NISIN AND THE MARKET FOR COMMERICIAL BACTERIOCINS

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Abstract: This report provides a background analysis of the market for nisin and other commercially available antimicrobial food additives. The report concludes that there is a potential role for a new U.S.-based entity to compete with a nisin product that is cost-competitive or provides quality guarantees to satisfy U.S. buyers who have tight specifications for ingredient sourcing and food safety and quality oversight.

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The **Texas Agribusiness Market Research Center (TAMRC)** *has been providing timely, unique, and professional research on a wide range of issues relating to agricultural and agribusiness markets and products of importance to Texas and the nation for thirty-five years.* **TAMRC** *is a market research service of the Texas Agricultural Experiment Station and the Texas Agricultural Extension Service. The main* **TAMRC** *objective is to conduct research leading to expanded and more efficient markets for Texas and U.S. agricultural and food products. Major* **TAMRC** *research divisions include International Market Research, Consumer and Product Market Research, Commodity Market Research, and Contemporary Market Issues Research.*

NISIN AND THE MARKET FOR COMMERICIAL BACTERIOCINS

Executive Summary

Nisin is the most commercially important member of a large class of bacteriocins produced by bacteria that can kill or inhibit the growth of other bacteria. This phase of the Nisin Market Study analyzes the characteristics of the current market for nisin and competing bacteriocins in four main sections highlighting: (1) the general market characteristics for antimicrobial preservatives; (2) current producers and sellers of commercial grade nisin; (3) current users of nisin and competing bacteriocins; and (4) implications for the market opportunities for nisin production in the U.S.

Nisin was developed in the early 1960s and is currently the most researched of all bacteriocins. Recognized as a food preservative by FAO/WHO in 1969, the FDA approved the use of nisin as an additive in canned products in the United States to inhibit the growth of *C. botulinum* in 1988. However, the FDA's zero tolerance policy toward Listeria contamination of food has motivated the search for new variations of bacteriocins in the global research community.

Concern about the resistance of emerging pathogens to conventional food preservation techniques and consumer resistance to chemical forms of control are increasing private and public research interest in expanding the opportunity for layers of protection, a "multi-hurdle" approach, rather than relying on a single method. Antimicrobial bacteriocins are among the several "hurdle" technologies and methods that have proved to be effective separately but even more effective in some combination. Multi-hurdle strategies incorporated into innovative delivery mechanisms are critical to the market expansion of the Ready-To-Eat (RTE) food products sector. The use of multi-hurdle preservative strategies provides incentive for expanded and innovative applications for nisin rather than for research on other antimicrobials due to the existing regulatory acceptance of nisin as GRAS and the time and resources needed to gain new approvals.

There are thousands of natural antimicrobials but most do not have sufficient activity to be considered for commercial development. Some have sufficient activity for inclusion in formulated, multi-hurdle, preservative systems. Bacteriocins are currently used to control microbial growth in a wide variety of food and beverage products. The preferred use of bacteriocins in these applications is in part due to their heat resistance since thermal processing is used extensively in food processing. Bacteriocins have narrow activity spectra against gram-positive bacteria, however. Nisin's preferred status among bacteriocins is a reflection of its relatively broader activity but also of indications of gram-negative bacteria control through multi-hurdle use.

Three typical applications of bacteriocins for the bio-preservation of food include: (1) the addition of purified bacteriocins to food products; (2) the inoculation of a food product with lactic acid bacteria that will manufacture bacteriocin in the product itself; and (3) the use of an ingredient in food processing that has been previously fermented with a bacteriocin-producing bacteria. Also, several new applications of nisin have demonstrated significant antimicrobial activity in controlled research studies.

Bacteriocin preservatives are part of the $22 billion global food additives market that has grown at an average annual rate of 2.4% per annum between 2001and 2004. This market is expected to continue growing at 2-3% per annum through 2007 to $24 billion. The preservatives market is expected to

experience steady to strong growth potential associated with the RTE market and the need to provide associated food product stability since many RTE products are sold in kiosks, food courts, and other venues which may have fewer conventional methods of stabilizing food.

Consolidation in the preservatives industry through mergers and acquisitions is rapidly creating a global supply market. The global leader in the antimicrobial preservatives industry is Danisco A/S, a Danish company, with Royal DSM (Netherlands), and Kerry Bio-Sciences (Ireland) considered to be their peer competitors in the bio-preservatives sector. Danisco's Nisaplin™ is generally considered to be the most commercially available form of nisin for food preservative uses. Danisco's strategic focus for their nisin product line is the U.S. meat and deli food sector in order to take advantage of the FDA approval status of nisin as a natural ingredient. Other players in the global nisin market include Rhodia, S.A. (France) along with numerous producers and providers of various antimicrobial products based in China. Some of these Chinese sources are in joint ventures or alliances with European-based corporate entities.

Most U.S. users of nisin appear to purchase the product from European sources. Some reports suggest that U.S. firms avoid purchasing nisin from Chinese companies because of a current lack of regulatory oversight in China, an insufficient cost differential between European and Chinese sources, and/or a lack of a competitive U.S. market strategy by Asia-based nisin sources. Three key U.S. users of nisin (Sysco Foods, Kraft, and Schreiber Foods) provided some insight into the use of nisin in the U.S. Their information suggests that certain buyers consider the incorporation of nisin in their multi-hurdle food preservation strategies as beneficial and, indeed, are seeking additional ways to incorporate nisin into their operations.

An analysis of the number of U.S. RTE deli meat producing establishments (likely to be the highest demander of nisin or similar agents to control spore growth of listeria) identified 143 large firms out of nearly 5,000 producing RTE deli meats. Key large players could be selected for marketing a lower-cost antimicrobial product. Numerous other market opportunities exist. For example, the cheese industry has over $22 billion in total industry value. Certain cheeses are high-value product types and might support price mark-ups for quality-enhancing additives. There are relatively few large companies in the U.S. cheese industry, allowing opportunity for focused commercialization of a lower-cost nisin-based preservative in a concentrated industry.

In conclusion, this study suggests substantial near-term commercial opportunities for nisin within a global market for antimicrobial food additives in which Danisco appears to operate with little competition currently. This following are some insights regarding market potential for nisin based on the findings of the study. First, the only characteristics that make nisin in general preferred to other more cost effective multi-hurdle modes of food preservation are its "natural" ingredient designation in the U.S. (but not necessarily in the EU) and a broader antimicrobial activity range than other bacteriocins in the same class. Beyond these two characteristics, the commercial viability of nisin is determined largely by the economic, social, technological, and regulatory considerations outlined in the report.

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Nisin is the most commercially important member of a large class of bacteriocins produced by bacteria that can kill or inhibit the growth of other bacteria. Approved by the U.S. Food and Drug Administration (FDA) in 1988 for use in pasteurized processed cheese spreads, nisin is currently the only purified bacteriocin approved for food use in the U.S. However, nisin has been used for several decades as a food preservative in more than 50 countries.

