

# Using Lactic Acid Bacteria to Improve the Safety of Minimally Processed Fruits and Vegetables

The objective in using biocontrol cultures is not to ferment foods, but to control the microbial ecology through competitive inhibition of pathogenic bacteria

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**L**actic acid bacteria (LAB) have for centuries been responsible for the fermentative preservation of many foods, including fruits and vegetables. Currently, there is interest in possible use of LAB as biocontrol agents to ensure safety of minimally processed, refrigerated (MPR) foods which are not acidified. Such applications of LAB have been widely studied in meat and dairy products, but only recently have been considered for use in MPR fruit and vegetable products.

Preservation of minimally processed fruit and vegetable products presents unique challenges to food microbiologists. Microbial populations on (and in) fresh vegetables can range from as low as  $10^2$  cfu/g to as high as  $10^9$  (Lund, 1992; Nguyen-the and Carlin, 1994). Predominant microflora on fresh fruits and vegetables typically are Gram-negative rods, including *Pseudomonas* spp., *Enterobacter* spp., and others; the LAB responsible for fermentation of brined vegetables usually make up 1% or less of the total aerobic plate count (Lund, 1992; Nguyen-the and Carlin, 1994).

Technological developments to prolong the shelf life of minimally processed vegetable products include various washing treatments to reduce the numbers of microorganisms (Adams et al., 1989), as well as modified-atmosphere packaging during storage and refrigeration (Zagory and Kadar, 1988; Hotchkiss and Blanko, 1992). Washing procedures, including adding chlorine, chlorine dioxide, or other compounds to the wash water, generally have not been very successful at reducing the indigenous microflora (Adams et al., 1989; Brackett, 1992; Nguyen-the and Carlin, 1994).

The ineffectiveness of washing or sanitizers to remove bacteria from produce is likely due to microorganisms located in protected regions near the surface of the plant material. Bacteria can be harbored within stomata or under trichosomes or

other surface features of plant material (Fig. 1). The hydrophobic nature of many plant surfaces may protect bacteria on these surfaces from contact with aqueous sanitizing solutions. Bacteria may also be within the flesh as a result of tissue damage, or reside within otherwise healthy tissue (Meneley and Stanghellini, 1974).

Altering the normal microbial ecology of these products through cutting, processing, modified-atmosphere packaging, and refrigerated storage may have the unintended effect of allowing the growth of pathogenic bacteria (Gould, 1992; Hotchkiss and Banco, 1992; Sofos, 1993). Cutting of produce may exacerbate problems due to bacterial pathogens, and cross-contamination of the produce may occur in the released cell sap.

## Food Safety Considerations

Pathogenic microorganisms may be present on fresh fruits and vegetables and in related MPR products (Nguyen-the and Carlin, 1994; Schofield, 1992; Madden, 1992). Vegetable products have been implicated in at least two outbreaks of listeriosis (Ho et al., 1986; Schlech et al., 1983), along with other refrigerated foods (Farber and Peterkin, 1991), and the U.S. Dept. of Agriculture has declared a zero tolerance for *Listeria monocytogenes* in foods (USDA, 1989). A variety of other pathogens (Nguyen-the and Carlin, 1994) may be found on fruit and vegetable products, including *Salmonella* and *Shigella* spp., enteropathogenic strains of *Escherichia coli*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Staphylococcus aureus*, and others. Whether these pathogens grow and cause disease depends on the type of product, conditions of storage (time, temperature, atmosphere, etc.), and competing microflora.

The extended shelf life of some MPR vegetable products may result in an undesirable "safety index," a concept developed by Hintlian and Hotchkiss (1988) to define the microbial risks associated with modified-atmosphere-packaged foods. This index is defined as the ratio of spoilage bacteria to pathogenic bacteria in foods, measured as their

relative cell concentrations.

The technology involved in producing MPR fruit and vegetable products may affect the safety index, and the microbial ecology of these products has not been thoroughly explored. Modified-atmosphere packaging, with elevated levels of CO<sub>2</sub> and reduced levels of O<sub>2</sub>, has been found to be an effective means of increasing product shelf life from a sensory quality perspective (Zagory and Kadar, 1988). Hao and Brackett (1993) found that the primary effect of modified-atmosphere packaging was to decrease the metabolic activity of the vegetable material itself. The growth rates of the bacteria present in these products were relatively unaffected.

