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Effect of Sodium Hypochlorite for Controlling Bacterial Blotch on *Pleurotus ostreatus*

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Sodium hypochlorite alkaline was tested against *Pseudomonas tolaasii* causing bacterial blotch on cultivated oyster mushroom (*Pleurotus ostreatus*). The minimum inhibitory concentration of sodium hypochlorite against *P. tolaasii* contained active chlorine (AC) at 1.4 mg/l on plate assay. The highest cultivation yield was obtained from the treatment of AC 5.7 mg/l. Treatment of sodium hypochlorite at the rate of higher than AC 11.4 mg/l resulted in reduced yields at the harvest. However, the population of total bacteria on the bed surface treated with AC 5.7 mg/l of sodium hypochlorite was maintained to some extent. Inhibitory concentration against total bacteria on the bed surface was over AC 22.8 mg/l. Mushroom mycelium was damaged and its growth strongly inhibited at the concentration of AC 200 mg/l. Mushroom caps showed yellowish symptom by chemical injury by treatments of AC 74.1 mg/l or higher. Sporocarps infected by *P. tolaasii* were irrevocable at any concentration of sodium hypochlorite. Routine watering with AC 5.7 mg/l from mushroom initiation to the end of picking resulted in reduced bacterial blotch incidence of 40% and 86% at two mushroom farms. The treatment resulted in higher quality mushroom production compared to that conventionally watered with tap water alone.

KEYWORDS: Bacterial blotch, Infection, *Pleurotus ostreatus*, *Pseudomonas tolaasii*, Sodium hypochlorite

Pseudomonas tolaasii causes brown, slightly sunken blotches on maturing sporophores and brown streaks has been observed along the stipes of severely infected mushrooms. Wong and Preece (1980, 1982) reported that *P. tolaasii* causing bacterial blotch usually existed in casing layer. They also found that *P. tolaasii* disappeared in the casing layer where mycelium of *Agaricus bisporus* was absent. Fruitbodies on the bed are often threatened by bacterial blotch under poor conditions. The cultivation methods for *Pleurotus ostreatus* are quite different from *A. bisporus* in that no casing layers are used. Waterlogging often occurs on oyster mushroom beds, causing the development of bacterial disease. Moreover, the deviation of temperature between indoor and outdoor generates condensed moisture on the surface of bed that accelerates proliferation of the pathogenic bacteria. Oh *et al.* (1999, 2000) found that vinyl covering on oyster mushroom bed reduced waterlogging.

Routine watering with chlorinated water was effective to control bacterial blotch and soft rot of pinheads of *A. bisporus* (Ayers and Lambert 1955; Royse and Wuest, 1980). Treatment by chlorinating water reduced severity and incidence of brown blotch on *A. bisporus* caused by *P. tolaasii* (Wong and Preece 1985a, 1985b) and on *P. ostreatus* caused by *P. agarici* (Shin *et al.*, 1994). The successive treatment of activated chlorine dioxide reduced bacterial blotch incidence on *A. bisporus* (Geels *et al.*, 1991). Treatment with chlorinated lime in *Flammulina velutipes* cultivation showed increased yield as well as reduced bacterial disease incidence (Lee *et al.*, 1999). However, there has been no trial to control the bacterial blotch on *P. ostreatus* caused by *P. tolaasii* using sodium hypochlorite (SH).

The objective of this experiment was to determine the efficient use of SH for controlling bacterial disease of oyster mushrooms caused by *P. tolaasii* and report the results obtained using the chemical at commercial mushroom farms.

Materials and Methods

Microorganisms and chemicals: *Pseudomonas tolaasii* (ATCC 33618) was obtained from American Type Culture Collection. It was maintained at 25°C on *Pseudomonas* Agar F (PAF, Difco). *Pleurotus ostreatus* (You *et al.*, 1993) was obtained from the National Institute of Agricultural Science and Technology (NIAST, RDA, Suwon 441-707, Korea) and maintained on potato dextrose agar (PDA). Sodium hypochlorite (AC 10%, Ducksan Co. Ltd. Korea, and AC 5%, Hayashi pure chemical industries Ltd. Japan) was used in this experiment.

