EXOCELLULAR POLYSACCHARIDES FROM *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* AND *STREPTOCOCCUS SALIVARIUS* SUBSP. *THERMOPHILUS*

By

RILEY MCNEIL PIGEON

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1997

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2000

EXOCELLULAR POLYSACCHARIDES FROM *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* AND *STREPTOCOCCUS SALIVARIUS* SUBSP. *THERMOPHILUS*

Thesis Approved:

Stanley E. Buckenmaier
Thesis Adviser

Peter M. Muriana

Patricia Rayas Duarte

Wayne B. Powell
Dean of the Graduate College

ACKNOWLEDGEMENTS

I express sincere thanks to Dr. Stanley Gilliland for serving as my graduate and undergraduate advisor for the past seven years. The support and guidance that he provided was instrumental in the completion of my college degrees. Appreciation is also extended to my other committee members, Dr. Peter Muriana and Dr. Patricia Rayas.

I would like to express a huge amount of gratitude to my parents, Jim and Ruth Pigeon, they never failed to love, encourage, and support me as I strived to reach my goals and dreams. My father- and mother-in-law, Charles and Linda Tye, are also deserving of thanks for their support during my college years.

A tremendous amount of thanks goes to my wife Teryl, who always provided love, understanding, and encouragement. She never failed to support me through college and to help me with typing papers. I appreciate her understanding during those late nights that I spent studying or writing. I hope to repay her support by helping her to reach her new goals.

My children have been a wonderful addition to my family. I would like to thank both Trevor and Trenton for keeping me on my toes and for allowing me some "play" breaks while studying. I hope to provide love, support and encouragement as they go through life just as my parents did.

I would also like to thank my friends and co-workers in the research lab. Thanks for providing assistance when needed. It would have been impossible to work full time in the industry and do academic research without this type of support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Cholesterol has an essential metabolic role in our bodies. It functions as an important precursor compound in the body. Vitamin D, steroid hormones and bile acids all have cholesterol as a precursor. Despite these functions, excessive cholesterol levels can lead to the development of several complications including stroke and coronary heart disease.

There has been extensive interest in the potential health or nutritional benefits that can be derived from certain lactic acid bacteria, especially lactobacilli. Potential benefits include antitumoral activity, improved lactose digestion, improved immune response, antipathogenic activity and hypocholesterolemic activity. The mechanisms of cholesterol lowering effects of lactobacilli have been the focus of several research studies. Proposed mechanisms include cholesterol assimilation into cellular membranes of lactobacilli and deconjugation of bile acids by the action of bile salt hydrolase produced by the lactobacilli. Both of these mechanisms have the potential to decrease serum cholesterol. Assimilating dietary cholesterol into bacterial cellular membranes during growth of the lactobacilli in the intestine would make it unavailable for absorption. Bile acid deconjugation would be effective in that the free bile acids produced in the reaction are

much more easily excreted via the feces. Cholesterol would be utilized in the replacement of the lost bile acids, thus lowering the level of the cholesterol pool.

Many lactic acid bacteria produce capsular or free exocellular polysaccharides (EPS). There have been hundreds of studies focusing on the structure and characterization of these polymers, however there are very few that studied the potential health promoting effects of exocellular polysaccharides. Among them is a study by Nakajima et al. (96) that focused on the cholesterol lowering activity of milk fermented with an exocellular polysaccharide producing lactic acid bacteria. The consumption of this milk decreased serum cholesterol levels in rats while the consumption of milk fermented with a non-EPS producing strain failed to be significantly different than the control group.

Overall, there is a lack of studies that attempt to link the production of bacterial exocellular polysaccharides to the serum cholesterol-reducing phenomenon that can result from the consumption of fermented milk products. There is potential for these polymers to interfere with the absorption of cholesterol or bile acids by binding the sterol and removing it from the body. This would put a continuous drain on the cholesterol-bile acid pool and thus reduce serum cholesterol levels.

The objective of this study was to determine if exocellular polysaccharides produced by different strains of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* could bind bile acids *in vitro*.

CHAPTER II

REVIEW OF LITERATURE

Bile Acids and Cholesterol

Hypercholesterolemia and Coronary Heart Disease

The ill effects of cholesterol in the human diet has been extensively studied and reported. Elevated cholesterol levels have been implicated in the onset of cardiovascular disease or coronary heart disease, one of the leading causes of death in the United States (65, 100). Excess serum cholesterol can build up on artery walls, creating lesions or deposits. These deposits become calcified and harden resulting in atherosclerosis or hardening of the arteries. As the openings of the inner walls of the arteries become narrow, blood flow is restricted. The probability of blood clots increase, creating the potential of complete blockage of an artery. Elimination of blood flow causes death to the tissues involved. If the blockage occurs in the coronary arteries a myocardial infarction (often called a heart attack) can occur. Strokes are caused by blockages of the blood flow to the brain tissues (121).

Total cholesterol is commonly found associated with proteins in moieties called lipoproteins. Total cholesterol can be divided into groups based on the density of these lipoproteins. Very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) all vary in composition and atherogenic risk. Studies have shown that elevated LDL cholesterol is highly correlated to the risk of coronary

heart disease (65, 100). Conversely, HDL's are reported to have a protective effect (65, 121)

To best describe the association between LDL's and coronary heart disease, familial hypercholesterolemia is a genetically inherited disease related to lipoprotein dysfunction. This condition is caused by a lack of functional LDL receptors in body cells. These receptors are responsible for the uptake of LDL's for degradation and utilization. Cholesterol rich LDL's then build up in the blood serum to levels 3-5 fold that of normal levels (121). Affected individuals rapidly form atherosclerosis and coronary heart disease and often die at an early age (121).

Role of cholesterol and bile acids

Cholesterol is an essential constituent of our bodies. Its structure gives fluidity to cellular membranes and it serves as a precursor to several steroid hormones, vitamin D and bile acids (121). There are two sources of cholesterol in the body. Dietary cholesterol is absorbed from the intestinal lumen and transported to the liver. The liver may also obtain cholesterol through synthesis beginning with acetyl-CoA (5, 27, 36, 85, 121).

Bile acids are the end product of the oxidative catabolism of cholesterol in the liver (26). After being transformed, bile acids are usually conjugated with the amino acids glycine or taurine. Glycine conjugated bile acids are dominant in the human due to the minimal amount of taurine in the normal diet (29). These bile acids are then excreted into the bile and eventually into the intestinal lumen where they function as emulsifying agents. They aid in the digestion of fats through lipase action by improving the solubility

of fats and the formation of micelles. Bile acids also facilitate the transport of fatty acids and monoglycerides across the mucosal membranes through the formation of micelles. In addition, bile acids are needed for absorption of other fat-soluble dietary compounds such as fat-soluble vitamins, cholesterol, and steroid hormones (25, 26). Bile acids that are absorbed are transported back to the liver to be recycled. Some bile acids are not absorbed and are excreted from the body. Lost bile acids are replaced through synthesis from cholesterol. According to Voet and Voet (121), this is the only way for the body to rid itself of excess cholesterol.

Absorption of lipids from the GI tract

Bile acids are required for the effective absorption of lipids from the small intestine (60, 61, 80). Emulsification of lipids facilitate digestion and absorption of triglycerides, fatty acids, monoglycerides, vitamins A, D, E, and K, cholesterol, sterols and other fat soluble dietary compounds. Bile acids are soluble in both aqueous and non-polar solvents. They form micelles with fat-soluble materials. These micelles are able to pass through the mucosal membranes thus being absorbed into the body . There are reports of a critical micellular concentration of 2 mM bile acid that must be maintained for proper lipid absorption. At concentrations less than 2mM, the formation of large micellular aggregates may be reduced and cholesterol may not be absorbed (26, 39, 61).

In addition, conjugated bile acids are believed to better facilitate the absorption of lipids through increased stability of the micellular aggregates (39, 61).

Enterohepatic Circulation of Bile Acids

The liver and the small intestine are involved in an efficient exchange of bile acids in a cycle called the enterohepatic circulation of bile acids. After the liver produces bile acids, either by synthesis from cholesterol or absorption from the blood, they are stored in the gall bladder at concentrated levels and excreted as a component of bile into the small intestine. There they act as emulsifiers aiding in fat absorption. Once the bile acids pass through the mucosal cell membranes they are transported back to the liver to be recycled. This cycle is so efficient that the total bile acid pool is cycled many times over on a daily basis (26).

Re-absorption of bile acids can occur in all segments of the small intestine. The mechanism of absorption depends highly on the location in the intestine and the type of bile acid being absorbed. Passive diffusion takes place in all segments of the small intestine while active transport against an electrochemical gradient occurs in the ileum. The duodenum and jejunum are involved only in the passive transport of bile acids (25, 26,107).

Molecular charge also plays a role in the absorption of bile acids. The pKa of the acids dictate the net charge on the molecule at a given pH. Unconjugated bile acids, such as cholic acid, have a pKa around 6.0. Conjugated bile acids such as taurocholic and glycocholic, have much lower pKa's of 2.0 and 4.0 respectively (26). The pH of the small intestine ranges from 4.5- 6.0. At this pH the unconjugated bile acids are free of negative charge and are allowed to easily diffuse down a concentration gradient in a non-ionic passive diffusion process. At this same pH, the conjugated bile acids are ionized

and negatively charged and can diffuse slowly by ionic passive diffusion or much more quickly by active transport mechanisms (25, 26, 107). The net effect of these transport systems is the re-absorption of primarily conjugated bile acids. Small amounts of unconjugated or free bile acids are also absorbed with the rest escaping the body via the feces.

Effects of Probiotic Cultures on Serum Cholesterol Levels

Beneficial effects of probiotic cultures

Several researchers have focused on the potential health and nutritional benefits that can be derived from some species of lactic acid bacteria. This area of probiotics is recognized worldwide and is very popular in European and Asian countries. The potential benefits include improved nutritional value of some foods, control of intestinal infection by pathogens, improved lactose utilization, inhibition against some types of cancer and control of serum cholesterol levels (50).

The nutritional value of many foodstuffs can be improved by controlled fermentation of lactic acid bacteria. Production of stimulating cellular factors, increases in B-vitamins and alterations in amino acid content have all been hypothesized (50).

Some probiotic lactic acid bacterial cultures exhibit antagonistic action towards spoilage organisms in food as well as pathogens that contaminate our foods supply. Pathogens such as *Staphylococcus aureus*, *Salmonella* spp., *Clostridium* spp., *Vibrio* ssp., *E. coli* and *Streptococcus* spp. can cause food borne illnesses. Many scientists believe that the inhibitory effect toward these cultures is due to the production of such material as

hydrogen peroxide and bacteriocins during growth of the lactic acid bacteria (44). In a study by Brashears et al. (9) *Lactobacillus delbrueckii* subsp. *lactis* was shown to inhibit *E. coli* O157:H7 during refrigerated storage of raw chicken meat. This inhibition appears to be associated with the production of hydrogen peroxide. Several studies were conducted by groups using animal models to test *Lactobacillus acidophilus* for therapeutic and prophylactic effects in controlling intestinal pathogens. Watkins and Miller (126) used gnotobiotic chicks to demonstrate that *L. acidophilus,* when fed as a prophylactic prior to being challenged with *Salmonella typhimurium* or *Staphylococcus aureus,* could more effectively control mortality from intestinal pathogens than those treated with a therapeutic type diet containing *L. acidophilus*. Fuller (38) also used gnotobiotic chicks to model antagonistic effects of *L. acidophilus* towards *Escherichia coli*.

Some people experience problems with lactose utilization. The primary cause of this problem is a deficiency of the enzyme lactase in the digestive system (50). This condition is known as lactose maldigestion. Consumption of lactose containing dairy products by these persons can lead to abdominal complications such as diarrhea, flatulence and bloating (73). Some probiotic cultures produce β-galactosidase which breaks down lactose into monomeric units. Some research groups have reported that some species of lactobacilli used to make cultured or culture containing dairy products, have sufficient amounts of the enzyme β-galactosidase to provide an improvement lactose utilization in lactose maldigestors (46). However, there is a high amount of variation in enzyme produced by different species and strains(48). There also is variation in the stability of the enzyme during storage (48). These factors must be considered

when selecting an organism to function effectively in improving the digestion of lactose (50).

Some lactic acid bacteria are also reported to have an anticarcinogenic effect. In a review article, Gilliland (50) indicates that this effect may be due to the inhibition of organisms that convert procarcinogens into carcinogens in the intestines. In a study by Shahani et al. (108), *L. acidophilus* was found to reduce the proliferation of cancer cells in rats. It has also been shown that cells of *L. acidophilus* can reduce the activity of enzymes associated with the genesis of carcinogenic compounds (50).

Additionally, serum cholesterol levels can be controlled through the administration of probiotic cultures. This phenomenon will be discussed in detail in the following section.

