Biological Control of Olive Green Mold in *Agaricus bisporus* Cultivation

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Successful methods to control the damaging weed mold *Chaetomium olivaceum* (olive green mold) in mushroom beds are not presently known. An attempt was made to control *C. olivaceum* by biological means. A thermophilic *Bacillus* sp. which showed dramatic activity against *C. olivaceum* on Trypticase soy agar (BBL Microbiology Systems)—0.4% yeast extract agar plates was isolated from commercial mushroom compost (phase I). When inoculated into conventional and hydroponic mushroom beds, the bacillus not only provided a significant degree of protection from *C. olivaceum*, but also increased yields of *Agaricus bisporus*.

The commercial cultivation of the white mushroom, *Agaricus bisporus* Lange (*Agaricus brunnescens*), continues to rank third overall in economic importance as a vegetable crop in Canada (1). Although traditionally accepted as a horticultural craft, mushroom production is in essence an industrial fermentation process (10, 13). Presently, mushroom culture represents the only major process in biotechnology which successfully converts cellulosics into useful foods and by-products. The methods used in this solid-state fermentation have not changed significantly over the years. Because of the current nature of the growing conditions (i.e. use of animal manures, plant materials, chemical fertilizers, and other agricultural residues as a substrate), the cultivation of the mushroom is susceptible to many competitive organisms and weed fungi (6, 7), causing substantial loss of dollars to the grower due to decreased yields (15). One such organism is *Chaetomium olivaceum*, more commonly known as olive green mold. Olive green mold is a weed mold which frequently occurs after pasteurization (phase II) of the compost and severely inhibits mushroom mycelial development by competing for nutrients (3, 8). Consequently, spawn growth is retarded or fails altogether. The growth of *C. olivaceum* in mushroom beds is very rapid and has been known to spread onto sterilized soil and throughout wooden shelving (2). One reason for the invasion of *C. olivaceum* into mushroom beds is the presence of free ammonia in the compost after the phase II pasteurization (4, 17). The presence of free ammonia in compost, although toxic to the mushroom mycelia, results in production of compounds which readily support growth of olive green mold (19). As yet, there are no known methods to successfully control this pest (22).

From earlier research (11, 16, 20, 21) it was suggested that a certain degree of protection from invasion of the mushroom compost by disease-causing organisms may be obtained by prior fermentation of the compost with selected microorganisms. Huhnke (11) states that, by inoculating specific thermophilic microorganisms into sterilized compost (i.e. Till substrate), the cultures are capable of causing a selective protection of the substrate against diseases and pests. Hence, the objectives of this investigation were to select thermophilic microorganisms which would not only support the mushroom, but also protect it from damage by *C. olivaceum*. Furthermore, it was hoped that this thermophilic organism could be used in the preparation of a hydroponic system utilizing synthetic substrates (thereby eliminating the potential hazards of compost) as a growth medium for *A. bisporus*. If successful, the results of this research should facilitate the emergence of the mushroom industry into a level of technology experienced in modern industrial fermentations.

MATERIALS AND METHODS

Selection of thermophiles for activity against *C. olivaceum*. Representative (45-g) samples of commercial compost which had been passed through the initial standard mushroom composting stage (phase I) before delivery to the grower for pasteurization (phase II) were received on a weekly basis from the Fraser Valley Mushroom Growers Association. Upon receipt, these samples were immediately incubated for 2 days at 55°C under aerobic conditions and high humidity (Fisher Isotemp incubator). This incubation provided a controlled duplication of the final thermophilic
treatment given by the mushroom grower before inoculation which mushroom spawn.

For bacteriological analysis, 10 g of the thermophilic compost was mixed with 90 ml of sterile distilled water, and the sample was kneaded thoroughly to place sufficient microflora into suspension. Thereafter, 10-fold dilutions, using sterile distilled water, were prepared to a 10⁻⁵ dilution. Samples of 0.1 ml from the 10⁻² to 10⁻⁵ dilutions were spread plated in duplicate on TSA agar (Trypticase soy agar [BBL Microbiology Systems] + 0.4% yeast extract [Difco Laboratories]) and incubated at 55°C for 16 to 20 h. After incubation and the appearance of colonies, all plates were sprayed with a spore suspension of *C. olivaceum*. The treated plates were then incubated at 25°C and examined daily for the presence of zones of fungal inhibition.

