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DEVELOPMENT OF NONFERMENTED ACIDOPHILUS MILK AND TESTING ITS PROPERTIES

by

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TITLE

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This dissertation is dedicated

to my daughters, Swati and Smriti

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INTRODUCTION

Discovery of fermented milks is among the most important contribution to civilization. These foods provide nutrition, vital elements to health and make them desirable staples in man's daily diet. Fermented milk foods are made from readily accessible raw materials. The method of manufacture is simple to adopt, and the product is of better keeping quality and usually safe. The evolution of fermented milk foods began many centuries before Christ, probably in the warm climate of the Mediterranean Sea Basin. The first of these products was undoubtedly discovered by accident. The methods of treating the milk differed from one tribe of people to another and naturally the products differed too. Thus, there originated a large number of fermented milks, which are known by different names.

Additional interest in fermented milk has stemmed from the publication of the book "Prolongation of Life," during the early part of the 20th century by Nobel Laureate Metchnikoff (76). He stated that man is the victim of an auto-intoxication from "wild bacilli," which inhabit the large intestine and cause hardening of the arteries and premature aging. He knew that this putrefaction may be prevented if an acid producing microflora were established in the human intestine. He also pointed out that the longevity of the Balkan is due to the large consumption of sour milk and to the establishment in their intestine of an acid producing microbial population.

Acidophilus milk is one of the most important fermented milks. It is usually thought of as the product prepared by growing a selected

culture of Lactobacillus acidophilus in boiled skim milk, partially skimmed milk or whole milk until large numbers of the microorganisms are present and sufficient lactic acid has been formed to cause curd formation. Since L. acidophilus does not multiply rapidly in milk and is easily overgrown by contaminating bacteria, the milk is given a rather high heat treatment before inoculation. This process leaves a flavor defect in the final product, which is not relished by most consumers. Furthermore, this product possesses other defects such as high acidity, high cost of production due to tedious manufacturing process and a low viable L. acidophilus cell count after fermentation. Several studies have been made concerning the therapeutic value of acidophilus milk (36, 69, 72, 103). More recently, emphasis has been placed on studies relative to the antibacterial properties of L. acidophilus (94, 113, 138, 141).

Hence, there appears to exist a need for the development of a product which is free from those inherent defects of acidophilus milk and still provides all the therapeutic properties of the L. acidophilus culture. The major departure from conventional acidophilus milk was first reported by Myers (83) in which he grew L. acidophilus in a sterile medium, harvested the cells and added them to pasteurized milk to obtain a population comparable to that in regular acidophilus milk. More recently, Speck (120) developed a similar product known as sweet acidophilus milk. However, no information is available in the literature about the therapeutic properties of nonfermented acidophilus milk. The purpose of this research was to develop a "me too" type product and evaluate its therapeutic properties.

REVIEW OF LITERATURE

A. Nutritive Value of Milk and Cultured Dairy Products

Milk, one of man's oldest foods, is still one of the most widely used foods. Milk products constitute only about 12% of the calories in the American diet, yet they provide 76% of the calcium, 44% of riboflavin, 30% of phosphorus, 23% of protein, 12% of vitamin A and 10% of thiamine (45). An important characteristic of milk sugar (lactose) is its ability to promote the growth of a lactobacillus flora in the gut with possible displacement of undesirable forms of microorganisms in the intestinal tract. Duncan (32) has reported that rats and chicks receiving lactose showed improved retention of dietary calcium. Block and Weiss (12) have reported that milk proteins contain very low amounts of cystine and methionine but are rich in lysine. They also reported that the extent of cystine deficiency was much more marked in casein than in α-lactalbumin and β-lactoglobulin. In experiments with calves, Blaxter and Wood (11) obtained biological values of 40.84 and digestibility coefficients of 91.97 for milk proteins. The digestibility coefficient provides one of the indices of the nutritional value. All natural animal and vegetable fats melting below 50 C are completely digested by normal individuals (26). It is also reported that butterfat has a digestibility coefficient of 97. There has been a long continued discussion on the question of whether butterfat *per se* possesses a specific nutritional value not possessed by other animal fats or vegetable fats. Schantz et al. (108) stated that rats grew better over the period of 6 weeks following weaning if they received a liquid diet of skim milk

