Identification and Thermal Sensitivity of Two Bacterial Pathogens of *Agaricus bisporus*

D. J. ROYSE, Assistant Professor, and P. J. WUEST, Professor, Department of Plant Pathology, The Pennsylvania State University, University Park 16802

ABSTRACT

ROYSE, D. J., and P. J. WUEST. 1980. Identification and thermal sensitivity of two bacterial pathogens of *Agaricus bisporus*. Plant Disease 64:455-457.

The bacterial blotch pathogen was recovered from horticultural grade peat moss treated with aerated steam for 30 min at 52.2 C (126 F). A 30-min treatment with aerated steam at 52.5 C eliminated the bacterial blotch pathogen from mineral soil and the pathogen of mummy disease from artificially infested soil. In general, as the treatment temperature increased from 43.8 C (111 F), the number of bacteria recovered from soil or peat decreased. Both bacterial species were eliminated at 55 C (131 F), regardless of substrate, when moisture contents were maintained at field capacity before and during the treatment. Our studies resulted in a technique whereby these two pathogens can be quantified in mushroom casing, either soil or peat moss.

Additional key words: *Agaricus brunnescens*, mushroom diseases, *Pseudomonas tolaasi, P. fluorescens* biotype Va, selective medium

Casing is a material such as soil or peat moss spread over compost colonized by the mycelium of *Agaricus bisporus* (Lange) Sing (= *A. brunnescens* Peck). This material usually is applied to a depth of 20–40 mm and serves as the substrate for the sporocarps.

Sphagnum peat moss, neutralized or made slightly alkaline with limestone, is used extensively. Acid peats might be considered unlikely substrates for bacterial mushroom pathogens, but disease occurrence has been reported (5).

Loam soil as casing was preferred to peat in North America until recently (12). Unlike peat, soil seems a potential source of common pests and pathogens to which *A. bisporus* is susceptible. Pasteurizing soil before using it as casing was suggested in the 1930s, and steam pasteurization is now used widely in Pennsylvania.

Microorganisms in the casing layer are reported to be essential for sporocarp primordia initiation (3). The casing thus can be considered a substrate supporting both *A. bisporus* and other microflora. The goal of casing treatment is pasteurization; sterilization would eliminate beneficial organisms.

In studies of the effects of aerated steam on selected fungal pathogens and weed molds of mushroom (8,13), exposure of casing soil to 54.4 C for 30 min eliminated the fungi. Studies of other mushroom pathogens were needed, however, to determine the minimum soil treatment temperature required to optimize the ecosystem for *A. bisporus*.

This study considers the effect of aerated steam on two bacterial pathogens in soil and neutralized peat casing and describes a method for quantifying the two pathogens.

MATERIALS AND METHODS

Soil and peat substrate. A Hagerstown silty clay loam, used for casing at the Mushroom Research Center of The Pennsylvania State University, was one substrate for the test bacteria. The soil, pH 7.5, had cation exchange capacity of 19.6, 2.2% organic matter, and 0.49 meq of potassium, 1.1 meq of magnesium, and 18.0 meq of calcium per 100 g of soil. A Canadian (horticultural grade) sphagnum peat (Atkins & Durbrow, New Brunswick, Canada) with 18.1 kg of dolomitic limestone added per 0.17 m^3 of peat moss was the second substrate. The sphagnum