Preparation of *P. tolaasii*: *P. tolaasii* was cultured on PAF at 25°C for 24 hrs. Bacteria were collected in 5 ml of distilled sterile water after soaking for 2 hours. The suspension was centrifuged at 8,000 rpm for 15 min (Hitachi SCR20BA). After discarding supernatant, *P. tolaasii* cells were harvested, re-suspended, and centrifuged with 1 ml of distilled sterile water using Eppendorf tubes. The cells were washed once more as described above. Finally cells were suspended in 100 μl distilled sterile water and used for bacterial inoculum. Concentration of *P. tolaasii* was measured

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on PAF using the dilution drop method as described by Miles and Misra (1938).

Minimum inhibitory concentration: SH (AC 10%, Ducksan Co. Ltd. Korea) was diluted to determine the minimum inhibitory concentration (MIC) value. Concentrations of SH used were AC 0.5 mg/*l*, AC 0.8 mg/*l*, AC 1.1 mg/*l* and AC 1.4 mg/*l*. SH dilutions (10 m*l*) were mixed with 100 μl of *P. tolaasii* suspension (9.0×10^9 cfu/m*l*) and incubated at 25°C for 5, 10, or 15 min. Samples of 20 μl were withdrawn from each tube and dropped onto PAF. MIC was determined after incubation at 25°C for 24 hrs.

Effect of sodium hypochlorite on the growth of *P. ostreatus*: To determine the effect of SH on mycelial growth of *P. ostreatus*, 20 μl of each diluted solution ranging from AC 57 mg/*l* to AC 228 mg/*l* of SH (AC 5%, Hayashi Ltd. Japan) by two-fold dilution, were dropped on the mycelium and 0.5 m*l* plated around colony of *P. ostreatus* grown on PDA for 4 days. Plates were sealed and incubated at 25°C for 3 days before our observations were recorded.

To obtain an optimum concentration for applying to mushroom beds, SH was sprayed on cultivated oyster mushroom beds (W 45 × L 45 × H 15 cm) at several concentrations, ranging from AC 2.8 mg/*l* to AC 16.8 mg/*l*. Sporophores were inspected for chemical damage after 24 hrs. The mushroom growing substrate was cotton waste, weighing 2.5 kg per bed.

Dirty condition tests: A sample of 1.5 cm^2 was collected from the bed surface in the 3rd flush, and placed in a 100 m*l* flask containing 10 m*l* of sterile water. The flask was kept at room temperature for 30 min. Flasks were then shaken by hand and 1 m*l* mixed with 9 m*l* of SH solution at the concentration of AC 5.7 mg/*l*, AC 11.4 mg/*l*, AC 17.1 mg/*l* and AC 22.8 mg/*l*. Each mixture (0.5 m*l*) was dropped on PAF and incubated 25°C for 24 hrs. Bacterial populations grown on PAF were investigated.

Effect of sodium hypochlorite on mushroom yield: Two oyster mushroom farms were chosen to determine the effect of SH on controlling bacterial disease. One had been suffering devastating bacterial blotch problems and low yields for two years. The other had estimated around 50% yield reduction due to bacterial blotch. Water containing AC 5.7 mg/*l* of SH was applied to the mushroom beds and primordia at the mushroom farms, without adjustment of pH. Watering was maintained whenever surface of the bed slightly dried during cultivation. Effect of SH was assessed based on total yields of oyster mushroom.

Results and Discussion

Minimum inhibitory concentration of sodium hypochlorite against *P. tolaasii*: *P. tolaasii* was completely inhibited at the concentration of AC 2.8 mg/*l* independently of contact time. MIC of SH against *P. tolaasii* was AC 1.4 mg/*l* at contact time of 5, 10 and 15 min (Fig. 1). Wong and Preece (1985a) described that it was sufficient to be destructive against *P. tolaasii* cells within seconds of contact time at the concentration of 5 mg per litre of Free Available Chlorine. Shin *et al.* (1994) described that MIC was 40 ppm of total sodium hypochlorite against *P. agarici*, which was equivalent to AC 2.25 mg/*l* in our experiment. Compared to these results, MIC in this experiment was lower against *P. tolaasii*.

