Improvement of Agrobacterium-Mediated Transformation of Medicinal Mushrooms

Jang-Won Choi¹ · Hee-Sung Park²*

¹Department of Bioindustry, Daegu University, Gyungsan 712-714, Korea
²Department of Biotechnology, Catholic University of Daegu, Gyungsan 712-702, Korea

Received: OCT. 2, 2013, Revised: NOV. 28, 2013, Accepted: NOV. 29, 2013

ABSTRACT

Wounding to the mycelia of five mushroom species caused them to be susceptible to Agrobacterium-mediated transformation. The high transformation rate indicated that the wounds generated by mechanical means were a highly conclusive for agroinfiltration. Some transformants of Ganoderma lucidum were distinctive from the wild type in their morphology and antioxidative activity.

Key words - Agrobacterium-mediated transformation, insertional mutagenesis, mechanical wounding, medicinal mushrooms, mycelia

*Corresponding author: Hee-Sung Park
Tel: +82-53-850-3245
Fax: +82-53-850-3459
E-mail: hspark@cu.ac.kr
I. Introduction

Mushrooms have long been recognized as a powerful resource associated with health, vigor and long life (Wasser & Weis, 1999). Numerous bioactive compounds have already been identified but a plethora of compounds still await discovery. In order to exploit the biosynthetic potential of medicinal mushrooms, studies of functional genomics has the potential of being highly informative. However, DNA delivery into these mushrooms may be a challenge, and a practical transformation method should be outlined in advance (Meyer, 2008).

*Agrobacterium tumefaciens* has a natural capability to transfer its T-DNA into plant genomes. It has also been developed to transform yeast (Bundock et al., 1995) or fungi (de Groot et al., 1998), or even human cells (Kunik et al., 2001). Despite success in some fungi, transformation efficiency has always posed a challenge. At this point, no general rule can be applied to predict the effectiveness of the *Agrobacterium*-mediated transformation (AMT) technique and therefore, the need for developing an optimized AMT method independently for the fungus of interest persists.

To transfer the T-DNA fragment into the plant genome, the wounding of plant cells has been recognized as a critical prerequisite. In some instances, wounds deliberately generated in vitro by mechanical means can facilitate the T-DNA transfer regardless of the susceptibility or recalcitrance of the host plants (Singh & Chawla, 1999; Flores Solis et al., 2003; Kim et al., 2007). In this work, we assessed mechanical wounding, and herein describe its significance for achieving a high frequency of mushroom transformation mediated by *Agrobacterium*.

II. Materials and Methods

2.1 Mycelia growth

*Ganoderma lucidum* (KACC42231), *Lentinula edodes* (KACC42378), *Grifola frondosa* (KACC50027), *Schizophyllum commune* (KACC43373), and *Lyophyllum decastes* (KACC41766) were obtained from the Korean Agricultural Culture Collection. Mycelia growth was maintained in potato dextrose agar (PDA) or broth (PDB) medium at 25°C.

2.2 Mycelia Transformation

To transform the mycelia, pCambia1300 (CAMBIA, Australia) carrying genes coding for hygromycin phosphotransferase (*hph*) under the CaMV35S promoter and neomycin phosphotransferase was introduced into *A. tumefaciens* LBA4404. *Agrobacterium* cells were grown (18 h, 28°C, 180 rpm) in Luria-Bertani (LB) broth containing antibiotics (50 mg/mL of kanamycin and 50 mg/mL of streptomycin), collected by centrifugation (2 min, 10,000 rpm) and then resuspended in MS medium at pH 5.7 (Murashige and Skoog, 1962) to a final density of OD₆₀₀=0.5. Fungal discs (5 mm in diameter) prepared from the PDA culture using a steel loop were mixed with an equal volume of bacterial suspension. Agroinfiltration was performed for 5 min under vacuum followed by co-cultivation (22°C, 2 day). Subsequently, fungal discs were transferred to the selective PDA medium containing hygromycin and 250 mg/mL of cefotaxime and incubated (10-14 day, 25°C). In a wounding experiment, the fungal discs mixed with an equal volume of aluminum oxide particles (60 µm in average size) in a 50 mL tube were vigorously shaken up and down for 2-3 min before agroinfiltration and subsequent co-cultivation (Kim et al., 2007).