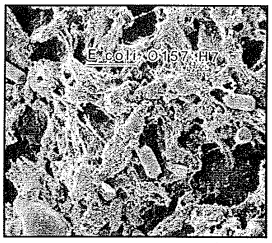
Dry Fermented Sausage and *E. coli* O157:H7



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Cover: Rod shaped bacteria, *Escherichia coli* O157:H7, attached to ground beef (magnification x 15,000). Electron micrograph image provided by T. S. Schwach and E. A. Zottola, Department of Food Science and Nutrition, University of Minnesota.

UPDATE ON DRY FERMENTED SAUSAGE *ESCHERICHIA COLI* O157:H7 VALIDATION RESEARCH

An Executive Summary Update
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DRY FERMENTED SAUSAGE AND *E. COLI* O157:H7

INTRODUCTION

In December 1994 an outbreak of foodborne disease caused by the enterohemorrhagic *Escherichia coli* O157:H7 was linked to the consumption of one of the oldest foods known to mankind — dry fermented salami.

The Blue Ribbon Task Force on *E. coli* O157:H7 at the National Cattlemen's Beef Association (NCBA) responded by evaluating the immediate research needs to address this new problem. The task force and other industry scientists agreed that research was needed regarding the survival/destruction of *E. coli* O157:H7 in dry and semi-dry fermented sausage products.

Within days, a request for proposals to validate various process parameters was sent to 16 research organizations. The response period was short and the Christmas holidays were approaching. Of the five proposals received, the one from the University of Wisconsin's Food Research Institute (FRI) was selected based on scientific merit, experience, turn-around time and budget projections.

A research team worked with the primary researchers, John Luchansky, Ph.D., Charles Kaspar, Ph.D., and Eric Johnson, Ph.D., to "fine-tune" the protocol. The team was comprised of Forrest Dryden, Ph.D., and Daniel Brown, from Hormel Foods Corp.; Bruce Tompkin, Ph.D., and Lee Christiansen, Ph.D., from Armour Swift-Eckrich; John Cerveny, from Oscar Mayer Foods Corp.; John Piccetti, from Columbus Salame Company; and Larry Hand, Ph.D., from Diversitech, Inc.

The purpose of the research team was to make sure the research would meet as much of industry's needs as possible. This team has continued to be actively involved in research reviews and consultations.

Prior to the project's initiation, two important requirements needed to be met — undergoing a U.S. Department of Agriculture Food Safety Inspection Service (USDA/FSIS) review and locating a funding source. By February 1995, the project was underway. The informal review by USDA/FSIS was favorable and a campaign for funding had identified enough dollars to begin the project. The overall coordination and support of the research has been an outstanding example of industry, government and scientific cooperation in finding a solution to a public health challenge. Contributors to the research funding are listed in Table 1.

TABLE 1.

CONTRIBUTORS TO DRY FERMENTED SAUSAGE *E. COLI* O157:H7 VALIDATION RESEARCH

American Association of Meat Processors
American Meat Institute
Armour Swift-Eckrich
Beef Industry Council, Meat Board
California Beef Council
Diversitech, Inc.
Doskocil Foods
Hormel Foods Corp.
National Meat Association
Organon Teknika Corp.
Oscar Mayer Foods Corp.
Pork Industry Group, Meat Board
Columbus Salame Company
Viskase Corp.

OBJECTIVES

The purpose of the research has been to provide industry and public health authorities with the scientific information needed to validate multiple procedures for control of *E. coli* O157:H7 in dry and semi-dry fermented sausage. Portions of the research could then be applied by industry in "like procedures" to eliminate excessive validation studies.

PROJECT OUTLINE

Realizing that many variations exist in processing of dry and semi-dry fermented sausages, the research team attempted to identify the processing parameters that were most common in industry.

The project was conducted in two phases. Phase I evaluated acid sensitivity. Low (4.4-4.6) and high (5.0-5.3) pH targets were evaluated at three fermentation temperatures (70°F, 90°F, and 110°F). *Lactobacillus plantarum* was employed as the starter culture for the 70°F fermentation, whereas *Pedicoccus acidilactici* was used for both the 90°F and 110°F fermentation (see Figure 1, page 7).

