Effect of 10-oxo-trans-8-decenoic acid on growth of several mushroom mycelia

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ABSTRACT

10-Oxo-trans-8-decenoic acid (ODA) was supplemented to potato dextrose yeast agar or broth at 0, 2.5, 5 and 10 ppm. With ODA, the relative linear growth rates increased in mycelia of Yun Chih (Coriolus versicolor (Fr.) Quel.), winter mushrooms (Flammulina velutipes (Curtis: Fries) Sing.) and shiitake (Lentinula edodes (Berk.) Pegler) Tainung 1. When ODA was spread on top of the agar, no effects on mycelial growth were observed in Brazilian mushrooms (Agaricus blazei Murrill), winter mushrooms and shiitake. In shaken flasks, as evidenced by the biomass, ODA affected the mycelial growth of Yun Chih and winter mushrooms especially in the log phase. The residual sugar content reduced significantly faster for ODA added Yun Chih and winter mushrooms. The laccase activities peaked at day 10 for all treatments of Yun Chih and winter mushrooms. ODA affected slightly the pH values in shaken flasks. Summarily, ODA was found to be stimulatory to the mycelial growth of Yun Chih and winter mushrooms on agar plates and shaken flasks, and to shiitake Tainung 1 in agar plates.

Key words: Biomass, Coriolus versicolor, Flammulina velutipes, Lentinula edodes, laccase.

Introduction

10-Oxo-trans-8-decenoic acid (ODA) produced in Agaricus mushrooms was first reported as one of enzymatic breakdown products of linoleic acid (Tressl et al., 1982). Later this compound was isolated as its corresponding methyl ester to study the biosynthetic formation of 1-octen-3-ol in common mushrooms (Wurzenberger and Grosch, 1982). An enzymatic pathway was then established for the breakdown of linoleic acid to form 1-octen-3-ol and ODA via lipoxygenase and hydroperoxide lyase (Grosch and Wurzenberger, 1984). Recently, ODA was proved to stimulate mycelial growth in Agaricus bisporus (Mau et al., 1992), Auricularia spp. (Mau et al., 1999a), Agrocybe cylindracea (Mau and Li, 1999) and Pleurotus eryngii (Mau and Ma, 2000). In addition, 1-octen-3-ol has been found in other fungi, such as Aspergillus and Penicillium spp. (Kaminski et al., 1974; Borjesson et al., 1989), thus by inference it is apparent that ODA is also concurrently produced. In fact, ODA could not only promote the growth of both Aspergillus niger and A. oryzae mycelia, but also increase activities of major enzymes including protease, cellulase, α-amylase and β-amylase (Li, 1997).

Agrocybe cylindracea strain B produced the

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highest amount of 1-octen-3-ol (337 µg/g fresh wt) than any other mushrooms (Mau et al., 1999b). Therefore, ODA could be better prepared using the enzyme system in *A. cylindracea* strain B for the formation of 1-octen-3-ol. Like its effect on several mushrooms mentioned above, ODA might be expected to have similar physiological effects on other mushrooms. Accordingly, our objective was to elucidate the physiological effects of ODA on mushrooms by examining its influences on the mycelial growth on agar plates and in shaken flasks. The mushrooms studied herein included Brazilian mushroom (*Agaricus blazei* Murrill), Yun Chih or turkey tail (*Coriolus versicolor* (Fr.) Quel.), winter mushrooms (*Flammulina velutipes* (Curtis: Fries) Sing.), shiitake (*Lentinula edodes* (Berk.) Pegler) and paddy straw mushrooms (*Volvariella volvacea* (Bulliard: Fries) Sing.).