There are conflicting reports relating the influence of modified-atmosphere packaging on growth and survival of pathogens and spoilage microorganisms (Nguyen-the and Carlin, 1994). Competing populations may respond differently to environmental factors, such as changing gas concentrations due to the respiration of plant material within packaging. Bennik et al. (1995) demonstrated in a model system that growth rates for *L. monocytogenes*, *A. hydrophilia*, and *Bacillus cereus* may be reduced by modified-atmosphere conditions, but final cell density was not affected. One major concern is that *Clostridium botulinum* spores have been isolated from a variety of vegetables and this organism may, under appropriate conditions of temperature, pH, and atmosphere, grow and produce toxin in MPR vegetable products if the packaging permits the O<sub>2</sub> concentration to drop below 1% (Nguyen-the and Carlin, 1994).

Fermented vegetables, however, his-

torically have been considered safe, because of the low pH eventually attained in these products and the inability of pathogens, such as *C. botulinum*, to grow under acidic conditions. LAB species can produce a variety of metabolites (Table 1), including lactic and acetic acids which lower pH, that are inhibitory to competing bacteria, including psychrotrophic pathogens (DeVuyst and Vandamme, 1994; Vandenberg, 1993). The inhibition by organic acids has been attributed to the protonated form of these acids, which are uncharged and may therefore cross biological membranes. The resulting inhibition of growth may be due to acidification of the cytoplasm and/or accumulation of anions inside the cell (Russel, 1992).

LAB in general are much more resistant to low pH than are other bacteria. Because of these attributes, and the common use of LAB in food fermentations, it is not surprising that LAB have been proposed for use as biocontrol agents in nonfermented foods, including MPR fruit and vegetable products (Gombas, 1989; Holzapfel et al., 1995; Stiles, 1996). In the event of prolonged storage or temperature abuse of MPR foods, the biocontrol culture (LAB) should grow, and prevent the growth of pathogenic microorganisms by competitive inhibition. As suggested by Holzapfel et al. (1995), the use of protective cultures should only be considered as a supplement to good manufacturing practices, not as a substitute for the proper handling and packaging of MPR products. The use of biocontrol cultures may therefore be considered to enhance existing hurdle technology to prevent the growth of pathogens in MPR foods.

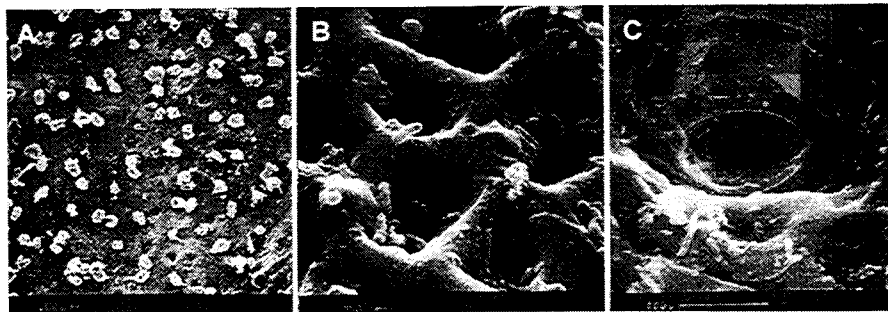


Fig. 1—Scanning electron micrographs of the surface of pickling cucumber fruit: (A) fresh fruit showing numerous trichomes and stomata, (B) fermented fruit showing lactic acid bacteria adhering to epidermal cells, and (C) fermented fruit showing lactic acid bacteria at the entrance of a stomate. The electron micrographs (Smith and Fleming, unpublished) were taken from a study by Smith et al. (1979).