Consumption of fermented and nonfermented cultured dairy products

Lactic acid bacteria potentially can provide many benefits to the health of consumers of cultured products (35, 50). Among these benefits is control of serum cholesterol levels. Researchers first documented this observation after studying young Maasai tribesmen in Africa. Mann and Spoerry (81) were attempting to demonstrate that the addition of a surfactant to the diet of the tribesmen would increase the absorption of lipids and cholesterol. However, both the control groups and the surfactant treated group gained weight and exhibited lower cholesterol levels when fed a diet of meat and fermented milk (fermented with a strain of lactobacilli). The researchers hypothesized that the fermented milk harbored some factor that inhibited cholesterol synthesis or uptake.

Since the publication by Mann and Spoerry, many researchers have focused attention on the possible role of fermented dairy products in control serum cholesterol. Among them, Mann persisted in research to identify the factor associated with this phenomenon. He (82) applied the same type of experiment to U.S. subjects using a commercial yogurt culture and observed similar effects. Significantly lower cholesterol levels were obtained by individuals consuming the yogurt versus those consuming a non-fermented milk during a 12 day trial (82, 83).

Other studies involving yogurt consumption followed (6, 58, 64, 70, 86, 103, 104, 106, 114, 115). In a study by Hepner et al. (58), human subjects were given unpasteurized yogurt, pasteurized yogurt and plain milk for a period of 12 weeks. Both of the yogurts were shown to reduce the serum cholesterol levels. The authors hypothesized that a metabolite from the fermentation process was responsible for the reduction.

Thakur and Jha (114) reported reductions in cholesterol levels of rabbits fed yogurt. Milk and calcium supplemented diets were also evaluated and determined each to have a hypocholesteremic activity. However, the results indicated that the yogurt was the most effective in reducing the evidence of aortic atherogenic lesions.

In a study by, Rao et al. (104), milk fermented with *Streptococcus thermophilus* was fed to rats. The results indicated a lowering of plasma cholesterol levels versus rats fed milk or no dairy products. In a second experiment by the same researchers methanol solubles were extracted from fermented milk and nonfermented milk. These were fed to rats in a manner similar to that of the first experiment and resulted in cholesterol reduction in the fermented milk methanol solubles group.

Different responses to yogurt consumption by male and female were observed by Bazzarre et al (6). The females in the study significantly lowered their serum cholesterol and increased the ratio of high density lipoproteins: total cholesterol by consuming yogurt. Females not consuming the fermented dairy product and all males did not exhibit the same effect.

Some studies focused on the action of *L. acidophilus* toward increased cholesterol levels. Gilliland et.al. (47) compared two strains of *L. acidophilus* on their ability to significantly inhibit increases in cholesterol levels of pigs being fed a high cholesterol diet. Variability among the two strains were reported and correlated to differences of the two strains with respect to cholesterol uptake in laboratory media. Anderson and Gilliland (4) concluded that a particular strain of *L. acidophilus* of human origin significantly reduced serum cholesterol levels of hypercholesterolemic individuals. The 2.4 - 3.2% reduction in cholesterol levels correlated to a 6-10% reduction in the risk of coronary heart disease. In a study by Danielson, et al. (21), mature boars were divided into two groups after 56 days on a high cholesterol diet. One group remained on the high cholesterol diet, while the other received an acidophilus diet supplemented with yogurt containing a similar amount of energy and cholesterol. After 56 days the group consuming the yogurt had significantly lowered serum cholesterol levels. A study by Grunewald (55) reported the same results with rats fed milk fermented with *L. acidophilus*. In another study by De Rodas et al (24), pigs were shown to have significantly lower serum cholesterol levels when fed a diet containing cells of *L. acidophilus*. In addition, LDL levels were also reduced.

Reports by several researchers have disputed the hypercholesterolemic effects of cultured dairy products. Thompson et al. (115), reported no significant difference between the cholesterol lowering ability of whole milk, 2% milk , skim milk, sweet acidophilus milk, buttermilk or yogurt when fed to healthy individuals with normal cholesterol levels. Pulusani and Rao (103) examined whole body lipids, liver cholesterol and serum cholesterol in rats. Three groups of rats were fed either *L. acidophilus* milk, *Streptococcus thermophilus* milk or *L. bulgaricus* milk. All were compared to a control group and all diets consisted of normal rat chow without excess cholesterol. The researchers found no evidence in the alteration of bodily lipid or cholesterol levels. Other studies have also shown similar results (64, 70, 86, 106). Jaspers et al. (64) concluded that yogurt consumption did not significantly reduce cholesterol or blood lipid levels in adult males having normal levels of serum cholesterol. McNamara et al. (86) published similar results from a study in which yogurt intake in normolipidemic males failed to decrease plasma lipid levels. Kiyosawa et al. (70) even reported that consumption of skim milk had a greater hypocholesteremic effect than yogurt. The authors believed the differences to be associated with decreased lipid consumption in the skim milk treated group. It is important to note that all of these studies involved humans or animals having normal cholesterol levels and thus it is not likely that any decreases in serum cholesterol should have been expected.

According to Gilliland (49, 50) it is important to consider the strain of the culture being used in the study. Factors such as cholesterol assimilating ability, ability of culture to survive and grow in the intestine, bile tolerance and host specificity all can play a

significant role in determining the potential hypocholesterolemic effect of a probiotic culture.

Assimilation of cholesterol by lactic acid bacteria

Attempts to explain the hypocholesterolemic effect of cultured dairy product consumption have been made by several researchers. In a study by Eyssen (34), conventional animals held in a nonsterile environment excreted much higher levels of cholesterol in the feces than germ-free animals held in a sterile environment. When these groups of animals were challenged with a high cholesterol diet, the group with the "normal" flora had nearly half the cholesterol in the blood as the germ-free group. This study demonstrated that the intestinal flora might interfere with cholesterol uptake from the intestines. In a similar study by Danielson and Gustafsson (22), rats were used to demonstrate the hypocholesterolemic effect of intestinal microflora versus the absence of such colonization.

Furthermore, a study by Mott et al. (89) also found the development of a natural intestinal microflora in pigs greatly influenced the serum cholesterol levels and excretion of bile acids. The authors suggested that microbial alteration cholesterol and bile acid absorption might have been responsible for the effect rather than cholesterol alteration or metabolism by microbes in the gut.

Cholesterol assimilation was the focus of several researchers. Gilliland et al. (47) found that *L. acidophilus* could remove or assimilate cholesterol from laboratory media. Anaerobic conditions, as well as the presence of bile, were required for the organism to assimilate the cholesterol and remove it from the cultured media. Evaluation of several

strains of *L. acidophilus* isolated from pig feces for assimilating ability revealed highly variable results. *Lactobacillus acidophilus* RP32 (ATCC43121) removed a significantly higher amount of cholesterol from the broth than did other strains. Pig feeding trials were performed involving this organism and *L. acidophilus* P47, which did not assimilate cholesterol during growth in laboratory media. After 10 days on a high cholesterol diet supplemented with *L. acidophilus* RP32, the serum cholesterol levels were significantly lower than were those for the pigs not receiving lactobacilli. In contrast, the group fed strain P47 had cholesterol levels comparable to pigs not receiving lactobacilli.

Three strains of *L. acidophilus* isolated from mature boars in a study by Danielson et al. (22) were determined to be bile tolerant and able to assimilate cholesterol in vitro. A subsequent feeding trial was performed using the most active assimilating strain. Boars on a high cholesterol diet were given yogurt containing this strain and compared to a control group on the same high cholesterol diet. The boars receiving the lactobacilli had significantly lower serum cholesterol levels than the boars in the control group.

Buck and Gilliland (10) isolated 123 strains of *L. acidophilus* from 9 human subjects. They were evaluated for bile tolerance, cholesterol assimilation and bile deconjugating ability. High variability among strains was reported for all 3 measures. The authors indicated that cholesterol assimilation and bile tolerance were critical factors that must be considered when selecting a strain to provide potential benefits to consumers.

In a study by Noh et al. (97), it was shown that some of the cholesterol removed from the culture media could be recovered in the cellular membranes of *L. acidophilus*. In addition, it was reported that the assimilation occurred during growth at a pH

maintained a 6.0. This result disputed the report of Klaver and Van der Meer (71) that the observed assimilation was actually coprecipitation of cholesterol with deconjugated bile salts that tend to precipitate below pH 6. Reports from Walker and Gilliland (123) also indicate that there is no statistical relationship between bile salt deconjugation and cholesterol assimilation among 19 strains of *L. acidophilus*. Similar results were reported by Dambekodi and Gilliland (20) from studies involving *Bifidobacterium longum*. However, the inclusion of sodium taurocholate enhanced the ability of *B. longum* to assimilate cholesterol. Brashears et al. (8) compared strains of *Lactobacillus casei* to *L. acidophilus* for the ability to assimilate cholesterol into cellular membranes and bile salt deconjugation. Both organisms were active in bile deconjugation, however the *L. casei* strains could only remove small amount of cholesterol through assimilation compared to *L. acidophilus* strains.

Deconjugation of Bile Acids by Lactic Acid Bacteria

Bile acids can under go several biotransformations in the intestine by the action of microbes. These transformations include deconjugation, desulfation, dehydroxylation and epimerization. Among these, deconjugation is most frequently observed (63).

Deconjugation of bile acids involve the removal of taurine or glycine from the free bile acid by hydrolysis. Free or deconjugated bile acids are more easily excreted from the body. (25, 26, 107). Thus, an increase in the deconjugation of bile acids in the small intestine could result in increased excretion of bile acid putting a continuous drain on the cholesterol pool as more bile acids are synthesized.

Bile salt hydrolase is the enzyme that is responsible for the conversion of conjugated bile acids into free acids and amino acids. Bile salt hydrolase activity is found in many bacterial genera such as *Bacteriodes, Streptococcus, Lactobacillus, Clostridium, Fusobacterium, Peptostreptococcus* and *Bifidobactium* (16, 51, 72)

Interestingly, in a study by Kobashi et al. (72), specificity of some species of *Lactobacilli* toward bile type was observed. For example some species would deconjugate both glycocholic and taurocholic acid, while others deconjugated specifically glycocholic or taurocholic. In addition, bile salt hydrolase enzymes were isolated from these organisms and found to have specific action depending on bile type.

Several researchers have studied and detected the presence of bile salt hydrolase in *L. acidophilus* (17, 23, 45, 113). In a study by Tannock et al. (113), the effect of lactobacilli on bile salt hydrolase activity was evaluated in the small intestines of mice. Conventional mice were found to have nearly double the bile salt hydrolase activity than mice devoid of lactobacilli. After the lactobacillus free mice were inoculated with the organism there were no significant differences between the groups with respect to this enzyme activity. Corzo and Gilliland (19) measured the bile salt hydrolase enzyme activity of 3 strains of *L. acidophilus*. One strain in the study (ATCC 43121) had bile salt hydrolase levels 14-fold higher than the two other strains. This strain was also found to deconjugate both sodium taurocholate and sodium glycocholate more effectively than the other strains.

Consumption of ropy fermented milk

Some bacterial cultures produce slime-like exocellular polysaccharides during growth. When lactic acid bacteria that produce these polymers are allowed to ferment milk, the viscosity of the product is increased. In some cases the milk may be thickened to the degree that the term "ropy" is applied due to the stringy, slimy texture produced in the milk. Many researchers agree that the use of these exocellular polysaccharides can improve the texture of fermented milk and decrease the susceptibility to syneresis or separation of the whey from the curd (12, 15, 53, 88, 91, 118, 122).

The physiologic effects, of ropy fermented milk, has been the focus of many research studies. Among them, Kitazawa and coworkers (68,69) have demonstrated antitumural activity of slime producing lactococci. Forsen et al. (37) pointed out immune biological effects associated with these types of organisms.

In a study, by Nakajima et al. (96) the effects of ropy fermented milk consumption on serum cholesterol levels and other blood lipids were evaluated. The study included three groups of rats being fed a high cholesterol diet. One group was fed a diet supplemented with ropy fermented milk produced by a slime forming strain of *Lactococcus lactis* subsp. *cremoris*. Another group received non-ropy milk fermented with a non-ropy strain of *L. lactis* subsp. *cremoris*. A third group received a non-fermented, acidified milk as a control. The results indicated that there was a significant decrease in the serum cholesterol levels during the consumption of the ropy milk versus the acidified control. The non-ropy milk failed to show this difference. In addition, the group that was fed the ropy fermented milk had significantly higher HDL-cholesterol than the control group. However, the average serum cholesterol levels and the HDL level

among the fermented milk groups were not significantly different, although the ropy milk did produce a numerically lower cholesterol level and a higher HDL level. The researches suggested that the observed differences were due to the effect of the water soluble extra-cellular polysaccharide on cholesterol or bile acid absorption from the intestines. Due to resistance of the polysaccharide to digestive enzymes and its water holding capacity, they hypothesized that the bacterial polymer acted in a manner similar to dietary fiber.