Effect of sodium hypochlorite on the growth of *P. ostreatus*: SH did not affect the mycelial growth of oyster mushroom at the low concentration below AC 171 mg/*l*. However, treatment with AC 200 mg/*l* showed inhibitory effect on the mycelial growth (Table 1). Several researchers (Ayers and Lambert, Royse and Wuest, Wong and Preece) reported that treatment with 100 to 200 ppm of available chlorine or FAC 150 mg per litre was adequate for controlling bacterial blotch in *A. bisporus* cultivation. This seems to

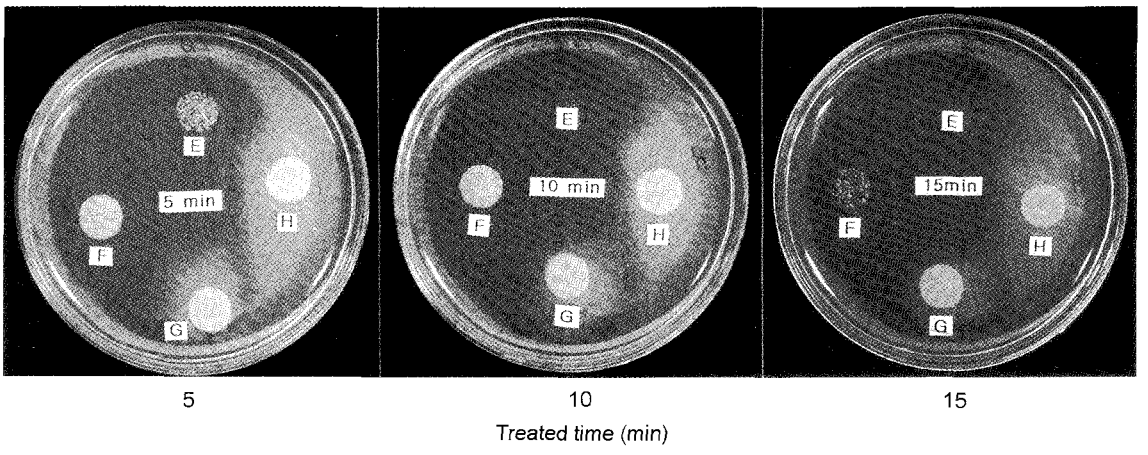

Fig. 1. Determination of minimum inhibitory concentration on contact time against *Pseudomonas tolaasii*.
*E : AC 1.4 mg/*l*, F: AC 1.1 mg/*l*, G: AC 0.8 mg/*l*, H: AC 0.5 mg/*l*.

Table 1. Critical concentration of sodium hypochlorite on *Pseudomonas tolaasii and Pleurotus ostreatus*

	Concentration of sodium hypochlorite (Available Chlorine mg/*l*)														
	0.5	0.8	1.1	1.4	2.8	5.7	8.5	11.4	14.3	17.1	22.8	68.4	74.1	171	200
A*	-	-	+**	+++											
B	-	-	-	-	-	+	+	+	+	++	+++				
C	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+++
D	-	-	-	-	-	-	-	-	-	-	-	+	+++		
E	-	-	-	-	++	+++	++	++	++	+					

*A : Determintion of Minimum Inhibitory Concentration against *Pseudomonas tolaasii*.
B : Inhibitory concentration of bacterial population on the bed surface by SH.
C : Inhibitory effect of SH on inhibition of mycelial growth.
D : Chemical damage on sporophore by SH.
E : Increasing effect of SH on yield of *Pleurotus ostreatus*.
**Degree of reaction; -, no reaction; +, low; ++, moderate; +++, high.

Table 2. Effect of sodium hypochlorite on yield of oyster mushrooms

	Concentration (Available Chlorine mg/*l*)						
	2.8	5.7	8.5	11.4	14.3	17.1	Ctrl
Yield (g/0.16 m^2)**	1008AB*	1172A	985AB	940AB	719B	688B	1110A

*Means followed by different letters are significantly different at P = 0.05.
**Mean of total mushroom producted, 1st to 3rd flush on 2.5 kg substrate per box.

be too high concentration to grow *P. ostreatus* due to there being no casing used in *Pleurotus* cultivation. SH caused slight yellowish discoloration on the surface of mushroom cap at the concentration of AC 74.1 mg/*l*. However, there was not any chemical damage of oyster mushroom cap at the concentration of below AC 68.4 mg/*l* (Table 1). These agree with the results reported by previous researchers (Ayers and Lambert, 1955; Wong and Preece, 1985a).