Although the effectiveness of nisin and other bacteriocins as food preservatives has been well documented in the research literature, cost remains an issue impeding broader use of bacteriocins as food additives. A key factor in the location of market expansion is the acceptance of nisin as a "natural preservative" in the U.S. whereas the European Union (EU) market has reservations about this designation[1]. Thus, there is an ongoing search for new and more effective methods of manufacturing nisin and other existing bacteriocins and means for their commercial development to address both biologic and economic concerns. Pimaricin, an antimycotic preservative, is the only other antibiotic-like, naturally occurring compound that has similar FDA approval.

As new, lower cost and more efficient means are developed to produce nisin, the primary concerns become those of identifying potential market opportunities as well as the competitive pressures from the growing commercialization of other bacteriocins. This study focuses on these two issues in four main sections highlighting (1) the general market characteristics for antimicrobial preservatives; (2) current producers and sellers of commercial grade nisin; (3) current users of nisin and competing bacteriocins; and (4) implications regarding the market opportunities for nisin.

General U.S. and World Market Parameters for Antimicrobial Preservatives

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Understanding the commercial market opportunities for nisin first requires an understanding of the world market characteristics for nisin and competing bacteriocins to provide some context to the analysis of market opportunities. Accordingly, this section of the report provides a history of the development of the market for nisin, issues related to safety, stability and food product use, competing commercially viable bacteriocins, and relevant characteristics of the production of nisin.

History of Nisin Market Development

Bacteriocins comprise a subgroup of preservatives that are produced by bacteria and possess antibiotic attributes that differ from therapeutic antibiotics in that they possess a narrow specificity of action against similar or closely related strains. The use of therapeutic antibiotics in food is prohibited but the use of additives that possess natural preservative or antimicrobial properties has become a strategic, and in some cases trademark, component of food safety. To avoid confusion with therapeutic antibiotics,

[1] Personal communication with K. Haugen, Danisco (Denmark), July 6, 2005.

which are sometimes known to cause allergic reactions in humans, bacteriocins are more generally referred to as antimicrobials rather than antibiotics.[2]

Although their antimicrobial activity was first discovered in 1928, bacteriocins were not used in food products until 1951. The antibiotic characteristics of lactic acid bacteria (LAB), for example, have been known for over half a century. The ability of LAB to control growth of gram-positive microorganisms has become increasingly important globally due to the improved documentation of illnesses and deaths associated with pathogen-contaminated food.

Nisin, produced by *Lactococcus lactis subsp. lactis*[3], was developed in the early 1960s and is currently the most researched of all bacteriocins. Nisin was recognized as a food preservative by FAO/WHO in 1969. In 1988, the FDA approved the use of nisin as an additive in canned products in the United States to inhibit the growth of *C. botulinum*. However, the FDA's zero tolerance policy toward contamination of food by *Listeria monocytogenes*, a resilient pathogenic bacterium common in the environment, has motivated the search for new variations of bacteriocins in the global research community.

Outbreaks of listeriosis, a serious foodborne disease, have attracted public attention and placed increased pressure on food manufacturers with respect to food safety.[4] Although the incidence of the disease declined in the late 1990s and reached a plateau of 0.3 cases per 100,000 of population in 2001, the fatality rate from listeriosis is high compared with other foodborne illnesses.[5] During 2000-2003, 182 food product recall incidents were linked with contamination by listeria species.[6] Most (72) of these product recall events involved meats, 32 involved cheeses, and 22 involved seafood. Producers of these higher-risk product types are likely to consider the use of bacteriocins for enhanced food safety and shelf-life stability to meet FDA's zero tolerance standard and may also be motivated by the high cost of recalls and the potential negative impact on product brand or firm reputation.

Food Safety and Stability Issues

The Joint Expert Committee on Food Additives (JECFA) of the U.N. Food and Agriculture Organization (FAO) and the World Health Organization (WHO) indicated the benefits of nisin specifically, and other bacteriocins, in providing antibiotic activity in 1969.[7] Although the JECFA recommended daily intake limits of 60 mg of pure nisin for a 70 kg person, there is no maximum limit to the use of nisin in processed cheese in Australia, France, or Great Britain.

Although the FDA set a maximum limit of 10,000 IU/g in the U.S., the use of nisin-producing starter cultures is unregulated since Lactoccocus species are generally regarded as safe (GRAS).[8] The FDA no-

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[2] Chen, H. and D.G. Hoover, "Bacteriocins and their Food Applications," *Comprehensive Reviews in Food Science and Food Safety* 2:82 -100, 2003.

[3] In early research *Streptococcus lactis (now L. lactis)* was classified as Lancefield serological group N Streptococcus and documented as inhibiting streptococci in milk. Subsequently, Mattick and Hirsch referred to this bacteriocin as Nisin; a word derived from "N inhibitory substance."

[4] Mead P.S., Slutsker L., Dietz V., McCaig L.F., Bresee J.S., Shapiro C., et al., "Food-related Illness and Death in the United States," *Emerg Infect Dis* 5:607–25, 1999.

[5] http://www.cdc.gov/foodnet/pub/publications/2000/pass_2000.pdf and http://vm.cfsan.fda.gov/~dms/lmr2-su.html

[6] Authors' analysis of FDA and USDA press releases. Unpublished.

[7] WHO. 1969. Specifications for Identity and Purity of Some Antibiotics. World Health Organization/ Food Additives. 69.34:53-67.

[8] FDA. Federal Register. 1988. Nisin Preparation: Affirmation of GRAS Status as a Direct Human Food Ingredient. 21 CFR Part 184, Fed. Reg. 53:11247-11251.

action status, based on GRAS, set the legal precedent in the U.S. for the use of bacteriocins as food additives. The maximum dose limits for use are 200 mg/kg in canned and plant protein foods and 500 mg/kg in dairy and meat products. However, the more typical dose is 100-200 mg/kg, depending on the extension of shelf-life, or microbial control, desired. Although the FDA established the U.S. precedent for use of nisin in food products, the Food Safety and Inspection Service (FSIS) has jurisdiction over the safety and effectiveness of bacteriocins in upstream commodity segments of the market, such as meat and poultry. Thus, oversight depends on where the bacteriocin is incorporated into the food supply chain - upstream where the product is still considered a commodity or downstream during further processing and manufacturing into intermediate or complete meal products.

Concern about the resistance of emerging pathogens to conventional food preservation techniques and consumer resistance to chemical forms of control are increasing private and public research interest in expanding the opportunity for layers of protection, a "multi-hurdle" approach, rather than relying on a single method. Concurrently, the concern that automatic addition of bacteriocins to the manufacturing process has the potential to increase the rate of resistance adds to the incentive to identify a broad spectrum of commercially producible bacteriocins. However, evidence from research studies indicates that *L. monocytogenes* resistance to nisin does not appear to be stable, providing additional support for the use of nisin over other antimicrobials.