Other researchers have recognized the possible effects of intestinal organisms on the binding of bile acids and cholesterol and subsequent interference of absorption in the intestine. Mott et al.(89), while studying the effects of intestinal bacteria on fecal sterol composition, concluded that mechanisms other than biotransformation of bile acids were responsible for the observed serum cholesterol reduction in pigs. They found that conventional piglets experienced a four-fold increase in fecal cholesterol excretion accompanied by the decreased cholesterol levels. Germ-free piglets did not exhibit this same excretion rate and cholesterol reduction while on the same high cholesterol diet. The authors cited other factors such as bulk of the feces, intestinal transit time, and binding of bile acids by intestinal bacteria as being responsible for sterol balance in the body.

Chikai et al. (16) studied the bile deconjugation ability of several species of bacteria. They found that increases in bile deconjugation resulted in increased bile acid excretion. They speculated that adhesion of free bile acids to bacteria or dietary fibers might also contribute to the large amount of excretion.

Overall, there are very few studies that attempt to link the production of bacterial exocellular polysaccharides to the binding of bile acids or other sterols. Several researchers have studied the effects of lactic acid bacteria and other intestinal micro-organisms on cholesterol levels and fecal sterol excretion, however, few attribute it to the presence of bacterially produced polymers.

Other Methods to Control Serum Cholesterol Levels

Dietary control of cholesterol

Cholesterol, if present in the diet, can be absorbed into the body from the intestinal lumen. Conner et al. (18) demonstrated a positive correlation between dietary intake of cholesterol and cholesterol levels in the blood. By decreasing cholesterol in the diet it is believed that serum cholesterol levels can be controlled to a certain degree.

Hypocholesterolemic Milk Constituents

Some research studies have indicated that cow's milk contains natural substances that are known to reduce or inhibit cholesterol synthesis in the body. Among these substances is orotic acid, a pyrimidine derivative, which exerts an inhibiting effect towards β-hydroxy-β-methyl-glutaryl – Coenzyme A (HMG-CoA) reductase. This enzyme functions as the rate limiting factor in the biosynthetic pathway of cholesterol (124). According to Harden and Robinson (57), rats on diets containing up to 1% orotic acid had lower cholesterol levels and a higher amount of HDL cholesterol than did those in the control groups. Ahmed et al. found that orotic acid is converted to uracil which also inhibits cholesterolgenesis (2). In addition, Ward (125) et al. discovered the presence of

uric acid in human milk that exhibits similar properties. Haggerty et al. (56) studied the effects of milk fermentation on levels of orotic and uric acids. They found that fermentation reduced the levels of orotic acid, which indicated the presence of another mechanism of hypocholesterolemic activity produced by probiotic bacterial cultures.

Drug Therapy in Cholesterol Reduction

Drug therapy is another alternative in controlling cholesterol levels. Many drugs have been introduced that either prevents the formation of cholesterol or remove bile acids from the body through physical binding. Dujovne et al. (30) tested the combined use of probucol and cholestipol in cholesterol therapy. These drugs had two completely different mechanisms of cholesterol reduction and opposite side effects. The combination of the two drugs was much more effective than the use of either drug alone. In addition, fewer side effects were observed.

Bile acid sequesterants also may be included in this type of therapy. Cholesterylamine is a resin that is commonly used to bind and remove bile acids from the intestines (77). However sequesterants can cause increases in cholesterol synthesis and plasma triglycerides soon after the beginning of the therapy (98). This can be overcome by the use of nicotinic acid or clofibrate.

Side effects from the use of drugs in cholesterol therapy include constipation, diarrhea, nausea, and interactions with other drugs and decreased absorption of fat-soluble vitamins and nutrients (77). Alternative methods in cholesterol control would allow many to avoid such undesirable side effects.

Use of Dietary Fiber to Sequester Bile Acids

The effects of dietary consumption of food fiber has been extensively studied and documented. Consuming dietary fibers has been associated with several healthy improvements in the gastro-intestinal tract. Many authors report decreases in fecal transit time, suppression of serum cholesterol levels and reduction in the glycaemic response (66, 87, 102). In a review article by Kelsay (66), dietary fiber intake was reported to be associated with improvements in coronary heart disease, diabetes mellitus, diverticular disease, cancer and other gastrointestinal diseases.

Dietary fiber can be defined as "those remnants of vegetable cell walls that are not hydrolyzed by the digestive enzymes of man" (79). A similar definition is given by Topping (116). Dietary fiber can be classified into two categories based on their solubility in water. This separation causes problems or controversy determining the effects of dietary fiber on health and nutrition. For example, Garg (42) stated that the lack of cholesterol lowering activity of insoluble fibers are sometimes incorrectly interpreted and applied to all fibers. The author further clarifies that most soluble dietary fibers exhibit some hypocholesterolemic activity. The fibers that tend to show the effect usually produce highly viscous solutions. Examples of these are guar gum, pectin, and some hemicelluloses. Examples of insoluble fibers are cellulose, brans and lignin. Topping (116) also made similar comparisons.

Many research studies have focused on the mechanism behind the hypercholesterolemic attributes of dietary fiber consumption. Most authors tend to report the binding or sequestering of bile salts/acids as the most plausible explanation behind the observed effects of dietary fiber consumption on cholesterol reduction in man. (42,

66, 76, 87, 102) Increased concentration of bile acids excreted via the feces is cited as the major evidence to support this mechanism. In addition, those consuming high amounts of soluble fiber exhibit reductions in fecal transit times and increased fecal weight that may also contribute increase bile acid excretion. This effect would therefore be analogous to bile-sequesterant drugs such as cholesterylamine. Other documented mechanisms include the possible alteration of lipid metabolism by short chain fatty acids formed during colonic fermentation of dietary fibers (116).

In an attempt to characterize the biological functions of dietary fibers, Adiotomre et al. (1) compared *in vitro* results to that of actual cholesterol reductions in human feeding trials. The researchers calculated a predictive index using data from dialysis binding studies and concentrations of propionate from fermented fibers. The index was calculated by multiplying the percent bile acid bound vs. the control (no fiber) by the magnitude of propionate increase vs. the control. The index results for each fiber seemed to correlate with *in vivo* cholesterol reduction very strongly, however no statistical analyses were performed to verify this correlation.

Eastwood and Boyd (32) were the first to demonstrate the binding of bile salts to dietary fiber. The study was carried out by monitoring changes in the distribution of bile salts in the small intestine of rats consuming dietary fiber. Eastwood and Hamilton (33) confirmed this, when they found that lignin was very efficient in binding bile salts *in vitro*. Additionally, the effects of pH were studied by the researchers and it was shown that the magnitude of binding was enhanced in an acid environment.

Kritchevsky and Story (74) investigated several different bile acids and salts for their ability to adhere or bind to fibers including alfalfa, wheat straw and sugar beet pulp.

The researchers compared the results with synthetic sequesterant drugs such as cholesterylamine and cholestipol. The drugs bound significantly more bile acid than the fibers. However, the drugs bound higher amounts of taurocholate while the natural fibers bound more glycocholate. The authors concluded that it is important to consider the type of bile acid or salt, as well as the type of fiber, when evaluating the binding capability.

Further studies (111) by the same researchers yielded similar results. Alfalfa, bran, cellulose, lignin and cholesterylamine were used in binding experiments with 10 different conjugated and deconjugated bile acids. The highest amount of binding was with cholesterylamine followed by lignin, alfalfa, bran and cellulose. The authors concluded that the findings were consistent with the serum cholesterol lowering effect of lignin and alfalfa and the absence of the effect while consuming bran and cellulose. The authors further concluded that the binding process is complex mechanism and the true mechanisms vary with each fiber source and type.

Vanhouny et al. (119) performed binding studies using radioisotope methods with taurine conjugated bile salts complexed with cholesterol, lecithin and fatty acids in micelles. They found that commercial binding resins such as cholesterylamine bound 81-92% of the bile salts. In addition to the insoluble fiber fractions evaluated in previous research studies, soluble guar gum was included. Of all the fibers tested, guar gum sequestered the greatest amount (31-38%) of the bile acids. The lignin and alfalfa removed 20-38% and 6-15% respectively. Wheat bran and cellulose were both ineffective in the removal of the bile salts from the micelle mixture.

Kern et al. (67) compared different types of vegetable fiber residues and the binding of bile acids of each *in vitro* and *in vivo*. The absorption of bile salts was found

to increase with increased concentrations of bile acid and decreased pH. The fiber residues of corn, string bean, and potato all exhibited potential for removal of bile acid while that of apple, celery and lettuce showed minimal removal. The *in vivo* studies were performed with human subjects. It was found that the dihydroxy bile acid chenodeoxycholic was preferentially bound to fecal particulate matter as opposed to cholic acid. Other studies have revealed similar binding of bile acids and increased excretion of fecal bile acids with diverse sources of fiber including psyllium (11,117), carrot fiber (59) and cereal food fibers (62).

Some studies focused on the use of minerals to enhance the binding of bile salts. Hoagland and Pfeffer (59) discovered that the binding of bile acids to carrot fibers was enhanced by the presence of Ca^{2+} ions. The authors concluded that the binding of bile acids to the fiber is accomplished through Ca^{2+} salt bridges that occurs during ionization of carboxylate groups on the fiber. Similarly, Pandolf and Clydesdale (99), studied the use of iron and/or calcium to increase the magnitude of binding bile salts. The results indicated that iron and calcium both enhanced the binding of certain specific bile acids to certain fibers.

Some researchers dispute the binding mechanism being responsible for the hypocholesterolemic effect. Moundras et al. (90) points out that some fibers are partially or completely degraded by bacteria in the cecum, yet the bile acids are not re-absorbed. The authors further provided results of studies showing that consumption of fermentable polysaccharides lead to significant increases in short –chain fatty acid production, increased propionate levels and increases in enzymes that are believed to decrease cholesterol synthesis.

Lampe et al. (76) documented research that involved a three week feeding trial of three different types of fiber at varying levels in the diet. Fecal bile acids were measured, as well as serum cholesterol levels. The results indicated that the reductions in serum cholesterol levels could not be explained by fecal bile acid excretion. The authors suggested the regulation of cholesterol synthesis was decreased by short chain fatty acids, however, they indicate that more research is needed.

As indicated in this section, the effects of fiber on the reduction of serum cholesterol levels have been well documented. The mechanisms are not fully understood, however, most evidence seem to support the binding or sequestering of the bile acids. The observed effects of fiber may be an additive combination of binding, short-chain fatty acid production and physical entrapment of bile acids.

Bacterial Exocellular Polysaccharides (EPS)

Characteristics of bacterial exocellular polysaccharides

Bacterial cells produce a large variety of polysaccharides that may perform different functions in bacterial cultures. For example, they produce teichoic acids and lipopolysaccharides that are important in the formation and structure of prokaryotic cell walls. In addition to these types of polymers, some bacteria including Gram-positive and Gram-negative excrete polysaccharides outside the perimeter of the cell wall hence the term "exocellular polysaccharide" or EPS. Exocellular polysaccharides typically are characterized by a slimy appearance or thickening of the growth medium. They can be

excreted in two forms, capsular and slime. Capsular polysaccharides are found closely associated with the cell wall of the bacteria. In this case polysaccharides form a layer that encapsulates the cell. Slime polysaccharides produced by some bacteria are found unassociated with the cell wall of the bacteria. They are found as free particles in the medium and are also known as "free" exocellular polysaccharides (112). Isolation or recovery of free EPS involves precipitation by various organic solvents. Capsular EPS is more difficult due to the nature of adherence to the cell wall (112).

The structure and monomeric composition of these polymers vary greatly which make characterization studies tedious to perform and evaluate. The most common monosaccharide units present in exocellular polysaccharides are hexoses including D-glucose, D-galactose, and D-mannose. Methyl pentoses such as fucose and rhamnose also have been found (112).

Functions of exocellular polysaccharides

The functions of exocellular polysaccharides are obscure. There have been several proposed theories on why some bacteria expend an enormous amount of energy producing these polymers. It is believed that they provide protective mechanisms as well as sites for nutrient storage or absorption. Protective functions are believed to inhibit desiccation, phagocytosis, engulfment from amoeba and harm from lysozyme (112). Furthermore, Gilliland and Speck (43) reported a significant correlation between increased survival rate and increased amounts of capsular polysaccharides among different strains of lactic streptococci stored for 7 days at –17 degrees C. This provides evidence for a possible protective mechanism against death during freezing.

It has also been hypothesized that these bacteria produce EPS as a means of storing excess carbohydrate for later use by the cell or the EPS may act as a mediator in the absorption of nutrients and ions by using the carboxylic acid and ketal groups present on the polymer. The absorption of water may also be enhanced by the presence of capsular EPS (112).

Application and uses of exocellular polysaccharides

The major applications and uses of bacterial exocellular polysaccharides are very similar to those of plant based soluble polymers such as guar gum, locust bean gum and carrageenan. They can be used as bio-thickeners, gelling agents and binding agents or to stabilize emulsions, create foams, and suspend particles in foods or other applications (88,110).