Phase II dealt with heat sensitivity. For each of the pH and fermentation temperature combinations, the product was either not cooked, cooked or held at the fermentation temperature after the target pH was reached. The cook was a modified Method 7 as shown in Table 2. Other variables studied included casing size (small 55 mm and large 105 mm) and state of drying (moisture/protein ratios of 2.3, 1.9 and 1.6).

In all validation protocols, a five-strain mixture of *E. coli* O157:H7 was inoculated into the raw batter at levels of at least 10^7 cfu/gm. Each process run was conducted in triplicate. The USDA/FSIS challenge study design recommendations were followed precisely.

TABLE 2.

COOKING PROTOCOL*	
Large Diameter	Small Diameter
1 h @ 100°F**	1 h @ 100°F
1 h @ 110°F	6 h @ 125°F
1 h @ 120°F	
7 h @ 125°F	

*house temperatures
**omitted for 110°F fermentation

RESULTS

The research consists of approximately 30 trial runs with more than 170,000 pieces of data. Results listed in Tables 3, 4 and 6 are those the research team felt were most significant and should be provided to industry and USDA/FSIS as soon as possible. Although all processes yielded an average of at least a 2D decrease in pathogen numbers, some did not achieve the desired 5D reduction.

Table 3 gives examples of the processes that are shown to cause less than a 5D reduction in *E. coli* O157:H7. This information is useful as follows:

1. There may be no need to validate these or less stringent processes (higher pH, lower fermentation temperature, no starter culture, etc.).
2. The processes provide some lethality to *E. coli* O157:H7 and, modified or used in combination with other process controls, could be used to collectively create a 5D control (see Table 6, page 8).

TABLE 3.

EXAMPLES OF PROCESSES THAT YIELD LESS THAN A 5D REDUCTION OF *E. COLI* O157:H7

- Ferment at 70°F to pH 4.6 and dry or hold at 70°F for 7 days then dry (small casing). See Figure 2, page 10.
- Ferment at 90°F to pH 4.6 and hold at 90°F for 7 days then dry (large casing).
- Ferment at 90°F to pH 5.3 and hold at 90°F for 7 days then dry (large casing).
- Ferment at 110°F to pH 4.6 and dry (small and large casing). See Figure 3, page 10.

Table 4 should provide the validation information needed by industry for like processes and processes more severe (lower pH, higher cook, etc.).

TABLE 4.

EXAMPLES OF PROCESSES THAT YIELD A 5D OR MORE REDUCTION OF *E. COLI* O157:H7.

- Ferment at 90°F to pH 5.3 and apply cook, then dry for ≥ 7 days (large casing). See Figure 4, page 11.
- Ferment at 90°F to pH 4.6 and hold at 90°F for ≥ 6 days (small casing).
- Ferment at 90°F to pH 4.6 and apply cook (small and large casing).
- Ferment at 110°F to pH 4.6 and hold at 110°F for ≥ 4 days (small and large casing). See Figure 5, page 11.

Table 6 (page 8) summarizes the *E. coli* O157:H7 reduction for the various processes evaluated in this project. This information on the actual log reduction of certain processes may be very useful in evaluating certain operations. The USDA/FSIS has indicated that a process less than the 5D could be used, if appropriate quality control programs could ensure the use of high quality raw ingredients.

Table 5 may be useful in evaluating greater (more severe) or lesser processes when evaluating a lower or higher risk. The risk evaluation is based on results of the July 11, 1995 meeting with USDA/FSIS.

TABLE 5.

PROCESS/STEP	RISK
1. Heat Processed	Lower
2. High pH	Higher
3. Beef Ingredient	Higher
4. High Initial Coliform Count — Ingredient	Higher
5. Low Water Activity/ Moisture — Protein Ratio	Lower
6. Low Fermentation Temperature	Higher

As another component of this study, the effectiveness of the EHEC-Tek™ (Organon Teknika) assay in detecting *E. coli* O157:H7 was evaluated on 315 of the various process samples. Results from this phase of the project indicate that the antibody-based rapid method is comparable (91 percent agreement) to the standard culture-based method that was used by FRI.

POSSIBLE APPLICATIONS

In August 1995, the USDA/FSIS clarified four options for addressing the *E. coli* O157:H7 problem in dry and semi-dry fermented sausage. As this research project has matured an additional "Option 5" has been recommended by the Blue Ribbon Task Force and accepted by USDA/FSIS. The five options are:

1. Utilize a heat process as listed in 9 CFR 318.17 (145°F for 4 min).
2. Include a validated 5D inactivation treatment.
3. Conduct a "hold and test" program for finished product.
4. Propose other approaches to assure at least a 5D inactivation.
5. Initiate a Hazard Analysis Critical Control Point (HACCP) system that includes raw batter testing and a 2D inactivation.

