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BARD

FINAL REPORT

PROJECT NO. US-865-84

Development of Lactic Cultures to Produce Fermented Mutton and Poultry Sauages

B. Ray, R.A. Field, M.C. Johnson, B. Juven

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BARD project No. US-865-84

Title

Development of lactic cultures to produce fermented mutton and
poultry (turkey) sausages

Investigators' Names
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Project's starting date: September, 1985

Type of Report: 1st Annual _____ 2nd Annual _____ Final XX

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הספריה המרכזית
למדעי החקלאות
ב'ח-711

Meat-Preservation
Sausages
Fermentation
Bacterial starter cultures

630.72
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II. Abstract

The major objective of this project was to develop self-stable high quality fermented sausage products from low quality, perishable mutton and turkey mechanically deboned meat (MDM). To achieve this goal the following areas were studied: selection of starter cultures to be used in the fermentation of mutton and turkey MDM; study genetic control and physiological parameters associated with some important traits in these starter strains; and develop processing and storage parameters for the production of mutton and turkey fermented sausages. Results suggested that selection of a proper culture should be based on rate of acid and flavor compounds production in sausage mix, levels of H_2O_2 and biogenic amine production during storage and also ability to produce specific bacteriocins that are capable of inhibiting growth of many spoilage and pathogenic bacteria. This later trait is present in some strains, and is plasmid linked. Processing parameters were developed initially to produce stick and bologna types products. But the taste panelling data indicated that a stick type product would be preferred over bologna type product as a snack food. These products were self-stable and at room temperature retained their quality for 120 days.

III. Objectives

A. INTRODUCTION

In the United States, Israel and some other countries, mutton and turkey meat, especially mechanically deboned (MD) meat, are low in value compared to red meat and poultry products. Undesirable flavor and shorter shelf-life are major reasons for this lower value. Both the flavor and spoilage problems can be overcome by producing desirable fermented products (such as fermented sausages) from mutton and MD-

turkey. The fermented products may have a market, especially in countries where slight mutton flavor is not objectionable and refrigeration to extend shelf-life of fresh meat is lacking. In addition, these products could be good sources of animal protein in many developing countries.

The desirable fermented mutton and turkey sausages can be produced through: (1) formulation of the sausage mixes in relation to protein, fat and spices; (2) use of suitable starter cultures that not only produce acid and flavor compounds in the sausage mix but also have the ability to inhibit growth of spoilage and pathogenic bacteria; (3) producing final products that have long shelf-life.

These aspects were studied under this project.

B. OBJECTIVES OF ORIGINAL RESEARCH

The objectives of this proposal were:

1. Survey of strains of Pediococcus species and Lactobacillus plantarum for some desirable and undesirable metabolic traits for their selection as starter cultures in sausage fermentation.
2. Studies on the plasmid controls of some desirable and undesirable traits in selected starter cultures.
3. Development of parameters for sausage mix formula, fermentation steps, subsequent processing steps and storage conditions of the finished products.

IV. BODY OF THE REPORT

1. Survey of strains of *Pediococcus* species and *Lb. plantarum* for the stability as starter cultures in sausage fermentation.

a. Carbohydrate metabolism patterns

A total of 50 strains (*P. acidilactici*, 6; *P. pentosaceus*, 14 and *Lb. plantarum*, 30) were tested for utilization of different carbohydrates. These cultures were obtained from the following sources: own stock collections, different research laboratories in the U.S., Israel and other countries, and commercial culture manufacturers in the U.S. and Europe. Most of the strains showed species specific carbohydrate fermentation patterns; however, a few strains in each species showed some variation. In general, the strains fermented glucose, fructose and galactose; all *Lb. plantarum* and *P. pentosaceus* fermented lactose; sucrose fermentation varied with strain.

These results showed that glucose and fructose can be used in the sausage mix in fermentation with any of the above strains. Suitability of use of lactose and sucrose will be dependent upon a particular strain.

b. Rate of acid production of selected strains of *Pediococcus* and *Lactobacillus plantarum* species.

(i) Rate of acid production in broth containing glucose:

A total of 8 *P. pentosaceus*, 6 *P. acidilactici*, and 7 *Lb. plantarum* strains were examined for the rate of acid production (by measuring reduction in pH) in a nutritional

broth supplemented with 1% glucose. To determine the influence of growth temperatures, strains were grown at 7 different temperatures between 20 to 45°C.

The time requirements to reduce the initial pH of 6.5 to final pH 4.8 to 5.0 at different growth temperatures varied considerably with species and strains. Among the pediococci 10 strains reduced pH to 4.8 to 5 within 4 h at 45°C, at 40°C 8 reduced with 4 h, at 35°C all reduced within 5 h, at 30°C 10 reduced within 6 h, at 25°C 10 reduced within 8 h and at 20°C 8 reduced within 24 h. Among the Lb. plantarum strains at 40°C 5 reduced in 8 h, at 35°C all reduced in 8 h, at 30°C all reduced in 10 h, at 25°C 3 reduced in 12 h and at 20°C all required 24 h.

These data suggested that at a given incubation temperature strains differ in their rate of acid production. Thus, selection of strain(s) for use in sausage fermentation should be based on the ability of a strain to reduce the pH at a specific temperature within a given period of time.

(ii) Growth of selected strains in broth containing spice mix (to be used in sausage).

Selected strains of Pediococci and Lb. plantarum strains were grown in 1% glucose broth supplemented with spice mixtures (Table 1), in concentrations to be used in sausage fermentation, at 30 and 37°C and examined for rates of acid production. The cultures reduced the pH to

4.8 to 4.9 within 10 h incubation at 30 and 37°C.

(iii) Rate of acid production in meat and spice mixture.

Selected strains of Pediococcus and Lb. plantarum were inoculated in ground mutton containing dextrose and spice mixtures at a level of 10^6 cells/g, incubated at 30 and 37°C, and monitored for drop in pH. At 30°C pH dropped from original 5.9 to 4.9 in 16 h by P. acidilactici and in 20 h by Lb. plantarum. At 37°C, P. acidilactici reduced the pH in 8 h but Lb. plantarum took about 16 h.

In meat mixtures, compared to broth, the starter cultures took longer time to reduce the pH to 4.8 to 4.9.

c. Production of organic acids and volatile compounds by meat starter cultures.

Methods to detect organic acid and volatile compounds produced by meat starter cultures by HPLC was developed. Main metabolic products were found to be lactic, acetic and pyruvic acids. The presence of spice mix in the turkey meat (MDM) did not affect the production of these acids. Detailed results are included in Appendix 1.

d. Production of hydrogen peroxide by meat starter cultures.

A total of 12 strains of Pediococcus spp and Lb. plantarum were tested for their production of hydrogen peroxide. All the strains produced H_2O_2 in vitro; however, the amounts varied widely among the strains.

Results of these studies are presented in Appendix 2 (The article has been accepted for publication in J. Appl. Bacteriol.)

e. Production of biogenic amines by meat starter cultures.

Selected pediococci and Lb. plantarum strains were used to determine their ability to produce biologically active amine during growth in bacteriological media and in sausage mix. The levels of biogenic amines produced by these starter cultures were very low and below the levels that is considered health hazard. These results are presented in Appendix 3 (paper in preparation for publication).

f. Production of large volume of cultures in a fermentor.

For inoculation in sausage mix we need to grow starter cultures at relatively large volumes. Selected strains of Pediococcus species and Lb. plantarum were grown in table top fermentor in broth supplemented with 1% glucose. The influences of rate of agitation, growth temperatures, constant pH, partial oxygen pressure and flow of nitrogen and air on growth and acid production were monitored. Viable cell count, optical density and acid production at selected intervals were measured. At 40°C P. acidilactici grew much faster while Lb. plantarum and P. pentosaceus grew better at 30 to 35°C.

2. Plasmid control of some desirable traits.

a. Occurrence of plasmids in *Pediococcus* species and *Lb. plantarum*.

A total of 10 *Pediococcus* species and 7 *Lb. plantarum* strains were examined for the presence of plasmid by modification of the current isolation method. All *Lb. plantarum* strains were found to harbor plasmids but some *pediococci* strains did not have plasmid (Appendix 4).

b. Production of bacteriocin by the meat starter cultures.

A total of 12 *P. pentosaceus*, 6 *P. acidilactici* and 15 *Lb. plantarum* strains were examined for the production bacteriocin against three indicator strains of lactic acid bacteria. Four *P. acidilactici* and one *Lb. plantarum* were found to inhibit growth of one or more strains of the indicators.

c. Physical and chemical characteristics of bacteriocin from different producing strain.

The inhibitory compounds were protein in nature, active at wide ranges of pH, after heat treatment, refrigeration and freezing. Purification from one strain revealed that it is a small peptide of about 2700 Md. Results are presented in Appendices 5,6,7,8 (one paper has been published, two have been accepted, another one is in preparation).

d. Host range and mode of action.

The bacteriocins from these strains were effective against many bacteria involved in food spoilage and food related health hazard. Initial studies showed that bacteriocin from one

strain is bactericidal and probably impares the energy synthesis of the sensitive species by binding at a specific site on cell. Results are presented in Appendices 7 and 8.

e. Genetic control of bacteriocin production.

In P. acidilactici strains the genetic determinants for the synthesis of bacteriocins are plasmid linked. Mutants lacking in bacteriocin production showed the loss of a specific plasmid. Bacteriocin plasmid was also conjugally transferred from a donor to a recipient. In Lb. plantarum, mutants lacking in this trait could not be isolated, probably because the trait is chromosomally linked. Results of these are presented in Appendices 8,9,10 (one paper has been accepted for publication in J. Industrial Microbiol., one has been submitted and the other one is in preparation).

f. Other plasmid control traits.