There are currently two commercially available purified exocellular polysaccharides approved for food use in the United States. Xanthan gum is a polymer produced by several species of the genus *Xanthomonas*. Gellan gum, which was just recently approved for limited use in foods, is produced by *Pseudomonas elodea*.

There has been a considerable amount of interest in the use of exocellular polysaccharide producing starter cultures in the dairy industry. Currently, a large amount of the yogurt produced in the United States contains increased milk solids and stabilizing polymers to provide improved texture and body and to reduce syneresis. The use of starter cultures that produce exocellular polysaccharides could provide these same properties to the dairy product as it is being cultured without the need for adding excess

milk solids or stabilizers . This would be beneficial in areas of Europe where use of stabilizers is not allowed in any foods (12).

EPS production by Lactic Acid Bacteria

Several species of lactic acid bacteria possess the ability to produce both capsular and free exocellular polysaccharides. Due to the potential benefits of these bacteria to the quality of fermented dairy food products, several thermophilic and mesophlic lactic acid bacteria and the polymers they produce have been studied (14). The organisms included in these studies were *Streptococcus salivarius* subsp. *thermophilus* (13, 28, 40, 75, 78, 101, 118, 122), *Lactobacillus debrueckii* subsp. *bulgaricus* (7, 12, 41, 52, 53, 54, 122), various members of the genus *Lactococcus* (15, 84, 120) other *Lactobacilli* (15, 31, 92, 93, 94, 109, 105, 127) and *Bifidobacterium longum* (3, 95).

In an in depth review article, Cerning (14) details the polymer production capabilities of several lactic acid bacteria. The article discussed *Leuconostoc mesentroides, Streptococcus mutans, Streptococcus sobrinas, Streptococcus salivarius* subsp. *thermophilus, Lactobacillus hilgardii* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The author states that polymers are produced under a wide range of conditions, however optimum conditions are required to maximize the production.

The polymers can be found as slime produced in liquid media causing increased viscosity or on solid medium. The production of large amounts of polymer is usually apparent by visual assessment. Stringy, ropy filaments, increased viscosity and reduced flow characteristics are all cited as identifiers (14). Ropy fermented milk is caused by the

presence of these substances after fermentation with a polymer producing lactic acid bacteria.

Cerning (14) further describes the wide amount of variation in production capabilities, polymer type and structure and physical characteristics. For instance, some types of bacteria have been found to lose some of their production ability over a period of time during many subcultures in laboratory media. It is believed that some mesophilic organisms that have plasmids coding for this trait are susceptible to this loss. However, thermophilic lactic acid bacteria are thought to have chromosomal DNA encoding for EPS. Some mesophilic bacteria produce homopolysaccharides such as dextrans and mutans while thermophilic and mesophilic organisms produce heteropolysaccharides of varying composition. The unique properties of EPS produced by lactic acid bacteria has prompted many dairy researchers to study characterization, isolation, quantification and optimization to analyze the potential for use in dairy foods. Wacher-Rodarte et al. (122) compared ropy yogurt starters to nonropy strains and discovered significant reduction in syneresis in the yogurt. The level of syneresis or "wheying off" was nearly half that observed in yogurt made using nonropy starters. The authors also found a highly significant correlation between the lower levels of syneresis and apparent viscosity.

Low et al. (78) found that the moisture retention in low fat mozzarella cheese was significantly improved when a starter known to produce a capsular polysaccharide was used. Improved water-binding in this product increases quality and gives the processor a competitive advantage in the market.

Other desired characteristics include reductions in the amount of added milk solids and stabilizers to achieve a smooth viscous yogurt with desirable mouthfeel (13, 14

122). This has led to research to study the rheological behaviors of these polymers (12, 13, 15).

Structural characteristics of exocellular polysaccharides

There are numerous documented studies outlining the structural properties of exocellular polysaccharides produced by lactic acid bacteria. The structures may include repeating units of the same monosaccharides such as glucose in a homopolysaccarhide. Heteropolysaccharides contain repeating units of different monosaccharides. The two most commonly reported monosaccharide units are glucose and galactose. Galactose seems to dominate in most polymers (14). Other sugar residues such as rhamnose, arabinose, mannose and other neutral sugars may be found in small concentrations. Cerning et al. (15) reported 47% Galactose, 45.4% Glucose and 7.6% other sugars in the EPS produced by a strain of *Lactococcus lactis* subsp. *cremoris*. A galactose concentration of 52% was reported for a different strain of the same bacteria. There also were differences in percentages of sugar in EPS based on the carbon and energy source available.

Most exocellular polysaccharides are found to contain repeating units of a given structure of 4-6 monosaccharides. These units may include various types of linkages and branching structures depending on the polymer. The exact structure of these repeating units have been reported for strains of several organisms including *Lactobacillus paracasei* (105), *Lactobacillus helveticus* (109), *Bifidobacterium longum* (95), and *Streptococcus salivarius* subsp. *thermophilus* (28, 75). These repeating units link together

to form polymers that can have a very high molecular weight ranging from 0.5×10^6 – 1.2×10^6 daltons (7).

Bouzar et al. (7) studied strains of *L. delbrueckii* subsp. *bulgaricus* and reported changes in the sugar composition of EPS at different stages of fermentation. Changes in the ratio of galactose to glucose were observed. However, the final composition after 24 hours was about 38.6% glucose and 57.4% galactose.

Uemura et al. (118) have studied two different polymers from one strain *L. delbrueckii* subsp. *bulgaricus*. Both included a repeating pentasaccharide unit and galactose and glucose in a molar ratio of 3:2. The molecular weights of which were reported to be 1.1×10^6 Da – 1.2×10^6 Da. One structure was identified as neutral polysaccharide while the other was classified as acidic due to the presence of a small amount of phosphorus.

Cerning et al. (12) reported a smaller EPS of 500,000 Da produced by a strain of *L. delbrueckii* subsp. *bulgaricus*. It was composed of galactose, glucose and rhamnose in a 4:1:1 ratio.

Streptococcus salivarius subsp. *thermophilus* also has been the focus of research on EPS formation. Lemoine et al. (75) investigated the polymers produced by two different strains. They found that the two strains produced polymers that were similar in chemical composition but very different in structural formation. One strain contained galactose and glucose in 1:1 ratio in a branched 4 monosaccharide repeating unit. The other contained galactose, rhamnose and glucose in a 3:2:1 ratio in branched hexamer repeating pattern. Both compounds were found to have molecular weights exceeding 2.0×10^6 Da.

Doco et al. (28) also studied strains of *Streptococcus salivarius* subsp. *thermophilus* and reported an exocellular polysaccharide produced that contained glusose, galactose and n-acetyl-glucosamine . The polymer was structured in a tetrapolysaccharide arrangement.

Variations in conditions for EPS production

Exocellular polysaccharide producing strains differ in their reaction to environmental changes during growth. Carbon source, mineral requirement, pH and temperature all effect the efficiency at which polymers are produced (112). There are a multitude of research articles describing conditions required to maximize the yield of EPS during growth.

Garcia-Garibay et al. (41) reported that *L. delbrueckii* subsp. *bulgaricus* produced the greatest amounts of EPS at higher temperature in the range of 40 – 48 ° F. They also indicated a reduction in polymer production capability upon continued subculture, especially when incubated at these higher temperatures. Similar results were reported by Grobben et al. (52).

Grobben et al. (53) studied the effect of changing carbon source on EPS production and composition. Results indicated nearly 3 times the quantity was produced when a strain of *L. delbrueckii* subsp.*bulgaricus* was grown on a combination of fructose and glucose versus fructose only. It also was observed that the basic chemical composition was altered due to carbon source. The EPS was found to contain only galactose and glucose when grown in the presence of fructose. When grown with the mixed sugars the EPS contained galactose, glucose and rhamnose. Grobben et al. (54)

demonstrated that EPS production by a strain of *L.delbrueckii* subsp. *bulgaricus* was affected by nutrients such as essential and non-essential vitamins. They found that a certain vitamin combination retarded growth but EPS production per cell was nearly doubled.

Gancel and Novel (40) found that some temperatures and sugars decrease growth rate but increase the rate of polymer synthesis. The authors concluded that complex regulatory pathways are activated during the stationary phase of growth to govern EPS production.

Several research articles have been published by Mozzi et al. (91) that outlined production conditions required to increase polymer production in *Lactobacillus casei*. Culture pH was found to effect production. A broth culture initially at pH 5.0- 6.0 exhibited high amounts of growth and lactic acid production, but low EPS production. In broth with an initial pH of 4.0 the specific EPS production per cell was enhanced. The same researchers also indicated a stimulatory effect of $MnSO_4$ and triammonium citrate (92). Other salts such as acetate, K_2HPO_4 and $MgSO_4$ did not stimulate EPS production. Additional studies by these authors found enhancement from the use of galactose as a carbon source (93) and growth at a constant pH of 6.0 (94).

There are numerous factors that effect the overall production of EPS. Usually, the conditions of the highest amount of production per cell are those that reduce growth rates. Mineral supplementation as well as excess carbohydrate is also known to enhance production (112).

Use of bacterial exocellular polysaccharides in binding of bile acids

Most articles documenting the hypocholesterolemic effects of plant based polysaccharides cite the binding of bile acids/salts as the most plausible mechanism for the observed effect. There is currently a lack of research studying the possibility of bile acid binding through contact with exocellular polysaccharides produced by lactic acid bacteria. The focus of this research study is to test the ability of capsular exocellular polysaccharide material produced by commercial yogurt starter cultures to bind bile acids *in vitro*. The findings may suggest an alternative mechanism for lowering serum cholesterol levels through consumption of fermented yogurt products made using starter cultures that produce EPS. In addition, the results might explain the findings of Nakajima et al. (96) that showed ropy fermented milk significantly reduced cholesterol levels in rats versus the consumption of milk fermented with a nonropy strain.

REFERENCES

1. Adiotomre, J., M.A. Eastwood, C.A. Edwards, and W.G. Brydon. 1990. Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans. Am. J. Clin. Nutr. 52:128-134.

2. Ahmed, A.A., R.D. McCarthy, and G.A. Porter. 1979. Effect of milk constituents on hepatic cholesterolgenesis. Atherosclerosis. 32:347-357.

3. Andaloussi, S.A. H. Talbaoui, R. Marczak and R. Bonaly. 1995. Isolation and characterization of exocellular polysaccharides produced by *Bifidobacterium longum*. Appl. Microbiol. Biotechnol. 43:995-1000.

4. Anderson, J.W. and S.E. Gilliland. 1999. Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolemic humans. J. Am. Coll. Nutr. 18:43-50.

5. Aries, V., J.S. Crawther, B.S. Drasar, and M.J. Hill. 1969. Degradation of bile salts by human intestinal bacteria. Gut. 10:575-581.

6. Bazzarre, T.L., S.L. Wu, and J.A. Yuhas. 1983. Total and HDL-cholesterol concentrations following yogurt and calcium supplementation. Nutr. Rep. 28:1225-1232.

7. Bouzar, F., J. Cerning, and M. Desmazeaud. 1996. Exocellular polysaccharide production in milk by *Lactobacillus delbrueckii* subsp. *bulgaricus* CNRZ 1187 and by two colonial variants. J. Dairy Sci. 79:205-211.

8. Brashears, M.M., S.E. Gilliland, and L.M. Buck. 1998. Bile salt deconjugation and cholesterol removal from media by *Lactobacillus casei*. J. Dairy Sci. 81:2103-2110.

9. Brashears, M.M., S.S. Reilly, and S.E. Gilliland. 1998. Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157:H7 on refrigerated raw chicken meat. J. Food Prot. 61:166-170.

10. Buck, L.M. and S.E. Gilliland. 1994. Comparisons of freshly isolated strains of *Lactobacillus acidophilus* of human intestinal origin for ability to assimilate cholesterol during growth. J. Dairy Sci. 77:2925-2933.

11. Buhman, K.K., E.J. Furumoto, S.S. Donkin and J.A. Story. 1998. Dietary psyllium increases fecal bile acid excretion, total steroid excretion and bile acid biosynthesis in rats. J. Nutr. 128:1199-1203.

12. Cerning, J., C. Bouillanne, M.J. Desmazeaud, and M. Landon. 1988a. Isolation and characterization of exocellular polysaccharide produced by *Lactobacillus bulgaricus*. 8:625-628.

13. Cerning, J., C. Bouillanne, M.J. Desmazeaud, and M. Landon. 1988b. Exocellular polysaccharide production by *Streptococcus thermophilus*. 8:625-628.

14. Cerning, J. 1990. Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol. Rev. 87:113-130.

15. Cerning, J., C. Bouillanne, M. Landon, and M. Desmazeaud. 1992. Isolation and characterization of exocellular polysaccharides from slime-forming mesophilic lactic acid bacteria. J. Dairy Sci. 75-692-699.