Use of *Bacillus AOG* (see below) in conventional mushroom production. A 500-g portion of standard phase II mushroom compost was spawned with 9.0 g of *A. bisporus* spawn grains (obtained from Fraser Valley Mushroom Growers Association) and placed in plastic growing trays (8 by 15 by 23 cm). This spawned mushroom compost was then subjected to various experimental treatments: (i) control—no further addition to the compost bed; (ii) 100 ml of a 10⁷/ml thermophilic *Bacillus AOG* culture grown in TSY broth for 96 h at 55°C (New Brunswick Psychotherm shaker) was added; (iii) 100 ml of a 10⁷/ml thermophilic *Bacillus AOG* culture grown in TSY broth for 96 h at 55°C was added, followed by spraying 2 ml of a *C. olivaceum* spore suspension on top of the bed; (iv) 2 ml of a *C. olivaceum* spore suspension was sprayed on top of the bed only. All experiments were conducted in triplicate (except iv, which was done in duplicate).

The growing trays were then incubated at 25°C for 12 days to promote spawn growth. During this incubation, the mycelial diameters of the spawn in each tray were measured on a daily basis (for 7 days) as an indication of the mycelial development under the various treatments (5). After 12 days, each of the trays was cased with 2.5 cm of a sterile peat-sand-ground limestone (dolomite) mixture in a ratio of 5:5:1 and placed in a 16°C incubator to promote pinhead formation. Air was pumped in at 550 ml/min to keep CO₂ levels at a minimum. Relative humidity was maintained at 80%. As the mushroom fruiting bodies appeared (harvested just before opening of gills), yields from each tray were determined over a 1-month period.

Use of *Bacillus AOG* in hydroponic mushroom production. A 2% (wt/vol) malt extract (Difco) solution containing 2% CaCO₃ and 0.4% yeast extract (pH 7.0) or a liquid compost solution was chosen as the liquid nutrient for mushroom hydroponic culture. The liquid compost was prepared by adding 5 liters of distilled water to 1 kg (wet weight) of mushroom compost. The mixture was agitated vigorously, and extract was collected by filtration through cheesecloth. The extract was then sterilized at 15 lb/in² for 15 min and was called liquid compost (final pH, 7.2). The *Bacillus AOG* culture was inoculated into this liquid compost and into the 2% malt extract. These inoculated liquid nutrients were then incubated at 55°C for 96 h to yield a final concentration of 10⁶ cells per ml. The experimental treatments were similar to the solid compost plan previously described. A 500-ml amount of a 10⁴/ml *Bacillus AOG* culture in either liquid compost

![FIG. 1. Inhibition of olive green mold by the isolated Bacillus sp.](http://aem.asm.org/)

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or 2% malt extract was added to 180 g of sterile vermiculite (inert carrier material, no less than 2.0 mm in size) within a plastic tray. A sterile glass-wool plug approximately 20 cm long was placed on the bottom of the tray to ensure aerobic conditions within the bed. After the spawning, representative trays with or without the bacillus were sprayed with 2 ml of a C. olivaceum spore suspension and incubated at 25°C for 12 days. All trays were then cased with 2.5 cm of a sterile peat-sand-ground limestone mixture and maintained at 16°C for fruiting body formation. Yields of mushrooms were recorded during a 30-day cropping period. All hydroponic experiments were done in triplicate.

RESULTS AND DISCUSSION

After approximately 4 months of phase I compost examinations, a thermophilic Bacillus sp. was isolated which showed dramatic activity against C. olivaceum on TSY agar plates (Fig. 1). The Bacillus sp. (Bacillus AOG ["anti-olive green"]) isolated was a facultatively anaerobic, sporeforming, gram-positive rod, motile, catalase positive, Voges-Proskauer positive, and capable of growth in 10% NaCl and at pH 5.7. It resembles the thermophile Bacillus coagulans in that it was resistant to 0.02% sodium azide when grown at 55°C and was acidophilic (9). The isolation procedure indicated that Bacillus AOG was capable of producing a potent antibiotic against C. olivaceum (as shown on TSY agar). The chemical identity of this bacillus-produced inhibition is now under study.