Effect of sodium hypochlorite on mushroom yield: To determine the effect of SH on oyster mushroom yield, several concentrations (AC 2.8, 5.7, 8.5, 11.0, 14.0 and 16.8 mg/*l*) were examined. Treatment with AC 5.7 mg/*l* resulted in the highest yields in bed cultivation. The higher concentration of SH was applied to the bed, the less yield of mushrooms was produced (Table 2). By treating AC 5.7 mg/*l*, mushroom yield was increased by 10 kg/m^2 compared to non-treated (Table 3). At the point of this result, it is thought that SH has effect on yield by inhibiting multiplication of microorganisms undesirable for oyster mushroom.

Table 3. Effect of sodium hypochlorite available Chlorine 5.7 mg/*l* on mushroom yield and bactrerial population on bed surface

Treatment	Bacterial population* (cfu/1.5 cm^2)	Yield (kg/m^2)**
Treated	2.9 × 10^7B***	26A
Non-treated	4.7 × 10^7A	16B

*Bacterial population of bed surface.
**Mean of total mushrooms produced from 1st flush to 5th flush.
***Means followed by different letters are significantly different at P = 0.05.

Ayers and Lambert's (1955) recommended concentration was 100 ppm to 200 ppm of available chlorine which reduced bacterial blotch disease on *A. bisporus*. Royse and Wuest (1980) also reported that acidified (pH 3.5) reduced severity and incidence of brown blotch of *A. bisporus*, but there was no effect of acidification. Wong and Preece (1985a) designated that *P. tolaasii* was markedly affected by pH adjusted chlorinated water, but there was no significant effect of altering pH in commercial mushroom (*A. bisporus*) beds. According to Shin *et al.* (1994), spraying SH 40-50 ppm reduced the incidence of the yellow blotch caused by *P. agarici* without impairing the growth of oyster mushroom. In our experiment bed surfaces were treated with chlorinated water (AC 5.7 mg/*l*) intermittently or daily, immediately after pinning on oyster mushroom farms. Yield was inreased up to 10 kg/m^2. It is difficult to compare our results with those of Shin *et al.* (1994) because the culture used was *P. agarici*. Bacterial infection was assessed by mushroom yield. On a farm suffering 84% crop loss for two years, no difference in the yield was seen when treated with SH. On another farm, suffering 50% crop loss, the effect of controlling bacterial disease was an 86% reduction in diseased mushrooms (Table 4).

Wong and Preece (1985a) also reported that only the highest concentration (FAC 150 mg/*l*) was able to prevent the multiplication of *P. tolaasii* cells present in the casing layer. However, based on our results, we recommend that the effective concentration for controlling bacterial disease in oyster mushroom cultivation should be, either SH AC 5.7

Table 4. Bacterial disease incidence and mushroom yield after treating with chlorinated water at two farms

Mushroom farms*	Disease incidence (%)		Yield (kg/m^2)	
	Treated	Non-treated	Treated	Non-treated
A	60a**	66a	5.1a	4.4a
B	14b	40a	10.4a	7.2b

*Farm A and B suffered 84% and 50% crop loss respectively, due to bacterial blotch infection before treating sodium hypochlorite from '98 to '99.

**Means followed by different letters are significantly different at P = 0.05.

mg/*l*, or AC 11.4 mg/*l*. It could be conferred that applying SH AC 11.4 mg/*l*, may have no significant difference in oyster mushroom yields.

Determination of bacterial population on the concentration of sodium hypochlorite: Most bacteria were killed by the treatment of AC 22.8 mg/*l* of SH on the surface of mushroom beds (Table 1). Bacteria existed on the bed surface to some extent when treated with AC 5.7 mg/*l* (Table 3). Masaphy *et al.* (1987) reported that mycelium of *A. bisporus* may be stimulated by microorganisms affecting sporophore formation. It seems that other beneficial bacteria may stimulate the pinhead initiation of oyster mushrooms. Therefore, it should be considered that non-pathogenic bacteria might survive on mycelial surface of *P. ostreatus* by the above concentration. If necessary, it may apply one or two times of this concentration (AC 22.8 mg/*l*) during cultivation. It is recommended that this chemical should be applied by all farmers seeing bacterial blotch during *Pleurotus* spp. cultivation according to this dose.

