Applications in the dairy industry

Last year at the World Conference on Biotechnology for the Fats and Oils Industry, AOCS and the German Society for Fat Research (DGF) explored the potential for biotechnology in the fats and oils industry. In related industries, there are many exciting biotechnology possibilities as well. Associate Editor for JAOCS *News for Biotechnology J.B.M. Rattray asked Susan Harlander, assistant professor of food biotechnology at the University of Minnesota's Department of Food Science and Nutrition, to prepare the following overview on biotechnology and the dairy industry to show biotechnology's impact in another important area of food science.*

Biotechnology has been broadly defined as a collection of technologies that employ living organisms, or substances derived from living systems, in industrial processes. The roots of biotechnology are founded in animal husbandry, plant breeding and food fermentation practices that have been used for thousands of years. Biotechnology is not new to the dairy industry where dairy starter cultures have been and are being used to produce a host of fermented products including cheese, yogurt, buttermilk and sour cream.

Discovered in the 1970s, techniques for splicing and recombining fragments of DNA from different organisms (recombinant DNA technology or genetic engineering) serve as the foundation for the new era of modern biotechnology. Although the term "biotechnology" often is thought to be synonymous with genetic engineering, it, in fact, encompasses a much broader set of technologies including cell fusion, protein engineering, enzyme immobilization, bioreactor design and fermentation technology, and rapid detection systems based on the use of monoclonal antibodies and DNA probes.

To date, the primary industrial focus of biotechnology has been its use in the development of human pharmaceutical products such as insulin, human growth hormone, interferons, interleukin-2 and tissue plasminogen activator produced by genetically engineered microorganisms, and for the development of diagnostic kits for detecting disease. However, the potential for genetically engineering dairy starter cultures with desirable functional,

chemical or nutritional properties is destined to have a major impact on the dairy industry in the next decade.

The lactic acid bacteria have been utilized for the production of fermented foods for thousands of years and are recognized by the U.S. Food and Drug Administration (FDA) as safe for this use in food systems. Within this group of organisms, individual strains possess characteristics essential for successful dairy fermentations. Genetic engineering is a powerful tool for identifying, isolating and combining these desirable properties into one strain—in essence "tailor-making" a strain for a specific purpose. In most cases, the DNA to be inserted into a starter organism comes from another food-approved organism. Unlike techniques that utilize various mutagens to induce random changes in microorganisms, genetic engineering allows for targeted, specific, controllable and precise changes to be made.

Impact on fermentation processes

Phage-resistant starter cultures. The lactic acid bacteria utilized in dairy fermentations include various species of streptococci and lactobacilli. These strains ferment lactose, the primary carbohydrate source in milk, to lactic acid and other end products of fermentation that contribute to the flavor, texture and keeping quality of cultured dairy products. Lysis of starter cultures by bacteriophage is a primary cause for slow acid production and failure of lactic streptococci during dairy fermentations and results in economic losses to the cheesemaker.

Various techniques to prevent bacteriophage attack have been devised. These methods include sanitation, use of concentrated cultures, elimination of the ripening period, use of phage-inhibitory media, rotation of cultures, and use of a blend of multiple strains with different phage-resistance phenotypes. However, these systems involve maintenance of a large number of cultures and phage-sensitive strains can eventually develop that necessitate replacement of one or more of the individual starters in the blend.

Numerous investigators have demonstrated the association of phage-resistance phenotypes with plasmid DNA in the lactic streptococci. Mechanisms of plasmid-mediated phage resistance include: interference with phage adsorption; restriction/modification systems; and abortive phage infection which may include reduced burst size, temperature effects and reduced or extended latent periods.

Genetic engineering currently is being used to identify and isolate the gene(s) responsible for phage resistance. The ultimate goal is to combine several "cassettes" of DNA which code for different phage-resistance mechanisms onto a high copy number plasmid or onto a genetic element which can be targeted to the chromosome of the recipient organism. This single strain would possess multiple mechanisms for resisting phage attack, and could be used reliably and continuously for the production of consistently high quality fermented products.

Accelerated ripening of cheese. The flavor and texture associated with a good quality cheddar develops during the ripening or aging process. During storage, the starter organisms slowly lyse in the curd matrix, releasing cell-associated proteases and lipases. This process can take from 12 to 36 months, and results in considerable cost to the cheesemaker. Attempts have been made to accelerate this process by adding exogenous nonstarter proteases and lipases; however, this technique is riddled with technical difficulties and is not well accepted by the cheesemaker. A more natural approach involves the construction or selection of autolytic starters that are capable of lysing at the cook temperature, thus prematurely releasing the enzymes into the curd. One could then clone specific proteolytic enzymes into these strains to further accelerate the ripening process. In addition to the economic savings due to shorter storage time, this approach would not alter the cheesemaking process, nor trigger labeling requirements.

Fermentation-derived rennet. Another enzyme used extensively in the dairy industry is the coagulant rennet, obtained by aqueous extraction of calf stomachs. The supply and price of rennet are determined by the availability of suitable calf stomachs, which often are in short supply. Microbial enzymes developed over the years to supplement the supply of rennet have deficiencies that relate to lower yields and in some cheese flavor defects.

To meet the demand for a consistent supply of high purity rennet, several laboratories have used genetic engineering techniques coupled with fermentation technology to produce the enzyme. The calf rennet gene has been isolated, sequenced, synthesized in vitro, inserted into an expression vector and cloned into a nonpathogenic strain *of Escherichia coli.* This strain is capable of producing large quantities of the enzyme, which is identical in structure and function to calf-derived rennet. A Food Additive Petition and a petition requesting that fermentation-produced rennet be affirmed as GRAS (generally recognized as safe) has been filed with FDA, making this one of the first products of biotechnology to enter the regulatory arena.

