

Thesis Title STUDIES ON FORMATION AND REGENERATION OF
 Agaricus bitorquis PROTOPLAST
Name Upatham Pengpara
Degree Master of Science (Environmental biology)
Thesis Supervisory Committee
 Preecha Klingasorn, Ph.D.
 Sanchai Tantyaporn, Ph.D.
Date of Graduation 21 March B.E.2533 (1990)

ABSTRACT

The protoplast fusion technique is one of the several ways of mushroom strain improvement. This research deals with the formation and regeneration of A. bitorquis protoplasts. Some factors affecting the liberation of Agaricus bitorquis (KB 629) mycelium were investigated to maximize the protoplast yield. The condition of protoplast formation are important in determining the regenerative potential. The 0.6 M mannitol pH 6.8 (Man 6.8) was the best osmotic stabilizer for the formation of them compared with 0.2 M sodium phosphate buffer pH 6.8 (SPB 6.8) and SPB 6.8 containing 0.2, 0.6, 1.0 M NaCl and KCl. The 10-20 days old cultured mycelium in Malt extract liquid medium (MELM) on shaker gave the highest yield of protoplasts. The combination of 1.5% w/v NovozymTM 234 and 0.5% w/v β -Glucuronidase at 2-6 hrs

incubations time was the suitable condition for the protoplast formation in comparison to various concentration of single or combination of enzyme such as CELLULASE, HEMICELLULASE, CHITINASE, NOVOZYMTM 234 and β -GLUCURONIDASE . Regeneration of protoplast was shown to be influenced by the status of media, only in liquid regeneration medium (LRM) at 35° C and in malt extract liquid medium (MELM) later.