Analysis of mutants from the P. acidilactici strains indicated that sucrose fermentation is also plasmid linked. This trait is located in one of the two plasmids of 24 and 40 Md. Efforts to determine which of the two plasmids harbor the gene for sucrose fermentation is not yet successful (Appendix 10).

In Lb. plantarum a 47.1 Md plasmid is associated with the production of extracellular polysaccharide (Mucoid characteristics)(Appendix 11).

None of the plasmids so far examined harbor gene(s) for antibiotic resistance and metabolism of several carbohydrates

that were tested.

3. Production of fermented sausages from mutton and MD turkey meat.

a. Composition of spice mixture. A spice mixture, presented in Table 1 was formulated by the researchers at the University of Wyoming and Volcani Research Institute, mostly from their experience and published information.

b. Meat composition. Mutton was obtained from ewes slaughtered at the University of Wyoming Meat Laboratory. The meat was hot boned, trimmed and frozen immediately. Depending upon the need, required amounts were thawed overnight and used. The meat was used without any added fat.

Turkey deboned meat, heart and dark meat (from thigh) were obtained from a local processor. They were frozen immediately. Before use they were thawed as above and mixed as follows: 50% deboned meat + 25% heart and 25% dark meat.

c. Selection of starter culture strains. A decision was made by the researchers from both institutes that the fermentation will be carried out at 80 to 86°F for a time not exceeding 16 h to drop the pH to 4.9 ± 0.1 . It was also decided that one strain of P. acidilactici, one strain of P. pentosaceus and one strain of Lb. plantarum will be used and if possible one of these should be a commercial culture. A commercial culture of P. pentosaceus produced by Microlife in the U.S. (lactacele 75) was selected as it fits with the set criteria. P. acidilactici H and Lb. plantarum 27 from our stock were used as they not

only meet the above criteria, but both of them are capable of producing bacteriocin that inhibited growth of many food spoilage and pathogenic bacteria (Appendices 1 to 11). They also do not produce appreciable amounts of biogenic amines and hydrogen peroxide. In addition, earlier trials indicated that they were not inhibited by the spice mixes, and produce products of desirable texture, flavor and taste.

d. Product types. Initial decision by the researchers from both institutes was to produce two types of products: a stick type product (for snack food) in a 25 mm casing and a bologna type product (for cold cuts or sandwiches) in a 65 mm casing. However, after 3 trials with both types of products from mutton and one trial with MD turkey and evaluating the taste panel data and surveying the similar types of products in the U.S. market, the researchers agreed that snack type products will have better commercial success. So in latter trials, stick type products were tested (fermented "mutton-stick" and fermented "turkey-stick").

e. Processing of fermented products. Thawed meat (mutton or turkey deboned meat + heart + red meat) was coarsely ground using a 1/2" plate, mixed with spice mixture for 3 min at $\leq 10^{\circ}\text{C}$, reground through a 1/8" plate. The mixture in 5 kg portions were mixed uniformly with one of three starter cultures (P. acidilactici H, or P. pentosaceus 75, or Lb. plantarum 27) at a concentration of 10^6 cells/g at $\leq 10^{\circ}\text{C}$ for 5 min in a mixer. Each mix was then stuffed into collagen casing

(10 mm for stick, 65 mm for bologna) using a vacuum stuffer. The weight of each product was taken and they were hung on stainless steel rods inside a computer controlled smokehouse. The products were incubated at 30°C with RH 92% and checked for pH drop. It took about 16 to 20 h for the pH to drop to 4.8 to 4.9 from initial pH of 5.9 to 6.0 of the sausage mix. The products were then cooked at 59 to 60°C internal temperature and smoked (computer operating at cook cycle and smoke cycle). Total time for this cycle was 4 h. The products were then showered with cold water for 1 h and left in the smokehouse overnight for initial drying. The next morning the products were weighed, then removed to a drying chamber at 4 to 5°C and 65% RH and left until the total weight loss had reached 45% of initial weight. The products were then cut into 8 to 10" size, vacuum-packaged and held either at 4 to 5°C or at 20 to 25°C for taste paneling and other analysis (see enclosed photographic illustration).

f. Microbiological analysis. The products were analyzed after drying for the enumeration of Staphylococcus aureus and coliforms and presence of salmonellae. A 25 g sample was blended in sterile water and enumerated for S. aureus in Vogel Johnson agar plate and for coliforms in Lauryl Sulfate tryptose broth (for MPN). The blended materials were also preenriched in lactose broth, enriched in selenite-cystine broth and streaked on XLD agar plates for salmonellae. None of the samples were positive for salmonellae (in 25 g), S. aureus and

coliforms (in 0.1 g).

g. Taste paneling. Both fermented stick and bologna samples were used for taste paneling after 50 to 60 d storage at 4 to 5°C. The products did not have any mold growth on the surface. However, in some stick products made with P. pentosaceus 75 and Lb. plantarum 27 white deposition was found on the surface which were found to be not mold but some salts.

The panelists included both trained and untrained individuals about 12 to 14 per sitting. Each product was given two times on separate days. A copy of the format used for scoring is included in Table 2. Average score for each characteristic and overall desirability were listed in Table 3 and Fig. 1 and 2. The general comments for mutton sausage were: did not taste like mutton, quite acceptable in texture, flavor and appearance, prefer the stick over bologna; for turkey product: prefer turkey sticks over mutton because of flavor and texture.

h. Physical and chemical analysis of the products. The fermented products were analyzed for pH, water activity and moisture loss at the time of vacuum-packaging (Table 4). The average values for mutton stick ranged from 4.9 to 5.0 for pH, 0.85 to .93 for A_w and 45 to 47% for moisture loss. During 120 d vacuum-storage both at 4°C and 20°C very little changes occur in these (Table 5). Also there was no major changes among the different cultures used. The analysis of the turkey stick was not completed yet.

i. Commercial production of fermented mutton stick snacks.

The increased popularity of the commercial fermented beef stick in the U.S. over last few years suggested that similar products made from mutton and turkey meat may have a good market. In an effort, a flyer was prepared for the turkey stick and was mailed to several commercial turkey processors in Israel and the U.S. (Appendix 12).

Table 1. Composition of spice mixture used in sausage.

Composition	Amount/5kg meat
Salt	140.0 g
Dextrose	35.0 g
Black pepper (ground)	17.0 g
Cardamon (ground)	0.6 g
Nutmeg (ground)	0.6 g
Mace (ground)	0.6 g
Na-erythorbate	0.6 g
Na-nitrite	12.0 g (144 ppm)
Water	450.0 ml

Photographic display of processing of fermented sausages
at the Meat Processing Laboratory at the University of Wyoming

1. Raw meat (Turkey deboned meat, heart and red meat)
2. Grinding meat through a coarse grinder
3. Grinding sausage mix (meat and spices) through a fine grinder
4. Stuffing sausage mix (meat + spices + starter culture) in 25 mm casing for snack type products
5. Stuffing sausage mix for bologna (65 mm casing)
6. Hanging sausages in steel hanger
7. Computerized Smokehouse
8. Sausages were put inside Smokehouse for fermentation and cooking
9. Sausages after cooking and showering
10. Fermented sticks are cut into 8 to 10" sizes after drying
11. Vacuum-packaging of fermented sticks
12. Vacuum-packaged finished products (first three from left are turkey sticks, last one on the right are mutton sticks)
13. Taste paneling of the fermented sticks.

Table 2. Score card used for Taste Paneling (0 to 6)

Name _____ Date _____

Sample No. _____

ACID FLAVOR (TANGINESS)

extremely
bland

extremely
intense

MUTTON FLAVOR

extremely
bland

extremely
intense

TEXTURE

lacks
objectionable
fat

extremely
greasy

APPEARANCE

attractive
color

unattractive
color

OVERALL DESIRABILITY

extremely
desirable

extremely
undesirable

OTHER COMMENTS:

Table 3. Taste panel data of the mutton stick and bologna^a

Cultures used and product type	Acid flavor	Mutton flavor	Texture	Appearance	Overall acceptance
<u>Ped. acidilactici</u> H					
Stick	3.5 $\pm$ 1.1	2.5 $\pm$ 1.0	2.0 $\pm$ 0.9	2.5 $\pm$ 1.1	2.4 $\pm$ 0.9
Bologna	3.4 $\pm$ 1.1	2.7 $\pm$ 1.4	2.4 $\pm$ 1.0	2.0 $\pm$ 0.9	2.6 $\pm$ 1.0
<u>Lb. plantarum</u> 27					
Stick	3.5 $\pm$ 0.9	2.7 $\pm$ 1.1	2.1 $\pm$ 0.9	2.8 $\pm$ 1.2	2.5 $\pm$ 0.9
Bologna	3.3 $\pm$ 1.0	3.4 $\pm$ 1.1	2.6 $\pm$ 1.0	2.6 $\pm$ 1.1	2.7 $\pm$ 1.1
Lactacel 75					
Stick	3.4 $\pm$ 0.9	2.7 $\pm$ 1.0	2.2 $\pm$ 0.9	2.9 $\pm$ 1.2	2.4 $\pm$ 0.9
Bologna	3.5 $\pm$ 1.0	2.3 $\pm$ 1.0	2.7 $\pm$ 1.1	2.1 $\pm$ 0.9	2.6 $\pm$ 1.0

^aMedium value for acid flavor and lower values for all other characteristics, in a scale of 0 to 6, are desirable.

Fig. 1. Taste panel score.

Fig. 2. Taste panel score.

FERMENTED BALOGNA

MADE: 6/10, TESTED: 8/6 & 8/10

Table 4. Some physical characteristics of the final products (after drying and before vacuum-packaging).