Improving organoleptic and nutritional qualities

Enhancing the flavor and texture of fermented dairy products. Through genetic engineering, it will be possible to construct starter cultures that confer unique qualities to fermented products. For example, it may be possible to develop cultures capable of producing natural flavor compounds (peach, banana, berry, citrus) or proteins with intrinsic, noncaloric sweetness properties (aspartame, thaumatin, stevioside) to enhance the flavor and eliminate the need to add sucrose during the manufacture of yogurt or flavored cheese spreads. Construction of strains capable of producing extracellular polysaccharides would eliminate the need for the addition of stabilizing or thickening agents in yogurt.

Altering the nutritional properties of fermented dairy products. Dairy products serve as the primary source of calcium in the U.S. diet. In addition, they are an excellent source of protein and fat-soluble vitamins. However, most dairy products, with the exception of yogurt and cottage cheese, are perceived by the consumer as being high in saturated fat

and cholesterol. In the future, it may be possible to engineer starter cultures with the capability of enzymatically modifying cholesterol to nonatherogenic steroid derivatives. Similarly, it may be possible to enzymatically modify the saturation level of fat or to alter the absorbability of the saturated fat fraction.

Products for lactose-intolerant individuals. A large percentage of the adult population not of Northern European descent are unable to metabolize lactose, the primary carbohydrate in milk, due to a deficiency of the enzyme lactase in the brush border of the gut. Several investigators have demonstrated that, although lactose-intolerant individuals are unable to tolerate milk and certain fermented dairy products, they are able to consume yogurt without experiencing the typical associated symptoms (nausea, flatulence, gas).

It has been suggested that the beta-galactosidase produced by yogurt starter culture organisms, *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* survives passage through the stomach and continues to digest the lactose in the gut. Using natural selection and generic engineering, it should be possible to select strains that overproduce the enzyme and create more digestible products for lactose-intolerant individuals.

Engineering probiotic lactobacilli. Members of the genus *Lactobacillus* have been implicated in the enhancement of human health. Lactobacilli are commensal colonizers of the human and animal gastrointestinal track. Strains of lactobacilli have been shown to detoxify potential carcinogens; compounds with antitumor activity have been isolated from other strains.

As mentioned, certain strains produce beta-galactosidase which might facilitate lactose digestion in less tolerant individuals. Other enzymes responsible for digestion of proteins, fats and carbohydrates could be cloned into lactobacilli to facilitate digestion of these dietary components in aging or otherwise compromised individuals with digestive disorders. One could even create a strain capable of inhibiting the colonization of pathogenic microorganisms encountered during travel to foreign countries.

Certain lactobacilli are capable of metabolizing dietary cholesterol, although the mechanism is not well understood. The tissue-specific colonization of these organisms could be extended into the area of antigen secretion for the purpose of vaccination against mucosal diseases. The lactobacilli are clearly an exciting group of organisms with tremendous potential for enhancing the health of mankind.

Ensuring safety of fermented products

Inhibition of pathogens and spoilage organisms. There is increasing concern in the dairy industry over the emergence of pathogens not previously associated with fermented dairy products. The identification of soft Mexican-style cheese as the vehicle of transmission for *Listeria monocytogenes,* coupled with the ubiquitous presence of this organism in the dairy processing plant, has led to investigations of alternative methods for controlling potential pathogens in dairy products.

An exciting alternative that deserves further study takes advantage of the ability of certain lactic acid bacteria, including members of the genera *Streptococcus, Lactobacillus* and *Pediococcus,* to produce antimicrobial substances called bacteriocins. Bacteriocins usually are protein in nature and frequently inhibit only a narrow range of related or closely related organisms. However, certain of these "natural" antibiotics such as nisin, which was recently approved by the FDA for use in cheese spreads, are capable of inhibiting a fairly broad spectrum of potentially pathogenic and spoilage organisms.

Nisin is just one example of a host of bacteriocins produced by various members of the lactic acid bacteria. In many cases, these bacteriocins and the gene(s) that code for immunity to the bacteriocin are plasmid-mediated. Some have been demonstrated to inhibit strains of *Listeria monocytogenes.* In the future, it will be possible to either transfer the plasmids coding for bacteriocin production and immunity into commercial starter organisms via conjugation or transformation, or clone the gene(s) responsible for this phenotype into starters. It also would be desirable to clone the ability to produce mold and yeast inhibitors into dairy starters to extend the shelf-life of products.

Rapid detection methods. It is essential that we be able to monitor fermented dairy products for the presence of pathogenic microorganisms. Current microbiological methods for emerging pathogens like *Listeria monocytogenes* are tedious, time-consuming, operator-dependent and unreliable. To ensure the safety of dairy products, it is essential that simple, rapid, sensitive, reliable and reproducible detection methods be developed.

Biotechnology-based detection systems that utilize DNA probes and monoclonal antibodies currently are being developed and will revolutionize how we do quality control/quality assurance testing in dairy processing plants. Reducing current technologies, which require relatively sophisticated equipment and specialty chemicals and reagents, to dip-stick methodology will enhance our ability to detect, identify, quantify, monitor and epidemiologically track potentially pathogenic microorganisms on the farm, in the processing plant, in the final product or in the infected consumer.

Conclusions

Biotechnology is destined to have a major impact on many facets of our daily lives, from the food we eat to lifesaving drugs and vaccines. This article touches on just a few of the potential applications of biotechnology in the dairy industry. What biotechnology could do for the dairy industry in the future is limited only by the boundaries of our imaginations.