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The Thesis Submitted for the Degree of Ph.D of Plant Pathology

Identification of fungi associated with *Agaricus bisporus* and evaluation of antifungal activity of the best mycoendobiont isolates against some pathogens by protoplast fusion method

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October 2022

Abstract:
White button mushroom (*Agaricus bisporus*) is one of the most important types of edible mushrooms and one of the most economical food and medicine products. Due to low calorie and high content of carbohydrates, proteins, fiber, phenolic, unsaturated fatty acids, vitamins and minerals are significant sources of food. Infection of white button mushroom by *Mycogone perniciosa*, *Lecanicillium fungicula* and *Trichoderma harzianum* as its most important pathogenic fungi, reduces the quality and yield of the product which are not marketable and cause significant damage to breeders. Due to the detrimental effects of pesticides such as environmental pollution, concerns about human and animal health, and pathogen resistance to pesticides, emphasis is on biological control of plant diseases with antagonists and bio-controllers. To identify mycoendobiont fungi from button mushroom, samples were obtained from the cap, gills and stalk of healthy and infection mushroom, which collected from major mushroom growing farms in Iran. A total of 310 fungal isolates were obtained, 144 isolates were isolated from the cap, 64 isolates from the gills and 102 isolates from the stalk. In addition, 90 pathogenic isolates were isolated from infected cap tissues. Finally, 30 mycoendobiont taxa and 10 pathogenic taxa were identified based on morphological characteristics and rDNA-ITS region sequencing at the species level. Species of *Cephalotrichum purpureofuscum*, *Cladosporium allicinum*, *Clonostachys pseudochroleuca*, *Paecilomyces sinensis*, *Penicillium parvum*, *Peziza ostracoderma* and *Scedosporium apiospermum* are as new taxa for mycobiota of Iran. Primary screening of mycoendobiont isolates against button mushroom pathogens was performed based on the dual culture and mixed-medium methods and 12 isolates were chosen. Secondary screening of the selected isolates was performed with the assessment of antifungal activity of mycoendobiont isolates against button mushroom pathogens in greenhouse conditions. Eventually, two isolates of *Fusarium venenatum* KS-S32 and *Clonostachys rosea* KS-C22, which showed the highest inhibitory effect against pathogen were chosen for protoplast fusion. The best incubation time for the release of protoplasts from both parental isolates was determined to be 4 hours. The maximum protoplast yield was gained with a concentration of 6 mg per ml of glucanex cell wall degrading enzyme using 0.6 M KCL. The highest protoplast fusion rate was achieved with 40% Polyethylene glycol. Evaluation of antifungal activity of fusants and parent isolates on dual culture, mixed-medium methods and greenhouse conditions revealed that FC2 fusant significantly controlled pathogens. Isolation and identification of the generated metabolites affecting FC2 fusant and parental isolates were done based on the preparative TLC and HPLC. The highest activity was observed in FC2 fusant. The effective compound against *M. perniciosa*, *L. fungicula* and *T. harzianum*, 9-octadecanoic acid or oleic acid was identified. In total, protoplast fusion between *F. venenatum* KS-S32 and *C. rosea* KS-C22 causes an increase of antifungal activity, growth and yield in white button mushroom.
Keywords: Edible mushroom, mycoendobiont, symbiosis, biocontrol, spawn, protoplast, oleic acid.