<u>Culture used</u>	<u>Product type</u>	<u>pH</u>	<u>A_w</u>	<u>% weight loss</u>
<u>P. acidilactici</u> H	Mutton stick	5.0	.85	46
	Turkey stick	4.9	.91	45
<u>P. pentosaceus</u> 75	Mutton stick	4.9	.88	47
	Turkey stick	4.9	.92	45
<u>Lb. plantarum</u>	Mutton stick	4.9	.90	46
	Turkey stick	4.9	.93	46

Table 5. Proximate analysis of the fermented sausage.

Product type	Culture used	Time of Storage	Moisture	Protein	Fat	Ash
Raw mutton	-	-	68.0	21.5	12.2	1.0
Seasoned mix	-	-	67.0	18.7	12.0	3.3
Fermented stick	<i>E. acidilactici</i> H	0 d	38.6	33.0	23.0	6.0
		120 d, 4°C	39.4	31.9	22.2	6.0
		120 d, 20°C	37.7	31.6	23.4	6.1
	<i>E. pentosaceus</i> 75	0 d	39.2	33.3	22.4	6.0
		120 d, 4°C	40.6	31.4	22.7	5.8
		120 d, 20°C	38.8	31.6	22.3	6.1
	<i>Lb. plantarum</i> 27	0 d	37.8	34.7	23.6	5.9
		120 d, 4°C	39.5	31.8	22.2	5.7
		120 d, 20°C	36.1	33.2	24.1	5.9
Average (3 cultures)		0 d	38.5	33.7	23.0	6.0
		120 d, 4°C	39.8	31.7	22.4	5.8
		120 d, 20°C	37.5	32.1	23.3	6.0

Results for turkey stick are not completed yet.

V. Cooperation

The research proposal was developed by the principal and cooperating investigators. After the project was funded the researchers from both countries read the reviewers comments and made necessary changes in the research program. During research activities the researchers through letters and phone calls discussed the research findings and the future direction of research. Dr. Juven from Bet Dagan visited the University of Wyoming two times (1986 & 1987). The researchers not only trained each other some research techniques, but also made decision, especially in the area of product development, culture selections, testing parameters, etc. In addition, the researchers exchanged starter cultures to conduct studies in the area of their expertise as well as exchanged results between the two laboratories. Dr. Juven, during his last visit at the University of Wyoming Meat Lab undertook the processing of fermented turkey stick in our modern facility.

VI. Benefit to Agriculture

Mutton in the U.S. and mechanically deboned turkey meat in Israel as well as in the U.S. are low value products. Undesirable flavor and shorter shelf-life, especially for turkey meat, are often the reasons of their reduced value. In many developing countries lack of refrigeration may also contribute to the short shelf-life and lower cost of these products.

The project was aimed in the production of fermented meat products using these low priced raw materials for the following reasons: (a) to make self-stable products which will not need refrigeration but yet will have long shelf-life, (b) convert the low priced raw material into high priced fermented products, (c) produce products that are high in good quality protein and can be enjoyed as snack food, especially by the growing children in the developing

countries, (d) use system to ensure safety of the product and (e) undertake studies on physiological criteria and genetic control of some important traits in the starter cultures to be used in the processing of the fermented products.

Through our studies we have been able to determine the factors to be considered in selecting cultures for their use in meat fermentation. We have also shown how certain strain of cultures are capable of producing bacteriocins, that have inhibitory effects on many undesirable bacteria including those that are associated with food spoilage and food related health hazard. Use of these specific strains will be an advantage to control the growth of undesirable bacteria during fermentation of sausages. We have also been able to develop processing and storage parameters for the fermented sausages from mutton and turkey MDM and we believe that both mutton and turkey stick can be popular if commercially available.

VII. List of Publications

1. Bhunia, A.K., M.C. Johnson and B. Ray. 1987. Direct detection of an antimicrobial peptide of P. acidilactici in SDS-PAGE. J. Industrial Microbiol. 2:319. (2 copies enclosed).
2. Bhunia, A.K., M.C. Johnson and B. Ray. 1988. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by P. acidilactici H. J. Appl. Bacteriol. (accepted)
3. Ray, S.K., M.C. Johnson and B. Ray. 1988. Plasmid associated bacteriocin activity in P. acidilactici strains. J. Industrial Microbiol. (accepted).
4. Ray, S.K., W.J. Kim, M.C. Johnson and B. Ray. 1988. Conjugal transfer of a plasmid associated with bacteriocin production and immunity in P. acidilactici H. J. Appl. Bacteriol. (submitted).
5. Kim, W.J., M.C. Johnson and B. Ray. 1988. Genetic control of mucoid characteristics and bacteriocin production in Lb. plantarum 27. (In preparation).

6. Wu, C., R.A. Field, J. Busboom and B. Ray. 1988. Physical and chemical characteristics of fermented mutton sausage during storage (In preparation).
7. Harduf, Z., T. Nir and B.J. Juven. 1988. HPLC of biogenic amines, derivatized with 9-Fluorenylmethyl chloroformate. J. Chromatography. 437:379-386. (2 copies enclosed).
8. Juven, B.J., H. Weisslowicz and S. Harel. 1988. Detection of H₂O₂ produced by meat lactic acid bacteria. J. Appl. Bacteriol. (accepted).
9. Juven, B.J. 1988. Detection of biogenic amines in fermented meat sausage. (In preparation).

APPENDIX 1

2.2 Production of organic acids and volatile compounds by lactic starter cultures

INTRODUCTION

The traditional process of meat fermentation relied on the "natural" fermentation performed by the inherent flora of lactic acid-producing bacteria, or by back-inoculating a portion of recently fermented meat into the freshly prepared batch. With the development of starter cultures for the sausage industry, more uniform production methods against failures due to the occurrence of off-flavors, gassiness, proteolysis and lack of acid production were realized (Everson, 1971; Deibel, 1974).

Suppliers of starter cultures for sausage fermentation recommend the use of at least 0.75% glucose in meat mixtures for proper culture growth. Other carbohydrates, primarily sucrose and corn syrups, are suggested ingredients in some formulations for various fermented sausages. Tandler (1963) stated that the quantity of sugar needed depends on the type of sugar added, the curing substances used and the ripening process followed. About 1% sugar is needed to yield a reduction of 1 pH unit.

European-style manufacturers have maintained lower temperatures and extended fermentation schedules. Straight lactic acid production is minimized, and since lower fermentation temperatures are employed, food pathogens are not a significant problem.

The main product of carbohydrate fermentation in ripening sausages is lactic acid. Fermentation products other than lactic acid (e.g. acetic, butyric and propionic acids, ethanol, acetoin,

2,3-butane-diol and diacetyl), if formed, are only formed in minor amounts.

The variety and amount of added carbohydrate influences the course of the lactic acid formation during sausage fermentation. With glucose, sucrose or maltose, the rate and extent of lactic acid production is higher than with lactose, starch and dextrin (Klettner and List, 1980). A slower rate of lactic acid formation is desirable during the manufacture of many high-quality dry sausages because acid-sensitive bacteria contributing to flavor and curing color are enabled to "keep up" with the lactobacilli to some extent. The fermentation of slowly degradable carbohydrates usually stops at higher pH values because the water activity decreases during prolonged fermentation and becomes inhibitory to lactic acid formation at values of about 0.91 (List and Klettner, 1978)

MATERIALS AND METHODS

Test organisms and inoculum preparation

Lactobacillus plantarum (strain # 27), was used in this study. It was grown in APT broth at 20°C, the cells harvested at mid-logarithmic phase and washed with physiological saline. Inocula were prepared by suspending the cells in saline; the suspensions were standardized to approximately 10^8 colony-forming units (cfu)/ml.

In-vitro studies

In-vitro growth and fermentation studies were carried out in APT broth at 20°C, using a computerized bio-reactor. These experiments allowed us to study, prior to product development work: (a) growth and acids/flavor compounds production; (b) biogenic amines production. They also permitted us to produce the amounts of bacterial cells needed for product development work.

Meat samples

Mechanically deboned turkey meat (MDTM) was obtained from a local processing plant and stored at -40°C , before and after irradiation, until needed.

Irradiation

MDTM samples, packaged in sealed polyethylene bags, were placed in insulated boxes containing five times their weight of dry ice and sterilized by irradiation. The samples were irradiated with a ^{60}Co source at a dose of 1.4-1.8 KGy per h with totals of 25-30 KGy (2.5-3.0 Mrad).

Supplements

The samples of sterilized MDTM were supplemented with the following (amounts per kg meat): D-glucose, 7 g; sodium chloride, 28 g; sodium nitrite, 140 mg; sodium isoascorbate (erythorbate), 500 mg; manganese sulphate, 75 mg; ground black pepper, 2.5 g; ground cardamon, 500 mg; ground nutmeg, 500 mg; water, 80 g. With the exception of sodium chloride and the spices, which were added dry, the supplements were aseptically added to the meat as filter-sterilized concentrated solutions. Meat samples were prepared with one of more of the supplements. Unsupplemented meat samples were also tested. The addition of the supplements did not cause any significant changes in the initial pH of the meat (pH 6.5-6.6).

Meat inoculation and enumeration of the lactic starter culture

No viable bacteria were found in the irradiation-treated meat samples. Inocula were added to the irradiated supplemented or unsupplemented meat samples to yield a final concentration of ca. 10^5 cfu/g. Analysis of inoculated meat sub-samples showed an even distribution of the inoculum.

The inoculated samples were cooled to ca. 5°C and then stuffed into 20-mm diameter natural (collagen) casing. The products, thus prepared, were immediately stored at 20°C in an environment with

air relative humidity set at 94% by a saturated solution of potassium nitrate. Bacterial counts were performed in APT agar.