**Materials and Methods**

**Mushroom mycelia**

Mushroom mycelia studied were obtained from the following: *A. blazei*, the Biotechnology Center of Grape King Inc., Chungli, Taiwan; *L. edodes* (strains 271 and Tainung 1) and *C. versicolor*, Tien-Shan Mushroom Farm, Hsinshe, Taichung County, Taiwan; *F. velutipes* white strain, Lung-Kuo Mushroom Farm, Shihkang, Taichung County; *V. volvacea*, Hisng-Te Co., Tsaotun, Taichung County. Mycelia were inoculated on to potato dextrose yeast (PDY) agar (Difco) containing 0.5% yeast extract, and incubated at 25°C. After pure culture was obtained, the mycelium was re-inoculated into PDY agar and broth, respectively, and maintained at 25°C before use.

**Medium preparation**

The mushroom culture was grown on PDY agar or in the growth broth, which was prepared according to Schindler (1989). The growth broth was dispensed into 250-mL flasks prior to autoclaving, 95 ml per flasks, while 5 ml of autoclaved ODA solutions were aseptically added into flasks before inoculation. ODA solution was prepared as described earlier (Mau et al., 1993), except for using fruiting bodies of *A. cylindracea* strain B, which were obtained from Lung-Kuo Mushroom Farm. In a laminar flow cabinet, autoclaved ODA solution were aseptically added into the autoclaved PDY agar medium to meet the desired final ODA concentrations, and the medium was dispensed into petri dishes, ca. 20 mL per plate. The levels of ODA tested included 0, 2.5, 5, 10 ppm for both PDY agar and the growth broth. In addition, autoclaved ODA solutions were aseptically spread to the top of solidified PDY agars, 100 and 200 µg per plate, respectively.

**Culture incubation**

These agar plates with different concentrations of ODA were inoculated with 7.5-mm-punched mycelial plugs from 20-day old plates and incubated at 25°C for 10 days for linear growth study. In addition, the 20-day old culture in PDY broth was blended for 15 sec and added into the growth broth with different concentrations of ODA at the rate of 5% (v/v). These inoculated flasks were incubated at 25°C in a shaking incubator at 120 rpm for 14 days. Three shaken flasks of each ODA concentration were taken randomly at 2-day intervals for the determination of dry biomass, residual sugar, pH value and laccase activity.

**Determination of growth, dry biomass, pH, reducing sugar and laccase activity**

The mycelial diameter of each plate was measured daily using calipers to calculate the linear growth of the culture. The mycelial biomass were determined at 2-day intervals by vacuum filtering liquid culture in the growth broth through weighed filter paper, washing with reverse osmosis water
Effect of 10-oxo-trans-8-decenoic acid

several times, and drying to constant weight at 60°C. The residual sugar amount was determined using dinitrosalicylic acid method (James, 1995). The pH value of the growth broth was measured using a Hanna 8521 pH meter.

The activity of laccase was assayed as described in Leonowicz and Grzywnowicz (1981). The reaction mixture consisted of 1.0 mL of 0.04 M MES (2-[N-morpholino] ethanesulfonic acid, Sigma)-sodium hydroxide buffer, pH 5.3, 200 µL of 0.05 mM syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine, Sigma) in 95% ethanol, and 100 µL of culture broth from the shaken flasks, making up a final volume of 1.3 mL. The control mixture contained no culture broth. Changes in absorbance were monitored for 5 min at 525 nm. One unit (U) of the laccase activity was defined as a change in one absorbance unit (0.001) per min at 25°C, which was calculated from the initial linear portion of the curve obtained.

Statistical analysis

The linear growth rate for each ODA concentration was examined using six replicates (plates). The dry biomass, residual sugar and laccase activity and pH value were measured in triplicate (3 flasks) for each ODA concentration at 2-day intervals. The experimental data were subjected to an analysis of variance for a completely random design (Steel et al., 1997), to determine Fisher's least significant difference among means at the level of 0.05.

