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Investigations of Genotypes and Substrates for the Fruitbody Production of *Grifola frondosa* (Dicks.:Fr.)

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ABSTRACT: Three substrate formulations containing beech sawdust supplemented with either 10% wheat bran, 20% corn meal, or 10% wheat bran and 5% corn meal were tested for their ability to produce *Grifola frondosa*. The medium (2 kg wet weight) containing 20% corn meal produced an average of 310 g mushrooms per block for one stain (Gf 1). In submerged culture growth tests, a medium containing 6 g biomalt/liter water produced the most mycelial growth.

1 INTRODUCTION

Grifola frondosa is also known as klapperschwamm, maitake or hen of the woods. Maitake grows in Europe on stumps of broadleaf trees and is edible when young. Japanese researchers found that an extract of maitake is a more powerful anticancer agent than other medicinal mushrooms (Nanba 1993). In comparison with *Ganoderma* extract and lentinan, *Grifola frondosa* extract shows a high rate of inhibition against sarcoma 180 tumors in mice (King 1993). Also, polysaccharides from maitake mycelium which was cultivated in liquid medium showed high antitumor activity (Mizuno *et al.* 1995). Jong *et al.* (1990) and Weil (1993) mention antivirus, anticancer and antibiotic substances in the fruitbodies or mycelium of *Grifola frondosa*. The Japan National Institute of Health also confirmed the anti-virus activity of *Grifola frondosa* (Nanba 1995). In the USA mushroom growers use oak sawdust with some supplements for the production of fruitbodies on sterile sawdust blocks (Lee 1994).

2 MATERIALS AND METHODS

2.1 *Substrates*

Three different substrate formulations containing beechwood sawdust and either 10% wheat bran (WB), 20% corn meal (CM) or 10% wheat bran with 5% corn meal (WB+CM) were investigated for mycelium growth and fruitbody production of *Grifola frondosa*. Water was added to 63% substrate moisture content. The sterilisation process of the 2000g substrate blocks was 120°C for 2 hours. After cooling the blocks were inoculated with 2% grain spawn which was produced 2 weeks earlier. Mycelial growth time was 77 days at 25°C. The cultivation environment then was changed to 16°C, 90% RH and less than 800ppm CO_2 in the harvest room. Substrate and strain tests were completed with ten replications.

2.2 *Strains*

Two strains of *Grifola frondosa* we obtained from Japan (Gf2) and the USA (Gf1) were tested for their potential to produce fruitbodies on 2kg sterile substrate blocks. The sawdust was enriched with 20% corn meal and adjusted to 63% water content. The filled bags were autoclaved at 120°C for 2 hours. After the blocks cooled down to 28°C they were inoculated with 2% grain spawn. The mycelium growing time was 84 days at 25°C, 80% rel. humidity and less than 2000ppm CO_2. The cultivation conditions in the harvest room were then changed to 16°C, 95% RH and less than 800ppm CO_2.

2.3 *Submerged cultures*

The *Grifola frondosa* mycelial biomass production was accomplished with six replications in different nutrient solutions at 25°C and 40rpm in 150ml Erlenmeyer flasks (75ml solution) as shaking cultures. The solutions were 20g molasses (M), 6g biomalt (B) and 20g malt extract with 5g pepton (MP) per liter water. The nutrient solutions were sterilised by autoclaving the flasks for two hours at 120°C. After cooling they were inoculated with three 0.3g mycelium agar pieces. The mycelial growth time was 28 days.

3 RESULTS

3.1 *Substrates*

All substrates showed good mycelial growth but only on the sawdust with 20% corn meal (CM) could we obtain fruitbody development with an average yield of 310g/block. On sawdust blocks of WB and WB+CM we observed primordia which failed to develop into mature fruitbodies.

3.2 *Strains*

Both strains showed good mycelial growth. Six weeks after inoculation we obtained a well formed white mycelial coating around the blocks. Two weeks later, the mycelial coat produced reddish water drops. This was more pronounced with Gf1. After one week in the harvest room, fruitbody development began. With the use of strain Gf2 we obtained under this cultivation condition, a lot of deformed fruitbodies. Furthermore, the yield of Gf1 was much better than Gf2 (Fig. 1).