Each of these options will be discussed in their relation to this validation research summary.

Option 1. The inclusion of a heat process as described in 9 CFR 318.17 has no application to this summary. The processor need only provide documentation of the heat process.

Option 2. The validation of 5D inactivation processes was the purpose of this research. Using a 5D process as described in Table 6 will satisfy the requirement for validation.

Option 3. "Hold and Test" involves finished product testing, requires no knowledge of raw ingredients or the process, and is expensive. Testing finished product as the only means of assuring safety is contrary to the philosophy of HACCP. However, future research on the compositing of multiple sub-samples into larger analytical samples could reduce the cost of testing and provide a useful HACCP verification tool (see Additional Research Needs, page 5).

Option 4. Showing alternatives for a 5D control could include data from this research. Any proposed combinations that demonstrate a collective 5D control would require precise documentation.

Option 5. A new option recommended by the Blue Ribbon Task Force and accepted by USDA/FSIS involves a HACCP plan combined with Good Manufacturing Practices (GMPs) for fermented sausage. This option combines raw batter testing and documentation of at least a 2D lethality of *E. coli* O157:H7 between stuffing and shipping.

Option 5 offers the most practical solution to assuring the safety of certain dry fermented sausage products. Several key points, however, must be considered by each processor prior to implementation:

- An analytical method equivalent to that used by USDA/FSIS must be implemented in the raw batter testing.
- The sample size and compositing procedure must ensure a detection level of 1/gram. Further research is needed to establish the limits of compositing. In the interim, it is recommended that fifteen, 25-gram samples be taken from across the lot. These could then be composited into five, 75-gram analytical samples.
- The definition of a "lot" for the purposes of sampling must be statistically sound.
- GMPs must be applied.
- Further data is needed to define more clearly the minimum 2D process. Processors with data that validates that their process provides a 2D destruction between stuffing and shipping have met the requirement.
- As in the case of options 1 through 4, the process must address *Salmonella, Trichinella* and *Staphylococcus*.
- A procedure for dealing with lots from positive batter samples must be defined in the HACCP plan. At a minimum, all positive lots must be subjected to conditions that will provide a total 5D process.

In all cases related to the production of dry and semi-dry fermented sausage, it is the processors' responsibility to ensure the food safety of their products.

ADDITIONAL RESEARCH NEEDS

Several additional research needs were identified by the working group. These included:

1. The influence of water activity, fermentation at 75°F to a pH of 4.8 ± 0.2, and refrigeration on the inactivation of *E. coli* O157:H7.
2. The feasibility of compositing larger samples for "hold and test" programs.
3. The combination of the data from this research project with the Agricultural Research Data (ARS) data on microbial modeling for *E. coli* O157:H7.
4. The influence of other starter cultures and ingredients on the destruction/survival of *E. coli* O157:H7.
5. The review of similar research being conducted internationally.
6. The role of irradiation technology as applied to ingredients and/or the finished product.

The two most immediate needs identified were objectives 1 and 2.

Option 5 will require a negative test of 375-gram of raw batter from stuffing plus a process that will ensure a 2D inactivation. Thus, more data are needed to show inactivation for various fermentation and drying conditions necessary for consistent 2D inactivations.

It was generally agreed at the July 1995 USDA/FSIS Industry Review that dry fermented sausage fell into case 13 or 14 of the International Commission on Microbiological Specifications for Foods' suggested sampling plans based on type of hazards (severe, direct) and risk of hazard (not changing). Case 13 involves n = 15 (number of samples per lot) and c = 0 (number of samples positive), and case 14 involves n = 30 and c = 0.

Based on limited data provided to USDA/FSIS by the NCBA, USDA/FSIS will accept no more than three, 25-gram samples composited at one time. Traditional *Salmonella* testing involves fifteen, 25-gram samples into a single analytical unit.

