Fruitbody quality and enzyme production of strains of Hericium erinaceus, an edible mushroom of medicinal relevance

A. PH. GRYGANSKI¹, B. KIRCHHOFF², and H. P. MOLITORIS³

¹M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Tereshchenkivska St. 2, 252601, Kyiv, Ukraine
²WESER-CHAMPIGNON, Neue Heerstr. 35, 31840 Hessisch-Oldendorf, Germany
³Botanical Institute, University of Regensburg, Universitátsstr. 31, 93040 Regensburg, Germany

Cultivation experiments of 14 heterokaryotic strains of the edible and medicinally relevant mushroom *Hericium erinaceus* have shown a large variation in yield, quality and colour of the fruitbodies (Kirchhoff 1996). To determine the reasons for different fruitbody colours, phenoloxydases in the vegetative mycelium on agar and liquid media were investigated. It was shown that the colour of the fruitbodies correlates with the presence and activity of the phenoloxidase laccase. There is no correlation between fruitbody colour and presence of the phenoloxidase tyrosinase, responsible for browning of white button mushroom fruitbodies, *Agaricus bisporus*. The data obtained are important for selection or breeding of new *H. erinaceus* strains with good fruitbody qualities.

**Key words:** Fruitbody colour, fruit body quality, *Hericium erinaceum*, laccase, tyrosinase.

INTRODUCTION

The introduction of new species of edible mushrooms for the market is difficult because of the well developed market structure and high quality standards of...
commercial mushrooms. Consumers are used to the high quality of the white button mushroom, *Agaricus bisporus* (J. Lge) Imbach, and its established quality standards (Peters 1997). Colour is considered to be one of the most important factors in consumer perception of fresh mushroom quality. Mushrooms with discolouration are judged to be of lower quality and hence of lower commercial value (Burton 1988). Whiteness, compactness, fruitbody form, density and texture, and length and thickness of spines are also of importance to make *Hericium erinaceus* (Bull.: Fr.) Pers. more attractive for consumers. The colour of *H. erinaceus* fruitbodies, like that of other edible mushrooms, has two important aspects, namely colour of fruitbodies and its changes during fruitbody development, maturation and shelf life. For *H. erinaceus* it is important to produce clear white or snow-white fruitbodies, that do not change colour after mechanical damage, during storage, transport and sale (Figs. 1, 2). The main factor considered to be responsible for the browning by *A. bisporus* and, possibly, of other mushrooms, is tyrosinase. The browning of mushroom tissue is caused by the oxidation of phenols by tyrosinase to produce melanins (Rama et al. 1995). In addition to tyrosinase, laccase, another phenoloxidase, has also been studied, and may also be responsible for browning of harvested fruitbodies of *H. erinaceus*. This paper deals with colour and the correlation of this feature with the presence and activity of the enzymes laccase and tyrosinase – important attributes of quality of fresh fruitbodies of *H. erinaceus*.

**MATeRIALS AND METHODS**

**Biological material**

Fourteen strains of *Hericium erinaceus* (Bull.: Fr.) Pers. (He = heterokaryon: He1-He2, He4-He15) of different origin were obtained from culture collections and companies (Kirchhoff 1996). Six homokaryons (h = homokaryon: h1-h6) were obtained from the rarely occurring 1-nucleated chlamydospores of vegetative mycelium of strain He1. These cultures were maintained in the strain collection of WESER-CHAMPIGNON on malt peptone agar (MPA [malt extract 30 g, peptone 5 g, agar 16 g/1 H2O, pH 6]).

1. Qualitative laccase- and tyrosinase-tests on the surface of mycelial colonies on agar media (Molitoris and Schaaumann 1986)

Mycelium of the strains was cultivated for 2 weeks on agar media: MPA for tyrosinase-tests (application of p-cresol/glycine solution onto the colony surface), or MPA with guajacol (0.05 g/l) or with α-naphtol (0.05 g/l), for laccase-tests. The presence of laccase was determined by the appearance of brown-red (guajacol)
Fig. 1. White (good quality) fruitbodies of *Hericium Erinaceus*.

