DESTRUCTION OF SELECT HUMAN PATHOGENIC BACTERIA IN MUSHROOM COMPOST DURING PHASE II PASTEURIZATION

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ABSTRACT

Studies were conducted to investigate changes in microbial levels during mushroom composting and the destruction of human pathogens during Phase II pasteurization and conditioning. Total aerobic bacteria and coliform levels in compost prepared on a model mushroom farm were monitored throughout a complete crop cycle. There was little change in total bacteria levels. However, coliforms decreased to undetectable levels immediately following tunnel or tray Phase II composting. In a second experiment, Phase I compost was inoculated with high levels of *Listeria monocytogenes*, *Salmonella*, and *E. coli* O157:H7, filled into vented polypropylene bags, placed within trays of compost, and subjected to a standard 6-day Phase II protocol which included a 60oC/2h pasteurization treatment. Pathogen and coliform populations before and after Phase II were evaluated using standard microbiological enumeration and enrichment procedures. Initial levels for each pathogen ranged between 7.2 and 8.2 log CFU/g. Following Phase II, no human pathogens were detected in compost. A laboratory study was then conducted to determine the amount of time required to inactivate the 3 pathogens at 48.8oC (120^{o}F), 54.4^{o}C (130^{o}F), and 60^{o}C (140^{o}F). Complete inactivation of *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 at each respective temperature was observed at 36, 8, and 1 h. These studies demonstrate that complete inactivation of human bacterial pathogens in mushroom compost can be achieved during a standard 6-day Phase II protocol.

INTRODUCTION

The success of the mushroom industry in the last few decades can be attributed to consistent production of high quality and wholesome food products with tremendous consumer appeal. However, as consumer preference has shifted from processed to fresh market mushrooms, concerns about the safety of mushrooms has increased. As a result, mushroom growers are increasingly faced with demands for documented and verifiable food safety plans that demonstrate safe and sanitary production practices (LaBorde 2001, 2003).

Mushroom compost ingredients may include horse and poultry manure which are potential sources of human pathogenic bacteria (Heuvelink *et al.* 1996; USDA 1998; Himathongkham *et al.* 1999). However, mushroom compost is subjected to potentially lethal temperatures during Phase I and II treatments. During Phase I composting, temperatures may reach as high as 77-82oC (170-180oF) (Wuest *et al.* 1980). During a

standard Phase II pasteurization and conditioning protocol (Beyer 2003), temperatures typically reach at least 60°C (140°F) for 2 h. Because Phase II is carefully controlled and monitored, it could be used by mushroom growers as an effective food safety control measure. An understanding of microorganisms, including human pathogens, during mushroom production and verification of pathogen destruction during Phase II pasteurization will therefore be of value to mushroom growers as they develop their food safety plans.

OBJECTIVES

1. To study changes in microbial levels in compost prepared on a model mushroom farm.
2. To determine the effect of a standard Phase II pasteurization and conditioning protocol on select human pathogenic bacteria in inoculated mushroom compost.
3. To determine the temperature and time requirements for complete thermal inactivation of select human pathogenic bacteria using a laboratory model system.

MATERIALS AND METHODS

Sample preparation and treatments

1) Microbial levels in compost prepared on a model mushroom farm. Phase I composting of traditional horse manure compost (Beyer 2003) was conducted in a bunker system at the Penn State University Mushroom Test Demonstration Facility (MTDF). After Phase I, the compost was subjected to a 6-day Phase II pasteurization and conditioning treatment using either a tunnel or traditional tray system. After Phase II, both compost batches were used in a standard mushroom production cycle. Compost samples, taken over the course of the crop cycle, were enumerated for total aerobic bacteria and coliform levels.

2) Effect of Phase II pasteurization and conditioning on select human pathogens. Phase I compost (2500 g) was added into 4-L polypropylene mushroom cultivation bags (Unicorn Imp. & Mfg., Commerce, TX) and inoculated with 200 ml of a microbial composite consisting of two strains each of *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7. Gas exchange was facilitated by the presence of 2 filter patches (3 X 6 cm) on each bag. After thorough mixing, initial microbial levels were determined. Un-inoculated bags were used to follow changes in coliform bacteria.
Each bag was placed into a 61 X 61 cm (2 X 2 ft.) tray of compost and subjected to a standard 6-day Phase II pasteurization and conditioning protocol at the Penn State Mushroom Research Center (MRC). Pasteurization was completed when the temperature of the compost reached 60oC (140°F) for 2 h. Temperature profiles for compost and air were monitored using a Chameleon Total Climate Control (Groft Electronics, Landberg, PA). Temperatures within each bag were monitored using wireless temperature probes (HOBO Water Temp Pro, Onset Computers, Bourne, MA) placed in the center of each bag. Following each treatment, the contents of the bags were analyzed for residual pathogenic bacteria and coliforms.