To determine whether this thermophile would support growth of the mushroom, as well as

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conventional Yield (g, fresh wt)</th>
<th>Hydroponic Yield (g, fresh wt)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>172.1</td>
<td>9.0</td>
</tr>
<tr>
<td>Bacillus sp. added</td>
<td>171.0</td>
<td>10.5</td>
</tr>
<tr>
<td>C. olivaceum only</td>
<td>73.8(^b)</td>
<td>0.0</td>
</tr>
<tr>
<td>Bacillus sp. + C. olivaceum</td>
<td>138.0</td>
<td>16.5</td>
</tr>
</tbody>
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\(^a\) Average of three trays.
\(^b\) Average of two trays.
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FIG. 4. Poor spawn growth in hydroponic trays with only olive green mold present (a) and improved mycelial development due to the protection of the bacillus against olive green mold (b).

To protect it from damage by olive green mold, *Bacillus* AOG was inoculated into two kinds of culture medium. One medium was conventional, consisting of standard phase II mushroom compost, and the other was hydroponic, consisting of liquid substrates absorbed onto an inert physical support, vermiculite.

Figure 2 represents the rate of mushroom mycelial development in standard compost over a 7-day period. When the bacillus was added to the compost, the rate of mycelial development in the trays was enhanced. In addition, the bacillus exhibited a definite inhibitory effect on the development of olive green mold (F test on slopes, $P < 0.01$). This biological protection was further indicated (Table 1) by a significant 86.5% increase in mushroom yield of the trays containing bacillus and olive green mold over that of the trays containing olive green mold only (analysis of variance, $P < 0.05$). Furthermore, trays with only *C. olivaceum* produced fruiting bodies a full week later than those with *Bacillus* AOG and *C. olivaceum* together.

When the bacillus was added to hydroponic trays in 2% malt extract (Fig. 3), a remarkable improvement in the rate of mycelial development occurred ($P < 0.01$). The improved rate of mushroom mycelial growth is shown more vividly in photographs of the hydroponic trays (Fig. 4). Figure 4a represents a tray with only *C. olivaceum* added; relatively poor spawn growth is evident. Figure 4b represents the effect of the *Bacillus* AOG and olive green mold present at the same time. As these results show, the mushroom mycelia flourished in the presence of the bacillus.

![Fig. 5. Rate of mycelial development in liquid compost. Symbols: ■, control (sterile); ○, control (nonsterile); ●, Bacillus sp. added; ▲, C. olivaceum added; ♦, C. olivaceum and Bacillus sp. added.](http://aem.asm.org/)
Mushroom yields from the 2% malt extract experiments (Table 1) containing olive green mold only showed complete failure of any A. bisporus fruiting body formation. However, the crop yields from trays containing the bacillus produced maximum yields, even in the presence of the chaetomium mold. Analysis of variance indicated that treatments were significant in 2% malt extract (P < 0.05).

The results for hydroponic culture with liquid compost demonstrated significant biological control both during the mycelial growth phase (P < 0.01; Fig. 5) and during fruiting of the mushroom, as the yields demonstrate (Table 1) (analysis of variance, P < 0.05). Trays with only the competitor C. olivaceum added did not produce mushrooms. However, similar to the 2% malt experiments, the maximum yield occurred for trays containing the Bacillus AOG and C. olivaceum together. These experiments clearly show the benefits resulting from selective protection (biological control) through controlled fermentation of the nutrient substrate.

The successful use of hydroponics coupled with pure cultures of microorganisms (as demonstrated in these experiments) are important factors in the microbiological development of this fermentation process. Compost manufacture and materials are evaluated to cost 20 to 25% of the total mushroom production expenditures (18). Furthermore, on many larger intensive units, the disposal of spent compost frequently poses serious health hazards (e.g. mushroom workers’ lung [12]). Thermophilic bacterial liquid feeding of a supporting medium in mushroom cultivation offers a new approach to this fermentation, possibly leading to eventual continuous-culture techniques.

The isolation of a thermophilic microorganism antagonistic towards olive green mold is a unique finding. The application of this organism may eventually form an effective biological control method in the commercial cultivation of mushrooms. Furthermore, the use of this organism or its metabolites could potentially be extended for use in the preparation of differential media or in the protection of foods and plants from fungal invasion.

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LITERATURE CITED


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