Results and Discussion

On PDY agar plates, without the addition of ODA, mycelia of Yun Chih and paddy straw mushrooms grew faster than other mushroom mycelia (Table 1). With the addition of 2.5–10 ppm ODA to the agar (treatments B, C, and D), the relative linear growth rates significantly increased in mycelia of Yun Chih, winter mushrooms and shiitake Tainung 1. No effects on mycelial growth were observed in shiitake 271 and paddy straw mushrooms. However, the effect of ODA on Brazilian mushrooms was not consistent with its concentration tested. When ODA was spread on top of the agar, no effects on mycelial growth were observed in Brazilian, winter mushrooms and shiitake. When 200 µg ODA was spread on top of the plate, the mycelial growths of Yun Chih, shiitake Tainung 1 and winter mushrooms were significantly affected. However, among the ODA affected mushroom mycelia, ODA added into the agar was more effective than ODA spread on top of the agar. Similar result was observed in Mau et al. (1999a) and Mau and Ma (2000). In contrast, Mau and Li (1999) found that ODA spread on top of the agar was more effective.

Without the addition of ODA, the mycelial growth of Yun Chih was relatively higher as compared to other mushrooms excluding paddy straw mushrooms in Table 1. With the addition of ODA, the relative linear growth rates of Yun Chih were evidently higher (38.5–47.2%) than that of the control. However, although the increase in growth rates correlated with ODA concentration, treatments B, C and D were statistically similar. The effect of ODA on mycelial growth of winter mushrooms was significant and dose-dependent. At 10 ppm, 25.1% increase in the mycelial growth rate were observed in treatment D of winter mushrooms. Between two strains of shiitake, strain 271 grew faster in PDY agar than strain Tainung 1. However, the slightly faster grown strain 271 did not respond to ODA stimulation as did strain Tainung 1. The finding of less or no response to ODA in faster grown mushrooms was in general agreement with that found in Mau and Li (1999) and Mau et al. (1999a). Due to the fact that paddy straw mushrooms grew faster and did not respond
Table 1. Effect of 10-oxo-trans-8-decenoic acid on growth of mushrooms on potato dextrose yeast agar at 25°C.

<table>
<thead>
<tr>
<th>ODA Concentration*</th>
<th>Agaricus blazei</th>
<th>Coriolus versicolor</th>
<th>Flammulina velutipes white strain</th>
<th>Lentinula edodes 271</th>
<th>Lentinula edodes Tainung 1</th>
<th>Volvariella volvacea</th>
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<tbody>
<tr>
<td>A</td>
<td>3.1±0.2</td>
<td>8.9±0.6</td>
<td>5.9±0.4</td>
<td>4.5±0.4</td>
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<td>14.3±0.1</td>
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<tr>
<td>B</td>
<td>3.8±0.5</td>
<td>12.4±0.7</td>
<td>7.1±0.6</td>
<td>4.6±0.2</td>
<td>3.8±0.3</td>
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</tr>
<tr>
<td>C</td>
<td>3.4±0.5</td>
<td>12.7±0.8</td>
<td>7.3±0.6</td>
<td>4.4±0.3</td>
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</tr>
<tr>
<td>D</td>
<td>3.8±0.7</td>
<td>13.1±0.4</td>
<td>7.4±0.7</td>
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</tr>
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<td>E</td>
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<td>9.5±0.5</td>
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</tr>
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<td>F</td>
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Relative linear growth rate

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<tr>
<th>ODA Concentration*</th>
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<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<td></td>
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<td>1.00 c</td>
<td>1.00 c</td>
<td>1.00 a</td>
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<tr>
<td></td>
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<td>1.25 a</td>
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</tr>
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<td>1.17 b</td>
<td>0.95 a</td>
<td>1.04 d</td>
<td>0.96 b</td>
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<td>1.07 c</td>
<td>1.21 a</td>
<td>1.05 a</td>
<td>1.19 c</td>
<td>0.96 b</td>
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<td>1.07 b</td>
<td>1.15 b</td>
<td></td>
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</tbody>
</table>

* A: 0 ppm; B: 2.5 ppm (50 μg ODA/plate); C: 5.0 ppm (100 μg ODA/plate); D: 10 ppm (200 μg ODA/plate) in PDY agar; E: 100 μg ODA; F: 200 μg ODA spread on the surface of agar.