Fig. 2. Coloured and distorted (bad quality) fruitbodies of *H. Erinaceus*. 
or blue (α-naphtol) on the plates 1, 3, 5, 7 and 10 days after inoculation. Presence of tyrosinase (reddish-brown colour) was tested with the heterokaryons 10 and 20 days after inoculation and with the homokaryons after 20 and 60 days because of their slow growth rate.

2. Qualitative laccase- and tyrosinase tests of culture filtrate and mycelial extract from liquid (surface) cultures

Mycelium was cultivated in liquid MP medium. 100 ml Erlenmeyer flasks with 50 ml sterile nutrient solution were inoculated with 5 x 5 mm mycelial pieces from MPA cultures. After one week of growth mycelial biomass and culture filtrate were separated by filtration. The pH of the culture filtrate was around 6 for the heterokaryons and around 5 for the homokaryons. For growth determinations the vegetative mycelium was washed with distilled water and weighed (wet weight).

For preparation of the mycelial extract 1 g mycelium (wet weight) was initially disrupted using mortar and pestle with 2 g washed sea sand for 3 min. and after addition of 6 ml 0.05M/pH 6 phosphate buffer for another 5 min. The disrupted material was centrifuged in an Eppendorf centrifuge 5417R for 20 min. at 8000 xg and 5 °C. The supernatant was used both for qualitative and quantitative enzyme determinations. For homokaryotic strains enzyme determinations could be performed only with the culture filtrates owing to lack of mycelium.

In order to prove the presence of tyrosinase in the presence of laccase, culture filtrate and mycelial extract were treated at 60 °C for 10 min. whereby laccase should be destroyed and tyrosinase should be activated. For the qualitative determination of laccase or tyrosinase, 0.5 ml of culture filtrate or mycelial extract was mixed in test tubes with 1 ml of water (control), 2.6-dimethoxyphenol (DMOP), guajacol or p-cresol for laccase determination or with 1 ml of tyrosine for tyrosinase determination. Heat-treated material was used in the same way. Colour change of the solutions was observed at 19 °C after 1 and 12 hours following mixing.

3. Quantitative determination of laccase activity with DMOP in liquid culture

Culture filtrate or mycelial extract (0.4 ml) was mixed with 2.0 ml substrate (DMOP) and 0.3 ml Sörensen's buffer pH 5 in a 1 cm cuvette. DMOP solution: 100 mg 2.6-Dimethoxy-phenol was dissolved in 1 litre NaOH-citrate buffer 0.1 M, pH 5. Sörensen's buffer: A) 21.008 g citric acid + 200 ml 1N NaOH made up to 1 litre with distilled water; B) 0.1N NaOH; C) 1 litre of solution A was mixed with 41.7 ml solution B. The enzyme reaction was started by addition
of the enzyme solution. Readings were taken in an Eppendorf Spektrallinien Photometer with a 1101 M Cd-Lampe at 468 nm after 30 sec. reaction time at room temperature (19 °C).

RESULTS

1. Qualitative enzyme tests on agar media

Laccase (guajacol)

The colour reaction (brown-red colouring of the inoculum) of the heterokaryotic strains commenced usually 1 hour after inoculation, in some strains after 30 minutes. The enzyme reaction (brown-red zone in agar around inoculum or mycelial colony) (Fig. 3) appeared usually on the third day. After this time, colour intensity and size of the reaction zone did not change except for strain He10. The colour of the heterokaryotic fruitbodies correlated with the results of the guajacol laccase tests. The majority of strains with a white fruitbody (Table 1, colour intensity 1–3) had no positive enzyme reaction. All strains with yellow to brown fruitbodies (Table 1, colour intensity 4–6) had a strong positive reaction in the guajacol laccase test.

Only one out of six homokaryotic strains tested showed a positive reaction in the guajacol laccase test.