Stabilizing or controlling microbial activity is one of the many parameters considered in shelf-life stability. The strategic concept is to synergistically incorporate as many of the following five "hurdles" to microbial growth as is technically and economically feasible in order to eliminate that growth: (1) hydrogen ion concentration (pH); (2) water activity (aw); (3) oxidation-reduction potential (Eh); (4) the efficacy of antimicrobial ingredients; and (5) storage temperature.[9]

Possible "hurdle" technologies and methods that have been found effective separately but with improved efficacy in combination are as follows:

- Lowering the pH at ambient temperature through the use of organic acids such as lactic acid (considered one of the more effective techniques);
- Vacuum and modified atmosphere packaging (MAP) often including the use of nitrogen and CO_2 to minimize oxidation and thereby limit microbial spoilage by oxygen starvation;
- Heat treatment and Ultra Heat Treatment (UHT) used in conjunction with aseptic packaging and bottle filling;
- Pulsed Electric Fields (PEF) technology and High Pressure Processing (HPP), and Pulsed High-Intensity Light for use with juices, milks and liquid eggs;
- Hydrodynamic Pressure (HDP) using shock waves created in water medium surrounding the product to reduce, or eliminate, pathogens, tested for effectiveness with ground beef; and
- Antimicrobial bacteriocins that inhibit gram-positive spore activity.

Competing Available Bacteriocins and Their Commercial Uses

There are thousands of natural antimicrobials but most do not have sufficient activity to be considered for commercial development. Some antimicrobials have sufficient activity to be considered for inclusion in a formulated, multi-hurdle, preservative system. In the mid-1990's, Australian researchers identified Piscicolin 126, produced by the LAB *Carnobacterium piscicola* JG126, from 300 food bacteria that exhibited antimicrobial behavior as a highly competitive candidate to nisin in inhibiting growth of *L.*

[9] Deis, R.C., "The Complexity of Shelf-Life Stability," *Food Product Design*, Weeks Publishing, February 2002.

monocytogenes in deli and cheese products.[10] Chen and Hoover (2003) provide a review of the three classes of bacteriocins but those of primary consideration by food scientists fall under Class I (lantibiotics) and Class IIa. Appendix A reviews the bacteriocin producing bacteria and sphere of activity for these two classes of bacteriocins.

Bacteriocins are currently used to control microbial growth in a wide variety of food and beverage products (Table 1). The preferred use of bacteriocins in these applications, often in conjunction with other methods, is in part due to their heat resistance since thermal processing is used extensively in food processing. In fact, spores damaged by heat have heightened sensitivity to nisin providing improved efficacy against spores in low-acid, heat-processed foods such as canned vegetables. Nisin's mechanism in controlling spore outgrowth is sporostatic (inhibits growth of spores) not sporicidal (lethal to spores), requiring continued presence of nisin to maintain control. There are three typical applications of bacteriocins for the bio-preservation of food:

1. The addition, or application, of purified bacteriocins to food products;
2. The inoculation of a food product with lactic acid bacteria (LAB) that will manufacture bacteriocin in the product itself; and
3. The use of an ingredient in food processing that has been previously fermented with a bacteriocin-producing bacteria.

In 2003, Danisco's Nisaplin™ was considered to be the most commercially available form of nisin for food preservative uses although an increasing number of companies, particularly Asian manufacturers and distributors, were entering this market. The commercially offered product, Nisaplin™, has 2.5% nisin active ingredient, 77.5% NaCl (salt), and nonfat dry milk comprising 12% protein and 6% carbohydrate. However, there is a wide range of active ingredient (a.i.) content across the nisin-based products available commercially, generally in the range of 0.5% to 5% active ingredient. Nisin product form and strength is related to the intended use and desired level of microbial control (see Table 2 and Appendix B).

The use of multi-hurdle preservative strategies provides incentive for expanded and innovative applications for nisin rather than for research on other antimicrobials due to the existing regulatory acceptance of nisin as GRAS and the time and resources needed to gain new approvals. The following examples have all demonstrated significant antimicrobial activity in controlled research studies:

- The use of the bacteriocin nisin as a coating on edible packaging films, such as zein which is used to coat candies, has demonstrated control of listeria spore growth and the potential for reducing recontamination of food between the processing and packaging phases of manufacturing – a key control point for safety risk.[11]
- Incorporation of nisin and lauric acid into a soy-based plastic film that is subsequently laminated on to polyethylene packaging. Preliminary research studies at Clemson University's Packaging Technology Laboratories involve testing this technology for lunchmeat to control listeria spore growth.
- Oregon State University researchers have reported that nisin and lysozyme work as efficient sanitizers when applied to food contact surfaces by creating a biofilm.

[10] "Innovations: Fresh Ideas for Food Safety." Environmental Health Perspectives, Volume 108, No. 11, November 2000, p. A516-519.
[11] "Did you Know? http://www.foodtechsource.com/emag/014 Last accessed June 21, 2005.

Table 1: Areas of Antimicrobial Use in Foods and Beverages by Type of Microbe

Microbe	Meat	Culinary	Dairy	Bakery	Beverages
Listeria	Processed meats	Deli salads	Dressings Soft & smear cheeses		
Gram-positive pathogens Spore formers	Retorted meats	Egg products Canned vegetables	Processed cheeses	Bakery specialties	
Gram-positive spoilage	Processed meats	RTE meals Dressings Egg products Soups & Sauces	Fresh dairy products Cheeses Desserts	Bakery fillings	Fruit juices Soft drinks Alcoholic beverages
Gram-negative pathogens	Chicken & beef Whole carcasses				
Gram negative spoilage	Processed meats	Dressings			
Yeasts & mold spoilage	Processed meats	Dressings Mayonnaise Soups & sauces	Fresh dairy products Cheeses	Breads Pastries Tortillas Fillings	Fruit juices Wines

Source: Danisco website corporate section (http://www.danisco.com/antimicrobials)

Table 2: Commercial Nisin Methods of Use and Forms

Method of Use	Product Form	Food Products
Application	Dip Spray	Cheese slices Deli meats Cut fruit
Incorporation	Liquid Powder	Shredded cheese Ground meat Beverages
Edible Coatings		Candies
Packaging	Coated film Impregnated film	Cut meats Fish Seafood Ready-to-Eat (RTE) Foods

- Silver and zinc zeolites incorporated into packaging cloth, paper, and laminates. When the packaging material is in contact with the product, the zeolites release zinc and silver ions that disrupt the biochemistry of the microbial cell.

