Poster Presentation 2

P2-37

Isolation of genes differentially expressed during the fruitbody development of *Pleurotus ostreatus*

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To analyze genes involved in fruit body development of Pleurotus ostreatus(FMC456), mRNAs from three different developmental stages: vegetative mycelium, primordium, and mature fruit body, were isolated and reverse-transcribed to cDNAs. One hundred and fifty random PCR amplifications were performed with the cDNAs

Materials and methods: Pleurotus ostreatus(FMC456) was cultivated in a sawdust-medium containing beech sawdustand rice bran 3:1 (v/v). During the cultivation of FMC456, samples from three stages of development, i.e., mycelium, primordium(3-7 mm in diameter), and mature fruit body.

First-strand cDNA synthesis was performed in a reaction mixture containing 50 mM Tris-HCl (pH 8.5), 40 mM KCl, 5 mM MgCl2, 2 mM DTT, 850 uM each dNTP, 95 units of RNAase Inhibitor, 0.2 mM random primer, and 40 units of Superscript II Reverse Transcriptase.

10-mer RAPD primers were used to PCR amplify the second-strand cDNA. PCR was carried out as 45 cycles of the following thermal cycle: 30s at 95°C, 1 min at 50°C, and 2 min at 72°C.

Results: To detect changes in transcripts during fruit body development of Pleurotus ostreatus(FMC456), mRNAs were isolated from three stages of development. Then, reverse-transcribed cDNAs were used as templates for the following PCR.

A total of 150 PCR amplifications were performed with 10-mer RAPD primers. Each PCR product separated by the agarose gel was resolved into 1 to 9 distinct DNA bands. A total of 482, 484, and 483 cDNA fragments were identified in the mycelium, primordium, and mature fruit body, respectively.

The electrophoresis patterns of the PCR-amplified cDNA were confirmed as reproducible in two or three independent experiments.

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