Mushroom Food Safety Research – Past, Present, Planned

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Potential sources of human pathogens on mushroom farms

- Compost
  - Chicken manure
  - Horse manure
- Casing
  - Peat
  - Amendments
- People
  - Hands,
  - Bodily fluids
- The growing environment
  - Floors, drains, walls

"We must have strong minds, ready to accept facts as they are." — Harry S Truman
Listeria monocytogenes

- Multiple species within the genus *Listeria*
- Only *Listeria monocytogenes* is pathogenic
- Causes approximately 2,500 cases of listeriosis and result in 500 fatalities in the U.S. each year
- Isolated in chicken and horse manure, soil, water, and vegetation
- Occurs widely in both agricultural and food processing environments
- Grows in cool, moist environments
Salmonella spp.

- 2400 serotypes - all are considered potentially pathogenic
- Associated with the intestinal tract of warm and cold-blooded animals and found in chicken and other poultry products, contaminated water, untreated fertilizers, infected wildlife,
- *Salmonella* Enteriditis in 7.1% of egg layer houses (USDA 2000)
- *Salmonella* serotypes were isolated from 0.8% of the horses fecal samples taken from 8,417 horses on 972 operations (USDA 1998).
Strategies for Controlling Food Safety Hazards on Fresh Mushrooms

- **Pre-Harvest**
  - Physical
    - Compost pasteurization and conditioning
    - Casing heat treatments
  - Chemical
    - Disinfectants in irrigation water / casing
  - Biological
    - Bio-control agents in casing and on mushrooms to suppress growth of human pathogens

- **Post-Harvest**
  - Physical
    - Irradiation
    - Temperature effects
    - Packaging to control humidity, gas composition
  - Chemical
    - Disinfectants added to wash treatments
  - Biological
    - Role of background microflora on survival and growth of human pathogens
1) The Effect of Phase II Pasteurization on Populations of Select Human Pathogenic Bacteria in Mushroom Compost

- Inoculation studies at the Penn State Mushroom Research Center
- Survival of *Listeria monocytogenes*, Salmonella sp., and *E. coli* O157:H7 under a standard Phase II pasteurization and conditioning protocol
Destruction of select human pathogens and indicator microorganisms in mushroom compost during Phase II pasteurization and conditioning
(Target maximum temperature and time during 6-d protocol was 60°C for 2 h)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Before Phase II</th>
<th>After Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM</td>
<td>7.95 ± 0.21</td>
<td>0c</td>
</tr>
<tr>
<td>SS</td>
<td>7.40 ± 0.28</td>
<td>0c</td>
</tr>
<tr>
<td>EEC</td>
<td>7.95 ± 0.21</td>
<td>0c</td>
</tr>
<tr>
<td>TPC</td>
<td>8.75 ± 0.35</td>
<td>6.90 ± 0.43</td>
</tr>
<tr>
<td>TE</td>
<td>5.80 ± 0.28</td>
<td>&lt;1d</td>
</tr>
<tr>
<td>TC</td>
<td>5.10 ± 0.28</td>
<td>&lt;1d</td>
</tr>
<tr>
<td>EC</td>
<td>4.70 ± 0.14</td>
<td>&lt;1d</td>
</tr>
</tbody>
</table>

*a* Mean + standard deviation of the mean population of bacteria. Populations were significantly different before and after Phase II for all microorganisms.

*b* LM, Listeria monocytogenes; SS, Salmonella spp.; EEC, Escherichia coli O157:H7; TPC, mesophilic aerobic bacteria; TE, total enterobacteriaceae; TC, total coliforms; EC, generic Escherichia coli.

