Note

Relationships between Tyrosinase Activity and Gill Browning during Preservation of Lentinus edodes Fruit-bodies

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A correlation between tyrosinase activity and gill browning during preservation of Lentinus edodes fruit-bodies was observed. Latent-type tyrosinase was recognized in the precipitate of the gill homogenate, and activation of the tyrosinase was brought about by incubating the precipitate with acidic buffer. Changes in latent- and active-type tyrosinase content during gill browning indicated the possibility of a de novo synthesis of latent-type tyrosinase.

Key words: Lentinus edodes; gill browning; latent-type tyrosinase; active-type tyrosinase; de novo synthesis

The gill color of fruit-bodies of Lentinus edodes changes to brown during post-harvest preservation, followed by deterioration of the fruit-body. The mechanism of browning should be understood to find means for maintaining the quality of post-harvest fruit-bodies. There are a few reports on browning and tyrosinase activity of L. edodes, but latent- and active-type tyrosinases are not discussed. In this study, the correlation between tyrosinase activity and gill browning, and changes of latent- and active-type tyrosinase content during gill browning of the L. edodes were investigated.

Freshly harvested fruit-bodies of a dikaryotic strain of L. edodes (LE001-32) were used. This strain was obtained by crossing the monokaryotic stocks from spores of commercial strain 121 from Mori Sangyo Co. (Gunma, Japan) and that from strain 600 from Hokken Sango Co. (Tochigi, Japan). The dikaryotic strain was cultivated on sawdust medium for 50 days according to the method of Matsumoto, and the fruit-bodies harvested immediately after breakage of the veil.

To observe changes in gill browning, the preservation temperature was fixed at 25 °C, at which full browning occurred by incubating for 5 days. There was no browning from day 1 to day 2. Partially spotted browning was observed on day 3, and browning spread over the gills from days 4 to 5.

The distribution of tyrosinase in fruit-bodies during preservation was estimated by tissue printing, according to the method of Moore et al. As shown in Fig. 1, spots with tyrosinase activity were detected in the gills on day 3, and spread all over the gills and fruit-body on days 4 and 5, respectively.

The multiplicity of tyrosinases of L. edodes was confirmed by partially denaturing SDS polyacrylamide gel electrophoresis (PAGE) done by the method of Ingebrigtsen et al. The gels were stained for tyrosinase activity by the method of Jayaraman and Ramanuja. Figure 2 shows two bands with tyrosinase activity, and both increased in densities, indicating increased tyrosinase activity during gill browning.

Tyrosinase activity of the gill homogenate during preservation was measured by the method of Flurkey and Jen, and browning of the gill homogenate was assessed spectrophotometrically. As shown in Fig. 3, tyrosinase activity increased preceding gill browning.

The above results suggest a correlation between tyrosinase
activity and gill browning during post-harvest preservation.

Tyrosinase was activated by treatment with trypsin or SDS, and carboxypeptidase in Agaricus bisporus, and aspartic protease in Aspergillus oryzae, indicating the possible presence of a latent-type tyrosinase (protyrosinase). However, in these experiments there was no increase in tyrosinase activity when the gill homogenate was treated with trypsin, pepsin, or SDS, according to the method of Burton. As shown in Fig. 4, tyrosinase activity of the gill homogenate was increased by incubation with 50 mM acetate buffer (pH 5.0), thus indicating a latent-type tyrosinase to be present in the gills. By separating the gill homogenate into a supernatant and precipitate, latent-type tyrosinase was shown to be present in the precipitate, and it could be changed to soluble active-type tyrosinase by incubating with an acidic buffer. This is very much in contrast to A. bisporus, the latent-type tyrosinase of which was soluble in the supernatant.

Changes in latent- and active-type tyrosinase content during gill browning are summarized in Fig. 5. Increase of latent-type tyrosinase on days 3 to 4 suggests de novo synthesis of latent-type tyrosinase. Total tyrosinase content (latent-type tyrosinase content + active-type tyrosinase content) of days 4 and 5 was virtually the same. This result suggests the de novo synthesis of the latent-type tyrosinase ceased on day 4, and activation of the latent-type tyrosinase to the active type was presumed to occur following de novo synthesis of the latent-type tyrosinase.

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References