Water activity measurements

A_w measurements in meat samples were made at 20°C with a model DT Rotronic-Hygroscopic relative humidity meter (Rotronic AG, Zurich) calibrated with a saturated solution of sodium chloride (0.76 A_w at 20°C).

pH and titratable acidity

Five to 10 g of the sausage were homogenized in an Osterizer blender with 9 times its weight of distilled water and the homogenate used to measure pH and titratable acidity.

The amount of acid produced by the bacterial starter culture was determined from the amount of 0.1 NaOH required to adjust the pH to its original value.

Determination of organic acids and some flavor compounds

Efforts were made to analyze by gas chromatography, without previous esterification, the organic acids likely to be found in fermented growth media and meat samples (Carlsson, 1973; Playne, 1985; Theune, 1987). Chromosorb 101 of different mesh sizes was used, together with variations in sample preparation and operation conditions. Unsatisfactory (and irreproducible) results were obtained for the quantitation of lactic acid in standard solutions. Therefore, our efforts were conveyed to the use of high-performance liquid chromatography (HPLC) and only the results obtained with this technique are reported herein.

Sample preparation: Samples of fermented APT broth were analyzed directly after centrifugation and filtering through a 0.22 μ m membrane filter. Fermented meat samples were homogenized in a Osterizer blender with 9 times their weight of distilled water, centrifuge, perchloric acid added to the supernatant and the residual perchloric acid precipitated with KOH and removed by membrane-filtration.

HPLC standards: stock standard solutions (400 mM) were prepared in the same liquid used as mobile phase. The following compounds were tested: D-glucose, L(+) lactic acid, pyruvic acid, acetic acid, propionic acid, n-butyric acid, diacetyl, 3-hydroxy-2-butanone (acetoin). Succinic acid (with C₁₈ reverse-phase) or DL-malic acid (with Technicon resin C₃, H-form) were used as internal standards. Injected standards solutions contained 0.8 umoles of each compound.

Two HPLC columns were tested: (a) C₁₈ reverse-phase (Lichrospher RP-18, Merck), 5 um particle size, 125 x 8 mm at ambient temperature, with 0.05 M phosphoric acid with pH adjusted to 2.5 with concentrated NH₄OH as mobile phase; (b) Technicon resin C₃, (8% cross-linked cation exchange resin) in the H-form, in a water-jacketed glass column 300 x 6 mm maintained at 40°C; 0.01 N sulphuric acid as mobile phase. With both columns, a flow rate of 0.8-1.0 ml/min and a refractive index detector were used.

RESULTS AND DISCUSSION

Figures 1A and 1B show the separation of organic acids, glucose and diacetyl, from a standard solution, obtained with a C₁₈ reverse-phase and with a cross-linked cation exchange (H-form) column, respectively. Comparison of the retention times obtained is shown in Table 1.

Table 1. Chromatographic data obtained from a mixture of organic acids and specific flavor compounds (0.8 umoles each)

<u>Compound</u>	<u>Column</u>
	C ₁₈ RP Technicon

	RT ¹ (min)	RT (min)
Acetic acid	2.51	7.09
Acetoin	ND	ND
Butyric acid	18.67	10.04
Diacetyl	NT	7.02
Glucose	1.30	4.64
Propionic acid	6.06	8.17
Pyruvic acid	1.75	4.92
Lactic acid	2.28	6.43
Succinic acid	3.86	6.40
Malic acid	1.87	5.47

¹ RT= retention time

ND= not detected; NT= not tested

In contrast to results obtained with gas chromatography, no problems were encountered in the separation of organic acids in general and lactic acid in particular by HPLC. No derivatation was found to be necessary. In a comparison of several organic acids, succinic acid was selected as an appropriate internal standard for the C₁₈ RP column, and malic acid for the Technicon column.

Preliminary work done with samples of APT or sterile mechanically deboned turkey meat (MDTM), fermented by selected strains of lactic starters, allowed an easy detection of glucose and lactic acid. At this stage of the work, no additional compounds have been found in those samples.

The fermentation of MDTM by *Lactobacillus plantarum* (#27), as affected by various sausage ingredients, was tested at 20°C. Table 2 shows the composition of the meat mixes tested.

Table 2. Product formulations

Product #	Glucose	Mn ⁺⁺	NaCl	Erythorbate	Nitrite	Spices
1	+	-	-	-	-	-
2	+	+	-	-	-	-
3	+	+	+	-	-	-
4	+	+	+	-	-	+
5	+	+	+	+	-	+
6	+	+	+	+	+	+

Table 3. pH changes in sausages prepared from mechanically deboned turkey meat inoculated with *Lactobacillus plantarum* #27 and stored at 20°C and 94% R.H.

Product #	Days after inoculation ¹		
	1	3	4
1	4.9	4.3	4.4
2	4.6	4.4	4.5
3	5.3	4.5	4.7
4	5.2	4.6	4.7
5	5.2	4.6	NT
6	5.2	4.6	4.7

¹ Immediately after inoculation all the products had similar pH values (6.5-6.6).

As a general rule, pH is the most important and easily measured parameter used to monitor fermentation progress in sausages. Kramlich (1971) has stated that a pH near 5.1 is desirable; in practice, the pH of fermented sausages range from 4.7 to 5.4 (Kramlich et al., 1973). The rates of fermentation in sausage mixes containing glucose as fermentable carbohydrate is shown in Table 3, in terms of pH reduction. In meat samples that did not contain NaCl, the pH was reduced to values below 5.0 within 1 day of incubation, whereas in samples containing NaCl (2.8%) values below 5.0 were reached between 1 and 3 days. Addition of manganese seems to improve fermentation by *L. plantarum*.

Initially, sausage batter has a pH of 5.7 to 6.2. During fermentation, the lactic acid bacteria produce lactic acid from the added carbohydrate and the pH drops to a range of 4.6 to 5.3. The production of lactic acid and the resulting drop in pH is responsible for both the characteristic flavor and texture of the sausage and the inhibition of spoilage and pathogenic microorganisms. At the low pH of fermented sausage, the solubility of sarcoplasmic but mainly of myofibrillar proteins, decreases significantly. It is important to realize that although greater quantities of acid present in sausage will provide greater preservative qualities, too much acid will decrease palatability. The production of acid also helps the sausage to "squeeze" out moisture from its protein sponge. As the meat protein reaches its isoelectric point (pH 5.1-5.3) the water retaining capacity of the meat is sharply reduced.

ABSTRACT

Difficulties were encountered in the quantitation of lactic acid by gas chromatography. On the other hand, the main organic acids likely to be found in fermented sausages (e.g. lactic, acetic,

pyruvic, etc.) could be determined by either of two HPLC procedures.

The addition of salt, nitrite, erythorbate and spices had very little influence on the fermentation rate of mechanically deboned turkey meat by *Lactobacillus plantarum*.

REFERENCES

Carlsson, J. 1973. Simplified gas chromatographic procedure for identification of bacterial metabolic products. Appl. Microbiol. 25: 287-289.

Deibel, R.H. 1974. Technology of fermented, semi-dried and dry sausages. In "Proceedings of the Meat Industry Research Conference", p. 57, American Meat Institute Foundation, Chicago, Ill.

Everson, C.W. 1971. Use of starter cultures in sausage products. In "Proceedings 13th Annual Meat Science Institute", Ed. J.A. Carpenter and D.D. Brown, p. 11, National Independent Meat Packers Association and the University of Georgia, Athens, Georgia.

Klettner, P.-G. and D. List. 1980. Effect of the type of carbohydrate on the ripening process of fermented sausages. Fleischw. 60: 1589-1593.

Kramlich, W.E. 1971. Sausage products. In "The Science of Meat and Meat Products". Ed. J.F. Price and B.S. Schweigert. 2nd. ed., p. 484. W.H. Freeman & Co., San Francisco.

Kramlich, W.E., A.M. Pearson and F.W. Tauber. 1973. Sausage formulations. In "Processed Meats". p. 190. Avi Publishing Co., Westport, Conn.

List, D. and P.-G. Klettner. 1978. Effect of starter cultures on lactic acid production during the ripening of fermented sausages. Fleischw. 58: 136-139.

Playne, M.J. 1985. Determination of ethanol, volatile fatty acids, lactic and succinic acids in fermentation liquids by gas chromatography. J. Sci. Food Agric. 36: 638-644.

Tandler, K. 1963. The use of sugar substances in the manufacture of salami-type sausages. Fleischw. 43: 804-

Theune, H.H. 1987. Personal communication.

Fig.1. Chromatograms obtained with C₁₈ reverse-phase (1A) and with a cross-linked cation exchange resin (1B) for a standard solution containing the following compounds: acetic acid, acetoin, butyric acid, diacetyl, glucose, lactic acid, malic acid, propionic acid, pyruvic acid and succinic acid. For details, see Materials and Methods.

APPENDIX 2

Detection of Hydrogen Peroxide Produced by Meat Lactic Starter Cultures

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Detection of Hydrogen Peroxide Produced by Meat Lactic
Starter Cultures

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Key words: Hydrogen peroxide Meat Lactic starter cultures

ABSTRACT

Twelve strains of meat lactic starter cultures (Pediococcus spp. and Lactobacillus plantarum) were found to produce hydrogen peroxide in vitro. The (cumulative) amounts of H_2O_2 produced were measured through the peroxidative action of catalase on H_2O_2 and oxidation of added formate to CO_2 , by the H_2O_2 -catalase complex formed.

There was a problem in building a calibration curve for converting values of formate oxidation into amounts of H_2O_2 , either by adding H_2O_2 directly to the assay mixture or having it produced via a glucose-glucose oxidase system.