Bacteriocins have narrow activity spectra against gram-positive bacteria. Nisin's preferred status among Class I bacteriocins is a reflection of its relatively broader activity but also of indications of gram-negative bacteria control through multi-hurdle use with PEF (pulsed electric fields) after EDTA (ethylenediaminetetraacetic acid, a chelating agent used as a preservative) has been used to make the outer membrane of gram-negative bacteria permeable.[12]

Multi-hurdle strategies incorporated into innovative delivery mechanisms are critical to the market expansion of the Ready-To-Eat (RTE) food products sector. Listeriosis risk is greatest in this sector, particularly for refrigerated foods, because *L. monocytogenes* is capable of multiplying at refrigerator temperatures. Hurdle strategies using antimicrobials have demonstrated the capability of providing control of gram-positive and gram-negative bacteria, including *E. coli spp*. In addition to food uses, bacteriocins have been commercially available in non-food uses for the control of mastitis in livestock and, with enhancement by chelators, in control of ulcers in humans.

The Production of Nisin

The production of bacteriocins has conventionally occurred in a complex media making its manufacture economically infeasible for large-scale production. Extensive research studies have demonstrated that the titer amounts of bacteriocin produced depend on the composition of the medium. Batch or commercial production of bacteriocins by various LAB strains has received considerable attention for several decades due to their antibiotic properties. An economical nutrient source, however, was the primary constraint to developing a commercial system. At the same time, a search for a solution to managing whey as a co-product of cheese manufacture was ongoing which provided challenging environmental management problems due to whey's high biochemical oxygen demand of more than 75%.

By the late 1990's research studies indicated that whey permeate had the potential to provide an economical and successful medium for the growth of LAB strains that produce bacteriocins, in particular nisin.[13] Shimizu[14] et al. demonstrated in 1999 that the use of nisin-producing LAB in a mixed culture system, which included a grain-based fermentation extract, provided the opportunity for increased nisin production through control of the co-production of lactate which causes microbe growth inhibition. The conventional method of extracting lactate with organic solvents, however, cannot be used where nisin is intended for use as a food additive. Extraction of lactate using conventional separation techniques is effective but costly in extending the fermentation process. A mixed-culture that controls lactate production and enables the extension of the fermentation process has the potential to meet consumer demands for environmentally sensitive production techniques.

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[12] Smid, E.J. "Biopreservation of Foods Using Nisin." EU Project No: FAIR-CT96-1148. Agrotechnical Research Institute (ATO), Wageningen University, Netherlands.
[13] Flores, Simone Hickmann and Ranulfo Monte Alegre. "Nisin Production from *Lactococcus lactis* ATCC 7962 Using Supplemented Whey Permeate." Biotechnol. Appl. Biochem (2001) 34, 103-107.
[14] Shimizu, Hiroshi, Taiji Mizuguchi, Eiji Tanaka, and Suteaki Shioya. "Nisin Production by a Mixed-Culture System Consisting of *Lactococcus lactis* and *Kluyveromyces marxianus*." Applied and Environmental Microbiology, July 1999, p. 3134-3142, Vol. 65, No.7.

More recently, scientists at the Department of Food Research associated with the Universidad Autónomo de Querétaro in Mexico demonstrated that the use of predictable concentrations of sweet whey and yeast extract provides a bacteriocin yield of 20g per liter of fermentate, using a 3-liter (commercial) fermenter.[15]

These studies prove that bacteriocins can be produced using abundant, low-cost raw materials. The U.S. produces approximately 30 million tons of liquid whey annually from cheese manufacturing - approximately 9 pounds of whey by-product for each pound of cheese produced. For dairy product manufacturers, this by-product has become a costly waste management problem, particularly with increasingly stringent controls on environmentally sound methods of disposal. Despite the low cost of the materials needed to produce nisin, there is still a question of production scale since the available research reports only laboratory production at experimental levels.

Current Producers/Sellers of Commercial Grade Nisin

Bacteriocin preservatives are part of the $22 billion global food additives market that has grown at an average annual rate of 2.4% per annum between 2001and 2004. This market is expected to continue growing at 2-3% per annum through 2007 to $24 billion. However, this market includes the more uncertain 'fashion' products such as fat replacers, a sector that grew at nearly 7% over this period in addition to the more stable sweeteners, flavors, and preservatives market sectors. Although paralleling the overall sector growth during this period, the preservatives market is expected to experience steady to strong growth potential associated with the Ready-to-Eat (RTE) market and the need to provide associated food product stability since many RTE products are sold in kiosks, food courts, and other venues which may have fewer conventional methods of stabilizing food.

Consolidation in the preservatives industry through mergers and acquisitions is rapidly creating a global supply market. For example, in the preservatives sector, DSM acquired Roche's vitamin and food additives business while Danisco acquired most of Rhodia's ingredient operations.[16] Many western suppliers have formed joint ventures (JV's) and alliances with Chinese firms or moved their production operations to China, in response to the increasing influence on price from Chinese suppliers (see Appendix C).

The global leader in the antimicrobial preservatives industry is **Danisco A/S** with DSM and Kerry Bio-Sciences considered to be their peer competitors in the bio-preservatives sector. Danisco's primary line of business is the supply of ingredients to the global food industry, specializing in sugar and sweeteners. The Danish holding company recorded profits of U.S.$176 million on sales of U.S.$2.9 billion for the year ending April 30, 2004.

Danisco's bio-preservatives R&D were enhanced with the acquisitions of Aplin and Barrett of the UK, their source of antimicrobials research and primary production facility of nisin. Recent strategic alliances and acquisitions in 2003-04 include the 80% acquisition of Tianguan (Nanyang) Co. Ltd. in China, the purchase in 2004 of Rhodia's ingredient business, and the equity alliance with TMI Europe of France.

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[15] Pena-Gomar, G. "Produce Bacteriocin from *L. lactis* Using Alternative Culture Media." As reported in Food Technology Intelligence, Microbial Update International, April 2005, Vol 10, IS 6. www.ftipub.com
[16] "The Food Additives Market: Global Trends and Developments" 3rd Edition. Leatherhead Food International, June 2005.

For the last 5 years, Danisco held a substantial minority interest in Genencor, a venture established in 1982 between Genentech and Corning, Inc. Genencor provided the R&D basis for Danisco's bio-ingredients and brings over 3,000 owned and licensed commercial applications. In accordance with Danisco's corporate strategy of accomplishing growth through acquisition, Danisco purchased the remaining shares of Genencor in a cash transaction in May 2005.

The Genencor division of Danisco has manufacturing locations in the United States, Finland, Belgium, China, and Argentina.[17] More than half of Genencor's $410 million yearly sales are outside the United States.[18] Genencor's profits in 2004 were $26 million, having grown by nearly 15% from 2003. Key competitors to Genencor have been identified as Diversa, Novo Nordisk, and DSM (Royal DSM NV).[19] Among these biotechnology competitors, only DSM is involved substantially in the food ingredients and packaging materials subsectors. A key strategic addition in the full acquisition of Genencor is the infrastructure for Danisco's targeted expansion in the U.S.