*c* Negative by the direct plating method, negative by the enrichment method

*d* Negative by direct plating
Thermal inactivation of select human pathogenic bacteria in mushroom substrate at 48°C (120°F), 54.4°C (130°F), and 60°C (140°F)

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Pathogen</th>
<th>Initial population (log CFU/g)</th>
<th>Time for total inactivation¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>48.8°C (120°F)</td>
<td>L. monocytogenes</td>
<td>8.0</td>
<td>&gt;24 h, &lt;36 h</td>
</tr>
<tr>
<td></td>
<td>Salmonella spp.</td>
<td>8.0</td>
<td>&gt;12 h, &lt;24 h</td>
</tr>
<tr>
<td></td>
<td>E. coli O157:H7</td>
<td>7.7</td>
<td>&gt;24 h, &lt;36 h</td>
</tr>
<tr>
<td>54.4°C (130°F)</td>
<td>L. monocytogenes</td>
<td>8.1</td>
<td>&gt;6 h, &lt;8 h</td>
</tr>
<tr>
<td></td>
<td>Salmonella spp.</td>
<td>7.9</td>
<td>&gt;6 h, &lt;8 h</td>
</tr>
<tr>
<td></td>
<td>E. coli O157:H7</td>
<td>7.4</td>
<td>&gt;6 h, &lt;8 h</td>
</tr>
<tr>
<td>60°C (140°F)</td>
<td>L. monocytogenes</td>
<td>8.0</td>
<td>&gt;30 min, &lt;60 min</td>
</tr>
<tr>
<td></td>
<td>Salmonella spp.</td>
<td>7.9</td>
<td>&gt;3 min, &lt;6 min</td>
</tr>
<tr>
<td></td>
<td>E. coli O157:H7</td>
<td>7.9</td>
<td>&gt;3 min, &lt;6 min</td>
</tr>
</tbody>
</table>

¹none detected (<0.1 CFU/g)
Destruction of select human pathogens and indicator microorganisms in mushroom substrate heated at 54.4°C (130°F)

LM, *Listeria monocytogenes*; EEC, *Escherichia coli* O157:H7, SS, *Salmonella* spp.; TPC, mesophilic aerobic bacteria; TE, total enterobacteriaceae; TC, total coliforms; EC, generic *Escherichia coli*
2) Microbial Ecology of Mushroom Casing Soils and Preharvest Strategies to Enhance Safety and Quality of Fresh Mushrooms

- Microbial interactions between naturally existing harmless microorganisms in casing soils and human pathogens
- Effect of slicing on pathogen growth
Microbiology of the mushroom casing layer

Log CFU/gm (wet wt.)

Aerobic Plate Count

Yeasts

Molds

Actinomycetes
Listeria monocytogenes and Salmonella die rapidly in untreated casing soil but grow in sterile soil.

A control measure for casing soils that includes thermal pasteurization eliminates natural microflora that suppress pathogens.
Addition of *Penicillium* sp. inhibits pathogen growth but does not decrease effect of untreated casing soil.

Microbial ecology of casing soils not yet fully understood. What is the role of *Agaricus*?
Listeria monocytogenes grows on sliced mushrooms but not whole mushrooms at 12°C (54°F).

When mushrooms are cut, cellular nutrients that support microbial growth are released.
3) Development and Assessment of Pilot Food Safety Educational Materials for Hispanic Workers in the Mushroom Industry Using the Health Action Model

- Ph.D. thesis project by Sergio Nieto-Montenegro under supervision of Drs. J. Lynne Brown (advisor) and Luke LaBorde
- Direct observations of worker behaviors made at farm and packing house operations
- Results used to develop training curriculum
Mushroom Food Safety Training Kit

- Curriculum developed by the Penn State Department of Food Science and Hispanic Workforce Management
- Teach the basics of personal hygiene and practices that prevent contamination of mushrooms during harvesting and packing operations
- Includes hard copy and electronic versions of the lessons in English and in Spanish, PowerPoint slides, posters, and companion documents.

Nieto-Montenegro S, LaBorde, LF, Brown JL. 2006
Mushroom Good Agricultural Practices Standards and Guidelines

Mushroom Good Agricultural Practices Program

Industry-Wide Food Safety Standards for Fresh Mushroom Growing, Harvesting, and Shipping

2008

Mushroom Good Agricultural Practices Plan

For

Name of Farm

The information in this document is a true representation of the food safety conditions and practices followed at this location.