Hydrogen peroxide is one of the primary metabolites that may be produced by lactic acid bacteria and which may contribute to their antagonistic action. It is cytotoxic, apparently because of its capacity as an intermediate in oxygen reduction to generate more reactive and cytotoxic oxygen species such as the hydroxyl radical ($\text{HO}\cdot$), which is a powerful oxidant (Halliwell 1978; Fridovich 1981), and which can initiate oxidation of biomolecules (Willson 1979). Studying the mechanism of lipid oxidation in meats (one of the major causes of quality deterioration) Harel & Kanner (1985) have shown that H_2O_2 activates metmyoglobin, probably to a porphyrin cation radical which initiates lipid peroxidation.

Raccach & Baker (1978) examined the production of H_2O_2 in vitro by two commercial strains of meat starter cultures, Pediococcus cerevisiae and Lactobacillus plantarum, and found a maximal level of $0.85 \mu\text{g H}_2\text{O}_2$ per 10^9 cells, which they could not consider high enough for antimicrobial activity. They concluded that, under the conditions of their experiments, the meat starter cultures were unable to produce and accumulate H_2O_2 .

Knowledge about H_2O_2 formation in fermented meat products is of importance in relation to the explanation of (1) antimicrobial effects and (2) colour and flavour changes. Thus, the inability to produce H_2O_2 has been considered to be one of the main criteria in the selection of bacterial strains designed for the manufacture of fermented sausages. Once formed, H_2O_2 may react with chemical constituents of bacteriological media or food products and therefore be undetected by the conventional analytical methods.

The purpose of this study was to assess the production of H_2O_2 by meat lactic starter cultures. The method reported is

based on the (cumulative) oxidation of formate through the peroxidative activity of catalase on H_2O_2 (Portwich & Aebi 1960) according to the following reactions (Schonbaum & Chance 1976):

The method described here enables the accumulation of extracellular H_2O_2 to be measured.

MATERIALS AND METHODS

Organisms

Five strains of Pediococcus pentosaceus, three of P. acidilactici and four of Lactobacillus plantarum, suitable for the production of fermented sausages, were used. They were obtained from companies that market starter cultures (strains # P-60, P-100, P-164, P-166, P-167, L-6, L-51, L-151), manufacturers of fermented sausages (P-29), and research laboratories (P-163, P-171, L-159).

Bacterial growth and inocula

Bacterial cultures were grown in the APT medium of Evans & Niven (1951) in test tubes at 25°C, subcultured twice, and then used to inoculate 100 ml portions of APT broth in 250 ml Erlenmeyer flasks that were incubated at 25°C in a shaker bath. Cells were harvested at the late logarithmic phase. They were centrifuged at 8000 g, washed twice with 0.3 mmol/l Na-K phosphate buffer (pH 7.2), and resuspended in relatively small amounts of phosphate buffer (pH 6.2-6.4) to a final concentration of 10^9 - 10^{10} viable cells/ml. The dry weight of bacterial cells was determined by filtering a 10-ml volume of bacterial suspension through a 0.2 μm

membrane filter, and drying the filter to constant weight at 85°C in vacuo.

Chemicals

Catalase (thymol-free; 11,000 units/mg) and sodium formate were from Sigma; ^{14}C formate Na with a specific activity of 1.8 or 5 mCi/mmol was obtained from New England Nuclear, Boston, MA.; Aqualuma scintillation liquid was from Packard. When used, sodium azide (Sigma) was added at a concentration of 1 mmol/l.

Formate oxidation

The basic reaction mixture contained, in 4.0 ml of 0.05 mol/l phosphate buffer (pH 6.2-6.4) placed in a 25-ml Erlenmeyer flask: 2-4 mg (dry weight) of bacterial cells, 5 μmol of sodium formate (0.25 μCi , ^{14}C) and 40 μmol of glucose. The Erlenmeyer was stoppered with a rubber cap which held a 8-mm diameter centre well containing a filter paper (Whatman 3MMchr) previously soaked with 100 μl of 1 mol/l KOH. Catalase (1,000 U), dissolved in 1 ml, was then injected into the flask, and the flask placed immediately in a shaker bath for at ca. 25°C for 2 or 18 h. The reaction was stopped by the addition of 1.0 ml of 1N acetic acid (May & de Haen 1979). The $^{14}\text{CO}_2$ released was collected by KOH. The filter paper was transferred to a polyethylene scintillation vial, and 5 ml of Aqualuma was added. The radioactivity was counted with 70% efficiency with a model MR300 Kontron Scintillation Counter.

H_2O_2 production was expressed in values of formate oxidation.

RESULTS AND DISCUSSION

Studies of formate oxidation to carbon dioxide in biological material have demonstrated the involvement of catalase and H_2O_2

in the reaction (Aebi et al. 1957; May & de Haen 1979). The oxidation of added formate by endogenous or exogenous catalase may, therefore, be referred to as intra- or extracellular H_2O_2 production (May & de Haen 1979).

Preliminary work was carried out to establish the specificity of the assay for H_2O_2 . Formate oxidation by the lactic acid starter cultures tested was inhibited by the addition of sodium azide and did not occur in the absence of catalase (results not shown), indicating that in the assay described: (1) H_2O_2 is the compound involved in the oxidation of formate, and (2) the lactic cultures do not produce carbon dioxide directly from formate. Twelve strains of bacterial meat starter cultures (eight of Pediococcus spp. and four of L. plantarum) caused oxidation of formate to varying extents (Table 1). The growth of P. acidilactici at 25°C was significantly slower than that of P. pentosaceus. Lower levels of formate oxidation by P. acidilactici as compared with P. pentosaceus were also usually observed. The amounts of accumulated H_2O_2 increased significantly when the period of incubation was extended from 2 to 18 h (Table 2).

The sensitivity of the method can be explained by a continuous sequestration of the H_2O_2 formed. With a specific culture, increased weights of bacterial cells gave increased levels of formate oxidation (results not shown).

Trials to build a calibration curve of added H_2O_2 amounts against radioactivity counts, and therefore calculate the actual amounts of H_2O_2 produced by the bacteria, have failed. No significant differences in levels of formate oxidation (CO_2 production) were found in a system containing catalase, formate, glucose and increasing amounts of H_2O_2 . When H_2O_2 is low, i.e., at low rates of H_2O_2 formation (as probably occurs with the lactic cultures), the peroxidatic pathway will predominate; when H_2O_2 is high, the catalatic pathway will predominate (Chance et

al. 1979). It is possible that in a system containing catalase, H_2O_2 and formate only, an alternative reaction may occur which does not lead to the oxidation of formate and formation of CO_2 , but to the decomposition of residual peroxide by compound I (catalytic pathway) and production of O_2 and water (Schonbaum & Chance 1976). The bacteria added may contain certain chemical compounds (enzymes or others) which protect the peroxide formed and/or enhance the reaction rate toward formate oxidation (Portwich & Aebe 1960).

In a reaction mixture containing 4,500 U of catalase and in which H_2O_2 was generated by glucose oxidase (2,000 U), 260 nmol of CO_2 were produced from 500 nmol of glucose, via formate oxidation. Assuming that, under optimal conditions, one mole of H_2O_2 is produced from one mole of glucose by the action of glucose oxidase, these results indicate a maximum efficiency of ca 50% in catalase-mediated formate oxidation by H_2O_2 .

Harel & Kanner (1985) have found that, in a microsomal fraction isolated from chicken and turkey muscle tissues, 30 nmol of metmyoglobin pre-activated with 30 nmol of H_2O_2 was enough to initiate membranous lipid peroxidation. This suggests that the levels of H_2O_2 found in our work, as produced by some of the lactic starter cultures, might cause undesirable oxidative changes in the meat products to which they had been added.

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REFERENCES

- Aebi, H., Frei, E., Knab, R. & Siegenthaler, P. 1957.
Untersuchungen über die Formiatoxydation in der Leber.
Helvetica Physiologica Pharmacologica Acta 15, 150-167.
- Chance, B., Sies, H. & Boveris, A. 1979. Hydroperoxide metabolism
in mammalian organs. Physiological Reviews 59, 527-605.
- Evans, J. B. & Niven, C.F.Jr. 1951. Nutrition of
heterofermentative lactobacilli that cause greening of cured
meat products. Journal of Bacteriology 62, 599-603.
- Fridovich, I. 1981. The biology of superoxide and superoxide
dismutase. In: Oxygen and Oxy-radicals in Chemistry and
Biology ed. Rodgers, M.A.J. & Powers, E.L., pp. 197-204. New
York: Academic Press.
- Halliwell, B. 1978. Superoxide-dependent formation of
hydroxyl radicals in the presence of iron chelates. FEBS
Letters 92, 321-326.
- Harel, S. & Kanner, J. 1985. Muscle membranal lipid peroxidation
initiated by H₂O₂-activated metmyoglobin. Journal of
Agricultural and Food Chemistry 33, 1188-1192.
- May, J.M. & de Haen, C. 1979. Insulin-stimulated intracellular
hydrogen peroxide production in rat epididymal fat cells.
Journal of Biological Chemistry 254, 2214-2220.
- Portwich, F. & Aebi, H. 1960. Erfassung der Peroxydbildung
tierischer Gewebe mittels peroxydatischer Umsetzungen.
Helvetica Physiologica Acta 18, 312-327.
- Raccach, M. & Baker, R.C. 1978. Formation of hydrogen peroxide by
meat starter cultures. Journal of Food Protection 41:
798-799.
- Schonbaum, G.R. & Chance, B. 1976. Catalase. In: The Enzymes ed.
Bayer, P.D., 2nd ed., vol. 13, pp. 363-408. New York: Academic
Press.