Laccase (α-naphtol)

The colour reaction of the heterokaryotic strains started usually 1 hour after inoculation (blue colour of the inoculum). The enzyme reaction (blue zone around colony, fig. 4) appeared usually on the fifth day. Colour intensity and extent of the reaction zone did not change after day 5. However, after 10 days the mycelium overgrew the coloured zone, prohibiting further observation. There was no correlation between the laccase test (both presence and intensity of reaction) and fruitbody colour.

Only one out of six homokaryotic strains tested showed a positive reaction of laccase with α-naphtol. The reaction was finished after 3 days of growth and did not change thereafter.

Tyrosinase (p-cresol and glycine)

Tyrosinase activity was observed on the 10th day after inoculation for strains He2 and He14 and after 20 days for strains He4, He6, and He12–He15. Possibly, all *H. erinaceus* strains possess intracellular tyrosinase which would not allow observation of the reaction on the colony surface. Only old or destroyed cells may
Table 1. Correlation between colour of fruitbodies and qualitative tests for laccase (4 days, 25 °C) for the heterokaryotic strains (He) on agarised media.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Colour of fruitbodies*</th>
<th>Laccase (guajacol)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>He6</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>He2</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>He13</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>He1</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>He10</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>He9</td>
<td>3</td>
<td>++</td>
</tr>
<tr>
<td>He8</td>
<td>3</td>
<td>+++</td>
</tr>
<tr>
<td>He12</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>He14</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>He7</td>
<td>4</td>
<td>++</td>
</tr>
<tr>
<td>He4</td>
<td>4</td>
<td>+++</td>
</tr>
<tr>
<td>He11</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>He5</td>
<td>5</td>
<td>+++</td>
</tr>
<tr>
<td>He15</td>
<td>6</td>
<td>++</td>
</tr>
</tbody>
</table>

* Colour of fruitbody: 1 = white; 2-3 = white to yellow; 4-5 = yellow to brown; 6 = brown.
** Laccase intensity: – = no reaction; + = present; ++ = strong; +++ = very strong.

Table 2. Correlation between colour of fruitbodies and the results of qualitative laccase tests in culture filtrate (CF) and mycelial extract (ME) from liquid culture (4 days, 25 °C) of heterokaryotic *Hericium* strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Colour of fruitbodies*</th>
<th>Laccase (CF)** guajacol after 12 hours</th>
<th>Laccase (ME)** guajacol after 12 hours</th>
<th>Laccase (CF)** DMOP after 1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>He6</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>He2</td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>He13</td>
<td>2</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>He1</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>He10</td>
<td>3</td>
<td>++</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>He9</td>
<td>3</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>He8</td>
<td>3</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>He7</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>He4</td>
<td>4</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>He12</td>
<td>4</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>He14</td>
<td>4</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>He11</td>
<td>5</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>He5</td>
<td>5</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>He15</td>
<td>6</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

*, **, *** for explanation see Table 1
Fig. 3. Laccase reaction with guajacol on agar plates with different strains of *H. erinaceus* (after 4 days, 25 °C).

Fig. 4. Laccase reaction with α-naphthol on agar plates with different strains of *H. erinaceus* (after 4 days, 25 °C).
Fig. 5. Qualitative laccase tests (guajacol) with culture filtrate of different strains of *H. erinaceus* (from left to right: He9, He15, He7, He13, He1).

Fig. 6. Qualitative laccase tests (guajacol) with mycelial extracts of different strains of *H. erinaceus* (from left to right: He7, He8, He4, He6, He1).
therefore show a colour reaction with p-cresol as substrate and the rate of lysis of old cells may differ in various strains. There was no correlation between colour of fruitbodies and presence of tyrosinase in the strains investigated.

Except for the homokaryotic strain h6, the tyrosinase tests of all homokaryons were positive.