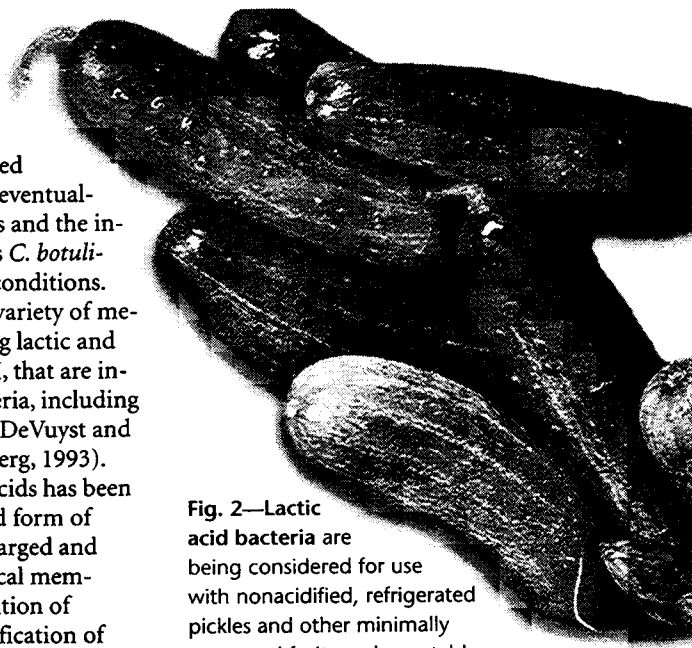


Fig. 2—Lactic acid bacteria are being considered for use with nonacidified, refrigerated pickles and other minimally processed fruits and vegetables

The hurdle concept (Leistner and Gorris, 1995) advocates use of multiple preservative factors to prevent growth of pathogens. In MPR fruit and vegetable products, the main factors (hurdles) affecting growth of the indigenous bacterial populations are sanitation, modified-atmosphere packaging, refrigeration, and the competitive interactions of the bacteria themselves. There may be significant variation in these factors, allowing opportunity for growth of pathogens, and indicating the need for additional hurdles.

### History of Biocontrol

The ecology of vegetable fermentations primarily is directed by the production of inhibitory metabolites. This process, known as amensalism or interference-type competition, may also be used in biocontrol applications. The objective in using biocontrol cultures is not to ferment foods, but to control the microbial ecology should spoilage occur. Spoilage would then be the result of the growth of the biocontrol culture, which is nonpathogenic.

The earliest references to competitive inhibition of pathogenic bacteria by competing microbial cultures relate to clinical applications that date back to the end of the 19th century and the beginning of the science of microbiology (Florey, 1946). One of the first reports of the use of LAB as biocontrol agents in foods was the work of Saleh and Ordal (1955), who demonstrated the antagonistic effects of a *Lactococcus lactis* (formerly *Streptococcus lactis*) inoculum on the growth of, and formation of toxin by, *C. botulinum* in a frozen chicken la king product. A more recent example is the Wisconsin process for ensuring the safety

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of bacon (Tanaka et al., 1985). Protective LAB cultures in bacon were found to be more effective than 120 ppm of sodium nitrite, the maximum amount of nitrite allowed on cured meats. The use of biocontrol LAB has been investigated for a variety of applications with refrigerated meat products (Holzapfel et al., 1995).

In addition to being used as competitive biocontrol cultures, LAB have also been added to nonfermented foods to extend shelf life. Gilliland and Speck (1975) and Raccach et al. (1979) both used large numbers of LAB ( $10^8$  or more) inoculated on meats to extend shelf life and prevent the growth of pathogens. They reported little or no acid development since the cultures do not undergo significant growth. The delayed onset of spoilage during extended storage or with temperature abuse was attributed to production of inhibitory metabolites, such as hydrogen peroxide and bacteriocins. Examples of this method in the literature are limited, however, possibly because adding large numbers of bacteria to meats may have an unacceptable sensory impact.

## Biocontrol of Vegetables

Romick (1994) used a *Lactobacillus plantarum* strain as a protective culture to prevent the growth of *L. monocytogenes* in brined, nonacidified, refrigerated pickle products. In these products, the cucumbers maintain a fresh appearance and texture and must be refrigerated to inhibit fermentation and spoilage. In inoculated pack experiments, a genetically marked *L. monocytogenes* strain grew in the brined cucumbers at both 5°C (increasing 1–2 log units in 20 days) and higher abuse temperatures (10°C and above). Growth of *L. monocytogenes* was limited, however, by the growth of naturally present LAB or an added *L. plantarum* culture. The limitation of growth correlated directly with the concentration of protonated lactic acid in the brine, with a limiting concentration of 4 mM (Romick, 1994).

Carlin et al. (1996) described the influence of the naturally occurring microflora of endive leaves on growth of *L.*

*monocytogenes*. The *L. monocytogenes* culture was inoculated onto the surface of leaves that were washed with hydrogen peroxide or untreated leaves. The peroxide treatment reduced the indigenous microflora by 1–2 log cycles and allowed growth of the added culture. A mixed population of microorganisms, isolated from bacteria naturally present on the endive leaves, was shown to competitively inhibit the growth of *L. monocytogenes*.