Danisco intends to spin off the health care business lines of Genencor and become a minority stakeholder in the resulting bio-pharma enterprise.[20] With this restructuring, Danisco will maintain its operating focus on food industry applications which offer more immediate returns than those from potential medical breakthroughs. Genencor's health care business lost $23 million (EBITDA basis) last year.[21]

Danisco's strategic focus for their nisin product line is the U.S. meat and deli food sector in order to take advantage of the FDA approval status of nisin as a natural ingredient, or additive (GRAS). In addition, the FDA's 'zero tolerance' position on *L. monocytogenes* has created an opportunity through industry demand for effective multi-hurdle preservative approaches, particularly those that are non-chemical and do not change the structure, taste, or texture of the food product. Each prospective use is designed based on food product type (for example, meat versus cheese) and form (whole cut, sliced, or shredded), level of control, and whether the objective is food safety, extended shelf-life, production efficiency, or a combination of objectives. Danisco provides validation of the preservation formulation through their R&D facilities. This validation may also serve as due diligence support in an increasingly accountable and litigious society. Support for this strategic focus is provided by the R&D and production facilities of Aplin and Barrett in the UK with additional production facilities, if needed, in Denmark. Although business and customer support is provided through Danisco's Copenhagen, Denmark operations, there is a regional support and business facility in Kansas.

In building this strategic focus, it is interesting to note that many of the research literature reports indicate that nisin samples originated from Aplin and Barrett. A further research expansion to support their multi-hurdle formulations for customer solutions is Danisco's recent patent application for a method involving pre-application of EDTA followed by a nisin-rosemary product combination which is effective against gram-negative spores.

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[17] http://www.genencor.com. Last accessed June 29, 2005.
[18] Ann Law, www.hoovers.com. Last accessed June 29, 2005.
[19] www.hoovers.com. Last accessed June 29, 2005
[20] *Chemical Market Reporter*, May 2, 2005 v267 i18 p6 (1), full text in Insite2, Reed Business Information, Record Number: A132557389. Accessed June 29, 2005.
[21] Kerri Walsh, *Chemical Week*, April 27, 2005 v167 i14 p22 (1), full text in Insite2, Reed Business Information, Record Number: A132164282. Accessed June 29, 2005.

Other competitors to Danisco are either involved with nisin or potentially could be a force in nisin production. **Royal DSM, NV**, based in the Netherlands, focuses on life sciences and performance materials.[22] The life sciences unit makes feedstock for pharmaceutical and food uses. The life sciences unit also produces sweeteners and food enzymes. The company's performance materials unit makes synthetic fibers, plastics, and resins used in coatings. DSM also produces fiber intermediates, melamine, and agrochemicals. DSM's financial performance in 2004 was remarkable with profits doubling to $357.4 million,on $10.6 billion in annual sales. DSM produces antimycotics, enzymes, and food additives but does not produce a nisin-based product at this time.

Kerry Group Plc is a major supplier of food additives and flavors as well as a producer of consumer foods. Its Kerry Bio-Sciences unit was established in 2004 upon completion of the acquisition of the food ingredient business of Quest International, a Netherlands-based unit of Imperial Chemical Industries, plc. The cash transaction continues an aggressive acquisition program globally since the incorporation of the Irish firm in 1985. Kerry Group's earnings exceeded $182 million for the year ended in 2004. Kerry Group revenues were $5.2 billion for the most recent year.[23] Before acquisition, the Quest food ingredients business had profits of $30 million on sales of $255 million.[24] Its assets included 9 production sites worldwide. Kerry Bio-Sciences' activities in the meat-processing sector include cultures and fermented shelf-life protectants,[25] making it a key competitor in the antimicrobials market. Proteins are also developed for use in pharmaceutical applications.

Rhodia, S.A., based in France, is another player in the specialty chemicals business. Rhodia was an innovator in nisin-using products, having successfully obtained FDA approval in 2001 for the use of nisin in processed meat production.[26] Rhodia's divestment strategy in recent years included the sale of the enzyme products business to Genencor and the sale of Rhodia's food ingredients business to Danisco.[27] Rhodia posted a net loss of $781 million for 2004 on more than $6.6 billion in sales.[28] As of January 2003, Rhodia's remaining food division was focusing on food additives, which could include nisin. The specialty chemicals lines remain independent under the Rhodia business, according to the most recent available information.

As indicated in Appendix C, there are numerous producers and providers of nisin and other antimicrobial products based in China. Some of these sources are in joint ventures or alliances with European-based corporate entities. There are some reports that at least U.S. firms consistently avoid purchasing nisin from Chinese companies. Reasons for this could include a current lack of regulatory oversight in China, an insufficient cost differential between European and Chinese sources for companies choosing to incorporate nisin in their preservative strategy, or whether the Asia-based nisin sources lack a competitive market strategy in comparison to their European counterparts such as Danisco. Further investigation on this issue is warranted.

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[22] Tim Walker, www.hoovers.com. Last accessed June 29, 2005.
[23] Financial information converted from euros to US dollars at exchange rate of 1.25 dollars/euro.
[24] ICI news archive, March 2, 2004, http://www.ici.com/ICIPLC/news/po_arch_story.jsp?archive=1&year=2004a&newsId=393
[25] *Kerry Group Annual Report and Accounts*, 2004.
[26] http://www.na.rhodia.com/cws/home.jsp?current Site=US&bm
[27] Ian Young, *Chemical Week*, Jan. 15, 2003, v165 i2 p13(1), full text in Insite2 Record No: A96961111.
[28] Financial information converted from euros to US dollars at exchange rate of 1.25 dollars/euro.

Leading U.S. and Multinational Food Industry Users of Nisin and Other Antimicrobials

A key factor in understanding the market potential for nisin is the wide variation in current manufacturing systems that result in reported prices ranging from $200/kg to $375/kg. However, translating the cost of nisin application into cost per unit weight of product depends on the target bacteria, the dosage needed to control spore growth for each specific product (such as meat, cheese, or fruit), the risk profile of the product, and whether the control objective is food stability or extended shelf life.

Sysco Foods' primary objective is stability and predictability of product shelf-life rather than extended shelf-life. The company requires suppliers to use a multi-hurdle approach to shelf-life stability. Although suppliers can choose among alternative synergistic approaches to food stability, according to their cost-benefit determination, the use of antimicrobials such as nisin is preferred by Sysco as a method for deli-meats, cheese, and RTE products[29]. According to Sysco, the cost to their supplier is generally in the range of 1.5-2¢/lb. of product. In the interest of transparency, Sysco requires that all labels list preservative ingredients, even though this is not strictly required for GRAS-designated ingredients. None of Sysco's product lines or ingredients can be sourced from China currently (June 2005) including ingredients of products supplied to Sysco. This U.S. foodservice leader is closely following the R&D on integrated antimicrobial packaging, particularly with respect to whether FDA will approve these products as GRAS or will require additional approvals.