If current *E. coli* O157:H7 methodology can be shown not to lose sensitivity in larger composites, USDA/FSIS will consider larger composites. The current hold and test requirement involves 15 or 30 analytical samples. The recently accepted three, 25-gram samples would reduce the number of assays to five or 10. If the larger plan works for *E. coli* O157:H7 as it does for *Salmonella*, the number would be reduced to either one or two.

The economic benefits of compositing would prompt more testing of raw and finished product and provide a useful HACCP tool.

Protocols for research objectives 1 and 2 have been developed and are being reviewed. Industry funding will again be needed to finance these important research objectives.

PROGRESS SUMMARY

As this research has progressed, the following efforts have been made to review and disseminate information:

- The research concept was presented to the American Meat Institute Foundation (AMIF) briefing in Chicago on Feb. 23-24, 1995.
- Results were shared with USDA/FSIS and industry in a technical meeting hosted by USDA/FSIS in Washington, D.C. on July 11, 1995.
- In August of 1995, the research team met in Madison, Wis., to review and modify the research protocol.
- An update was provided to the National Meat Association's (NMA) board of directors meeting in Aspen, Colo., on Aug. 28, 1995 and at a special meeting of contributing members in Burlingame, Calif., on Oct. 11, 1995.
- During the American Meat Institute (AMI) convention in September 1995, two presentations were made to update industry on the progress of the project.
- The latest update meeting was held at the then National Live Stock and Meat Board in Chicago on Dec. 19, 1995. This summary is largely a result of this meeting.

Data from this research was also shared with:

- The AMI's Scientific Advisory Committee in Washington, D.C. on Feb. 1, 1996.
- The USDA/FSIS Offices of Science and Technology and Inspection Services on Feb. 6, 1996.
- The 50th anniversary meeting of the NMA in San Francisco on Feb. 15-18, 1996.
- The 22nd Annual ABC Research Technical Seminar, Gainesville, Fla., on Feb. 20, 1996.

FUTURE INITIATIVES

As the data from this project is analyzed in more detail, the information will be disseminated to user groups, such as AMI, NMA, American Association of Meat Processors (AAMP) and USDA/FSIS for further distribution. Data from this research project will be presented to:

- The 50th anniversary meeting of the FRI in Madision, Wis., on May 29-30, 1996.
- The annual meeting of the International Association of Milk, Food and Environmental Sanitarians in July 1996 in Seattle, Wash.

SUMMARY STATEMENT

The research effort described in this publication represents a very proactive effort by industry scientists to further ensure the safety of meat and meat products. The full study will be published in a peer reviewed scientific journal.

APPENDIX

TABLE 6.

SUMMARY OF SALAMI PROCESSES

Temp	pH	Process	Casing	Log Reduction of O157:H7 Each trial	Average
70°F	≤ 4.6	dry	small	(2.07, 2.32, 1.86)	2.08 ± .23
	≤ 4.6	hold	small	(2.14, 2.80, 1.83)	2.26 ± .50
	≥ 5.0	hold	small	(4.78, 5.89, 1.66*)	4.11 ± 2.1
	≥ 5.0	hold	large	(4.03, 2.42, 2.34)	2.93 ± .96
	≥ 5.0	heat	small	(5.91, 6.05, 5.54)	5.83 ± .26
	≥ 5.0	heat	large	(5.91, 5.89, 5.03)	5.61 ± .50
90°F	≤ 4.6	hold	small	(5.91, 6.91, 6.46)	6.43 ± .50
	≤ 4.6	hold	large	(4.07, 4.52, 5.57)	4.72 ± .77
	≤ 4.6	heat	small	(6.93, 6.86, 6.46)	6.75 ± .26
	≤ 4.6	heat	large	(6.58, 6.91, 6.46)	6.65 = .23
	≥ 5.0	hold	large	(2.55, 3.27, 2.80)	2.87 ± .37
	≥ 5.0	heat	large	(5.81, 6.65, 6.84)	6.43 ± .55
110°	≤ 4.6	dry	small	(2.66, 2.90, 1.78)	2.45 ± .59
	≤ 4.6	dry	large	(2.00, 2.02, 2.35)	2.12 ± .20
	≤ 4.6	hold	small	(6.59, 6.51, 5.93)	6.34 ± .36
	≤ 4.6	hold	large	(6.46, 6.51, 6.28)	6.42 ± .32
	≥ 5.0	hold	small	(5.21, 6.50, 5.92)	5.88 ± .65
	≥ 5.0	hold	large	(5.91, 6.50, 5.69)	6.03 ± .42
	≥ 5.0	heat	small	(6.11, 3.09*, 5.77)	4.99 ± 1.66
	≥ 5.0	heat	large	(4.91, 3.41*, 5.53)	4.52 ± 1.09

* Smokehouse records are being evaluated for possible process deviations.