References

Ayers, T. T. and Lambert, E. B. 1995. Controlling mushroom disease with chlorinated water. *Plant Disease Reporter* **39**: 829-836.

Budavari, S. 1989. The Merk Index eleventh edition. Merk & Co., Inc. 1363 p.

Geels, F. P., Griensven, L. J. L. D. and Rutjens, A. J. 1991. Chlorine dioxide and the control of bacterial blotch on mushrooms, caused by *Pseudomonas tolaasii*. *Science and Cultivation of Edible Fungi* **13**: 437-442.

Lee, Hyun-Uk, Kim, Tae-Sung, Park, Hyean-Ceal, Song, Keun-Woo, Shin, Won-Kyo and Moon, Byung-Ju. 1999. Screening of chemicals on bacterial blotch caused by *Pseudomonas tolaasii* on *Flammulina velutipes*. *Kor. J. Mycol.* **27**: 164-169.

Long, P. E. and Jacobs, L. 1974. Aseptic fruiting of the cultivated mushroom, *Agaricus bisporus*. *Trans. Br. Mycol. Soc.* **63**: 99-107.

Masaphy, S., Levanon, D. and Henis, Y. 1987. Scanning electron microscope studies of interactions between *Agaricus bisporus* haphae and bacteria in casing soil. *Appl. and Environ. Microbiol.* **53**: 1132-1137.

Miles, A. A. and Misra, S. S. 1938. The estimation of the bactericidal power of the blood. Journal of Hygiene, *Cambridge* **38**: 732-749.

Nair, N. G. 1974. Methods of control for bacterial blotch disease of the cultivated mushroom with special reference to biological control. *Mushroom J.* **16**: 140-144.

Oh, Sejong, Chun, Changsung, Park, Jungsik, Kim, Heegyu and Fermor, T. R. 1999. Studies on the effect of vinyl mulching on *Pleurotus ostreatus* cultivation -quality and productivity of crop(1)-. *Kor. J. Mycol.* **27**: 107-111.

Oh, Sejong, Kong, Wonsik, Kim, Hankuong, Kim, Heekyu and Fermor, T. R. 2000. Studies on the effect of vinyl covering on *Pleurotus* spp. cultivation-Improved picking efficiency of *P. ostreatus* and *P. sajor-caju*. *Mushroom Science* **15**: 949-953.

Royse, D. J. and Wuest, P. J. 1980. Mushroom brown blotch: Effects of chlorinated water on disease intensity and bacterial populations in casing soil and on pilei. *Phytopathology* **70**: 902-905.

Shin, Gwan-Chull, Cho, Soo-Muk, Jeon, Nak-Beum and Ku, Ja-Hyeong. 1994. Effect of sodium hypochlorite (NaOCl) treatment on bacterial yellow blotch in oyster mushroom, *Pleurotus ostreatus*. *Kor. J. Mycol.* **22**: 190-195.

Sung, J. M., You, Y. B. and Cha, D. Y. 1998. Mushroom Science. Kyo-Hak Publishing Co., Ltd., pp. 247-332.

Wong, W. C. and Preece, T. F. 1980. *Pseudomonas tolaasii* in mushroom Crops: A note on Primary and Secondary Sources of the Bacterium on a Commercial Farm in England. *J. Appl. Bact.* **49**: 305-314.

Wong, W. C. and Preece, T. F. 1985. *Pseudomonas tolaasii* in cultivated mushroom (*Agaricus bisporus*) crops: effects of sodium hypochlorite on the bacterium and on blotch disease severity. *J. Appl. Bact.* **58**: 259-267.

Wong, W. C. and Preece, T. F. 1985. *Pseudomonas tolaasii* on mushroom (*Agaricus bisporus*) crops; bactericidal effects of six disinfectants and their toxicity to mushrooms. *J. Appl. Bact.* **58**: 269-273.

You Youngbok, You Changhyun and Cha Dongyeul. 1993. Strain improvement of the genus *Pleurotus* by protoplast fusion. *Kor. J. Mycol.* **21**: 200-211.