Studies on Laccase and Biomass Production *In Vitro* and Culture by a Mexican Wild Strain of *Agaricus bisporus* (J.Lge) Imbach: a Comparison with Commercial Strains

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Until recently, many scientists believed that *Agaricus bisporus* was an extracted European mushroom. However, recent findings showed the opposite. In Mexico, several strains of *A. bisporus,* growing under *Cupressus benthami* trees, have been isolated recently. Commonly known as the white button mushroom, most of the 2.4 million tons of cultivated *A. bisporus* around the world are still genetically closely related to the hybrids cultivated commercially. The improvement of genetic diversity of *A. bisporus* is essential for scientists to enhance productivity and diversification of cultivated mushrooms to meet the world market requirements. Some studies showed that wild strains of *A. bisporus* had better yield than several commercial strains. Moreover, some wild strains seem to be more resistant to brown blotch disease, produced by *Pseudomonas tolaasii,* than some commercial strains. Therefore, improvement of yield and natural resistance through wild strains can be considered in mushroom breeding programs. The aim of this paper is to evaluate comparatively three *A. bisporus* strains (two commercial and one Mexican wild strain) on *in vitro* enzyme laccase and biomass production as well as commercial mushroom production on compost.

Four different culture liquid media were used for the laccase and biomass experiment: malt extract (ME: 10 g/L malt), yeast malt extract (YME: 20 g/L malt, 2 g/L yeast), compost malt extract (CME: 10 g malt/600 mL compost extract and 400 mL distillated water), and yeast malt extract added with water soluble lignin derivates (YME-WSLD: 20

g/L malt, 2 g/L yeast, 3.7 g/L of Indulin AT fitted to a 1 μmol concentration of phenols/L of water). Strains were grown on static culture in glass of 250 mL containers, containing 10 mL of sterilized media. Ten replicates by each medium and strain were made. The samples were incubated for 7 and 14 days in the dark at 24°C. Biomass production was determined by weight differences filtering mycelia through filter papers (90 mm medium size pore). After filtration, the liquid medium was recuperated and used to determine laccase production with ABTS [2,2′-azino-bis (3 ethylbenz-thiazoline-6-sulfonic acid)] as enzymatic substrate.

For mushroom production, spawn was prepared on wheat grains, which were cooked for 15 minutes, left in hot water for 10 minutes, and then drained. The grains were added with 0.5% (dry weight) of a 1:1 proportion of calcium carbonate and calcium sulfate. The mixture was autoclaved at 121°C for 90 minutes in polyethylene bags (250 g). Strains were cultivated following the general commercial methodology. Commercial compost was inoculated with spawn of each strain at 1%. Plastic trays (3.8 cm long, 32 cm wide, and 16 cm high) with 5 kilograms of compost were used. Ten replications were made by strain. Mushrooms were harvested while the veil was still intact and were weighed once the stalk was cut off as for selling on the commercial market. Produced mushrooms were grouped by means of cap size: G1 1–5 cm and G2 5.1–10.0 cm. Yield was evaluated as means of fresh mushroom weight.

There were significant differences in biomass and laccase production of the three strains. The wild strain produced significantly less laccase and biomass than the commercial strains. Significant differences were found also regarding the culture media. Strains cultured on ME showed a higher production of laccase and biomass, followed by the MCE. Relationships among laccase, biomass, and yield cannot be determined. On the other hand, full substrate colonization was observed between the 11th and the 19th day (wild strain growth was faster). Although casing was made at the same time for the three strains, pinning of the wild strain was observed the next day after the casing. On average, the wild strain produced 1694.3 g for a 5 Kg sample, whereas commercial strains produced 1056.2 and 760.9 g. All three strains produced more fruit bodies on G1 than on G2.