3) Thermal inactivation of human pathogenic bacteria in compost using a laboratory model system. Phase I compost was inoculated with pathogens as previously described, and 75 g was weighed into polypropylene bags and vacuum-sealed. The dimensions of the filled and sealed bags were 12.7 X 22.9 X 0.5 cm. Samples (To=20oC) were heated in a temperature-controlled water bath adjusted to 48.8^{o}C (120^{o}F), 54.4^{o}C (130^{o}F), and 60^{o}C (140^{o}F). Temperature come-up times were 4.5, 5, and 5 min for each respective water bath temperature. A single bag was sampled for each time/temperature combination. After each treatment, the bags were immediately cooled in an ice water bath to room temperature and the contents were analyzed for residual pathogenic bacteria.

Microbial analysis

For the MTDF and laboratory experiments, 25 g of compost was combined with Buffered Peptone Water (BPW; Difco Laboratories, Detroit, MI) to yield a 1/10 dilution. For the MRC experiment, 250 g compost was sampled from each bag. After thorough mixing, the infusion was serially diluted and standard plating methods were used to enumerate total aerobic bacteria, coliforms, *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 (Merker 2001). Total aerobic bacteria were enumerated using Plate Count Agar (PCA; Difco) or Aerobic Plate Count Petrifilm (3M). Coliforms were enumerated using Violet Red Bile Agar (VRBA) or Coliform/E. coli Petrifilm (3M). The 3 respective pathogens were enumerated on Xylose Lysine Deoxychoalte Agar (XLD; Difco), Modified Oxford Agar (MOX, Difco), and MacConkey Sorbitol Agar (SMAC; Difco). When pathogens could not be detected by direct plating, a standard enrichment procedure (Merker 2001) (10 g compost in 90 g broth) was used to confirm complete thermal destruction.

RESULTS AND DISCUSSION

1) Microbial levels in compost prepared on a model mushroom farm.

- Reductions in total aerobic bacteria and coliform levels were not significantly (P>0.05) different between tunnel or tray Phase II treatments
- Total aerobic bacteria levels were reduced only slightly over a complete mushroom crop cycle.
- Coliform levels were reduced slightly during Phase I, but were not detected after Phase II.
- Coliform levels increased slightly after the incorporation of spawn.

2) Effect of Phase II pasteurization and conditioning on select human pathogens.

- Following Phase II, no human pathogenic bacteria or coliforms were detected with direct plating or enrichment.

3) Thermal inactivation of human pathogenic bacteria in compost using a laboratory model system

- *L. monocytogenes* appeared to be the most heat resistant and *Salmonella* the least resistant of the bacteria tested.

· The select human pathogenic bacteria were thermally inactivated within 36 h at 48.8oC (120°F), 8 h at 54.4°C (130°F), and 1 h at 60°C (140°F).

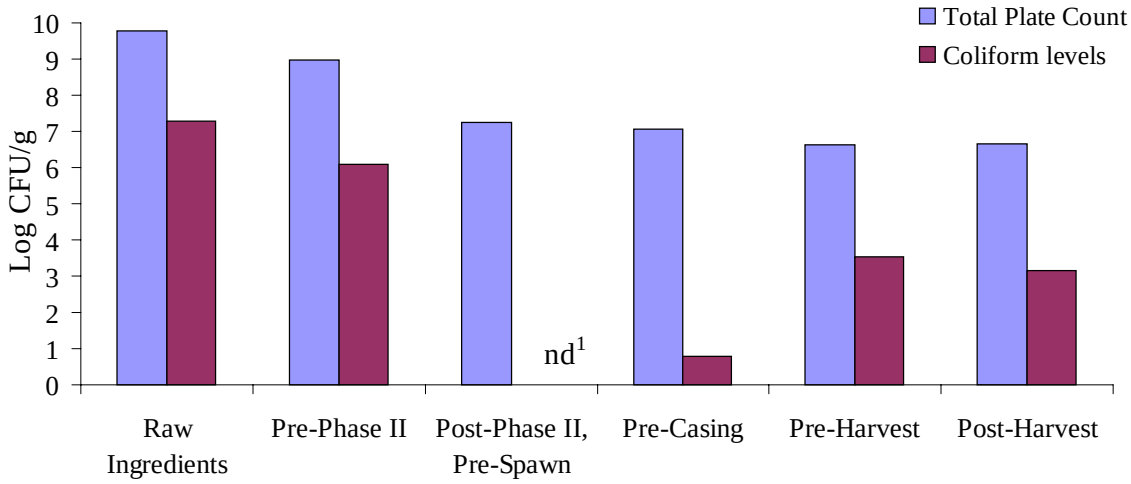

Figure 1. Changes in total aerobic bacteria and coliforms during a complete crop cycle. [1]none detected (<1 CFU/g)