2. Qualitative enzyme determination in liquid culture

2.1. Laccase

Guajacol

The majority of strains investigated showed a positive laccase reaction with guajacol. Strains producing white fruitbodies showed no laccase reaction in the culture filtrate or the mycelial extract. In contrast, the strains with coloured fruitbodies had distinct, strong or very strong laccase reactions in the culture filtrates (Fig. 5) compared with the mycelial extracts (Fig. 6). Generally, stronger reactions were observed in the culture filtrate than in mycelial extracts (Table 2). The reactions with guajacol became more distinct after 12 hours of incubation. Very often the reaction with guajacol gave an atypical colour, not red-brown but pink or grey. No differences in qualitative laccase tests were found between heat-treated and non-treated liquid samples. No positive laccase reaction was found with culture filtrates of homokaryons.

DMOP

The majority of the strains investigated showed a positive reaction for laccase with DMOP (yellow-orange, Table 2). The reactions with this substrate became more distinct after one hour. Most of the positive strains showed this reaction within 12 hours after addition of the reagent. In a number of cases temperature treatment prevented the laccase reaction (colour production with DMOP). Half of the homokaryons tested showed positive laccase reaction with DMOP.

p-cresol

No positive laccase reaction (white sediment) was found with p-cresol, neither in culture filtrate nor in mycelial extract under the experimental conditions used.

2.2. Tyrosinase

Tyrosine

No positive tyrosinase reaction with tyrosine (red, later changing to black) was found in the culture filtrate of heterokaryons or homokaryons. More than half of the strains investigated had a positive reaction for tyrosinase when the mycelial
extract was investigated. There were more strains with positive tyrosinase reaction after temperature treatment than without treatment. The tyrosinase reaction was more distinct after 12 hours as compared to 1 hr incubation time. Strain He8, He9, He12 and He14 had no positive reaction for tyrosinase with tyrosine.

p-cresol

With p-cresol no positive tyrosinase reaction (red) in the culture filtrate was observed with heterokaryons or homokaryons. In contrast, mycelial extracts of 10 strains studied showed distinct tyrosinase activity (red with p-cresol). As with tyrosine, the exceptions were the strains He8, He9, He12 and He14, which did not show any positive reaction. In some cases, after 12 hours of incubation the reaction intensity was stronger than after 1 hour, in other cases a positive tyrosinase reaction could be observed only after 12 hours. Generally, temperature treatment increased the intensity of the tyrosinase reaction.

3. Quantitative enzyme determination in liquid culture

Culture filtrate

Except for strains He1 and He6, the heterokaryotic strains investigated showed laccase activity with DMOP. Greatest enzyme activity was found in the strains He5 (0.98 E/ml), He12 (0.85 E/ml) and He14 (0.70 E/ml). Strains with white fruitbodies (colour intensity 1–3) had maximum laccase activity in the culture filtrate of only 0.6 E/ml. Except for the strains He7, He11, He15, strains with yellow and brown fruitbodies (colour intensity 4–6) had a laccase activity in the culture filtrate of more than 0.5 E/ml (Fig. 7).

Homokaryotic strains had no or only very low laccase activity (not higher than 0.10 E/ml).

Mycelial extract

Laccase activity in the mycelial extracts was usually stronger than in culture filtrates. The majority of the strains investigated showed laccase activity with DMOP. Only strain He1 did not possess laccase activity. The highest laccase activity was found in strains He14 (1.25 E/ml), He7 (1.05 E/ml) and He12 (1.00 E/ml). Except for strain He10, the strains with white fruitbodies (colour intensity 1–3) had a laccase activity in the culture filtrate of up to 0.6 E/ml. With exception of strain He4, the strains with yellow-and brown fruitbodies (colour intensity 4–6, Fig. 7) had a laccase activity in culture filtrate of more than 0.6 E/ml.
Fig. 7. Correlation between fruitbody colour (arranged for increasing colour intensity: white -> yellow -> brown) and laccase activity of *H. erinaceus* in liquid culture. The line indicates the mean of laccase activity of the culture filtrate (CF) and the mycelial extract (ME).