16. Chikai, T., H. Nakao, and K. Uchida. 1987. Deconjugation of bile acids by human intestinal bacteria implanted in germ free rats. Lipids. 22:669-671.

17. Cole, C.B. and R. Fuller. 1984. Bile acid deconjugation and attachment of chicken gut bacteria: Their possible role in growth depression. Brit. Poultry Sci. 25:227-231.

18. Conner, W.E., D.B. Stone, R.E. Hodges. 1964. The interrelated effects of dietary cholesterol and fat upon human serum lipids levels. J. Clin. Invest. 43:1691-1696.

19. Corzo, G. and S.E. Gilliland. 1999. Bile salt hydrolase activity of three strains of *Lactobacillus acidophilus*. J. Dairy Sci. 82:472-480.

20. Dambekodi, P.C. and S.E. Gilliland. 1998. Incorporation of cholesterol into the cellular membrane of *Bifidobacterium longum*. J. Dairy Sci. 81:1818-1824.

21. Danielson, A.D., E.R. Peo, K.M. Shahani, A.J. Lewis, P.J. Whalen, and M.A. Amer. 1989. Anticholesterolemic property of *Lactobacillus acidophilus* yogurt fed to mature boars. J. Anim. Sci. 67:966-974.

22. Danielson, H., and B. Gustafson. 1959. On serum-cholesterol levels and neutral fecal sterols in germ-free rats. Bile acids and steroids 59. Arch. Biochem. Biophys. 83:482-485.

23. Dashkevicz, M.P. and S.D. Feighner. 1989. Development of a differential medium for bile salt hydrolase-active Lactobacillus spp. Appl. Environ. Microbiol. 55:11-16.

24. De Rodas, B.Z., S.E. Gilliland, and C.V. Maxwell. 1996. Hypocholesterolemic action of *Lactobacillus acidophilus* ATCC 43121 and calcium in swine with hypercholesterolemia induced by diet. J. Dairy Sci. 79:2121-2128.

25. Dietschy, J.M., H.S. Salomon, and M.S. Siperstein. 1966. Bile acid metabolism. I. Studies on the mechanisms of intestinal transport. J. Clin. Inves. 45:832-846.

26. Dietschy, J.M. 1968. Mechanisms for the intestinal absorption of bile acids. J. Lipid Res. 9:297-309.

27. Dietschy, J.M. and D.J. Wilson. 1970. Regulation of cholesterol metabolism. New Engl. J. Med. 282:1179-1183.

28. Doco, T., J. Wieruszeski, and B. Fournet. 1990. Structure of an exocellular polysaccharide produced by *Streptococcus thermophilus*. Carbo. Res. 198:313-321.

29. Dowling, R.H. 1972. Progress In Gastroenterology: The enterohepatic circulation. Gastroenterology. 62:122-136.

30. Dujovne, C.A., P. Krehbiel, S. Decoursey, B. Jackson, S.B. Chernoff, A. Pitterman, and M. Garty. 1984. Probucol with colestipol in the treatment of hypercholesterolemia. Ann. Intern. Med. 100:477-482.

31. Dunican, L.K. and H.W. Seeley. 1965. Extracellular polysaccharide synthesis by members of the genus *Lactobacillus*: Conditions of formation and accumulation. J. Gen. Microbiol. 40:297-308.

32. Eastwood, M.A. and G.S. Boyd. 1967. The distribution of bile salts along the small intestine of rats. Biochim. Biophys. Acta. 137:393-396.

33. Eastwood, M.A. and D. Hamilton. 1968. Studies on the adsorption of bile salts to non-absorbed components of diet. Biochim. Biophys. Acta. 152:165-173.

34. Eyssen, H. 1973. Role of gut microflora in metabolism of lipids and sterols. Proc. Nutr. Soc. 32:59-63.

35. Fernandes, C.F., K.M. Shahani, and M.A. Armer. 1987. Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products. FEMS Microbiol. Rev. 46:343-354.

36. Field, F.J., N.T.P. Kim, and S.N. Mathur. 1990. Regulation of cholesterol metabolism in the intestine. Gastroenterology. 99:539-551.

37. Forsen, R., E. Heisha, E. Herva, and H. Arvilommi. 1987. Immunobiological effects of *Streptococcus cremoris* from cultured milk 'villi': Application of human lymphocyte culture techniques. Int. J. Food Microbiol. 5:41-47.

38. Fuller, R. 1977. The importance of lactobacilli in maintaining normal microbial balance in the crop. Br. Poult. Sci. 18:85-94.

39. Gallo-Torres, H.E., O.N. Miller, and J.D. Homilton. 1969. A comparison of the effect of bile salts on the absorption of cholesterol from the intestine of the rat. Biochim. Biophys. Acta. 176:605-615.

40. Gancel, F., G. Novel. 1994. Exocellular polysaccharide production by *Streptococcus salivarius* subsp. *thermophilus* cultures. 1.Conditions of production. J. Dairy Sci. 77:685-688.

41. Garcia-Garibay, M., V.M.E. Marshall. 1991. Polymer production by Lactobacillus delbrueckii subsp. bulgaricus. J. Appl. Bacteriol. 70:325-328.

42. Garg, A. 1994. Efficacy of dietary fiber in lowering serum cholesterol. Am. J. Med. 97:501-503.

43. Gilliland, S.E. and M.L. Speck. 1974. Relationship of cellular components to the stability of concentrated lactic streptococcus cultures at -17° C. Appl. Microbiol. 27:793-796.

44. Gilliland, S.E. and M.L. Speck. 1977a. Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures. J. Food Prot. 40:820-823.

45. Gilliland, S.E. and M.L. Speck. 1977b. Deconjugation of bile acids by intestinal lactobacilli. Appl. Environ. Microbiol. 33:15-18.

46. Gilliland, S.E. and H.S. Kim. 1984. Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. J. Dairy Sci. 67:1-6.

47. Gilliland, S.E. and C.R. Nelson, and C. Maxwell. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49:377-381.

48. Gilliland, S.E. and R.C. Lara. 1988. Influence of storage at freezing and subsequent refrigeration temperatures on β-galactosidase activity of *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 54:898-902.

49. Gilliland, S.E. 1989. Acidophilus milk products: A review of potential benefits to consumers. J. Dairy Sci. 72:2483-2494.

50. Gilliland, S.E. 1990. Health and nutritional benefits from lactic acid bacteria. FEMS Microbiol. Rev. 87:175-188.

51. Grill, J.P., C. Manginot-Durr, F. Schneider, and J. Ballongue. 1995. Bifidobacteria and probiotic effects: action of Bifidobacterium species on conjugated bile salts. Curr. Microbiol. 31-23-27.

52. Grobben, G.J., J. Sikkema, M.R. Smith, and J.A.M. de Bont. 1995. Production of extracellular polysaccharides by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772. J. Appl. Bacteriol. 79:103-107.

53. Grobben, G.J., M.R. Smith, J. Sikkema, and J.A.M. de Bont. 1996. Influence of fructose and glucose on the production of exocellular polysaccharides and the activities of enzymes involved in the sugar metabolism and the synthesis of sugar nucleotides in *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB. Appl. Environ. Microbiol. 46:279-284.

54. Grobben, G.J., I. Chin-Joe, V.A. Kitzen, I.C. Boels, F. Boer, J. Sikkema, M.R. Smith, and J.A.M. de Bont. 1998. Enhancement of exocellular polysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 with a simplified defined medium. Appl. Environ. Microbiol. 64:1333-1337.

55. Grunewald, K.K. 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci. 47:2078-2079.

56. Haggerty, R.J., L.O. Luedecke, C.W. Nagel, and L.K. Massey. 1984. Effect of selected yogurt cultures on the concentration of orotic acid, uric acid and hydroxymethylglutaric-like compound in milk after fermentation. J. Food Sci. 49:1194-1195.

57. Harden, K.K. and J.L. Robinson. 1984. Hypocholesteremia induced by orotic acid: dietary effects and species specificity. J. Nutr. 114:411-421.

58. Hepner, G., R. Fried, S. St. Jeor, L. Fusetti and R. Morin. 1979. Hypocholesterolemic effect of yogurt and milk. Am. J. Clin. Nutr. 32:19-24.

59. Hoagland, P.D. and P.E. Pfeffer. 1987. Binding of bile acids to carrot fiber. J. Agric. Food Chem. 35:316-319.

60. Hoffman, A.F. 1977. The enterohepatic circulation of bile acids in man. Clin. Gastroenterology. 6:3.

61. Holt, R. 1972. The roles of bile acids during the process of normal fat and cholesterol absorption. Arch. Intern. Med. 130:574-584.

62. Huang, C. and N.H. Dural. 1995. Adsorption of bile acids on cereal type food fibers. J. Food Proc. Engin. 18:243-266.

63. Hylemon, P.B. and T.L. Glass. 1983. Biotransformation of bile acids and cholesterol by the intestinal microflora. Pp. 189-213. In D.J. Hentages (Ed.), Human Intestinal Microflora in Health and Disease. Academic Press, Inc., N.Y., New York.

64. Jaspers, D.A.., L.K. Massey, and L.O. Luedecke. 1984. Effect of consuming yogurts prepared with three culture strains on human serm lipoproteins. J. Food Sci. 49:1178-1181.

65. Kannel, W.B., W.P. Castelli and T. Gordon. 1979. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. Ann. Intern. Med. 90:85-91.

66. Kelsay, J.L. 1978. A review of research on effects of fiber intake on man. Am J. Clin. Nutr. 31:142-159.

67. Kern, F., M.D. Herman, J. Birkner, M.D., and V.S. Ostrower. 1978. Binding of bile acids by dietary fiber. Am. J. Clin. Nutr. 31:S175-S179.

68. Kitazawa,H., T. Toba, T. Itoh, S. Adachi, and N. Kumano. 1988. Effect of ropy fermented milk on the metastasis of Lewis lung carcinoma in mice. Agric. Biol. Chem. 52:2331-2332.

69. Kitazawa,H., T. Toba, T. Itoh, N. Kumano, and S. Adachi. 1991. Anti-tumural activity of slime-forming encapsulated *Lactococcus lactis* subsp. *cremoris* isolated from Scandinavian ropy fermented milk, 'villi'. Anim. Sci. Technol. 62:277-283.

70. Kiyosawa, H., C. Sugawara, N. Sugawara, and H. Miyake. 1984. Effect of skim milk and yogurt on serum lipids and development of sudanophilic lesions in cholesterol-fed rabbits. Am. J. Clin. Nutr. 40:479-484.

71. Klaver, F.A.M., and R. Van der Meer. 1993. The assumed assimilation of cholesterol by Lactobacilli and *Bifidobacterium bifidum* is due to their salt-deconjugating activity. Appl. Environ. Microbiol. 59:1120-1124.

72. Kobashi, K., I. Nishizawa, and T. Yamada. 1978. A new hydrolase specific taurine-conjugates of bile acids. J. Biochem. 84:495-503.

73. Kolars, J.C., M.D. Levitt, M. Aoriji and D.A. Savaiano. 1984. Yogurt – an autodigesting source of lactose. New Engl. J. Med. 310:1-3.

74. Kritchevsky, D. and J.A. Story. 1975. In vitro binding of bile acids and bile salts. Am. J. Clin. Nutr. 28:305-306.

75. Lemoine, J., F. Chirat, J. Wieruszeski, G. Strecker, N. Favre, and J. Neeser. 1997. Structural characterization of the exocellular polysaccharides produced by *Streptococcus thermophilus* SFi39 and SFi12. Appl. Environ. Microbiol. 63:3512-3518.

76. Lempe, J.W., J.L. Slavin, K.S. Baglien, W.O. Thompson, W.C. Duane, and J.H. Zavoral. 1991. Serum lipid and fecal bile acid changes with cereal, vegetable, and sugar-beet fiber feeding. Am. J. Clin. Nutr. 53:1235-1241.

77. Lipid Research Clinics Program. 1984. The lipid research clinics coronary primary prevention trial results. I. Reduction in incidence of coronary heart disease. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. J. Am. Med. Assoc.251:365-384.

78. Low, D., J.A. Ahlgren, D. Horne, D.J. McMahon, C. Oberg, and J.R. Broadbent. 1998. Role of *Streptococcus thermophilus* MR-1C Capsular expolysaccharide in cheese moisture retention. Appl. Environ. Microbiol. 64:2147-2151.

79. Macdonald, I. 1976. Effects of dietary fiber: Are they all good? pp. 263-267 In G.A Spiller and R.J. Amen (Eds.), Fiber In Human Nutrition. Plenum Press. N.Y., New York.

80. Mallory, A., F. Kern, J. Smith, and D. Savage. 1973. Patterns of bile acids and microflora in the human small intestine. Gastroenterology. 64:26-33.

81. Mann, G.V. and A. Spoerry. 1974. Studies of a surfactant and cholesteremia in the Maasai. Am. J. Clin. Nutr. 27:464-469.