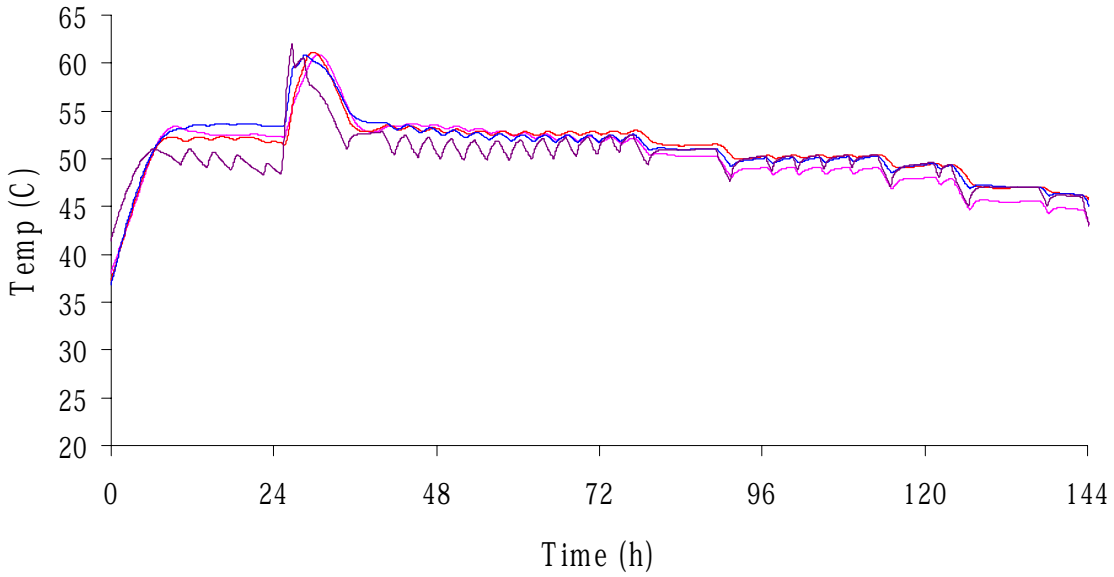

Figure 2. Temperature profile of compost in bags during a standard 6-day Phase II pasteurization and conditioning protocol.

Table 1. Effect of a standard Phase II pasteurization and conditioning protocol[1] on select human pathogenic bacteria and coliforms in mushroom compost.

	L. monocytogenes (Log CFU/g)		*Salmonella* (Log CFU/g)		*E. coli* O157:H7 (Log CFU/g)		Coliform Levels (Log CFU/g)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Initial level	8.1	7.8	7.6	7.2	8.2	7.7	5.3	4.9
After Phase II	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[3]	nd[3]

[1]Compost temperature was >60°C from 5.7 to 8.6 h during trial 1 and 10.3 to 10.8 h during trial 2.
[2]none detected, <0.1 CFU/g
[3]none detected, <10 CFU/g

Table 2. Thermal inactivation of select human pathogenic bacteria in mushroom compost at 48.8°C (120°F), 54.4°C (130°F), and 60°C (140°F).

Temp.	Pathogen	Initial population (log CFU/g)	Time for total inactivation[1]
48.8°C	*L. monocytogenes*	8.0	>24 h, <36 h
(120°F)	*Salmonella* spp.	8.0	>12 h, <24 h
	E. coli O157:H7	7.7	>24 h, <36 h
54.4°C	*L. monocytogenes*	8.1	>6 h, <8 h
(130°F)	*Salmonella* spp.	7.9	>6 h, <8 h
	E. coli O157:H7	7.4	>6 h, <8 h
60°C	*L. monocytogenes*	8.0	>30 min, <60 min
(140°F)	*Salmonella* spp.	7.9	>3 min, <6 min
	E. coli O157:H7	7.9	>3 min, <6 min

[1]none detected (<0.1 CFU/g)

CONCLUSION AND FUTURE RESEARCH NEEDS

The standard 6-day Phase II protocol used in this study was effective in inactivating *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 in mushroom compost. Additional studies are needed to correlate reductions of indicator organisms with the destruction of human pathogens so that each grower can validate Phase II pasteurization and

conditioning as an effective food safety control measure. Further work is also needed to observe changes in bacterial levels during Phase II pasteurization and conditioning using different types of commercial mushroom composting systems.

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