**Discussion and conclusion**

Laccase activity with guajacol as substrate was determined in both agar medium and liquid culture. The results obtained on the agar medium correspond generally with those in liquid culture. Qualitative laccase determinations in agar medium and liquid culture correspond with the quantitative determination of laccase activity with DMOP. An atypical colour of the reaction between laccase and guajacol can be explained where a pink colour can indicate an early or weak reaction, grey may be the result of (black) precipitated melanin as final reaction product.

Temperature treatment (10 min. at 60 °C.) of enzyme solutions did not destroy the laccase. It may be speculated that the laccase of *H. erinaceus* is more thermostable than in other fungi (Molitoris 1976).

No positive tyrosinase reactions were observed in young colonies on agar medium and in the culture filtrate of liquid cultures. An explanation for this could be the intracellular nature of this enzyme. Another possibility is that this enzyme is produced (and released) only in a later stage of cultivation. Late production of
tyrosinase has been shown in a number of fungi, e.g. by Turner (1974) who found that in *Agaricus bisporus* laccase predominated in the vegetative stage, whereas tyrosinase was found later during fruitbody development. The conditions in our experiments (7 days old liquid cultures) did not lead to the secretion or release of tyrosinase from the mycelium into the culture medium. No correlation between the qualitative tyrosinase tests and the colour of *H. erinaceus* fruitbodies was found. These results for *H. erinaceus* contradict the data obtained for fruitbodies of the white button mushroom *A. bisporus* where it was shown that the tyrosinase is responsible for browning of the fruitbodies resulting in a lower quality of this mushroom (Burton 1988, Rama et al. 1995, Jolivet et al. 1998). A quantitative determination of tyrosinase activity in fruitbodies could be a good method for determining promising strains of *H. erinaceus* with white, well-formed fruitbodies.

In most cases a close correlation between presence and activity of laccase and the colour of fruitbodies of *H. erinaceus* strains was found. However, strains He7, He11 and He15 with brown fruitbodies did not show high laccase activity in the culture filtrate (Fig. 5) although they produced high laccase activity in the mycelial extract. In contrast, strain He4, with brown fruitbodies, showed low laccase activity in the mycelial extract, but had relatively high laccase activity in the culture filtrate. It may be that strains of *H. erinaceum* have different rates of intracellular laccase production and release of this enzyme into the culture medium. This has been shown by Prillinger and Molitoris (1979) for strains of *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. where the secretion of laccase was shown to be under genetic control.

For production of white fruitbodies of *H. erinaceus* strains are recommended where laccase activity in liquid culture (culture filtrate or mycelial extract) is below 0.6 E/ml (in this study strain He1, He2, He6, He8, He9 and He13). An easier way to determine laccase activity would be the use of qualitative tests with guajacol in agar medium. Strains with low overall laccase activity showed no or only weak enzyme reaction in this test. Four strains of *H. erinaceus* with no or weak laccase reaction in qualitative tests (He1, He2, He6 and He13) produced fruitbodies of good quality in cropping experiments. Strains He1 and He2 showed both also rapid mycelial growth and a high yield of fruitbodies (Kirchhoff 1996). The latter two strains we therefore recommend for commercial cultivation.

Determination of the presence of laccase during growth on agar media and/or in liquid culture may constitute a good method to select better strains of *H. erinaceum* more suitable for commercial use. These strains should have good growth characteristics and a first grade fruitbody quality. The enzyme tests could also be important in selection and breeding experiments. In future experiments
for production of new strains that produce white, and thus high grade fruitbodies, the use of homokaryons with low laccase activity could reduce the number of trials and necessary investigations and thereby avoid expensive and time-consuming fruitbody tests.

ACKNOWLEDGEMENTS

The authors thank Ch. Thielke, Bovenden, for isolation and supply of homokaryotic strains used in this investigation, and I. Lauer and I. Kurchenko, Regensburg, for help in the experiments. Thanks are also due to S. T. Moss, Portsmouth, for critically reading the English text.

REFERENCES