Willson, R.L. 1979. Hydroxyl radicals in biological damage in vitro: what relevance in vivo?. In: Oxygen Free Radicals and Tissue Damage. CIBA Foundation Symposium (new series) 65.19-52. New York: Elsevier/North Holland.

Table 1. Catalase-mediated formate oxidation by Pediococcus spp. and Lactobacillus plantarum. The reaction mixture is described above. The reaction was stopped after 2 h incubation at 25°C. Values are $\bar{x} \pm$ S.D. from at least three separate experiments.

Bacterial strain	Formate oxidation (nmol/mg cells dry wt)
<hr/>	
<u>Pediococcus pentosaceus</u>	
P-29	39*
P-60	333 $\pm$ 30
P-100	261 $\pm$ 24
P-164	300 $\pm$ 36
P-167	330 $\pm$ 52
<u>P. acidilactici</u>	
P-163	34*
P-166	12 $\pm$ 2
P-171	38 $\pm$ 6
<u>Lactobacillus plantarum</u>	
L-6	109*
L-51	68*
L-151	260 $\pm$ 36
L-159	246 $\pm$ 39

* Results from individual experiments

Table 2. Effect of incubation period on catalase-mediated formate oxidation by Pediococcus acidilactici at 25°C. The reaction mixture is described above. Values are $\bar{x} \pm$ S.D. from at least three separate experiments.

Bacterial strain	Formate oxidation (nmoles/mg cells dry wt)	
	2 h	18 h
P-166	12 $\pm$ 2	315 $\pm$ 22
P-171	38 $\pm$ 6	613 $\pm$ 17

2.4A Detection of Biogenic Amines in Fermented Bacteriological Media and in Meat Sausages

INTRODUCTION

General

Biologically active amines (biogenic amines) are normal constituents of many foods and have been found in cheese, sauerkraut, wine and putrid, aged or fermented meats. Biogenic amines are generally either psychoactive or vasoactive. Psychoactive amines act on the neural transmitters in the central nervous system, while vasoactive amines act, either directly or indirectly, on the vascular system. Pressor amines are vasoactive amines that cause a rise in blood pressure. In 1901, Barger and Walpole (1) first identified tyramine, phenylethylamine, and isoamylamine as the pressor principles of putrid meat. Tyramine was the most active of the pressor amines. It is now well known that phenylethylamine derivatives including tyramine, dopamine and norepinephrine cause a marked increase in blood pressure when injected intravenously into mammals. Histamine is also strongly vasoactive, and, in contrast to tyramine, is a strong capillary dilator and can produce hypotensive effects.

Biogenic amines are normal constituents of many foods, but they usually do not represent any hazard to individuals unless large amounts are ingested, or the natural mechanism for the catabolism of one or more of the amines is inhibited or genetically deficient. Monoamine oxidases (MAO) and diamine oxidases (DAO) oxidatively deaminate the amines and play a major role in their degradation. Monoamine oxidase inhibitors (MAOI) are drugs that block the oxidative deamination of certain primary amines. These drugs have been primarily used for the treatment of depression in psychiatric patients.

In man, 20-80 mg of tyramine injected intravenously or subcutaneously cause a marked elevation of blood pressure. In individuals on MAOI, as little as 6 mg taken orally can cause a rise in blood pressure.

Following intravenous injection of 0.1 mg of histamine phosphate, pulse quickening and a fall in blood pressure occur within 20 sec. The onset of histamine headache occurs 1 min after injection.

Unlike tyramine, histamine acts as a powerful capillary dilator. Phenylethylamine, like tyramine, causes an increase in blood pressure by liberating norepinephrine from tissues. It is not as important as tyramine in precipitating hypertensive attacks.

Biogenic amines in fermented sausages

Dierick et al. (3) observed a general increase in free amino acids with little change in the concentration of free tyrosine during sausage ripening. However, these workers reported an increase in the presence of tyramine (at least tenfold) during sausage ripening, indicating that although free tyrosine is produced, it is decarboxylated to form tyramine.

Histamine has been associated with several outbreaks of food-borne illness. Apparently, microbial contaminants in the naturally fermented products account for the higher levels of histamine detected (14).

Histamine concentrations in fermented meats can be variable and dependent on the length of the aging process (14,11). Higher histamine levels have been found in dry sausages where a natural fermentation process is employed for an extended aging period. Rice et al (11) surveyed 13 commercial samples of meat sausages for tyramine, phenylethylamine and tryptamine contents and found tyramine levels to range from nondetectable levels to what might be considered physiologically dangerous levels in dry and

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semi-dry sausages. The highest tyramine concentrations reported by Rice et al. (11) was 123.7 mg/100 g of a Genoa salami. Low concentrations of histamine were found in the sausage products examined by Rice et al. (11). Tyramine occurred more often and its levels were generally higher than phenylethylamine or tryptamine. The average level of tryptamine in the sausages was less than 29 ug/g and it was present in only 38% of the samples. Vandekerckhove (15) examined 26 samples of dry fermented sausages and reported that histamine ranged from trace amounts to 19.74 mg/100 g dry matter. The mean histamine concentration for the sausages was 4.1 mg/100 g dry matter. Tyramine levels in the sausages ranged from 10.18 to 150.63 mg/100 g dry matter, and the mean tyramine concentration was 36.83 mg/100 g dry matter. Phenylethylamine concentrations ranged from trace amounts to only 6.06 mg/100 g dry matter and the mean concentration was 0.94 mg/100 g dry matter.

The use of commercial lactic starter cultures with limited tyrosine- and histidine-decarboxylating activities may reduce effectively tyramine and histamine formation through control of the natural fermentation. Through the control of undesirable microorganisms, *Lactobacillus* and *Pediococcus* cultures also appear to serve as an effective control agent against the production of the biogenic amines by naturally occurring microbial contaminants that possess amino acids decarboxylase activities. Similar results have demonstrated that the use of lactobacilli decreases the possibility of potentially hazardous tyramine concentrations that can result from a microflora that possess both tyrosine decarboxylase and proteolytic activities (5). Similar results have demonstrated that the use of a starter culture greatly decreases the possibility of development of microflora that possess both tyrosine decarboxylase activity and the proteolytic activity required to produce potentially

hazardous tyramine concentrations (5). Several reports have confirmed the lack of histamine and tyramine production by commercial available Lactobacillus and Pediococcus meat starter cultures (10).

Methodological problems

Derivatization of amines may be performed by various amine-specific reagents such as O-Phtalaldehyde(OPA), Dansyl-chloride (DnsCl) or 9-Fluorenylmethyl chloroformate(FMOC). The complexes formed can be detected by either light absorption or by fluorescence.

FMOC was chosen as: the derivative is stable, can be detected either by fluorescence or UV absorbance and derivatization can be automated. OPA and DnsCl give non-stable derivatives, and DnsCl derivatization is cumbersome, can not be automated and suffers from major interferences[2].

The FMOC reagent reacts with water to form FMOC-OH as an hydrolysis product, which appears as a large peak, and can be extracted with pentane[2].

The pH of the reaction mixture is critical to the formation of FMOC derivatives. At low pH the reaction is slow and incomplete. Amines which are rather hydrophobic require higher pH for quick and complete reaction[2].

Extraction of amines from meat samples may be performed with various solvents such as methanol, sodium hydroxide, trichloroacetic acid or perchloric acid. Perchloric acid was chosen as: amines solubility in acid is good, it is an efficient protein precipitant and potassium perchlorate, which forms by the addition of K⁺ ions, is easily removed as its solubility is 0.75% w/v at 0 C [7].

Purpose of the Study

The objective of this study was to optimize the separation and establish the optimum derivatization and detector conditions for the amines: tyramine (TYR), tryptamine (TRP), phenylethylamine (PEA) and histamine (HIS) in standards, in synthetic media and in meat products fermented by lactic starter cultures.

MATERIALS AND METHODS

Chemical analyses

The HPLC analytical procedures used in this work are presented in detail in the paper "High-performance liquid chromatography of biogenic amines, derivatized with 9-fluoenylmethyl chloroformate" (This report, pages).

Amino acids analyses were performed with an Amino Acid Analyzer, before and after hydrolysis of the samples.

At the time of preparation of this report we did not have a reliable HPLC method for the analysis of histamine. Results from histamine analyses are presented in the text only, whereas the Tables shown in this report refer to TRY, TRP and PEA.

Histamine analyses reported herein were done by the fluorometric o-phthalaldehyde (OPT) method of Shore (1971), using the homogenization and extraction procedures recommended by Taylor et al (1978). The fluorometric analyses were made with a model RF 540 Shimadzu recording spectrofluorometer, fluorescence (emission) being measured at 450 nm after excitation at 360 nm. In the determination of histamine recovery from non-inoculated and inoculated APT broth and meat sausage samples internal standards of 25, 50, 100 and 200 ng of histamine.HCL were used.

Starter cultures

The following bacterial strains were used in these studies:

Pediococcus acidilactici H (P-171)

Pediococcus pentosaceus 75 (P-164)

Lactobacillus plantarum 27 (L-159)

Overnight cultures in APT broth were added at ca. 1% concentration to either fresh APT broth or ground turkey meat to yield a final concentration of 10^4 to 10^5 viable organisms per ml or g.

Shore, P.A. 1971. The chemical determination of histamine. In O. Glick (ed.): Methods of Biochemical Analysis. Interscience Publishers, New York.

Taylor, S.L., E.R. Lieber and M. Leatherwood. 1978. A simplified method for histamine analysis in foods. J. Food Sci. 43: 247.

Amines production in APT broth

The starter cultures were grown in APT broth (pH ca. 7.0) in a bioreactor under controlled concentration of dissolved oxygen at either 20 or 30C and samples removed when the fermented medium reached pH 5.0. The samples were stored frozen until used.