Vescovo et al. (1996) isolated a series of psychrotrophic LAB strains from fresh vegetable salad ingredients. These LAB strains were screened to identify cultures producing bacteriocins, or forming an inhibition zone in agar diffusion tests, against a variety of Gram-negative and Gram-positive pathogens. The selected strains were then used in challenge studies in salad products with *A. hydrophilia*, *L. monocytogenes*, *Salmonella typhimurium*, and *S. aureus*.

While all of the pathogens were able to grow in the salad products (with an initial inoculum around  $10^5$  cfu/g) at 6 days, none were detected at 6 days when co-inoculated with the LAB cultures.

We used genetically marked *Leuconostoc mesenteroides*, *L. plantarum*, *L. lactis* (nisin-producing), and *L. monocytogenes* in (unpublished) mixed-culture experiments in cucumber juice and non-acidified, brined cucumber products. The bacterial strains were marked with plasmid-borne chloramphenicol or erythromycin resistance markers to allow their selective enumeration on MMRS (for LAB, Daeschel and Fleming, 1984) or MOX agar (for *Listeria*, Atlas, 1993) containing the appropriate antibiotic. In cucumber juice experiments, *L. lactis* predominated over *L. monocytogenes*, even if the initial inoculum level of the *L. lactis* culture was 2 log cycles lower than that for *L. monocytogenes*.

These results were similar to those of Buchanan and Klawitter (1992), who showed similar suppression of *L. monocytogenes* growth by a bacteriocin-producing *Carnobacterium piscicola* strain, when the initial inoculum of *C. piscicola* culture was 2 logs lower than that of the

**Table 1** Inhibitory metabolites of lactic acid bacteria<sup>a</sup>

Product	Main target organisms
<b>Organic acids</b>	
Lactic acid	Putrefactive and Gram-negative bacteria, some fungi
Acetic acid	Putrefactive bacteria, clostridia, some yeasts and fungi
<b>Hydrogen peroxide</b>	Pathogens and spoilage organisms, especially in protein-rich foods
<b>Enzymes</b>	
Lactoperoxidase system with hydrogen peroxide	Pathogens and spoilage bacteria (milk and dairy products)
Lysozyme (by recombinant DNA)	Undesired Gram-positive bacteria
<b>Low-molecular-weight metabolites</b>	
Reuterin	Wide spectrum of bacteria, yeasts, and molds
Diacetyl	Gram-negative bacteria
Fatty acids	Different bacteria
<b>Bacteriocins</b>	
Nisin	Some LAB and Gram-positive bacteria, notably endospore-formers
Other	Gram-positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type

<sup>a</sup>Adapted from Holzapfel et al., (1995)

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*L. monocytogenes* culture. In our (unpublished) challenge studies with *L. monocytogenes* in competition with *L. lactis*, *L. mesenteroides*, or *L. plantarum* (added separately) in nonacidified, refrigerated brined cucumber products, growth of the added *L. monocytogenes* culture was primarily inhibited by naturally present microflora rather than the added LAB strains from our culture collection.

The *L. monocytogenes* culture did not grow, but the added biocontrol cultures did not predominate over the indigenous LAB population, even though the biocontrol LAB culture had 100 times the initial cell concentration of the indigenous LAB.

## Selecting LAB for Biocontrol

An advantage of using biocontrol cultures is that the hurdles (including production of organic acids and bacteriocins) that inhibit growth of pathogens in a food product increase as conditions (e.g., storage time and temperature) become more favorable to growth of bacteria. Biocontrol cultures will likely be product specific, as growth of bacteria in plant materials may be affected by the availability of nutrients and naturally present inhibitors.

The competitiveness of biocontrol cultures may be enhanced by bacteriocin production. The competitive advantage of using bacteriocin-producing LAB has been demonstrated in cabbage and olive fermentations (Breidt et al., 1995; Ruiz-Barba et al., 1994).