82. Mann, G.V. 1977a. A factor in yogurt which lowers cholesteremia in man. Atherosclerosis. 26:335-340.

83. Mann, G.V. 1977b. Hypocholesterolemic effect of milk. Lancet ii:556-561.

84. Marshall, V.M., E.N. Cowie, and R.S. Moreton. 1995. Analysis and production of two exocellular polysaccharides from *Lactococcus lactis* subsp. *cremoris* LC330. J. Dairy Res. 62:621-628.

85. McIntyre, N. and K.J. Isselbacher. 1973. Role of the small intestine in cholesterol metabolism. Am. J. Clin. Nutr. 26:647-656.

86. McNamara, D.J., A.E. Lowell, and J.E. Sabb. 1989. Effect of yogurt intake on plasma lipid and lipoprotein levels in normolipidemic males. Atherosclerosis. 79:167-171.

87. Miettinen, T.A. 1987. Dietary fiber and lipids. Am. J. Clin. Nutr. 45:1237-1242.

88. Morris, V.J. 1995. Bacterial Polysaccharides. pp. 341-375. In A.M. Stephen (Ed.), Food Polysaccharides and Their Applications. Marcel Dekker, Inc. N.Y, New York.

89. Mott, G.E., R.W. Moore, H. Redmond, and R. Reiser. 1973. Lowering of serum cholesterol by intestinal bacteria in cholesterol-fed piglets. Lipids. 8:428-430.

90. Moundras, C., S.R. Behr, C. Demigne, A. Mazur and C. Remesy. 1994. Fermentable polysaccharides that enhance fecal bile acid excretion; lower plasma cholesterol and apolipoprotein E-rich HDL in rats. J. Nutr. 124:2179-2188.

91. Mozzi, F., G.S. De Giori, G. Oliver, and G.F. De Valdez. 1994. Effect of culture pH on the growth characteristics and polysaccharide production by *Lactobacillus casei*. Milchwissenschaft. 49:667-669.

92. Mozzi, F., G.S. De Giori, G. Oliver, and G.F. De Valdez. 1995a. Exocellular polysaccharide production by *Lactobacillus casei*. I. Influence of salts. Milchwissenschaft. 50:186-188.

93. Mozzi, F., G.S. De Giori, G. Oliver, and G.F. De Valdez. 1995b. Exocellular polysaccharide production by *Lactobacillus casei*. II. Influence of the carbon source. Milchwissenschaft. 50:307-309.

94. Mozzi, F., G.S. De Giori, G. Oliver, and G.F. De Valdez. 1996. Exocellular polysaccharide production by *Lactobacillus casei* under controlled pH. Biotechnol Lett. 18:435-439.

95. Nagaoka, M., H. Shibata, I. Kimura, S. Hashimoto, K. Kimura, H. Sawada, and T. Yokokura. 1995. Structural studies on a cell wall polysaccharide from *Bifidobacterium longum* YIT4028. Carbo. Res. 274:245-249.

96. Nakajima, H., Y. Suzuki, H. Kaizu, and T. Hirota. 1992. Cholesterol lowering activity of ropy fermented milk. J. Food Sci. 57:1327-1329.

97. Noh, D.O., S.H. Kim and S.E. Gilliland. 1997. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. J. Dairy Sci. 80:3107-3113.

98. Packard, C.J. and J. Shepard. 1982. The hepatobiliary axis and lipoprotein metabolism: Effects of bile acid sequesterants and illeal bypass surgery. J. Lipid Res. 23:1081-1096.

99. Pandolf, T. and F.M. Clydesdale. 1992. Dietary fiber binding of bile acid through mineral supplementation. J. Food Sci. 57:1242-1245.

100. Pekkanen, J., S. Linn, G. Heiss, C.M. Suchindran, A. Leon, B.M. Rifkind, and H.A. Tyroler. 1990. Ten-year mortality from cardiovascular disease in relation to cholesterol level among mean with and without pre-existing cardiovascular disease. N. Engl. J. Med. 322:1700-1707.

101. Petit, C., J.P. Grill, N. Maazouzi, and R. Marczak. 1991. Regulation of polysaccharide formation by *Streptococcus thermophilus* in batch and fed-batch cultures. Appl. Microbiol. Biotechnol. 36:216-221.

102. Potty, V.H. 1996. Physio-chemical aspects, physiological functions, nutritional importance and technological significance of dietary fibers – A critical review. J. Food Sci. Technol. 33:1-18.

103. Pulusani, S.R., and D.R. Rao. 1983. Whole body, liver and plasma cholesterol levels in rats fed Thermophilus, Bulgaricus, and Acidophilus milks. J. Food Sci. 48:280-281.

104. Rao, D.R., C.B. Chawan, and S.R. Pulusani. 1981. Influence of milk and Thermophilus milk on plasma cholesterol levels and hepatic cholesterolgenesis in rats. J. Food Sci. 46:1339-1341.

105. Robijn, G.W., H.L.J. Wienk, D.J.C. van den Berg, H. Haas, J.P. Kamerling, and J.F.G. Vliegenthart. 1996. Structural studies of the exocellular polysaccharide produced by *Lactobacillus paracasei* 34-1. Carbo. Res. 285:129-139.

106. Rossouw, J.E., E. Burger, P. Van der Vyver, and J.J. Ferreira. 1981. The effect of skim milk, yogurt, and full cream milk on human serum lipids. Am. J. Clin. Nutr. 34:351-356.

107. Schiff, E.R., N.C. Small, and J.M. Dietschy. 1972. Characterization of the kinetics of the passive and active transposrt mechanisms for bile acid absorption in the small intestine and colon of the rat. J. Clin. Inves. 51:1351-1362.

108. Shahani, K.M., B.A. Friend and P.J. Bailey. 1983. Antitumor activity of fermented colostrum and milk. J. Food Protection. 46:385-386.

109. Staaf, M., G. Widmalm, Z. Yang, and E. Huttunen. 1996. Structural elucidation of extracellular polysaccharide produced by *Lactobacillus helveticus*. Carbo. Res. 291:155-164.

110. Stephen A.M. and S.C. Churms. 1995. Introduction: III. Applications and uses. p. 7. In A.M. Stephen (Ed.), Food Polysaccharides and Their Applications. Marcel Dekker, Inc. N.Y, New York.

111. Story, J.A. and D Kritchevsky. 1976. Comparison of the binding of various bile acids and bile salts in vitro by several types of fiber. J. Nutr. 106:1292-1294.

112. Sutherland, I.W. 1972. Bacterial Exocellular polysaccharides. pp. 143-213. In A.H. Rose and D.W. Tempest (Eds.), Advances in Microbial Physiology. Academic Press. N.Y., New York.

113. Tannock, G.W., A. Tangerman, A. Van Scack., and M.A. McConnell. 1989. Deconjugation of bile acids by lactobacilli in the mouse small bowel. Appl. Environ. Microbiol. 60:3419-3420.

114. Thakur, C.P. and A.N. Jha. 1981. Influence of milk, yogurt and calcium on cholesterol-induced atherosclerosis in rabbits. Atherosclerosis. 39:211-215.

115. Thompson, L.U., D.J.A. Jenkins, M.A. Vic Armer, R. Reichert, A. Jenkins, and J. Kamulsky. 1982. The effect of fermented and unfermented milks on serum cholesterol. Am. J. Clin. Nutr. 36:1106-1111.

116. Topping, D.L. 1991. Soluble fiber polysaccharides: Effects on plasma cholesterol and colonic fermentation. Nutr. Rev. 49:195-204.

117. Trautwein, E.A., A. Kunath-Rau, and H.F. Erbersdobler. 1999. Increased fecal bile acid excretion and changes in the circulating bile acid pool are involved in the hypocholesterolemic and gallstone-preventative actions of psyllium in hamsters. J. Nutr. 129:896-902.

118. Uemura, J., T. Itoh, T. Kaneko, and K. Noda. 1998. Chemical characterization of exocellular polysaccharide from *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1. Milchwissenschaft. 53:443-446.

119. Vanhouny, G.V., R. Tombes, M.M. Cassidy, D. Kritchevsky, and L.L. Gallo. 1980. Dietary fibers: V. Binding of bile salts, phospholipids and cholesterol from mixed micelles by bile acid sequesterants and dietary fibers. Lipids. 15:1012-1018.

120. Vedamuthu, E.B., and J.M. Neville. 1986. Involvement of a plasmid in production of ropiness (mucoidness) in milk cultures by *Streptococcus cremoris* MS. Appl. Environ. Microbiol. 51:677-682.

121. Voet, D. and J.G. Voet. 1995. *Biochemistry*, 2nd ed. John Wiley and Sons, Inc. New York, New York. Pp 662-726.

122. Wacher-Rodarte, C., M.V. Galvan, A. Farres, F. Gallardo, V.E. Marshall and M. Garcia-Garibay. 1993. Yogurt production from reconstituted skim milk powders using different poymer and non-polymer forming starter cultures. J. Dairy Res. 60:247-254.

123. Walker, D.K. and S.E. Gilliland. 1993. Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. J. Dairy Sci. 76:956-961.

124. Ward, P.C., R.D. McCarthy, and A. Kilara. 1980. Inhibitor of cholesterolgenesis in human milk. J. Dairy Sci. 63 (Suppl. 1):42.

125. Ward, P.C., R.D. McCarthy, and A. Kilara. 1982. Isolation of an inhibitor of hepatic cholesterolgenesis from human milk. Atherosclerosis. 41:185.

126. Watkins, B.A., B.F. Miller. 1983. Competitive gut exclusion of avian pathogens by Lactobacillus acidophilus in gnotobiotic chicks. Poult. Sci. 62:1772-1779.

127. Yokoi, H., T. Watanabe, and Y. Fujii. 1990. Isolation and characterization of polysaccharide-producing bacteria from kefir grains. J. Dairy Sci. 73:1684-1689.

CHAPTER III

BINDING OF FREE BILE ACIDS BY CAPSULAR EXOCELLULAR POLYSACCHARIDES FROM *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* AND *STREPTOCOCCUS SALIVARIUS* SUBSP. *THERMOPHILUS*

Riley M. Pigeon and Stanley E. Gilliland

Department of Animal Science, Oklahoma State University

Stillwater, Oklahoma 74078

ABSTRACT

Several strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* were evaluated for their ability to produce capsular exocellular polysaccharides (EPS). There were high amounts of variation in the amount of capsular EPS produced by the organisms in this study. The streptococci tended to produce the most EPS/ml of culture, however when compared on the basis of amounts per 10^7 colony forming units (cfu), the results were quite different. The lactobacilli produced the most per 10^7 cfu. *L. delbrueckii* subsp. *bulgaricus* strains Lb-18 and Lb-10442 and *S. salivarius* subsp. *thermophilus* St-143 produced significantly higher amounts measured at 70.6, 47.4 and 47.0 μg / 10^7 cfu respectively than did other strains tested. Observations as low as 4.1 μg were obtained from other strains in the study.

Selected strains of both species were then tested for the ability to bind bile acids from laboratory media. Three strains of *S. salivarius* subsp. *thermophilus* (St-143, St-OSU1, and St-OSU3) and two *L. delbrueckii* subsp. *bulgaricus* cultures (Lb-18 and Lb-10442) that produced high levels of EPS were selected to test for the ability to bind bile acids. The two *Lactobacillus* cultures exhibited significantly higher amounts of binding activity for cholic acid measured in percentage of bile acid bound, μg bound per mg EPS and μg bound per 10^7 cfu than did the streptococci. These two strains were able to bind up to 15.33% of the cholic acid present in laboratory media, up to 452 μg/mg EPS and 2.9 μg/10^7 cfu. Binding of the conjugated bile acid, glycocholic acid, was also tested. The results indicated that the strains in this study did not possess the ability to bind glycocholic acid.

INTRODUCTION

Cholesterol assumes an essential role in our bodies. It serves as a vital precursor to several biologically important compounds including vitamin D, steroid hormones and bile acids. However, cholesterol can build to elevated levels in the blood leading to complications such as stroke and coronary heart disease. (24).

Certain lactic acid bacteria such as lactobacilli have the potential to aid in the control of serum cholesterol levels. Mann and Spoerry (19) first demonstrated that fermented milk might exhibit hypocholesterolemic effects in humans while studying Maasia tribesmen. Other studies have since found that some lactobacilli possess a cholesterol lowering ability in man (2,12) and animals (14,16,30).

Several researchers have attempted to elucidate the mechanism involved in the hypocholesterolemic action of these strains of lactic acid bacteria. One proposed mechanism is assimilation of cholesterol by the cells of *Lactobacillus acidophilus* during growth (4,14). Noh et.al. (23) demonstrated the ability of a strain of *L. acidophilus* to incorporate cholesterol from growth medium into cellular membranes during growth. Removal or assimilation of cholesterol by intestinal organisms in the small intestine could reduce the amount of cholesterol available for absorption from the intestine, thus exerting some control on serum cholesterol levels. Another plausible mechanism is deconjugation of bile acids by lactic acid bacteria that produce the enzyme bile salt hydrolase. Deconjugated or free bile acids are more easily excreted from the body (7, 26). Thus an increase in bile acid deconjugation in the intestines would continuously drain the cholesterol pool as more bile acids are synthesized. Many studies have found

bile salt hydrolase activity in strains of lactic acid bacteria, including *L. acidophilus* (13,17,29).