** Each value is expressed as mean ±SE (n = 6). Means not sharing the same letter within a column are significantly different (p < 0.05).
Effect of 10-oxo-trans-8-decenoic acid to ODA stimulation, and Brazilian mushrooms inconsistently grew as affected by ODA, these two mushrooms were not used to study ODA effect in shaken flasks.

Both strains of shiitake grew slowly in PDY broth regardless of ODA addition, of which strain 271 grew slightly faster than strain Tainung 1 (Table 2). This result was consistent with the finding in Table 1. At the end of incubation for 14 days, no stimulatory effect of ODA was observed for both strains. Like its growth on agar plates shown in Table 1, the mycelial growth of Yun Chih in shaken flasks was consistently fast. After inoculation for 2 days, the effect of ODA on mycelial growth in Yun Chih was observed as evidenced by its biomass and continued to be much more distinctly throughout the entire incubation time. At day 10, the mycelial biomasses of Yun Chih were 16.8 (100%), 19.2 (114%), 19.9 (118%) and 20.3 g/L (121%) for 0, 2.5, 5 and 10 ppm ODA. At day 4, the effect of ODA on the mycelial growth of winter mushrooms was observed. At day 12, the mycelial biomasses of winter mushrooms were 13.5 (100%), 15.0 (111%), 15.4 (114%) and 15.4 g/L (114%) for 0, 2.5, 5 and 10 ppm ODA. As evidenced by the biomass, ODA affected the mycelial growth of Yun Chih and winter mushrooms especially in the log phase. This is in general agreement with the findings of Mau et al. (1992, 1999a), Mau and Li (1999) and Mau and Ma (2000).

It was observed that at the end of the incubation (14 days), the biomass was slightly lower than the peaked value for Yun Chih and winter mushrooms with ODA added. However, the mycelia of the control of winter mushrooms and all treatments of shiitake continued to grow to day 14. This reduction in biomass might be due to the fact that autolysis occurred in the center of mycelial pellets as nutrients especially sugars were exhausted (Solomons, 1975). The reduction in biomass due to autolysis of mycelia in liquid cultures was also found in Agaricus campestris (Humpheld and Sugihara, 1952), A. bisporus (Hwang, 1996), A. cylindracea (Mau and Li, 1999), Auricularia spp. (Mau et al., 1999a), and P. eryngii (Mau and Ma, 2000). Camici and Sermonti (1952) explained that the autolysis was the formation of vacuoles, which would become larger and larger until the appearance of protoplasm, leaving only empty cell walls. However, the reduction in biomass was not observed in shiitake because the mycelia continued to grow, the maximal biomass was not reached, and the nutrients were still available.

Yun Chih and winter mushrooms not only differed in the rate of mycelial growth in shaken flasks but also in efficacy in biomass conversion from sugars. For the maximal biomass conversion without ODA stimulation, the biomass of Yun Chih at day 12 and that of winter mushrooms at day 14 were 18.7 and 13.9 g/L, respectively. In addition, for the maximal biomass conversion with 10 ppm ODA added, the biomass of Yun Chih at day 10 and that of winter mushrooms at day 12 were 20.3 and 15.4 g/L, respectively. However, their initial sugar contents were 22.1−22.2 for Yun Chih and 21.4−21.5 g/L for winter mushrooms.

The change in residual sugar inversely correlated with the increase in biomasses of mushrooms for all treatments (Table 3). During the mycelial growth of Yun Chih from day 2 to 8 and that of winter mushrooms from day 4 to 10, the residual sugar content dropped evidently, especially dramatically for treatments with 10 ppm ODA added. At the end of incubation time (14 days), the residual sugar contents in Yun Chih and winter mushrooms were below 2 g/L for all treatments. However, the biomasses produced were significant different. It revealed that ODA could stimulate the mycelia to increase the conversion ratio of sugar into biomass. This phenomenon was also observed in Mau et al. (1999a) and Mau and Li (1999).
Table 2. Effect of 10-oxo-trans-8-decenoic acid on dry biomass (g/L) of mushrooms in shaken flasks at 25°C.