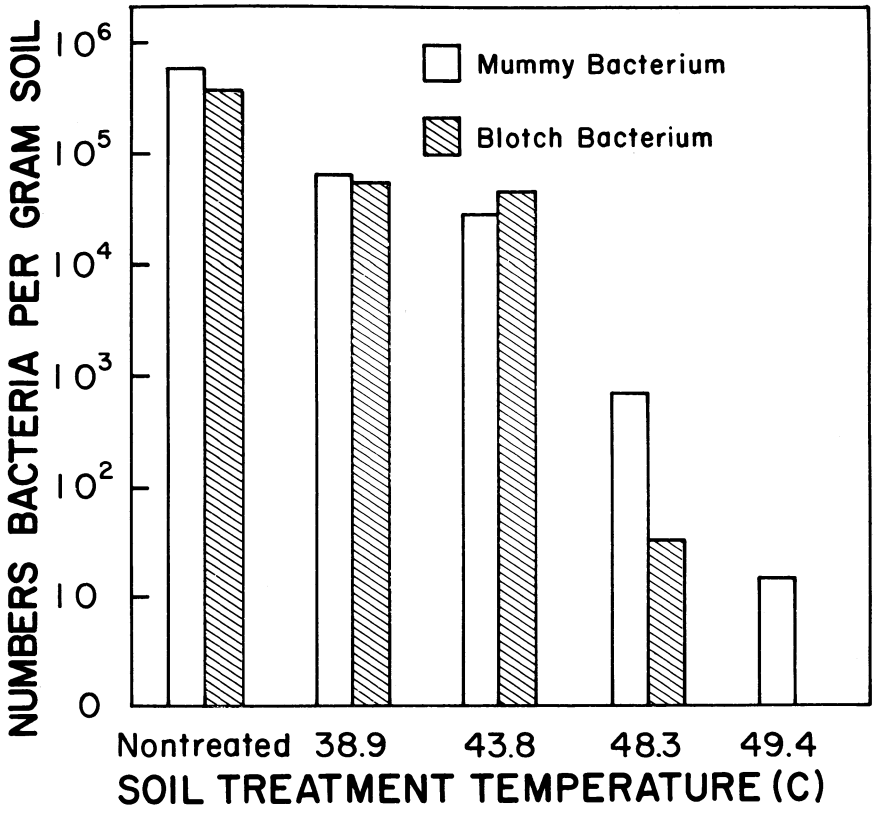

Fig. 1. Number of bacteria recovered on antibiotic selective medium from soil untreated or treated with aerated steam for 30 min at various temperatures.

Contribution 1138, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication October 1979 as Journal Series Paper 5858.

00191-2917/80/05045503/$03.00/0
©1980 American Phytopathological Society

peat, pH 7.2, had 431% water-holding capacity, 43.1% organic matter, and 56.9% ash. The soil and peat moss were screened through a 1-cm sieve before steam pasteurization for 2 hr at 68 C (155 F).

Bacterial seeding. A 1.5-L Erlenmeyer flask was partially filled with about 800 ml of pasteurized soil or peat. On dry weight basis, the moisture content of the soil was about 19% and that of peat was about 305%, before seeding with bacteria.

Flasks containing peat or soil were seeded with one of the test bacteria. The bacterial blotch pathogen was isolated from symptomatic mushrooms at the Plant Disease Clinic of The Pennsylvania State University and labeled 5462; the mummy bacterium (*Pseudomonas* sp.) was obtained from the American Type Culture Collection (ATCC 25415). Paine (10) named the blotch pathogen *P. tolaasi,* but more recently Lelliott et al (7) suggested it be considered a *P. fluorescens* biotype Va. Pathogenicity of the blotch organism was confirmed using Koch's postulates and a 24-hr culture of the bacterium in nutrient broth as inoculum; pathogenicity of the mummy bacterium could not be confirmed. The soil and peat were seeded with 20 ml of an aqueous bacterial suspension obtained by washing 24-hr cultures from slants of medium B of King et al (6) and adjusting the bacterial concentration to 5×10^7 with sterile distilled water. This aqueous addition raised the soil and peat moisture contents to about 26 and 322%, respectively, which are considered to be field capacity (1). The soil or peat in each flask was incubated at 25 ± 1 C for 5 days before aerated steam treatment.