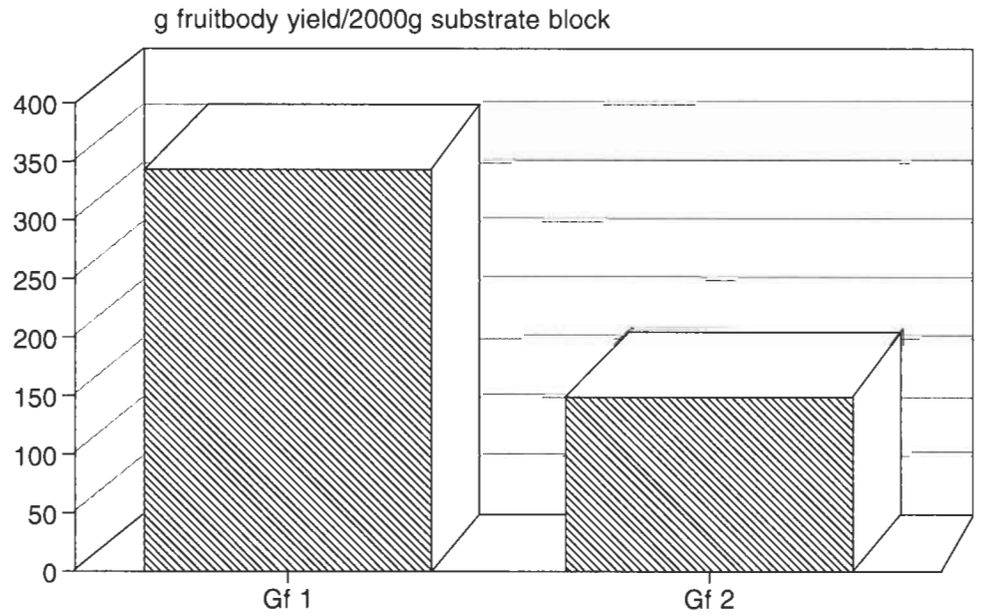

Fig. 1. Fruitbody yield of Gf1 and Gf2 *Grifola frondosa* strains produced on a medium of beech sawdust (80%) and corn meal (20%).

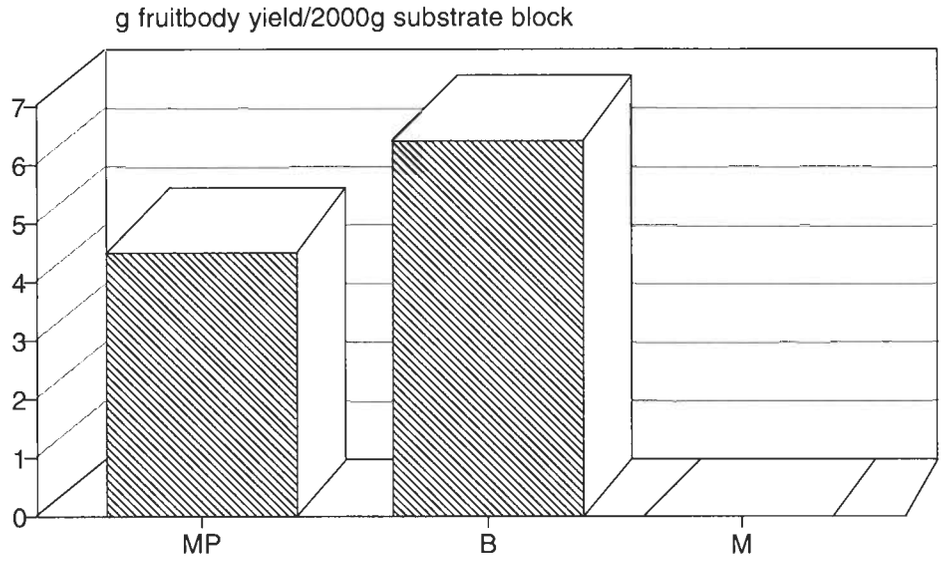

Fig. 2. Mycelium biomass production of *Grifola frondosa* in different nutrient solutions.

3.3 *Submerged cultures*

The mycelium of *Grifola* does not grow in the solution with molasses (Fig. 2). Remarkable was the strong mycelium growth on the surface of the nutrient solution B and MP. The highest mycelium production took place in a nutrient solution with 6g biomalt/l H_2O.

4 DISCUSSION

Beech wood sawdust supplemented with 20% corn meal served as a good substrate for the production of *Grifola frondosa* fruitbodies. The use of either a mixture of 10% wheat bran with beech wood sawdust or a mixture with 10% wheat bran and 5% corn meal does not produce good yield. Perhaps a lack of nitrogen supplementation is the reason for this reaction or the substrate decay is not well enough under the described conditions. Primordia death can also be caused by unfavourable climate conditions.

The reason for deformed fruitbodies with strain Gf2 could be due to a poor degradation of the substrate caused by an insufficient production of wood decay enzymes. Furthermore, it is possible that the strain Gf2 has genetically degenerated or the cultivation conditions are not well adapted to this strain.

The sugar beet molasses used in this test was more than one year old. It could be possible that after this time a nutrient fixing took place. A biomalt nutrient solution is a suitable medium to produce mycelium biomass of *Grifola frondosa* and it also is a good medium to produce liquid spawn in a fermenter. Liqiud spawn could have an advantage for the fruitbody production on sterile substrate blocks (Kirchhoff 1991, Kawai *et al.* 1995).

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