Publicly available information indicates that **Kraft** uses antimicrobials for a wide range of products and product forms including cheese slices, shredded cheese, and, more recently, has considered incorporating antimicrobials in a multi-hurdle approach to shelf stability on a broader range of further processed and refrigerated products. In November 2004, Kraft was issued a U.S. patent for a method of stabilizing fully cooked and refrigerated filled and unfilled pasta against pathogenic contamination for 120 days or longer. Kraft's method inoculates fortified cheese whey with a nisin-producing microorganism.[30] However, Kraft personnel will not reveal specifics on how they are using nisin or the product lines treated.

Schreiber Foods is a $2 billion privately held, global cheese company supplying foodservice, retail, and government with a broad range of dairy products. Schreiber has 13 production facilities and 4 distribution facilities in 7 states across the U.S. and facilities, or joint ventures, in Brazil, France, Germany, India, Mexico, and Saudi Arabia. Schreiber uses a multi-hurdle approach to controlling microbial contamination but uses non-nisin antimicrobials and antimycotics to control Clostridia species, which is their primary concern.

The details provided by these three companies suggest that certain buyers consider the incorporation of nisin in their multi-hurdle food preservation strategies as beneficial and, indeed, are seeking additional ways to incorporate nisin into their operations. While a comprehensive industry survey of users is outside the scope of this study, the possible extent of the market is suggested by industry-level statistics. Businesses that produce ready-to-eat (RTE) deli meat have been implicated in the most recent food safety crises relating to listeria species. The potential size of the market for nisin from the RTE deli

[29] Personal communication with Craig Watson, VP for Quality Assurance, Sysco Foods
[30] Food Ingredient News, November 2004.

meat segment can be estimated in terms of the number producing establishments (processing plants) which are the potential customers or in terms of the value of product shipments.

In 2001, there were 4,976 U.S. establishments producing RTE meat and poultry products[31] of which 1,630 were designated by the U.S. Economic Census as having RTE meat production as their principal activity (Table 3). The size distribution of these producers was skewed toward small operations. Only 143 producers were designated as "large" by USDA's definition (having more than 250 employees). In terms of production value of RTE deli meat, information from Neilson Co. as of 2001 indicated that U.S. sales of pre-packaged deli meat totaled $11.6 billion. This segment is likely to be the highest demander of nisin or similar agents to control spore growth of listeria species. Deli meats sliced at retail ($13.6 billion in annual sales) may also be a market for nisin.

The market potential for seafood and cheese producers cannot be defined as precisely as the RTE deli meat segment. The U.S. Economic Census provides information on the cheese industry in aggregate while the information for seafood is for both fresh and frozen products (Table 3). While all of these products are potential uses for nisin, this market size estimate is the broadest approach.

The $25 billion deli meat industry has the highest total potential value as a market for antimicrobials. The large number of total establishments indicates a fragmented industry as a customer base for a potential producer of nisin. However, it should be noted that there are 143 identified large firms producing RTE deli meats, so there is a strong likelihood that key large players can be selected for marketing an additive product. The cheese industry has over $22 billion in total industry value. Certain cheeses are high-value product types and might support price mark-ups for quality-enhancing additives. There are relatively few large companies in the U.S. cheese industry, allowing opportunity for focused commercialization of nisin-based preservatives in a concentrated industry.

Conclusions and Implications for Market Opportunities for Nisin

This report provides reviews the primary characteristics of the current markets for nisin and competing bacteriocins. The review suggests substantial near-term commercial opportunities for nisin within a global market for antimicrobial food additives in which Danisco appears to operate with little competition currently. The business entities involved in producing antimicrobial and antimycotic additives are strong competitors, billion-dollar firms, mostly based in Europe but producing and marketing globally. These firms can supply from low-cost locations relatively easily although the only specific information we have indicates that the market leader produces its branded nisin products (Nisaplin™ and Novasin™) in the UK and at a secondary facility in Denmark. In addition, a number of lesser-known players operate in China. There is a potential role for a new U.S.-based entity to compete with a nisin product that is cost-competitive or provides quality guarantees to satisfy U.S. buyers who have tight specifications for ingredient sourcing and food safety and quality oversight.

This study provides some insights regarding the market potential for nisin. The main characteristics that make nisin preferred in general to other more cost effective multi-hurdle modes of food preservation are its "natural" ingredient designation in the U.S. (but not necessarily in the EU) and a broader antimicrobial activity range than other bacteriocins in the same class. Beyond these two characteristics,

[31] USDA-FSIS, Appendix A to Final Regulatory Impact Analysis, 2003.

Table 3: Potential Markets for Antimicrobials in Food Use, Sales and Number of Establishments by Selected Product Type.

Product	NAICS[1]	Sales	Companies	Establishments
		$ millions	number	number
Cheese	311513	22,058	366	501
Seafood fresh and frozen	311712	7,567	531	606
Perishable processed food	311991	5,296	561	610
Deli meat, ready-to-eat		25,200		4,976

[1] North American Industry Classification System
Sources: US Economic Census and USDA FSIS, Final Regulatory Impact Analysis of Listeria Rule, http://www.fsis.usda.gov

however, the commercial viability of a nisin as compared to any other bacteriocin is determined largely by economic, social, technological, and regulatory considerations.

Economic cost considerations have constrained the adoption and expansion of nisin and other antimicrobials as effective options in multi-hurdle food preservation strategies. Nisin production formulation using low-cost ingredients may have potential in the U.S. market since the global market leader, Danisco, is manufacturing in the UK and Denmark. Danisco's pricing of nisin is based on "internal standards" which are not published or revealed. Prices are "negotiated" with customers incorporating the "complete solution" and, as such, appear to follow a cost-based pricing approach. Consequently, if nisin production in the U.S. is at least as cost efficient as Danisco's current method, there may be opportunities for the development of a commercial scale, U.S.-based facility to produce nisin to replace imports. A substantial initial investment in marketing the new product would also likely be necessary.

The **social acceptance** of nisin as a food additive is not currently a market constraint. The use of nisin in a multi-hurdle approach to food safety, stability, and shelf-life extension has the benefit of using a non-chemical, non-transforming technology that does not affect flavor, texture or product structure. In this case, the social acceptance of nisin might be even higher in the EU than in the U.S. Nevertheless, conventional methods of producing nisin that may include chemical processing could negate consumer acceptance and even the perception of nisin as an acceptable food preservation alternative.