Manager/Manager responsible for food safety

Signature

Date

Penn State University and the American Mushroom Institute © 2008
General Instructions:
- For clarification and guidance in answering these questions, please refer to the "Mushroom Good Agricultural Practices Standards".
- Place the point value for each question in the proper column (Yes, No).
- "*" In the Doc column means that documentation will be requested/reviewed by the auditor.
- Any "No" designation must be explained in the Comments section.

Conditions under Which an Automatic Audit Failure Will Occur

General conditions:
- An imminent food safety risk is present when mushrooms are grown or held under conditions that promote or cause the produce to become contaminated.
- The presence of evidence of violations, an excessive amount of insects or other pests in the mushrooms during packing, processing, or storage.
- Observation of employee practices (personal or hygiene) that have jeopardized or may jeopardize the safety of mushrooms.
- Verification of records.

Specific conditions:
- Answering "No" to any of the following questions.

| 1.1a | Does your farm have a documented food safety program manual? |
| 1.1b | Has the farm developed and implemented the food safety plan? |
| 4.2a | Are there adequate facilities for the number of employees? |
| 6.29 | Are appropriate measures taken to maintain aseptic conditions? |
| 7.1a | Are all cutting, cutting, and polishing equipment approved for use on mushroom farms, and are they maintained according to the instructions? |
| 9.11 | Are all equipment and facilities properly maintained? |
| 11.1b | Are all pest control devices placed so as not to contaminate product, packing materials, or other equipment? |
| 11.1c | Are the equipment and facilities properly maintained? |

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Future Research

• Microbial ecology of casing soils
  – More studies needed to understand basic microbial interactions in casing soil
  – General microbial inhibition vs. Formation of “antagonists”
  – Studies needed that take into effect the presence of actively growing Agaricus mycelium
  – Effect of partial replacement of traditional “light” peat with “dark” peat
  – Effect of sanitizing agents in irrigation water on microbial populations in casing soil and mushrooms
Future Research

• Disinfection treatments on mushrooms sold whole and prior to slicing
  – Effect on surface pathogens of whole mushrooms
  – Can microbial growth on sliced mushrooms be suppressed by pre-slice washing?
  – Chemically disinfection of sliced mushrooms?

• Physical treatments
  – Combined effect of UV induced Vitamin D formation and reduction in microbial populations

• Ionizing radiation
Current Research
Giorgi Mushroom Company Fund for Mushroom Research

• Microbial Ecology of Casing Soils and Food Safety Interventions to Reduce Mushroom Contamination Risks

• Rachel O’Patchen
M.S. student
Microbial Ecology of Casing Soils and Food Safety Interventions to Reduce Mushroom Contamination Risks

• Objective 1: Determine the fate of human pathogens in casing soil when held under commercial growing conditions
  – What is the effect of actively growing *Agaricus* on pathogen survival?
  – Does replacement of light (blonde) peat with dark peat affect pathogen survival?
Objective 2: Determine the effect of supplementing irrigation water with sanitizers on pathogens in inoculated casing soils and on mushrooms grown on inoculated soils.

– What is the effect of hydrogen peroxide in mushroom irrigation water on pathogens in casing soil and on mushrooms?

– Will the combined effect of pathogen reduction during the casing (pinning time interval + sanitizer irrigation) permit low levels of pathogens initially present in casing soils?
Mushrooms September 8 2009

#5 #7 2.5 compost 1.5 casing
#6 #8 2.5 compost 2.5 casing
#1 #3 3.5 compost 1.5 casing
#5 #7 3.5 compost 2.0 casing
#1 #3 4.4 compost 1.5 casing
#5 #7 4.4 compost 2.0 casing

All were scratched except those marked “u”
Upcoming study

• Longitudinal Monitoring of *Listeria monocytogenes* Contamination Patterns in Mushroom Farm
  – Monitor for contamination over a 1 year period
  – Identify persistent and sporadic strains using molecular subtyping techniques
  – Determine if disease producing strains are present
Resources

• http://foodsafety.psu.edu/mush/food safety.htm

• http://www.mgap.org/
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