homogenized with butterfat as compared with similar diets in which corn oil, cottonseed oil, coconut oil or soybean oil was employed in place of butterfat. They also observed that the difference in growth promoting capacity disappeared after a period of 6 weeks. Further, they noted that the unsaturated fatty acids of butterfat contained the effective fraction and afforded more growth than did a comparable amount of whole butter.

The importance of dairy products as a source of vitamins, especially vitamin B_{12}, in the human diet was observed by Woker and Picard (145). These workers reported that a vegetarian whose diet included dairy products showed no visible illness. However, another group of vegetarians who eliminated dairy foods from their diet developed certain illnesses which apparently were due to deficiency of vitamin B_{12}. Milk and milk products contain several minerals as major constituents. Calcium and phosphorus are considered dietary essentials and milk is a very rich source of these minerals (45). Nair and Mann (84) reported reduction of cholesteremia in rats due to inclusion of milk powder in their diet. A similar effect is obtained by feeding 0.1% β-hydroxy methyl glutaric acid (HMG) in the diet. This suggests that milk powder contain^d HMG.

Fermented dairy products display the same basic quantitative nutritional qualities as does milk or cream from which they are prepared. In addition, the microbial fermentation adds a new dimension of nutrition to cultured dairy products. The role of cultured dairy products in balancing the diet, particularly with respect to vitamins, was described in depth by Sullivan et al. (126). Shahani et al. (112) have

reported the B-complex vitamin (niacin, pantothenic and pyridoxine, biotin and folic acid) contents of 23 varieties of cheese and 8 varieties of processed cheese. They observed that microorganisms synthesized vitamins during ripening. Reif (99) reported the synthesis of niacin, B_6, B_{12} and folic acid by cheese starter organism.

Currently, where there was emphasis of protein and deemphasis of fat, both yogurt and buttermilk were welcomed. The average fat content of yogurt and buttermilk had been reported to be 1.7 to 3.3 and 1.1%, respectively, while each contained about 3.2% protein (59). In caloric value, yogurt was very similar to milk from which it was made. However, it was reportedly more easily digested and therefore more nutritious because the milk proteins, carbohydrates and fats were predigested by the bacterial cultures used in its manufacture (20, 59).

B. Biochemical Characteristics of *Lactobacillus acidophilus*

Bergey's Manual of Determinative Bacteriology outlines the typical biochemical characteristics of *Lactobacillus acidophilus* (10). These organisms are rods with rounded ends, generally 0.6-0.9 by 1.5-6 nm, occurring singly, in pairs and in short chains, are nonmotile, nonflagellated, and usually from rough colonies. Microscopic examination generally reveals twisted or fuzzy filamentous projections with a dark, felt-like mass in the center. Deep colonies are irregularly shaped with radiate or ramified projections. Glycogen is fermented by some strains and is generally weak. Some strains ferment melibiose, raffinose or both. It also ferments glucose, sucrose, galactose, mannose, maltose and lactose with acid but no gas production. Normally, they are

homofermentative producing DL-lactic acid. Ammonia is not produced from arginine. It produces acidity in the range of 0.3 to 1.9% in terms of lactic acid.

Cell wall peptidoglycan is of the L-lysine-D-aspartate type. Teichoic acid is generally absent. In some strains small amounts of glycerol teichoic acid is detectable. Cell walls do not contain any distinguishing hexoses or pentoses. Strains appear serologically diverse and no group reactions have been demonstrated. Growth requirements are acetate or mevalonic acid, riboflavin, calcium-pantothenate, niacin and folic acid. Exogenous thiamine, pyridoxal and thymidine are not required. Vitamin B_{12} (cyanocobalamins) are generally not required. Mutant strains may require deoxyribosides. It does not grow at 15 to 22 C, but grows at 45 to 48 C, with an optimum growth temperature of 35-38 C. It grows at an initial pH of 5-7, having an optimum pH of 5.5 to 6.0.