FIGURE 1.

SAUSAGE MANUFACTURE

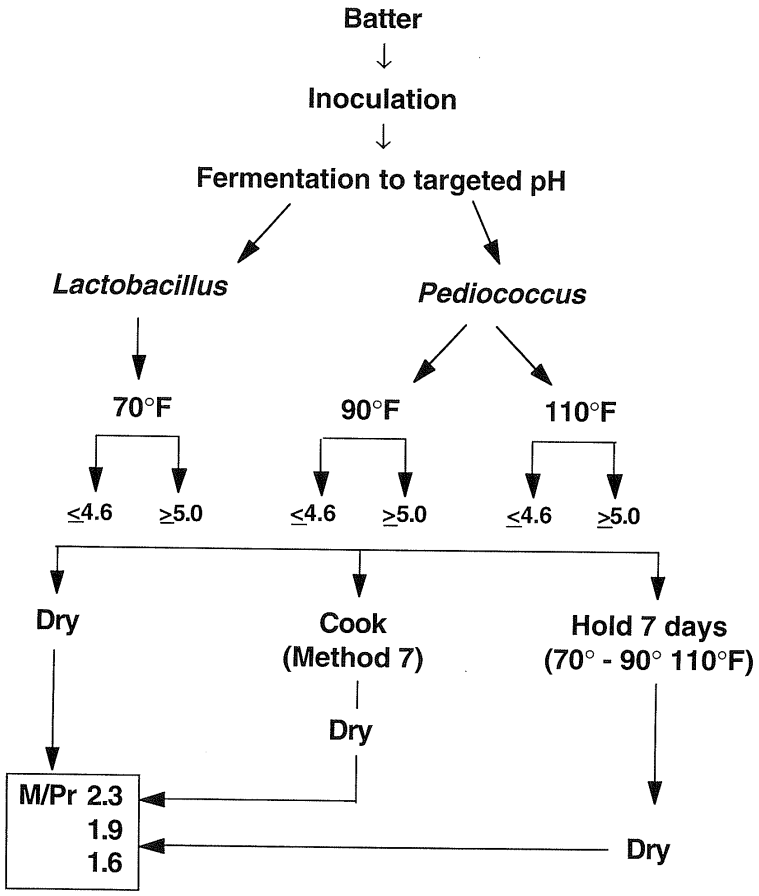


FIGURE 2.*

FERMENT AT 70°F TO PH 4.6 AND DRY OR HOLD AT 70°F FOR 7 DAYS THEN DRY (SMALL CASING).

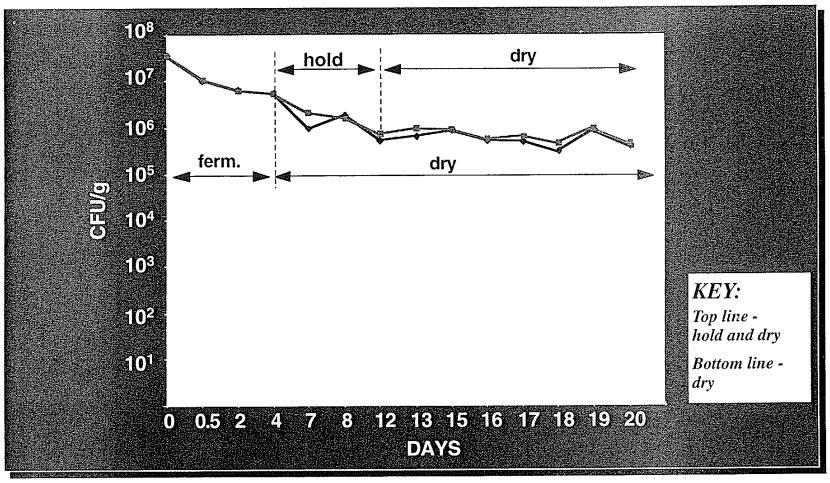
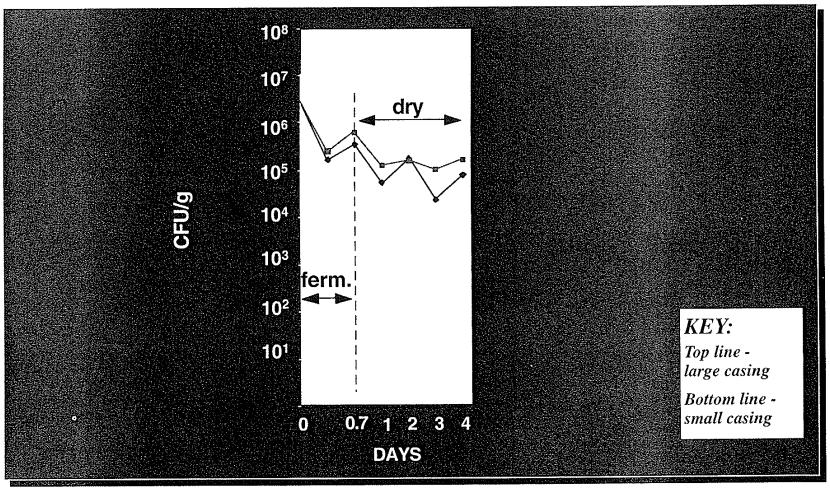