Amines production in turkey meat

The method used for the production of the fermented sausages is presented in p. of this report. Samples of radiation-sterilized mechanically deboned turkey meat (MDTM) were used in preliminary tests.

Extraction and deproteinization procedures

The following extraction mixtures (EM) were used for TYR, TRP and PEA:

- (a) 1M perchloric acid : isopropanol (1:1)
- (b) 1M perchloric acid : methanol (3:7)
- (c) 1M perchloric acid : methanol (1:1)

Bromcresol purple (0.1%) solution in 15 mM NaOH was used as a pH indicator.

The procedure used for spiking meat samples with the selected amines is presented in p. of this report.

For the preparation of standard amine solutions (SAS) in EM, 1 ml of the SAS plus 4 ml of EM were mixed in a 10-ml volumetric flask. One drop of the pH indicator was added and the contents

neutralized with 2 M KOH (until color changed from yellow to purple). Distilled water was added up to 10 ml and the contents cooled in a ice-water bath. The potassium perchlorate salt precipitates and 1 ml of the supernatant was centrifuged to remove any remaining precipitate (Sorvall SH-MT, 7000 rpm, 15 min at 2C). Five hundred ul of the supernatant were mixed with 500 ul of 0.4 M sodium bicarbonate and 500 ul of the mixture taken for derivatization.

RESULTS AND DISCUSSION

Amines extraction from meat samples

First results are shown in pages (1-10) of this report. The efficiency of various extraction solvent mixtures was tested in MDTM samples. Extraction with perchloric acid was selected because of (a) excellent amine solubility; (b) efficient protein precipitation and (c) the perchlorate salt formed by the addition of KOH precipitates readily at 0C and can be easily removed. In the comparison reported in pages (11-12), good results were obtained when the amines Fmoc derivatives had been extracted with methanol:perchloric acid. In order to obtain better linearity in the response for the various extracted amines, mixtures of perchloric acid with either methanol or isopropanol were compared.

In order to test the amines standards (for TYR, TRP and PEA) they were added to the various EM at the following final concentrations: 0.25, 0.50 and 1.0 nmoles (3 replicates each). The peak heights (in mAU; milli Adsorption Units) were measured

and the correlation coefficients calculated (Table 1). From Table 1 it is concluded that the mixture isopropanol:1 M perchloric acid is the most efficient, giving a correlation coefficient close to 1.

Table 1: Influence of various solvent mixtures (EM) on the efficiency of the amines extraction

Extraction Solvents	Correlation coefficient		
	TYR	TRP	PEA
	-----	-----	-----
Perchloric acid*	0.9922	0.9958	0.9954
Perchloric:methanol (1:1)	0.9850	0.9876	0.9788
Perchloric:methanol (3:7)	0.9971	0.9964	0.9964
Perchloric:isopropanol (1:1)	0.9997	0.9995	0.9988
-----	-----	-----	-----

* 1M perchloric acid in all cases

Extraction of amines from meat samples

Based on the results previously reported two EM were tested: perchloric:isopropanol (1:1) and perchloric:methanol (7:3). The average amount of the amine (nmol) was calculated by multiplying the ratio (peak height relative to the specific amine extracted from the meat sample/peak average obtained from the amine standard, after correction by the coefficient of the linear correlation) by the amount of the amine added. The

relative standard deviation (RSD, %) of the average amount of amine was determined from the replicates results. The recovery rates (%) were calculated by multiplying by 100 the ratio average peak height (corrected by correlation linear coefficient) /peak height relative to the amine standard.

Table 2 shows the amines recoveries from spiked meat samples, when isopropanol or methanol was added to the perchloric acid solution. The amine standard solution dissolved in (a) each of the two different EM and (b) 0.2M sodium bicarbonate, in concentrations similar to those added to the meat samples.

Results are average of 3 experiments.

From Table 2 it is apparent that the mixture isopropanol:perchloric is more efficient than methanol:perchloric. The highest recovery rates were obtained for TYR. For TRP and PEA, only small differences were observed, and the variation among replicates (RSD) was small. The RSD values varied from 1 to 3% (exceptions were 8.4% and 10.2% for 0.125 nmol of PE and TRYP, respectively) with isopropanol:perchloric as compared with 1% to 37% for methanol:perchloric acid.

The peak values for SAS obtained with 0.2 M sodium bicarbonate were lower than those obtained with isopropanol:perchloric (1:1). In the analyses of the meat extracts, several peaks were observed that were not amines derivatives but probably free amino acids.

Table 2. Recovery rates (R) obtained by extracting spiked meat samples with two different EM

(A) EM= isopropanol:perchloric acid (1:1)

amount of amine added (nmol)	TYR			TRP			PEA		
	amount rec'd (nmol)	RSD (%)	R (%)	amount	RSD	R	amount	RSD	R
1.0	0.90	2.1	90	0.75	2.2	74	0.91	3.0	90
0.5	0.48	1.6	94	0.33	3.1	70	0.44	1.8	89
0.25	0.25	0.9	101	0.16	1.6	63	0.22	2.1	88
0.125	0.14	2.5	111	0.07	10.2	57	0.11	8.4	84
Avg.			99			66			88
S.D.			9.2			7.5			2.6
C.C.	0.999			0.998			0.999		

(B) EM= methanol:perchloric acid (7:3)

1.0	0.80	9.0	82	0.70	5.6	71	0.86	4.7	87
0.5	0.46	2.3	86	0.38	2.5	70	0.46	1.1	88
0.25	0.25	11.1	93	0.18	16	67	0.30	6.5	88
0.125	0.11	19.7	102	0.06	37	62	0.10	20	90
Avg.			91			68			88
S.D.			8.8			4.0			1.3
C.C.	0.988			0.994			0.997		

Extraction of fermented sausage samples

The extraction was performed with isopropanol:1N perchloric acid (1:1). Table 3 shows the recovery rates obtained with sausage samples. In parallel, samples of amines dissolved in Isopropanol:Perchloric were also tested at similar concentrations. Results are average of 3 replicates.

The average amounts (nmol) of amines in the sausages was calculated as described for Table 2.

The amounts of amines in the sausages were calculated: average of measured peak heights (corrected on the basis of the average recovery percents) were converted into amounts of amine (nmol) by the linear correlation coefficient of the standard, and was multiplied by the dilution factor in order to obtain amounts of amine (nmol) per g of sausage meat. Multiplication by the molecular weights gives the results in mg of amine per g of meat. Of the amines tested (histamine not included), tyramine was the only amine found in the uninoculated MDTM samples in concentrations of 0.06-0.07 mg/g dry matter. Also in sausages fermented with the starter cultures, only tyramine, in concentrations of 0.31 to 0.44 mg/g dry matter, was detected (Table 4).

Vandekerchove (15) found tyramine in fermented sausages at concentrations of 0.1-1.5 mg/g dry matter.

Recoveries of histamine from spiked sausage amples, using the procedure of Taylor et al (1978), were low and inconsistent.

Table 3. Recovery rates (R) of amines from spiked sausage samples. The FMOC-amine derivative was extracted with isopropanol:perchloric acid.

amount of amine added (nmol)	TYR			TRP			PEA		
	amount	RSD	R	amount	RSD	R	amount	RSD	R
	rec'd (nmol)	(%)	(%)						
1.0	0.92	3.1	92	0.80	3.0	79	0.98	3.0	98
0.5	0.47	2.8	92	0.38	3.8	79	0.47	2.9	95
0.25	0.25	0.8	93	0.21	2.4	80	0.23	1.2	91
0.125	0.11	8.5	95	0.10	4.1	81	0.11	7.3	84
Avg:			93			80			92
S.D.			1.4			1.0			6.1
C.C	0.994			0.998			0.999		

Table 4. Amines in sausages fermented with selected starter cultures

Starter culture	umol/g	TYR		TRP & PEA
		mg/g	RSD (%)	
P-164	0.95	0.17	2.9	not detected
P-171	1.17	0.20	1.5	not detected
L-159	1.36	0.24	1.6	not detected

Amines production by starter cultures in APT broth

The starter cultures were grown in APT broth at 20C and 30C until the pH changed from ca. 6.7 to 5.0. Extraction of the amines TYR, TRP and PEA was done with isopropanol:perchloric (1:1).

Table 5 shows the recovery of amines added to APT samples. No detectable amounts of TY, TRP or PEA were found in the inoculated APT samples neither at 20 nor at 30C. Recoveries of the amines from APT (spiking tests) varied from 97 to 115%.

Table 5. Recovery rates (R) of amines from spiked APT broth. The FMOC-amine derivative was extracted with isopropanol:perchloric acid.

amount of amine added (nmol)	TYR			TRP			PEA		
	amount	RSD	R	amount	RSD	R	amount	RSD	R
	rec'd	(%)	(%)						
	(nmol)								
1.0	1.03	4.2	103	0.97	1.4	97	1.14	2.8	115
0.5	0.54	2.5	103	0.50	1.8	99	0.59	2.4	115
0.25	0.26	5.1	103	0.25	7.8	101	0.28	7.1	114
0.125	0.12	3.8	103	0.13	16	105	0.13	14	113
AVG:			103			101			114
S.D.			0.0			3.4			1.0
C.C.	0.993			0.999			0.998		

Recoveries of histamine from spiked APT fermented and non-fermented samples were satisfactory and varied from 96 to 110%. In APT samples fermented at 20 C, the following concentrations of histamine were found (ug histamine per 100 ml APT): P-167 (not detected); P-171 (120-175); L-159 (70-80). Lower concentrations were found in samples fermented at 30 C.