The exocellular polysaccharides produced by lactic acid bacteria are either excreted into the growth medium as free exocellular polysaccharides (EPS) or they adhere to the cell as capsular EPS (28). Due to the potential benefits of these bacteria to the quality of fermented dairy food products, several thermophilic and mesophilic lactic acid bacteria and the polymers they produce have been studied. *Streptococcus salivarius* subsp *thermophilus* (5,8), *Lactobacillus delbrueckii* subsp *bulgaricus* (3,15,31), other lactobacilli (10,21) and *Bifidobacterium longum* (1) have been included in these studies. Most of these focused on the structure and characterization of the EPS. Very few have studied the potential health promoting effects of exocellular polysaccharides. Among these, Nakajima (22) focused on the cholesterol lowering activity of milk fermented with an exocellular polysaccharide producing lactic acid bacteria. The consumption of this fermented milk significantly decreased serum cholesterol levels in rats, where as the consumption of milk fermented with a non-EPS producing strain did not. There is potential for these polymers to interfere with the absorption of cholesterol or of bile acids from the intestines by binding them and removing them from the body similar manner to that reported for plant based polysaccharides or dietary fiber (18).

The objective of this study was to determine if different strains *S. salivarius* subsp *thermophilus* and *L. delbrueckii* subsp *bulgaricus* could produce capsular exocellular polysaccharides with the ability to bind bile acids *in vitro*. The findings might suggest an alternative mechanism for lowering serum cholesterol levels through the consumption of fermented milk products.

MATERIALS AND METHODS

Source and maintenance of cultures

The cultures used in this study were obtained from two sources. *Lactobacillus delbrueckii* subsp. *bulgaricus* Lb-18 and Lb-10442, as well as *Streptococcus salivarius* subsp. *thermophilus* St-143 were from the culture collection of Chris Hansen Inc. (Milwaukee, WI)

Streptococcus salivarius subsp *thermophilus* St-OSU1, St-OSU2, St-OSU3, *L. delbrueckii* subsp *bulgaricus* Lb-OSU4 and Lb-OSU5 were all from the stock culture collection of the Food Microbiology Laboratory of the Food and Agricultural Products Center at Oklahoma State University. The cultures were maintained by subculturing weekly using 1 % inocula in lactobacilli MRS broth made from individual ingredients according to the manufacturer's directions (Difco Laboratories, Detroit, MI) except that 4% lactose was used in the place of glucose (MRSL). The inoculated broth was incubated for 18h at 37° C. The cultures were stored at 7° C between transfers. Stock cultures were stored in MRSL agar stabs (MRSL broth plus 1.5% agar). The cultures were subcultured at least three times immediately before experimentation . In addition, the cultures were subcultured in 1 % inocula in sterile 10% reconstituted non-fat dry milk (NFDM) at least three times prior to experimentation involving milk as the growth medium. The milk subcultures were incubated at 45 ° C for 12 hours and stored at 7° C between transfers.

Preparation of milk for EPS production

The milk was prepared by reconstituting a commercial instantized non-fat dry milk (NFDM) in deionized water at 10% (w/v). For use in experiments involving the measurement of EPS production the 10% NFDM was pasteurized at 80° C for 30 min and then refrigerated at 7° C until inoculation. It was prepared and used the same day.

Measurement of Capsular Exocellular Polysaccharide

The measurement of capsular exocellular polysaccharide material is complicated by its close association with the bacterial cells (28). The levels were determined by measuring the amount of glucose present in washed cell suspensions as described by Gilliland and Speck (11). The glucose concentrations were determined using either the variation of the anthrone method used by Gilliland and Speck (11) or the phenol-sulfuric method (9) using glucose as the standard. Glucose levels were determined and expressed as EPS μg/ml of culture (see Appendix A for standard curve information).

Plate Counts of Bacteria in Samples

The numbers of lactobacilli and streptococci in the experimental samples were determined using the pour plate method (32) on MRSL agar. Dilution blanks containing 99 ml of a sterilized solution of 0.1% peptone (Difco Laboratories) and 0.01% silicone antifoam (Sigma Chemical Co.) were used to dilute the samples. After solidification the plates were incubated at 37° C for 48 hours. A Quebec colony counter (American Optical Co. Buffalo, NY) was used to count the colonies to allow determination of the colony forming units (cfu) per milliliter or per gram.

Determination of culture identity

Initial confirmation of the identity of the cultures used was based on the Gram stain reaction and fermentation characteristics determined using API-50 CH kits (bioMerieux Vitek Inc., Hazelwood, MO) according to the manufacturer's directions except that mineral oil was not added to each test well. The inoculated kits were incubated in a Gas Pak anaerobic system for 48 hours. The results were compared to those given in *Bergey's Manual of Systemic Bacteriology* (27).

Initial Screening of Cultures for Capsular Exopolysaccharide Production

The strains of *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were initially tested to determine the level of capsular exocellular polysaccharide that could be produced in 10% reconstituted non-fat dry milk. Volumes of 10ml of freshly pasteurized NFDM was inoculated with each of the eight cultures at 1% inoculum and incubated at 45 ° C for 8 hours. The capsular exocellular polysaccharide content was determined similar to that of Gilliland and Speck (11). The milk proteins were solubilized by adding 1.1 ml of 20 % (w/v) sodium citrate to 5.5 ml volumes of each culture then, 4.4 ml of distilled water was added bringing the final concentration to 2% sodium citrate. The cells were collected by centrifugation at 12,000 g for 15 minutes at 0° C. Each pellet was washed once with 11ml of cold distilled water and resuspended in 5.5 ml of cold distilled water. The total sugar (EPS) content of the cell suspension using the anthrone method. The amount of capsular EPS was expressed as μg glucose/ml of original culture. In addition, plate counts were determined using the

pour plate method (32) as previously described. This allowed the amount of EPS per 10^7 cfu to be calculated and compared among strains.

Measurement of Bile Acids

The quantification of free bile acid (cholic acid) was accomplished using the method described by Walker and Gilliland (33). A standard curve was prepared using the sodium salt of cholic acid (Sigma Chemical Co.). (The standard curve and method is presented in Appendix A.)

Measurement of Bile Acid Binding

In order to determine if the strains were able to bind bile acids to the capsular EPS, an experiment was designed using cells grown in the MRSL broth. Large bottles containing 75ml of MRSL were inoculated (1%) with selected strains and incubated at 45° C for 8 hours. Following incubation, two 22.5 ml volumes of each culture were transferred into sterile 50 ml Erlenmeyer flasks. To one, 2.5 ml of a 15mM solution of cholic acid (sodium salt) was added bringing the final concentration of bile acid to 1.5mM (646 μg/ml). The second, received similar mM concentration of the sodium salt of glycocholic acid. These samples were held in a 37° C shaker water bath with gentle agitation for 2 hours to allow contact between the cells and the bile acids.

Following the shaking step, the pH of the mixture was adjusted to 7.0 and the cells removed by centrifugation (12,000 x g at 0° C for 10 min). Any bound bile acid would by removed when the cells were removed. The supernatant fluids were assayed

for bile salt using the colorimetric assay (33). An uninoculated control was included to permit a calculation of the amounts of bile salts removed (i.e. bound to the cells).

A portion of the initial broth culture was also analyzed for capsular EPS content using the same procedure as for the milk cultures except that sodium citrate was not necessary to recover the cells from the broth culture. The phenol-sulfuric method was used to quantify the capsular EPS content of the cells. This information allowed calculations of amount of bile acid bound per mg of EPS present.

Effect of initial pH on the bile binding assay

Cholic acid has a pKa around 5 making it less soluble when the pH is below 5. To test whether the pH of the broth cultures might have effected the removal of the bile acid , MRSL broth containing 1.5 mM cholic acid (sodium salt)was prepared and portions adjusted to the pH 6.5, 6.0, 5.0, 4.0, 3.0, 2.0, and 1.0. The broth was held in a shaker water bath for 2 hours, adjusted to pH 7.0, centrifuged (12,000 x g at 0° C for 10 min) and the supernatant analyzed for cholic acid following the same procedure described in the previous section.

Statistical Analyses

Experiments were conducted in triplicate. Each value was the mean of three independent trials. Exocellular polysaccharide concentration and the degree of binding of the bile acids were analyzed as independent variables in each respective experiment. The analysis involved the analysis of variance procedure of JMPIN statistical software (25).

The means with significant differences were separated using the least significant difference method within the statistical software.

RESULTS

Confirmation of identity of cultures

The identity of the cultures of *Streptococcus salivarius* subsp *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* used in this study were confirmed (Appendix C). The identity characteristics of each strain closely resembled those of the respective organism as listed in *Bergey's Manual of Systemic Bacteriology* (27). All strains of the lactobacilli used in the study matched the published reactions except for a positive reaction on mannose. The streptococci also closely matched of the published reactions. Each strain matched at least 16 of 17 reactions listed in Bergey's manual.

Initial Screening of Cultures for Capsular Exopolysaccharide Production

Significant differences ($P<.05$) were found among the strains in the amounts of exocellular polysaccharides produced in milk (Table 1). When considering the amount of EPS in μg/ml, *S. salivarius* subsp *thermophilus* culture strain St-OSU3 produced a significantly higher ($P<.05$) level than all other strains in the study. The other strains which were not significantly different, produced from 398-505 μg/ml EPS. Nearly twice as much was measured for St-OSU3 in some cases. The degree of difference among strains was greater compared on the basis of $\mu g/10^7$ cfu. This measurement was calculated to provide information on the amount of EPS produced on a per cell basis. *Lactobacillus delbrueckii* subsp *bulgaricus* Lb-18 produced 70.6 $\mu g/10^7$ cfu that was significantly greater ($P<0.05$) than for all other strains tested. Strains St-143 and Lb-10442 followed with 47.0 and 47.4 $\mu g/10^7$cfu respectively. These amounts were significantly higher ($P<0.05$) than the remaining five strains that produced 4.1 - 16.6

$\mu g/10^7$ cfu. Based on the amount of EPS produced per 10^7 cfu, three strains (Lb-18, Lb-10442, and St-143) were selected for further study. In addition strains St-OSU3 and St-OSU1 were included to compare strains with low production activity per cfu to the more active strains.

Measurement of Bile Acid Binding

The results of the statistical analysis of the binding data for the sodium salt of cholic acid indicated that there were significant differences ($P<0.05$) among the 5 strains in the comparison (Table 2). The degree of binding was measured in percent of cholic bound and μg cholic acid bound per mg EPS. Strain Lb-10442 bound the greatest amount ($P<0.05$) of bile acid measured at 15.33% which was nearly twice that of strain Lb-18 which bound 8.20%. St-OSU1 was able to remove 4.20% while the two remaining strains, St-143 and St-OSU3 were only able to bind minimal amounts. When the results of the μg cholic acid/ mg EPS measurement was analyzed very similar differences were observed. However, there was no difference ($P>0.05$) between strain Lb-18 (452 μg/mg EPS) and Lb-10442 (422 μg/mg EPS). Strains St-OSU1, St-143, St-OSU3 bound significantly less ($P<0.05$) than strains of *L bulgaricus* with 224, 86 and 56 μg/mg EPS respectively. In addition, strain Lb-18 was able to bind 2.9 $\mu g/10^7$ cfu, which was significantly higher than all the streptococci. The results of the glycocholic acid binding experiments indicated that the EPS associated with the strains in this study do not bind this conjugated bile acid. (See Appendix E).

Effect of initial pH on the Bile Binding Assay

In order to validate that sodium cholate was being associated with the EPS and not just precipitating due to low pH, an experiment was conducted to determine the effect of the pH on the removal of cholic acid from MRSL broth. The series of broth samples at various pH levels were treated and analyzed exactly as mentioned in the procedure of the bile binding assay for the cultures. The cholic acid values that were obtained ranged from 478 μg/ml to 530 μg/ml. A pattern associated was not associated with the results. For example, if some cholic acid precipitated at pH 3.0 one might expect some to be removed by the centrifugation step giving lower readings in the cholic acid measurement. However at pH 3.0, the cholic acid recovery was the highest 530 mg/ml.

DISCUSSION

It is well documented that some species of lactic acid bacteria, especially lactobacilli, can assist in the reduction of elevated serum cholesterol levels (2,12,19). Many researchers have shown that some lactic acid bacteria , such as the lactobacilli can assimilate cholesterol into cellular membranes (23).

Other studies have concentrated on potential of bile salt deconjugation being an explanation for the hypocholesterolemic activity of fermented milk (13,17,29).

