.
34. Urzúa, U., Larrondo, L. F., Lobos, S., Larrain, J., and Vicuna, R. (1995) *FEBS Lett.* 371, 132–136.
35. Rüttimann-Johnson, C., Cullen, D., and Lamar, R. T. (1994) *Appl. Environ. Microbiol.* 60, 599–605.
36. Mayfield, M. B., Kishi, K., Alic, M., and Gold, M. H. (1994) *Appl. Environ. Microbiol.* 60, 4303–4309.
37. Kuan, I. C., Johnson, K. A., and Tien, M. (1995) *J. Biol. Chem.* 268, 20064–20070.
38. Matsubara, M., Suzuki, J., Deguchi, T., Miura, M., and Kitaoka, Y. (1996) *Appl. Environ. Microbiol.* **62**, 4066–4072
39. Tien, M., Kirk, T. K., Bull, C., and Fee, J. A. (1986) *J. Biol. Chem.* **261**, 1687–1693
40. Farrell, R. L., Murthagh, K. E., Tien, M., Mozuch, M. D., and Kirk, T. K. (1989) *Enzyme Microb. Technol.* **11**, 322–328
41. Haemmerli, S. D., Schoemaker, H. E., Schindt, H. W. H., and Leisola, M. S. A. (1987) *FEBS Lett.* **220**, 149–154
42. Hu, Z. C., Korus, R. A., Venkataramu, C. R., and Crawford, R. L. (1993) *Enzyme Microb. Technol.* **15**, 567–574
43. Mester, T., Sierra-Alvarez, R., and Field, J. A. (1998) *Holzforschung*, in press
44. Goodwin, D. C., Aust, S. D., and Grover, T. A. (1995) *Biochemistry* **34**, 5060–5065
45. Schick Zapanta, L., and Tien, M. (1997) *J. Biotechnol.* **53**, 93–102
46. Koduri, R. S., and Tien, M. (1994) *Biochemistry* **33**, 4225–4230
47. Cancel, A. M., Orth, A. B., and Tien, M. (1993) *Appl. Environ. Microbiol.* **59**, 2909–2913
48. D’Annihale A., Crestini, C., Di Mattia, E., and Sermanni, G. G. (1996) *J. Biotechnol.* **48**, 231–239
49. Pease, R. A., Andrawis, A., and Tien, M. (1989) *J. Biol. Chem.* **264**, 13531–13535
50. Pribnow, D., Mayfield, M. B., Nipper, V. J., Brown, J. A., and Gold, M. H. (1989) *J. Biol. Chem.* **264**, 5036–5040
51. Lobos, S., Lairrain, J., Salas, L., Cullen, D., and Vicuña, R. (1994) *Microbiology (UK)* **140**, 2691–2698
52. Forrester, I. T., Grabski, A. C., Mishra, C., Kelley, B. L., Strickland, W. N., Leatham, G. F., and Burgess, R. R. (1990) *Appl. Microbiol. Biotechnol.* **33**, 359–365
53. Johansson, T., Welinder, K. G., and Nyman, P. O. (1993) *Arch. Biochem. Biophys.* **300**, 57–62
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Tünde Mester and Jim A. Field

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