Technological innovations in the production process and, perhaps more importantly, in the applications of nisin in food packaging, indicate expansion of market opportunities particularly in the ready-to-eat (RTE) food market. DuPont, for example, is a leading investor in corn-based biofilm packaging materials and indirectly supports research at Clemson's food packaging research institute. Clemson is a lead research location for nisin-coated and nisin-impregnated packaging film. DuPont's inherent interest in corn-based supply chains and value-added products indicates a second multinational corporate entity with the appropriate infrastructure to support innovation. Also, as consumers expand their food consumption options to include away-from-home as well as convenient in-home and take-home opportunities, the need to provide continued assurance of food stability in less-controlled environments is a strong motivating decision factor for food corporations.

Regulatory considerations also support the market potential of nisin. Having already been approved by the FDA for use as a naturally occurring antimicrobial and, thus, “generally regarded as safe” (GRAS) provides nisin an important regulatory advantage over competing bacteriocins

Finally, this study identifies a number of key issues relating to the potential market opportunities for nisin that must be addressed in developing a nisin production and marketing plan, including:

- The economic cost-benefit relationship between fermentate inputs and the titrated and purified nisin product.
- The lack of publicly available price quotations for a detailed commercial feasibility assessment.
- The value proposition to food manufacturers, foodservice, and retail associated with benefits of stabilizing food safety, extending shelf life, and reducing the number and cost of food recalls.
- The importance to the food supply chain of the geographic source of the preservative. Although nisin is not legally required to be listed on food labels, the increasing concern for transparency of ingredients is leading manufacturers and foodservice providers to list every additive. This is also a requirement for market access to certain global food retailers. Not all sources have a reputation for quality among buyers, particularly among U.S. buyers.
- The resolution of regulatory approval and oversight associated with incorporation of nisin in packaging films and materials, including the relationship between FSIS and FDA as the regulatory agency.

APPENDICES

Appendix A: Activity Spectra of Some Class I and Class IIa Bacteriocins

Appendix B: Current Uses of Nisin by Product Type

Appendix C: Known Nisin Manufacturers Worldwide

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Appendix A: Activity Spectra of Some Class I and Class IIa Bacteriocins

Bacteriocins	Producing Strain	Activity Spectra
Class I		
acidocin J1132	*Lactobacillus acidophilus* JCM 1132	Active against different species of *Lactobacillus*. Not active against *Lactococcus*, *Pediococcus*, *Streptococcus*, *Listeria monocytogenes*, *Bacillus* spp., and *Staphylococcus* spp.
lacticin 3147	*Lactococcus lactis* DPC3147	Active against different species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *L. monocytogenes*, *Listeria innocua*, *Staphylococcus aureus*, *Bacillus* spp. and *Clostridium* spp.
lactocin S.	*Lactobacillus sake* L45	Active against different species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *leuconostoc*, *Pediococcus*, *L. monocytogenes*, *L. innocua*, *Staphylococcus*, *Bacillus cereus*, and *Clostridium* spp.
Nisin	*Lactococcus lactis subsp. Lactis*	Active against different species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *L. monocytogenes*, *Listeria innocua*, *Listeria grayi*, *Listeria ivanovii*, *Listeria murrayi*, *Listeria seeligeri*, *Listeria welchimeri*, *Staphylococcus spp.* Prevents outgrowth of *Bacillus* spp. and *Clostridium* spp. and bactericidal to their vegetative cells.
plantaricin C	*Lactobacillus plantarum* LL441	Active against different species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, Staphylococcus carnosus, *Bacillus* spp. and *Clostridium* spp. Not active against *L innocua*.
thermophilin 13	*Streptococcus thermophilus* SFi13	Active against different species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *L. monocytogenes*, *L. innocua*, *S. carnosus*, *Bacillus* spp. and *Clostridium* spp. Prevents outgrowth of spores of *B. cereus* and *C. botulinum*
Class IIa		
acidocin A	*Lactobabillus acidophilus* TK9201	Active against different species of *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Streptococcus*, and *L. monocytogenes*. Not active against *Bacillus subtilis* and *S. aureus*.
bavaricin A	*Lactobacillus sake* M1401	Active against different species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *L. monocytogenes*. Not active against *Carnobacterium*, *Streptococcus*, *Brochothrix thermosphacta, Bacillus* spp. and *Staphylococcus* spp.

Bacteriocins	Producing Strain	Activity Spectra
Class IIa (continued)		
curvacin A	*Lactobacillus curvatus* LTH1174	Active against different species of *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Streptococcus*, *L. monocytogenes, L. innocua, L. ivanovii.* Not active against *Leuconostoc* and *Clostridium* spp.
divercin V41	*Canobacterium divergens* V41	Active against different species of, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *L. monocytogenes, L. innocua, L. ivanovii.* Not active against *Lactococcus* and *Leuconostoc.*
enterocin A	*Enterococcus faecium* CTC492	Active against different species of, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *L. monocytogenes, and L. innocua.*
lactococcin MMFII	*Lactococcus lactis* MMFII	Active against different species of, *Enterococcus*, *Lactobacillus*, *Lactococcus, L. ivanovii.*
mesentericin Y105	*Leuconostoc mesenteroides* Y105	Active against different species of, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *L. monocytogenes, L. innocua, L. ivanovii.* Not active against *Lactococcus.*
mundticin	*Enterococcus mundtii* AT06	Active against different species of *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *L. monocytogenes*, and *L. innocua.* Prevents the outgrowth of spores and vegetative cells of *C. Botulinum.*
pediocin PA-1	*Pediococcus acidilactici* PAC 1.0	Active against different species of *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *L. monocytogenes, L. innocua, L. ivanovii, Staphylococcus spp., B. cereus, and Clostridium spp.*
piscicocin V1a	*Carnobacterium piscicola* V1	Active against different species of *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *L. monocytogenes, L. innocu.* Not active against *Lactococcus, B. cereus, Clostridium spp, and S. aureus.*
piscicocin V1b	*Carnobacterium piscicola* V1	Active against different species of *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *L. monocytogenes, L. innocu.* Not active against *Lactococcus, B. cereus, Clostridium spp, and S. aureus.*
piscicolin 126	*Carnobacterium piscicola* JG126	Active against different species of *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *L. monocytogenes, L. grayi, L. ivanovii, L. seeligeri, and B. thermosphacta.* Not active against *Bacillus* spp., *Lactococcus, Clostridium spp, L. denitrificans, and Staphylococcus spp.*

Bacteriocins	Producing Strain	Activity Spectra
Class IIa (continued)		
sakacin A	*Lactobacillus sake* LB706	Active against different species of, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *L. monocytogenes, L. innocua*, and *L. ivanovii.* Not active against *Lactococcus and Leuconostoc..*
sakacin P	*Lactobacillus sake* LB674	Active against different species of, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *L. monocytogenes, L. innocua,* and *L. ivanovii.* Not active against *Lactococcus and Leuconostoc.*

Source: Chen, H. and D.G. Hoover, "Bacteriocins and their Food Applications," *Comprehensive Reviews in Food Science and Food Safety* 2:82 -100, 2003.