The guanine + cytosine content of the DNA of six strains was 36.7 ± 0.7 moles %. *L. acidophilus* was originally isolated from feces of infants and from mouths and vaginas of young adults.

C. Therapeutic and Nutritional Qualities of Lactobacilli

1. Lactobacilli in Gastrointestinal Disorders

Early work of Metchnikoff (74, 75, 76) and the clarification provided by Rahe (97) suggested that *L. acidophilus* rather than *L. bulgaricus* could be implanted successfully in the intestinal tract. Since then, lactobacilli have been used extensively in intestinal disease therapy. With the oral administration of antibiotics to cure systemic

infection of various types, patients often complain about the gastrointestinal discomfort. Yeast and mold infections are often diagnosed in these cases (48), and the standard therapy has been the use of concentrates of L. acidophilus (31, 40, 89, 91, 96, 144).

Studies in radiation biology with whole animals also have emphasized the importance of a balanced intestinal microbial flora in healthy animals. In this regard, Vincent et al. (140) substantiated earlier reports that lactobacilli constitute the predominant gut flora of small laboratory animals. They showed that post-irradiation bacteremia caused by coliform and pseudomonads results when lactobacilli decline in the small intestine of rats.

Gilliland and Speck (35) reported that lactobacilli isolated from the intestinal tract have the ability to deconjugate bile acids. The culture L. acidophilus more effectively deconjugates Na-tourocholate and Na-glycocholate under low oxidation reduction potential. Recent literature also attests to the value of using lactobacilli in the treatment of intestinal disorders, though there is a contrary report as well (79). Hawley et al. (44) have reviewed factors important in successful implantations. At least two considerations are important; that large numbers of viable cells be fed and that a fermentable carbohydrate be available to the cells in the intestinal tract. Beck and Nechels (9) used lyophilized cultures of L. acidophilus to treat different types of diarrhea, constipation and abnormal fermentations, and obtained excellent results with a majority of cases.

Recent reports by Dubos et al. (30), Savage and Dubos (106), Savage et al. (107) and Savage (105) have documented that an intimate

association existed between anaerobic streptococci, lactobacilli, fusiform bacteria and yeast particularly in an area of epithelium of gastro-intestinal tract of mice. These findings again emphasized the importance of a balanced population of microorganisms in healthy animals. Savage (104) found that administering penicillin to rats and mice resulted in a replacement of lactobacilli by torula yeasts, a situation which persisted as long as antibiotic was administered. The moment penicillin treatment was discontinued, indigenous lactobacilli again colonized the epithelium. *L. acidophilus* therapy in man and domestic animals is more widely practiced in other countries than the United States, especially in Europe (16, 19, 61, 63, 67, 77, 117, 119, 131, 133, 134, 135, 136, 137, 139). Feeding lactic acid bacteria, especially *L. acidophilus*, to swine as therapy by restoring a healthful microbial balance has been the subject of a few reports. Mollgarad (80) showed that the presence of lactic acid facilitated absorption of calcium. Pigs fed with cultures of lactobacilli were found to contain greater amount of lactic acid in the intestines and to grow better than the control groups which had not been given lactobacilli. These effects have been confirmed by Cole et al. (23) and Kershaw et al. (55) who also noted suppression of *E. coli*. Also, Leitgeb (66) observed that in growing, fattening pigs, the count of *E. coli* in the intestinal tract was inversely related to that of lactic acid producing bacteria, chiefly *L. bifidus*. Hill et al. (46) succeeded in reducing the intestinal amine level and scouring in weaned pigs by feeding acidophilus milk supplements. Pasicynj (91) and Nedyalkov et al. (86) also have noted a beneficial effect on swine in terms of weight gain and reduced enteritis.