<table>
<thead>
<tr>
<th>ODA concentration (ppm)</th>
<th>Day 0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
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<tbody>
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<td>0.5±0.1a*</td>
<td>1.6±0.1c</td>
<td>3.9±0.3c</td>
<td>7.2±0.5c</td>
<td>11.4±0.8c</td>
<td>16.8±0.9b</td>
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<td>0.5±0.1a</td>
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<td>4.7±0.3b</td>
<td>8.7±0.7b</td>
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*Flammulina velutipes white strain*

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*Leninula edodes 271*

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<th>ODA concentration (ppm)</th>
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*Leninula edodes Tainung 1*

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<th>ODA concentration (ppm)</th>
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* Each value is expressed as mean ± SE (n = 3). Means not sharing the same letter within a row are significantly different (p < 0.05).
<table>
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<tr>
<th>ODA Concentration (ppm)</th>
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<table>
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<th>Lentinula edodes Tainung 1</th>
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<tbody>
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<td>20.4±0.1b</td>
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<tr>
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<td>20.5±0.1a</td>
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</tbody>
</table>

* Each value is expressed as mean ± SE (n = 3). Means not sharing the same letter within a row are significantly different (p < 0.05).
The residual sugar contents in shiitake continued steadily to decrease with prolonged incubation time. The gradual decrease in sugar contents correlated inversely with the slow mycelial growth for both strains.

It is reported that laccase production correlated with mycelial biomass of *A. bisporus* (Wood, 1979; Matcham *et al*., 1985; Claydon *et al*., 1988). However, the pattern of laccase in shaken flasks of Yun Chih and winter mushrooms showed that its production did not correlate with their mycelial biomass (Table 4). Similar results were also observed in shaken flasks of *A. bisporus* (Hwang, 1996), *Auricularia* spp. (Mau *et al*., 1999a), *A. clyindracea* (Mau and Li, 1999) and *P. eryngii* (Mau and Ma, 2000). On the contrary, for both slow growing strains of shiitake, the laccase activities positively correlated with their mycelial biomasses. Therefore, it is envisioned that the laccase activity correlated with the mycelial biomass only before its peaked value.

The laccase activities peaked at day 10 for all treatments of Yun Chih and winter mushrooms. It is shown that the higher laccase correlated well with the higher ODA concentration added. However, the effect of ODA on laccase activities of two strains of shiitake was not obvious as expected. Laccase was considered an important enzyme for mycelia to obtain nutrients through its action on compost (Westermark and Ericksson, 1974a, b; Anders and Ericksoon, 1976; Haars and Hutterman, 1980). However, laccase seemed to be useless for mycelia grown in liquid culture containing almost defined substances. When mycelia grow in compost or on log or sawdust, the increased excretion of laccase as a result of ODA stimulation would acclimate the mycelia to obtain nutrients.

Generally, ODA affected slightly the pH values in shaken flasks, which followed the normal growth pattern to drop to a lowest value, and increased at the end of incubation time (Fig. 1). The pH of Yun Chih reached the lowest value of 3.3–3.6 at day 10 and returned to 4.0–4.4. The pH of winter mushrooms reached the lowest value of 5.3–5.4 at day 8–10 and then back to 6.2–6.5. The pH of strain 271 decreased to about 3.3 at day 8 and maintained this level to the end of incubation time, whereas that of strain Tainung 1 slightly decreased to about 5.3 at day 12–14. Most fungi produced acetic, oxalic and pyruvic acids during their fast growth period, and thereby decreased the pH values. However, the increase in pH values at the last stage of incubation time might be due to the production of ammonium-like substances, which neutralize the pH (Leu, 1992).

It was indicated that ODA at all concentration tested had no or slight effect on mycelial growth for two strains of shiitake, which was slow on agar plates and in shaken flasks. One possible explanation might be that shiitake produced high amount of 1-octen-3-ol. Shiitake 271 produced 30.7 µg/g 1-octen-3-ol (Yang, 1995). By inference it is apparent that ODA is also concurrently produced in high amount. Therefore, the concentration of ODA to stimulate the mycelial growth of shiitake might be higher than those used in this research.