Analyses of free amino acids in APT broth and meat samples indicated that tyrosine, tryptophane and phenylalanine (also histidine) contents were not a limiting factor for the production of the amine by the starter cultures.

The minimal amount of amine that could be detected by the HPLC method reported here were:

tyramine	0.005 mg	per ml broth or g meat
tryptamine	0.018 mg	" "
phenylethylamine	0.015 mg	" "

CONCLUSIONS:

Considering that 3 mg of phenylthylamine. may cause migrene in sensitive individuals, and that 6 mg tyramine, given orally, may cause increase in blood pressure in individuals taking MAOI, the sensitivity of the method described here for the analysis of TYR, TRP and PEA may be considered satisfactory. Moreover, the levels amount of TYR found in the fermented sausages inoculated with starter cultures are lower than that which might be considered as a health hazard. If higher sensistivity is required from the method described here, a fluorescent detector, instead of the UV detector, can be used.

REFERENCES

- 1) Barger, G. & G.S. Walpole. 1901. Isolation of the pressor principles of putrid meat. J. Physiol. (London) 38: 343-352.
- 2) Cunico, R.L., A. Mayer., P. Dimson & C. Zimmerman. Precolumn derivatization of amino acids using amino TAG. in Varian Instruments Group, Walnut Creek Division, 2700 Mitchell Drive, Walnut Creek, Ca. 94598. Lc #163.
- 3) Dierick, N., P. Vandekerckhove & D. Demeyer. 1974. Changes in nonprotein nitrogen compounds during dry sausage ripening. J. Food Sci. 39: 301-304.
- 4) Einarsson, S., B. Josefsson & S. Lagerkvist. 1983. Determination of amino acids with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography. J. Chromatogr. 282: 609-618.
- 5) Eitenmiller, R.R., P.E. Koehler & Reagan, J.O. 1978. Tyramine in fermented sausages: factors effecting formation of tyramine and tyrosine decarboxylase. J. Food Sci. 43: 689-693.
- 6) Gomori, G. in Methods in Enzymology. 1955. P.146. Clowick, S.P. & N.O. Kaplan, editors, New York, Academic Press.
- 7) Handbook of chemistry. 1967. pp. 977, 308-309. Lange, N.A, editor, McGraw-Hill, Tenth Edition.
- 8) Hui, J.Y. & S.L. Taylor. 1983. High pressure liquid chromatographic determination of putrefactive amines in foods. J. Assoc. Off. Anal. Chem. 66: 853-857.
- 9) Koehler, P.E. & R.R. Eitenmiller. 1978. High pressure liquid chromatographic analysis of tyramine, phenylethylamine and tryptamine in sausages, cheese and chocolate. J. Food Sci. 43: 1245-1247.

- 10) Rice, S.L. & P.E. Koehler. 1976. Tyrosine and histidine decarboxylase activities of *Pediococcus cerevisiae* and *Lactobacillus* species and the production of tyramine in fermented sausages. *J. Milk Food Technol.* 39: 166-169.
- 11) Rice, S., R.R. Eitenmiller & P.E. Koehler. 1975. Histamine and tyramine content of meat products. *J. Milk Food Technol.* 38: 256-258.
- 12) Ronnberg, A.L., C. Hansson., T. Drakenberg & R. Hakanson. 1984. Reaction of histamine with o-Phthalaldehyde: Isolation and analysis of the fluorophore. *Anal. Biochem.* 139: 329-337.
- 13) Ronnberg, A.L., C. Hansson & R. Hakanson. 1984. High performance liquid chromatographic determination of histamine in biological samples after derivation with o-Phthalaldehyde. *Anal. Biochem.* 139: 338-344.
- 14) Taylor, S.L., M. Leatherwood & E.R. Lieber. 1978. A survey of histamine levels in sausage. *J. Food Prot.* 41: 634-637.
- 15) Vandekerckove, P. 1977. Amines in dry fermented sausage. *J. Food Sci.* 42: 283-285.

ANIMAL SCIENCE ROUNDUP REPORT

UNIVERSITY OF WYOMING 1986

DISTRIBUTION OF PLASMIDS AMONG LACTOBACILLUS PLANTARUM STRAINS USED IN MEAT FERMENTATION

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Summary

The presence of plasmid(s) in seven L. plantarum strains used in the fermentation of meat was investigated. All the strains were found to harbor plasmids ranging from 0.26 to 1.27 megadaltons.

Introduction

Lactobacillus plantarum (L. plantarum) strains are one of the commonly used lactic acid bacteria in the manufacture of fermented sausages. These cultures not only produce lactic acid during fermentation but also are responsible for the production of metabolites that impart desirable flavor in the product (El-Gendy et al., 1983). In addition some strains are known to produce antimicrobial compounds against food pathogenic and spoilage organisms (Bacus and Brown, 1981), and some strains are also known to produce hydrogen peroxide and biologically active amines that are undesirable in the products (Rice and Koehler, 1976). In several lactic acid bacteria many of these desirable and undesirable traits have been found to plasmid-linked, we are interested to study the plasmid involvement of the above traits in L. plantarum strains used in meat fermentation.

Materials and Methods

A total of seven L. plantarum strains, originally obtained from commercial culture producers and laboratories and now maintained in our culture collection, were used in this study. The strains were grown in a nutrient broth to mid logphase and used for the lysis and isolation of plasmid DNA by a mini preparation procedure (Klaenhammer, 1984). The individual plasmids in the partially purified sample were separated by agarose gel electrophoresis and stained with ethidium bromide. The molecular weight of each plasmid was determined against the plasmids of known molecular weight

isolated from E. coli V517.

Results and Discussion

The photographs of the agarose gel showing the plasmid bands of L. plantarum strains are presented in Fig. 1. The number of plasmids and their molecular weight are presented in table 1. These data showed that plasmid number varied from two to seven among the strains. Molecular weight indicated that all strains harbor plasmids of variable sizes. Our next objective is to determine plasmid-linkage of the desirable and undesirable traits as related to the characteristics of fermented sausage. This will help us in our future goal of isolating and developing strains that carry most of the desirable and some of the undesirable traits.

Literature Cited

- El-Gendy, S. M., H. Abdel-Galil, Y. Shahin, and F. Z. Hegazi. 1983. Acetoin and diaetyl production by homo and hetero-fermentative lactic acid bacteria. J. Food Protect. 46:420-425.
- Bacus, J. N., and W. L. Brown. 1981. Use of microbial cultures: Meat products. Food Technology. 35(1):74-77.
- Rice, S. L., and P. E. Koehler. 1976. J. Milk Food Technol. 39:166.
- Klaenhammer, T. R. 1984. A General Method for Plasmid Isolation in Lactobacilli. Current Microbiology. 10:23-28.

TABLE 1. SUMMARY OF NUMBER AND M.W. OF L. PLANTARUM PLASMIDS

Strain	Number of Plasmids	Molecular Weight (Megadalton)
B	5	1.12, 0.86, 0.45, 0.34, 0.26
E	2	0.72, 0.45
H	5	1.12, 0.86, 0.45, 0.34, 0.26
I	2	0.72, 0.45
J	5	1.12, 0.86, 0.45, 0.34, 0.26
27	4	1.27, 0.82, 0.58, 0.38
44	7	1.22, 1.17, 1.07, 0.97, 0.87, 0.72, 0.47

$\frac{f}{i} = 4.5$ $\lambda = 4$

Fig. 1.

Plasmid bands visualized in seven strains of L. plantarum and E. coli V517. Tracks: a, B; b, E; c, H; d, I; e, J; f, 27; g, 44; h, E. coli V517

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APPENDIX 11

Genetic control of bacteriocin production and mucoid traits in *Lactobacillus plantarum* 27.

Examination of several *Lb. plantarum* strains for the bacteriocin production indicated that strain *Lb.* 27, originally obtained from Dr. Lucke, West Germany, is capable of inhibiting growth of several strains of lactic acid bacteria. The antimicrobial activity was destroyed by proteolytic enzymes but remained active after heat treatment, at wide pH range and after treatment with catalase (Table 1). Treatment with several plasmid curing agents to isolate strain(s) that had lost bacteriocin capability was not successful. However, several mutants were isolated that did not produce slime or mucoid colonies, a trait of the wild strain (Fig. 1 and Table 2).. These mucoid-less strains retained the ability to produce bacteriocin (Fig. 2) as well as their resistance/sensitivity to several antibiotics (Table 3) and carbohydrate fermentation pattern of the nonmucoid wild strain. Plasmid analysis of the wild (S_{lm}^+) and nonmucoid (S_{lm}^-) strains revealed that the S_{lm}^- strains had lost a 41.7 Md plasmid (Fig. 3 and Table 3).

The bacteriocin producing trait in this strain is probably a chromosomally linked trait.

"All-Turkey" Fermented Sausage Snack

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An "all-turkey" fermented sausage snack product has been developed which has acceptable flavor, color and texture as well as chemical and microbiological stability at ambient temperature. The product is made from less expensive parts of turkey carcasses plus mechanically-deboned turkey meat, and is processed with special bacterial cultures developed at the University of Wyoming. The stability of the new product is due to the ability of these cultures to cause a rapid fermentation and to produce natural compounds inhibitory to pathogenic and spoilage microorganisms. The cultures produce turkey sausages with desirable organoleptic qualities. Preliminary sensory evaluation tests have indicated the "all-turkey" product has flavor characteristics similar to products made from beef and pork.

Considering the marketing trends to prefer poultry meat over pork or beef and considering its organoleptic characteristics, the researchers believe that there is a great marketing potential for the new product as a healthful high-protein snack food.

On a basis of mutual agreement, the researchers will be interested in assisting your company in the technical and scientific aspects related to the production and marketing of this product.