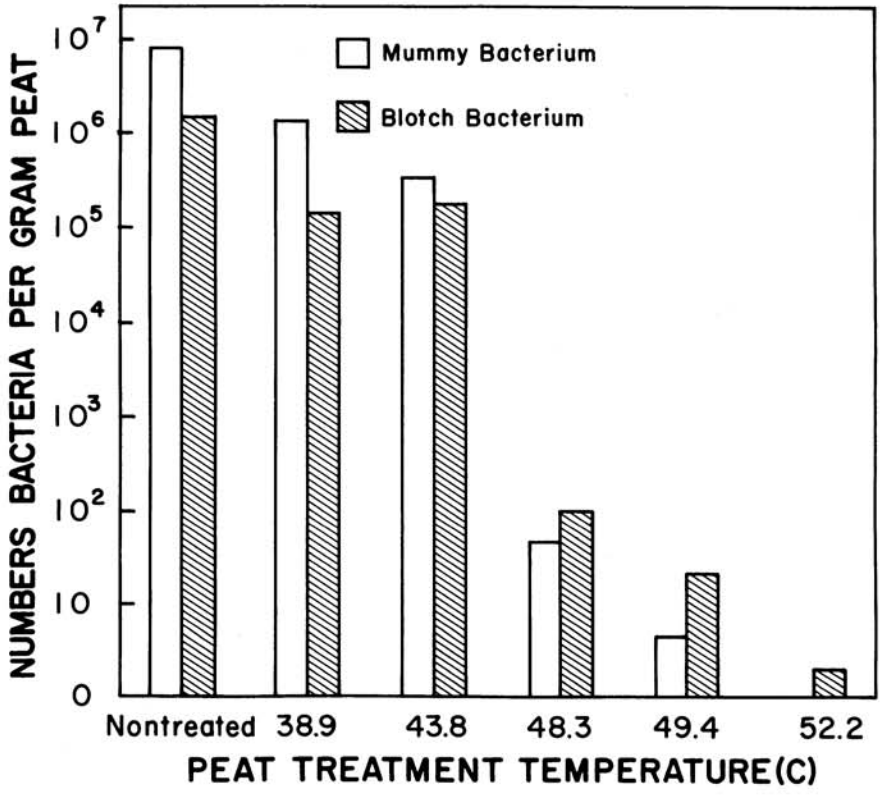

Fig. 2. Number of bacteria recovered on antibiotic selective medium from neutralized peat untreated or treated with aerated steam for 30 min at various temperatures.

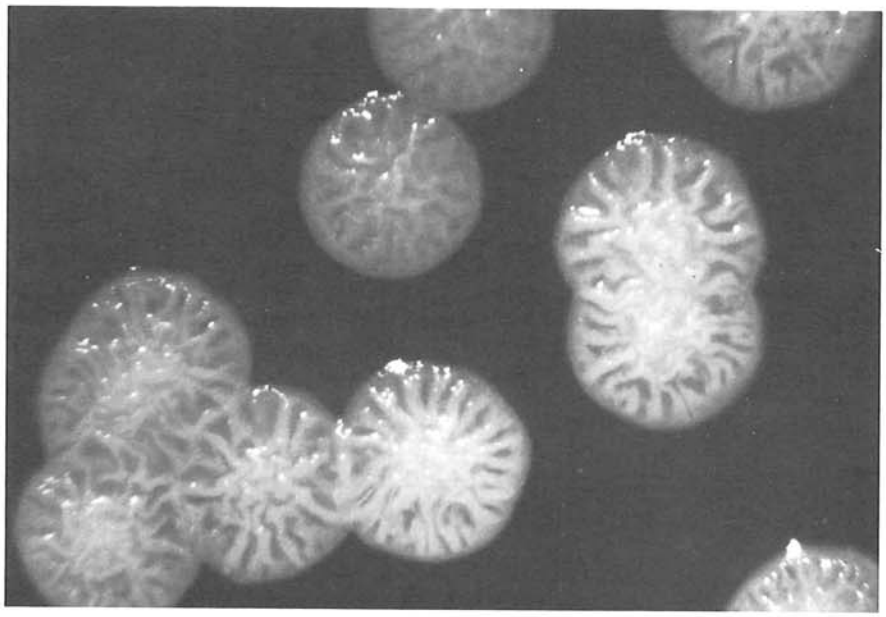

Fig. 3. Colony morphology of the bacterial blotch pathogen on antibiotic selective medium.

Soil treatment. Treatment with aerated steam was accomplished with the same equipment used by Moore and Wuest (8). Flour sifters were three-fourths filled with soil or peat and treated with aerated steam at 38.9, 43.8, 48.3, 49.4, 52.2, and 55 C for 30 min. A control was not treated. Temperatures were monitored with a multipoint potentiometric strip chart recorder, with one copper-constantan thermocouple placed centrally in soil in each treatment cylinder. Treatment temperature varied ± 0.5 C. Warm-up time before treatment and cooling time after treatment for soil and peat were 7 and 6 min and 3 and 2 min, respectively. After treatment, the casing materials were placed in unused polyethylene bags, stored at 4 C, and assayed for surviving bacteria within 6 hr.

Selective medium. The basal medium used for bacterial recovery consisted of medium B of King et al (6) to which 0.1N NaOH was added to adjust the pH to 7.2 before autoclaving. Penicillin G (75,000 units), novobiocin (45 mg), and cycloheximide (75 mg) (Sigma Chemical Co., St. Louis, MO 63178) were mixed together in 3 ml of 95% ethanol, then diluted with 50 ml of sterile distilled water (11), and added to 940 ml of melted (45 C) basal medium that had been autoclaved 15 min at 121 C. After 20 ml of the amended medium was added, the petri plates were allowed to dry overnight and refrigerated in polyethylene bags until use a few days later.