To be successful, the growth rates of a biocontrol culture presumably should be faster than that of the target pathogens, though the rates of production of inhibitory metabolites, and relative sensitivities of the pathogens and biocontrol cultures to these metabolites, will also affect the outcome of the competitive growth of these organisms. While rapid growth may be desirable from a safety standpoint, a liability may result regarding product quality. Reducing the initial population on MPR products may prolong the product shelf life and may be required to ensure the predominance of



Fig. 4—Products for salad bars—in addition to fresh-cut, prepackaged salads sold at retail—may be suitable applications for biocontrol cultures

a biocontrol culture. However, in the absence of biocontrol, reducing the competitive microflora may have the unintended effect of permitting growth of pathogenic bacteria.

A related point is that laboratory strains of LAB may not be ideally suited for competition with epiphytic LAB present on fruit and vegetable products. LAB isolates from the product in which the biocontrol culture will be used, such as the bacteriocin-producing isolates of Vescovo et al. (1996), may be best suited for growth in that product. Because there can be significant variation in the natural microflora on vegetables (Nguyen-the and Carlin, 1994), the epiphytic bacteria may be unreliable in controlling the growth of pathogens during refrigerated storage.

Biocontrol strategies for MPR fruit and vegetable products may include: (1) isolate potential biocontrol LAB from the refrigerated product; (2) reduce the total microflora in the vegetable product by one or more procedures, including heat, washing using chemical sanitizers, irradiation, or others; (3) add a bacteriocin-producing biocontrol culture, to achieve an appropriate initial cfu/mL, as determined experimentally; and (4) store the product under refrigeration. The product shelf life would then be dictated by growth of the biocontrol culture, and under temperature abuse conditions, the biocontrol culture will grow rapidly and prevent growth of pathogens.

Successful application of biocontrol cultures will require balancing quality (shelf life) and safety considerations. Measuring and understanding the factors affecting the competitive growth of bacteria will be required to make rational choices in selecting biocontrol cultures for food products.

## REFERENCES

- Adams, M. R., Hartley, A.D., and Cox, L.J. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiol.* 6: 69-77.
- Atlas, R.M. 1993. "Handbook of Microbiological Media," ed. L.C. Parks, p. 683. CRC Press, Boca Raton, Fla.
- Bennik, M.H., Smid, E.J., Rombouts, F.M., and Gorris, L.G.M. 1995. Growth of psychrotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. *Food Microbiol.* 12: 509-519.
- Brackett, R.E. 1992. Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *J. Food Protect.* 55: 808-814.
- Breidt, F., Crowley, K.A., and Fleming, H.P. 1995. Controlling cabbage fermentations with nisin and nisin-resistant *Leuconostoc mesenteroides*. *Food Microbiol.* 12: 109-116.
- Buchanan, R.L. and Klawitter, L.A. 1992. Characterization of a lactic acid bacterium, *Carnobacterium piscicola* 1k5, with activity against *Listeria monocytogenes* at refrigeration temperatures. *J. Food Safety* 12: 199-217.
- Carlin, F., Nguyen-the, C., and Morris, C.E. 1996. Influence of background microflora on *Listeria monocytogenes* on minimally processed fresh broad-leaved endive (*Cichorium endivia* var. *latifolia*). *J. Food Protect.* 59: 698-703.
- Daeschel, M.A. and Fleming, H.P. 1984. Selection of lactic acid bacteria for use in vegetable fermentations. *Food Microbiol.* 1: 303-313.
- DeVuyst, L. and Vandamme, E.J. 1994. Antimicrobial potential of lactic acid bacteria. In "Bacteriocins of Lactic Acid Bacteria," ed. L. De Vuyst and E.J. Vandamme, pp. 91-142. Blackie Academic and Professional, London.
- Farber, J.M. and Peterkin, P.I. 1991. *Listeria monocytogenes* a food-borne pathogen. *Microbiol. Rev.* 55: 476-511.
- Florey, H.W. 1946. The use of micro-organisms for therapeutic purposes. *Yale J. Biol. Med.* 19: 101-117.
- Gilliland, S.E. and Speck, M.L. 1975. Inhibition of psychrotrophic bacteria by lactobacilli and pediococci in nonfermented refrigerated foods. *J. Food Sci.* 40: 903-905.
- Gould, G.W. 1992. Ecosystem approaches to food preservation. *J. Appl. Bacteriol. Symp. Suppl.* 73: 58S-8S.
- Gombas, D.E. 1989. Biological competition as a preserving mechanism. *J. Food Safety* 10: 107-117.
- Hao, Y.-Y. and Brackett, R.E. 1993. Influence of modified