Summarily, ODA was found to be stimulatory to the mycelial growth of Yun Chih and winter mushrooms on agar plates and shaken flasks, and to shiitake Tainung 1 in agar plates. To cultivate mushrooms more economically and beneficially, the effect of ODA on mycelial growth of other mushrooms, and further study of ODA affected mushrooms in practical spawn making and mushroom production are needed.

**Acknowledgements**

The study was supported by National Science Council, R.O.C., project no. NSC88-2313-B005-093. We thank Chin-Chu Chen for providing *A.
Table 4. Effect of 10-oxo-trans-8-decenoic acid on laccase activity (U/ml) of mushrooms in shaken flasks at 25°C.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Day 0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>32±3b*</td>
<td>103±7c</td>
<td>172±13d</td>
<td>383±40c</td>
<td>613±48d</td>
<td>483±28a</td>
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<tr>
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<td>38±3a</td>
<td>115±10c</td>
<td>222±19c</td>
<td>455±26b</td>
<td>785±69c</td>
<td>216±30c</td>
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<tr>
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<td>39±4a</td>
<td>147±11b</td>
<td>256±23b</td>
<td>495±34a</td>
<td>824±56b</td>
<td>253±31bc</td>
<td>269±30b</td>
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<tr>
<td>10</td>
<td>0</td>
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<td>169±13a</td>
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<td>291±22a</td>
</tr>
</tbody>
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* Each value is expressed as mean ± SE (n = 3). Means not sharing the same letter within a row are significantly different (p < 0.05).
Figure 1. The change of pH value of mushroom mycelia in liquid culture containing various concentrations of 10-oxo-trans-
8-decenoic acid at 25°C. A: *Coriolus versicolor*; B: *Flammulina velutipes* white strain; C: *Lentinula edodes* 271; D: *Lentinula edodes* Tainung 1.

blazei; Hsi-Ming Chen for shiitake (271 and Tainung 1) and *C. versicolor*; Wen-Feng Chuang for *F. velutipes* and *A. cylindracea* strain B; and Chih-Yuan Chang for straw mushrooms.

**References**


Yang, M.S. 1995. Effects of gamma irradiation on the quality of shiitake (Lentinula edodes Sing.) and day lily (Hemerocallis fulva L.). Master’s Thesis, National Chung-Hsing Univ., Taichung, Taiwan, R.O.C.

10-氧-反-8-癸烯酸對一些菇類菌絲生長之影響

毛正倫 马榮村

國立中興大學食品科學系，臺中市，中華民國

摘要

10-氧-反-8-癸烯酸 (ODA) 以 0、2.5、5 和 10 ppm 之量加入馬鈴薯葡萄糖酵母瓊脂培養基 (PDA) 或培養液 (PDB)。在 ODA 存在下，在培養皿上雲芝 (Coriolus versicolor (Fr.) Quel.)、金針菇 (Flammulina velutipes (Curtis: Fries) Sing.) 和香菇 (Lentinula edodes (Berk.) Pegler) 臺農 1 號等菌絲之相對線性生長速率皆增加。ODA 散佈在瓊脂培養基表面對巴西洋菇 (Agaricus blazei Murrill)、金針菇和香菇之菌絲生長並無影響。在振盪培養瓶中，如由生物質所示，ODA 影響雲芝和金針菇之菌絲生長，特別在快速生長之對數期。在 ODA 添加之雲芝和金針菇菌絲培養瓶中，菌絲生長於第 10 天達到高峰。ODA 略影響振盪培養之 pH 值。簡言之，ODA 經發現能刺激在培養皿和振盪培養瓶中雲芝和金針菇之菌絲生長，以及在培養皿中香菇臺農 1 號之菌絲生長。

關鍵詞：生物質、金針菇、香菇、雲芝、漆酶。