Bacterial recovery. Two 1-g soil samples were collected from each treatment, and moisture content was determined in one sample. The second sample was placed in a 16 × 150 mm culture tube to which 10 ml of sterile distilled water was added. This sample was mixed on a Vortex mixer for 1 min, serial dilutions (1:10) were made, and 0.1-ml aliquots were plated on the selective medium. Plates were incubated for 4 days at 25 ± 1 C, and then bacterial colonies were counted. Each treatment was replicated twice, and the experiment was conducted twice. Raw data were transformed to raise all numbers to the

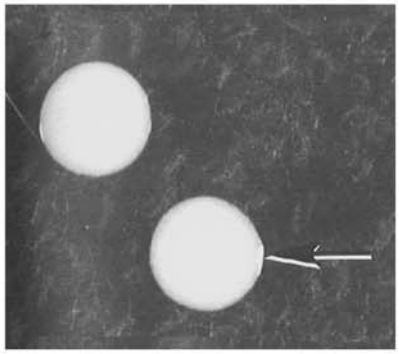

Fig. 4. Colony morphology of the mummy bacterium (indicated by arrow) on antibiotic selective medium.

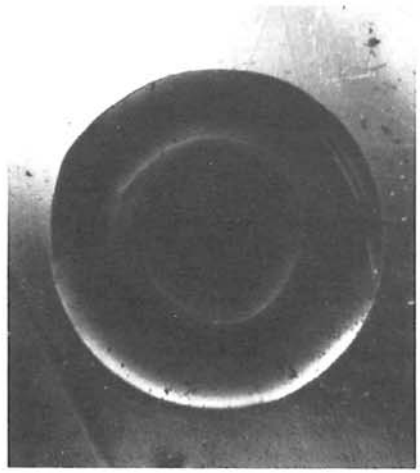

Fig. 5. Colony of mummy bacterium (indicated by arrow in Fig. 4) photographed under oblique lighting.

same basis, ie, bacterial cells per gram of dry soil or dry peat.

RESULTS

Thermal sensitivity of the pathogens. Aerated steam forced through the soil or peat at 55 C (131 F) and above for 30 min eliminated test bacteria from infested soil and peat. There were, however, some differences in survival between the two bacterial species, treatment temperatures, and casing materials (Figs. 1 and 2). In general, as exposure temperature increased above 43.8 C (111 F), the number of bacteria surviving decreased markedly.

Peat fostered higher populations of both organisms in the untreated controls, and the bacteria were more heat-tolerant in peat. The blotch bacteria withstood treatment at 52.2 C (126 F) in peat, but 49.4 C (121 F) was lethal when the organism was in soil. The mummy bacteria were reduced markedly as temperature increased from 43.8 to 52.2 C, but some survived 49.4 C in both soil and peat.

Characteristics of bacteria on selective medium. After 5–6 days of growth at 25 C, the blotch bacteria formed cream-colored, circular, umbonate colonies with curled margins (Fig. 3). Colony diameter ranged from 4 to 8 mm. The mummy bacteria formed circular, convex colonies with entire margins. (Figs. 4 and 5). Colonies were cream to tan in color and ranged from 5 to 8 mm in diameter. A typical colony produced by the mummy bacterium had a circular, opaque center surrounded by a translucent ring and an outer opaque ring (Fig. 5).

DISCUSSION

Blotch-causing bacteria and mummy-inducing bacteria recovered from steam-treated soil or peat were readily identifiable and distinguishable by colony morphology when grown on the test medium. Nair and Fahy (9) reported that the blotch organism produces a unique colony type but did not describe it. Our observation that the mummy bacterium also produces a unique colony type on the selective medium may provide the basis for a technique to assess the naturally occurring bacterial population and inoculum levels of either or both pathogens in soil or peat.

Paine (10) studied the thermal death point of *P. tolaasi* by immersing test tube cultures of the bacteria in a water bath at various temperatures. He showed that *P. tolaasi* cells were killed after 10-min exposure to 51 C. Because these conditions are optimum for heat transfer, valid inferences about the thermal sensitivity of naturally occurring bacteria in either soil or peat were lacking. Data regarding thermal sensitivity in vivo or in vitro on the mummy bacterium have not been published.