# Lactic Acid Bacteria

CONTINUED

- atmosphere on growth of vegetable spoilage bacteria in media. *J. Food Protect.* 56: 223-228.
- Hintian, C.B. and Hotchkiss, J.H. 1987. Comparative growth of spoilage and pathogenic organisms on modified atmosphere-packaged cooked beef. *J. Food Protect.* 50: 218-223.
- Ho, J.L., Shands, K.N., Friedland, G., Eckind, P., and Fraser, D.W. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. *Arch. Intern. Med.* 146: 520-524.
- Holzappel, W.H., Geisen, R., and Schillinger, U. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins, and food-grade enzymes. *Int'l. J. Food Microbiol.* 24: 343-362.
- Hotchkiss, J.H. 1988. Experimental approaches to determining the safety of food packaged in modified atmospheres. *Food Technol.* 42(9): 55, 60-64.
- Hotchkiss, J.H. and Banco, M.J. 1992. Influence of new packaging technologies on the growth of microorganisms in produce. *J. Food Protect.* 55: 815-820.
- Leistner, L. and Gorris, L.G.M. 1995. Food preservation by hurdle technology. *Trends Food Sci. Technol.* 6: 41-46.
- Lund, B.M. 1992. Ecosystems in vegetable foods. *J. Appl. Bacteriol. Symp. Supp.* 73: 115S-126S.
- Madden, J.M. 1992. Microbial pathogens in fresh produce—The regulatory perspective. *J. Food Protect.* 55: 821-823.
- Meneley, J.C. and Stanghellini, M.E. 1974. Detection of enteric bacteria within locular tissue of healthy cucumbers. *J. Food Sci.* 36: 1267-1268.
- Nguyen-the, C. and Carlin, F. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* 34: 371-401.
- Raccach, M., Baker, R.C., Regenstein, J.M., and Mulnix, E.J. 1979. Potential application of microbial antagonism to extended storage stability of a flesh type food. *J. Food Sci.* 44: 43-46.
- Romick, T.L. 1994. Biocontrol of *Listeria monocytogenes*, a psychrotrophic pathogen model, in low salt, non-acidified, refrigerated vegetable products. Ph.D. thesis. North Carolina State University, Raleigh.
- Ruiz-Barba, J.L., Cathcart, D.P., Warner, P.J., and Jimenez-Diaz, R. 1994. Use of *Lactobacillus plantarum* LPCO10, a bacteriocin producer, as a starter culture in Spanish-style green olive fermentations. *Appl. Environ. Microbiol.* 60: 2059-2064.
- Russel, J.B. 1992. Another explanation of the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. *J. Appl. Bacteriol.* 73: 363-370.
- Saleh, M.A. and Ordal, Z.J. 1955. Studies on growth and toxin production of *Clostridium botulinum* in a pre-cooked frozen food. *Food Res.* 20: 340-350.
- Schlech, W.F. III, Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, J.A., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S., and Broome, C.V. 1983. Epidemic listeriosis—Evidence for transmission by food. *New Eng. J. Med.* 308: 203-206.
- Schofield, G.M. 1992. Emerging foodborne pathogens and their significance in chilled foods. *J. Appl. Bacteriol.* 72: 267-273.
- Sofos, J.N. 1993. Current microbiological considerations in food preservation. *Int'l. J. Food Microbiol.* 19: 87-108.
- Stiles, M.E. 1996. Biopreservation by lactic acid bacteria. *Antonie Van Leeuwenhoek.* 70: 331-345.
- Tanaka, N., Meske, L., Doyle, M.P., Traisman, E., Thayer, D.W., and Johnston, R.W. 1985. Plant trials of bacon made with lactic acid bacteria, sucrose and lowered sodium nitrite. *J. Food Protect.* 48: 679-686.
- USDA. 1989. Revised policy for controlling *Listeria monocytogenes*. U.S. Dept. of Agriculture, Fed. Reg. 54: 22345-22346.
- Vandenberg, P.A. 1993. Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiol. Rev.* 12: 221-38.
- Vescovo, M., Torriani, S., Orsi, C., Macchiarioli, F., and Scolari, G. 1996. Application of antimicrobial-producing lactic acid bacteria to control pathogens in ready-to-use vegetables. *J. Appl. Bacteriol.* 81: 113-119.
- Zagory, D. and Kaday, A.A. 1988. Modified atmosphere packaging of fresh produce. *Food Technol.* 42(9): 70-77.

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