FIGURE 3.*

FERMENT AT 110°F TO PH 4.6 AND DRY (SMALL AND LARGE CASING).



*Figures are for illustration only.

FIGURE 4.*

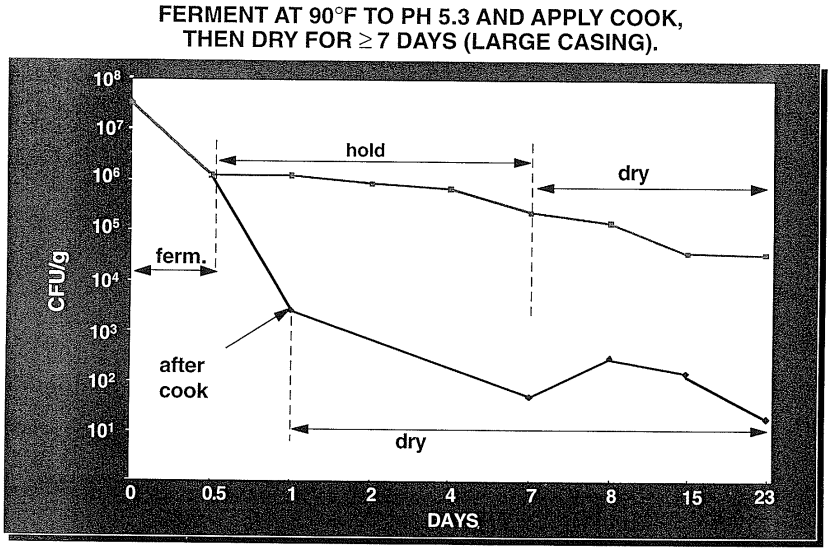
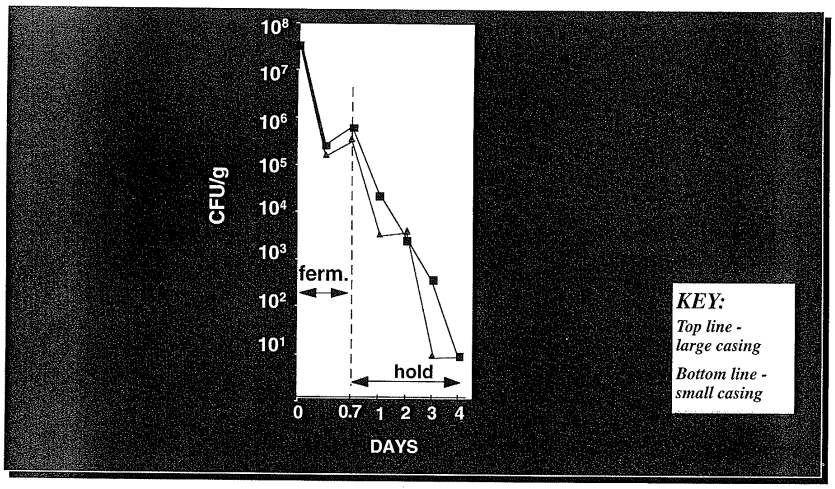


FIGURE 5.*



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