Optimum heat exchange vis-à-vis pasteurization occurs when soil or peat has a moisture content at which the microflora and pests are most active—generally when the soil moisture content is near field capacity (1). Aldrich et al (1) further reported that peat was more difficult to heat than soil because of a combination of physical characteristics, including specific heat, compression index, and moisture content at field capacity. This may explain in part the survival of *P. tolaasi* at temperatures nearly 3 C higher in peat than in soil. The mummy bacteria, however, were not recoverable from peat at a treatment temperature of 52.2 C (126 F).

Previous reports (2,12), focusing on the thermal sensitivity of *Pseudomonas* spp. invading or inhabiting greenhouse soil and casing soil, indicated that bacterial pathogens were eliminated after 30-min exposure of moistened soil to 48.9 C (120 F). Our results are in general agreement, except for those with the mummy pathogen, which was recovered from soil treated with aerated steam at 49.4 C (121 F) for 30 min.

Mushroom growers using aerated steam, or possibly other heat treatments to pasteurize casing, should know the physical characteristics of the casing, since all casings do not respond similarly. Happ (4) noted a similar phenomenon while working with Verticillium disease of mushrooms.

It should be noted that the soil and peat used in our study were at field capacity, which is considered to be optimum for pasteurization from a heat-exchange efficiency vantage. Treatment of casing that is too dry or too wet may influence the survival of bacteria in soil or peat. The moisture content of casing treated commercially in the mushroom industry rarely equals field capacity, suggesting that applicability of this newly derived information to existing commercial practice will require further study.

ACKNOWLEDGMENTS

We thank B. Myers, M. H. Jodon, and P. Zarkower for technical assistance.

LITERATURE CITED

1. ALDRICH, R. A., M. E. SCHROEDER, R. A. KEPPELER, and G. H. DIENER. 1975. Heating soils, soil mixes, and soil substitutes with saturated air. Pa. Agric. Exp. Stn. Bull. 800. 36 pp.
2. BAKER, K. F., and C. N. ROISTACHER. 1957. Principles of heat treatment of soil. Pages 138-161 in: K. F. Baker, ed. The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Manual 23. 322 pp.
3. EGER, G. 1962. Untersuchungen zur fruchtkorpergildung des kulturchampignons. Mushroom Sci. 5:314-320.
4. HAPP, A. C. 1974. Yield of *Agaricus bisporus* (Lange) Imbach and occurrence of Verticillium disease as influenced by aerated steam treatment of peat moss, spent compost or loam soil. M.S. thesis. Pennsylvania State University. 53 pp.
5. HAYES, W. A. 1978. Nutrition, substrates, and principles of disease control. Pages 219-237 in: S. T. Chang and W. A. Hayes, eds. The Biology and Cultivation of Edible Mushrooms. Academic Press, New York. 819 pp.
6. KING, E. O., M. K. WARD, and D. E. RANEY. 1954. Two simple media for the demonstration of pyocanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
7. LELLIOTT, R. A., E. BILLING, and A. C. HAYWARD. 1966. A determinative scheme for the fluorescent plant pathogenic Pseudomonads. J. Appl. Bacteriol. 29:470-489.
8. MOORE, R. K., and P. J. WUEST. 1973. Occurrence and severity of Verticillium disease of mushrooms produced on casing (soil) treated with aerated steam. Phytopathology 63:1368-1374.
9. NAIR, N. G., and P. C. FAHY. 1976. Commercial application of biological control of mushroom bacterial blotch. Aust. J. Agric. Res. 27:415-422.
10. PAINE, S. G. 1919. Studies in bacteriosis. II. A brown blotch disease of cultivated mushrooms. Ann. Appl. Biol. 5:206-219.
11. SANDS, D. C., and A. D. ROVIRA. 1970. Isolation of fluorescent pseudomonads with a selective medium. Appl. Microbiol. 20:513-514.
12. SCHISLER, L. C., and P. J. WUEST. 1972. Casing soil treatment in commercial mushroom production. Pa. Agric. Ext. Serv. Spec. Cir. 139. 12 pp.
13. WUEST, P. J., K. F. BAKER, and W. S. CONWAY. 1970. Sensitivity of four soil-borne mushroom pathogens to treatment with aerated steam. Phytopathology 60:1274-1275.