Appendix B: Current Uses of Nisin by Product Type

Product	Bacteriocin	Usage
	Food Uses	
Processed cheese	Nisin	Prevent growth of clostridia
Sliced cheese	Nisin Natamycin	Dipping, spraying of a liquid containing 200-300 ppm of the additive on surface of cuts and slices.
Shredded cheese	Nisin Natamycin	Incorporated in bagged, shredded cheese to prevent growth of spoilage bacteria.
Fresh and Mixed Milks	Vasilin™ (nisin)	Control of spoilage, particularly clostridia of the butirica and putrefascent types.
Flavored Pasteurized Milks and Dairy Products	Vasilin™ (nisin)	For use where the addition of flavorings such as cocoa powder increase the bacterial content.
Beer, wine, alcohol	Nisin	Control spoilage LAB.
Canned food	Nisin Vasilin™ (nisin)	Prevent growth of clostridia and botulinum. Vasilin™ can be used when heat treatment doesn't completely destroy thermophilic spores.
Salad dressings	Nisin	Control spoilage LAB in low pH foods
Deli meats – frankfurters/hotdogs	Nisin	Antilisterial
Cold-pack lobster	Nisin added to brine solution	Replace thermal processing to destroy *L. monocytogenes*, resulting in reduced product shrinkage
Cold Smoked Salmon	Nisin	Control *L. monocytogenes.*
Fresh-cut Cantaloupe and Honeydew melon	Nisin with EDTA	Control of yeasts, molds, and Pseudomonas spp.
Baked goods		
Pasta products	Nisin (whey derived)	Stabilize cooked, refrigerated, filled and unfilled pasta against pathogenic contamination.
	Non-Food Uses	
Pharmaceutical	Highly purified nisin with enhancement by chelators	Human ulcer therapy
Veterinary/Pharmaceutical	Highly purified nisin with enhancement by chelators	Mastitis control in livestock

Sources: Profood International, Inc. http://wwwprofoodinternational.com/Natamycin.html Last accessed June 21, 2005 and http://www.gov.pe.ca/ftc Applied Research Last accessed June 21, 2005.

Appendix C: Known Nisin Manufacturers Worldwide

Company	Geographic Location	Producer/Merchant	Products
Peer Manufacturing/Market Competitors			
Danisco Sales $2.9 billion in 2003/04 [Aplin and Barrett (UK), and Genencor, TMI Europe (French biotech)]	Antimicrobial operations in Copenhagen, Denmark Genencor Offices: Palo Alto, CA; Leiden, Netherlands Genencor Manufacturing: Cedar Rapids, Iowa; Rochester, NY; Beloit, WI; Hanko, Finland; Jämsänkoski, Finland; Brugge Belgium; Wuxi, Jiangsu Province, China; Arroyos Prov. de Cordoba, Argentina	Manufacturer R&D involving Nisin at their UK center formerly Aplin and Barrett.	Nisaplin™ (2.5% nisin) off-white powder 1kg polyethylene bottles. Novasin™ (2.5% nisin) light brown powder 550 gm polyethylene bottles.
DSM Sales $10.5 billion in 2004	Netherlands	Producer	Delvocid™ (Natamycin, Pimaricin, E235)
Kerry Bio-Science	Cork, Ireland Locations in Brantford, CA; Cebu, Philippines; Esterol, Malaysia; Menstrie, Scotland; Rochester (MN) and Norwich (NY), US; and Utrecht and Zwijndrecht, Netherlands.	Manufacturer R&D at 2 locations: Naarden, Netherlands and Chicago, US.	Antimicrobials
Other Nisin Providers			
Duke Thomson's International	Madhya Pradesh, India 0091-731-5066802 info@duketoms.com	Manufacturer	Nisin - grey or white powder Delvocid-Natamycin
Profood International, Inc.		Producer	
Sena Health Products and Nutritional Supplements			Nisin Clean – skin and environmental wipes
Xian Medihealth Company Ltd.			Distributor and Exporter
Abana Foodstuff Co., Ltd	Guangzhou, Guang Dong, China (8620) 85542625 gzken@vip.sina.com		Natural Antioxident Nisin

Company	Geographic Location	Producer/Merchant	Products
Other Nisin Providers (continued)			
Merchant Research and Consulting, Ltd.			Nisin, Natamycin, Pimaricin
Ecobio Biotech Co. Ltd.	China	Manufacturer	Nisin
Mayasan A.S.	UK?	International Manufacturer and Supplier	Vasilin – nisin concentrate
Acroyali Holdings	China	Supplier	Nisin in 500g, 1000g plastic bottles, and 10kg carton.[32]
FTZ United International, Inc.	Qingdao, China (0086) 532-6069596 chinachem@yahoo.com	Manufacturer and Wholesaler – exports to Europe, U.S., Korea, Japan	Nisin Natamycin Pimaricin (Kosher)
YP Bio-tech Co., Ltd.	Jinnan Economic Development Area (ShuangGang), Tianjin, China. (8622) 28594287 ypbio@china.com		YP-Nisin 1000 (nisin) Solubility: pH=7, 49mg Nisin/ml Ph=2, 117mg Nisin/ml. YP-50 Natamycin (Pimaricin) prepared as 50% a.i. and 50% lactose. Use in doses of 1-10mg/kg.
Zhejiang Silver-elephant Bioengineering Co. Ltd		R&D, production and marketing ITD is an exclusive dealer of Silver Elephant Nisin in N. America.	Silver Elephant Nisin
Beijing Oriental Rada Biotech Co., Ltd.	Haidian district, Beijing, China.	R&D, fermentation factory and sales network	Nisin (FCC)

Sources:
http://www.danisco.com/antimicrobials Last accessed April 4, 2005.
http://www.genencor.com Last accessed April 29, 2005.
http://www.dsm.com Last accessed June 23, 2005.
http://www.kerrygroup.com/bioscience Last accessed June 23, 2005.
http://www.duketoms.com/nisin.htm Last accessed June 21, 2005.
http://www.sednaproducts.com Last accessed June 21, 2005.
http://www.sototo.com Last accessed June 21, 2005.
http://www.abana.com.cn Last accessed June 21, 2005.
http://www.mcgroup.co.uk Last accessed June 21, 2005.
http://wwwecobio.com.cn Last accessed June 21, 2005.
http://wwwmayasan.com Last accessed April 28, 2005.
http://www.aisonschem.com/nisin.htm Last accessed April 4, 2005.
http://www.nisin.us Last accessed April 4, 2005.
http://www.bjrada.com Last accessed June 21, 2005.

[32] Recommendations: Nisin is useful in 10-50ppm. Nisin must be used in dosages of less than 0.5g/kg in meat products and dairy products, and less than 0.2 g/kg phytoprotein products. Apply as a 5% aqueous solution using sterile water.