Nakajima et.al. (22) found significant differences between serum cholesterol levels among rats consuming fermented milk with and without exocellular polysaccharides. The authors suggested that the observed differences were due to the effect of exocellular polysaccharides on cholesterol or bile acid absorption in the intestines. Due to the resistance of the polysaccharide to digestive enzymes and its water holding capacity, they hypothesized that the bacterial polymer acts similar to a dietary fiber.

Other researchers have recognized the possible effects of intestinal organisms on the binding of bile acids or interference of absorption in the intestine. Mott et al. (20) observed that fecal sterol composition is highly dependent upon the presence of bacteria in the intestines. The authors cited several factors including binding of bile acids by intestinal bacteria as being responsible for sterol balance in the body. Chikai et.al. (6) studied the bile acid deconjugation ability of several species of bacteria. They found that an increase in bile acid deconjugation resulted in increased bile acid excretion. They

speculated that adhesion of free bile acids to bacteria as well as dietary fibers might also contribute to the large amounts of bile excretion.

The results of this study indicate that some lactobacilli produce capsular EPS that can bind up to 452 μg of cholic acid per mg EPS or 2.9 μg/ 10^7 cfu from laboratory media. This indicates a possible action in enhancing increased excretion of free bile acids which could result in reduction of the cholesterol pool as cholesterol is used to synthesize more bile acid in the enterohepatic circulation to replace those excreted with the EPS via the feces.

In the screening portion of this study there were variable amounts of EPS production among strains. The greatest amounts of EPS in μg/ml were produced by the streptococci. However, the growth of the streptococci resulted in much higher cell numbers in cfu/ml than the lactobacilli. As a measure of how much EPS per cell was produced, μg EPS per 10^7 cfu was calculated. This allowed comparisons of strains on total amount produced, as well as amount surrounding each cell. The lactobacilli tend to produce the highest amounts with this measurement. The five strains that were selected for further study exhibited variable production capabilities in both the total production and amount produced per cell. These were included to determine if there are differences in bile acid binding properties that relate to the EPS production activity.

The bile acid binding experiment also resulted in highly variable activities that was dependent upon the strain. All five of the strains tested demonstrated the ability to bind cholic acid but not glycocholic acid from laboratory media.

The highest amount of cholic acid binding was achieved by the *Lactobacillus delbrueckii* subsp. *bulgaricus* strains Lb-10442 and Lb-18. Even though these strains

produced the lowest amount per ml in the screening phase of the experiment, they are capable of binding several-fold more cholic acid than some of the streptococci in this experiment. This provides evidence that the level of exocellular polysaccharide encapsulating the cells enhances binding of cholic acid. Thicker layers of capsular material may aid in the entrapment of cholic acid.

Figure 1 shows a comparison among strains on the amount of cholic acid that could theoretically be removed or bound when an 8 oz serving of the fermented milk or yogurt is consumed. As with the other results the most bile acid bound would be from the consumption of the *L. delbrueckii* subsp. *bulgaricus* strains Lb-10442 and Lb-18. Up to 45 mg of cholic acid could be removed by a serving containing Lb-10442. The strains of streptococci ranged from 9-26 mg of cholic acid/ 8 oz serving. The production of yogurt involves both *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. It may be reasonable to assume that if strains Lb-10442 and St-OSU1 were used to produce a yogurt product, it would bind up to 71 mg/ 8 oz portion if both organisms reach cell numbers similar to those of each individual culture in this study. In addition, other experiments in our laboratory indicate that some of these cultures produce free exocellular polysaccharides that are not associated with the cell (See Appendix D for data). This may also contribute to the total amount of bile acids bound due consumption of the yogurt.

The binding of bile acids to capsular EPS may contribute to the development of a probiotic that uses a variety of mechanisms to control serum cholesterol levels. Strain or strains of starter cultures that have the ability to bind free bile acid could be used to produce a yogurt product in conjunction with an organism known to deconjugate bile

acids and/or assimilate cholesterol. The strains in this study did not remove conjugated bile salts (sodium glycocholate) from laboratory media. Increasing deconjugation of glycocholic and taurocholic acids to release cholic acid would make more cholic available for binding and removal from the body. Even though the free bile acids are more likely to be excreted via the feces, the presence of an EPS that preferentially binds them over conjugated bile acids could enhance their excretion. Thus the combination of a probiotic culture such as *L. acidophilus* that deconjugates bile acids with strains of *L. delbrueckii* subsp. *bulgaricus* and *S salivarius* subsp. *thermophilus* could be used to manufacture cultured yogurt that would have enhanced hypocholesterolemic activity. Such a product would be useful for hypercholesterolemic individuals.

Table 1. Comparison of amounts of capsular exocellular polysaccharide produced by strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* grown in 10% NFDM.

Culture[1]	μg/ml[2,5]	cfu/ml[3]	μg/10^7 cfu[4,5]
St-OSU3	857[a]	$2.1x10^9$	4.1[c]
St-OSU2	505[b]	$9.3x10^8$	5.5[c]
St-OSU1	478[b]	$1.3x10^9$	4.4[c]
Lb-OSU4	471[b]	$3.9x10^8$	12.3[c]
Lb-OSU5	451[b]	$2.8x10^8$	16.6[c]
Lb-10442	448[b]	$1.3x10^8$	47.4[b]
St-143	439[b]	$9.6x10^7$	47.0[b]
Lb-18	398[b]	$5.8x10^7$	70.6[a]

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus.*

[2] EPS is expressed as glucose μg/ml of cell suspension isolated from 8-hour cultures grown at 45° C.

[3]cfu/ml of 8 hour cultures grown at 45° C. Values are the means of three replications.

[4]EPS is expressed as glucose μg/10^7 cfu in 8-hour cultures grown at 45° C.

[5]Values are the means of three replications. Means with no common superscript letters differ significantly ($P<0.05$).

Table 2. Binding of cholic acid (sodium salt) by capsular exocellular polysaccharide produced by strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* grown in MRS Broth containing 4% lactose as the source of carbohydrate.

Culture[1]	% Bound[2,5]	µg cholic acid/ mg EPS[3,5]	µg EPS/ ml	µg cholic acid/ 10^7 cfu[4,5]
Lb-10442	15.33[a]	422[a]	152	2.9[a]
Lb-18	8.20[b]	452[a]	76	1.1[ab]
St-OSU1	4.20[c]	224[b]	75	0.6[b]
St-143	1.50[d]	86[c]	75	0.4[b]
St-OSU3	0.63[d]	56[c]	54	0.2[b]

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus.*

[2]Values are expressed as a binding %, percentage of cholic acid removed by contact with cells from 8 hour cultures grown at 45° C.

[3]Values are expressed as µg of cholic acid bound per mg of Glucose(EPS).

[4] Values are µg of cholic acid bound per 10^7 colony forming units (cfu).

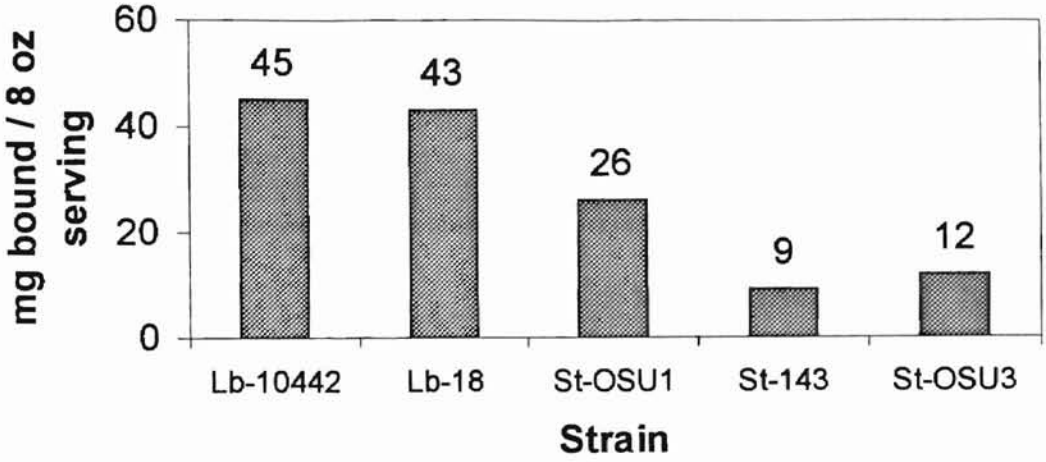
[5] Values are the means of three replications. Means within columns with no common superscript letters differ significantly ($P<0.05$).

Table 3. Effect of pH on the measurement of cholic acid using the bile acid binding assay.

pH[1]	µg/ml cholic acid
6.5	487
6.0	478
5.0	487
4.0	517
3.0	530
2.0	508
1.0	500

[1]Initial pH of MRSL broth before being adjusted to pH 7.0.

Figure 1. Comparison of the theoretical amounts of cholic acid that could be bound and removed from the body by consuming an 8 oz serving of cultured product containing each strain.



REFERENCES

1. Andaloussi, S.A. H. Talbaoui, R. Marczak and R. Bonaly. 1995. Isolation and characterization of exocellular polysaccharides produced by *Bifidobacterium longum*. Appl. Microbiol. Biotechnol. 43:995-1000.

2. Anderson, J.W. and S.E. Gilliland. 1999. Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolemic humans. J. Am. Coll. Nutr. 18:43-50.

3. Bouzar, F., J. Cerning, and M. Desmazeaud. 1996. Exocellular polysaccharide production in milk by *Lactobacillus delbrueckii* subsp. *bulgaricus* CNRZ 1187 and by two colonial variants. J. Dairy Sci. 79:205-211.

4. Buck, L.M. and S.E. Gilliland. 1994. Comparisons of freshly isolated strains of *Lactobacillus acidophilus* of human intestinal origin for ability to assimilate cholesterol during growth. J. Dairy Sci. 77:2925-2933.

5. Cerning, J., C. Bouillanne, M. Landon, and M. Desmazeaud. 1992. Isolation and characterization of exocellular polysaccharides from slime-forming mesophilic lactic acid bacteria. J. Dairy Sci. 75-692-699.

6. Chikai, T., H. Nakao, and K. Uchida. 1987. Deconjugation of bile acids by human intestinal bacteria implanted in germ free rats. Lipids. 22:669-671.

7. Dietschy, J.M., H.S. Salomon, and M.S. Siperstein. 1966. Bile acid metabolism. I. Studies on the mechanisms of intestinal transport. J. Clin. Inves. 45:832-846.

8. Doco, T., J. Wieruszeski, and B. Fournet. 1990. Structure of an exocellular polysaccharide produced by *Streptococcus thermophilus*. Carbo. Res. 198:313-321.

9. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350-356.

10. Dunican, L.K. and H.W. Seeley. 1965. Extracellular polysaccharide synthesis by members of the genus *Lactobacillus*: Conditions of formation and accumulation. J. Gen. Microbiol. 40:297-308.

11. Gilliland, S.E. and M.L. Speck. 1974. Relationship of cellular components to the stability of concentrated lactic streptococcus cultures at –17° C. Appl. Microbiol. 27:793-796.

12. Gilliland, S.E. 1979. Beneficial interrelationships between certain microorganisms and humans: candidate microorganisms for use as dietary adjuncts. J. Food Prot. 42:164-174.

13. Gilliland, S.E. and M.L. Speck. 1977. Deconjugation of bile acids by intestinal lactobacilli. Appl. Environ. Microbiol. 33:15-18.

14. Gilliland, S.E. and C.R. Nelson, and C. Maxwell. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49:377-381.

15. Grobben, G.J., J. Sikkema, M.R. Smith, and J.A.M. de Bont. 1995. Production of extracellular polysaccharides by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772. J. Appl. Bacteriol. 79:103-107.

16. Grunewald, K.K. 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci. 47:2078-2079.

17. Kobashi, K., I. Nishizawa, and T. Yamada. 1978. A new hydrolase specific taurine-conjugates of bile acids. J. Biochem. 84:495-503.

18. Kritchevsky, D. and J.A. Story. 1975. In vitro binding of bile acids and bile salts. Am. J. Clin. Nutr. 28:305-306.

19. Mann, G.V. and A. Spoerry. 1974. Studies of a surfactant and cholesteremia in the Maasai. Am. J. Clin. Nutr. 27:464-469.

20. Mott, G.E., R.W. Moore, H. Redmond, and R. Reiser. 1973. Lowering of serum cholesterol by intestinal bacteria in cholesterol-fed piglets. Lipids. 8:428-430.

21. Mozzi, F., G.S. De Giori, G. Oliver, and G.F. De Valdez. 1994. Effect of culture pH on the growth characteristics and polysaccharide production by *Lactobacillus casei*. Milchwissenschaft. 49:667-669.

22. Nakajima, H., Y. Suzuki, H. Kaizu, and T. Hirota. 1992. Cholesterol lowering activity of ropy fermented milk. J. Food Sci. 57:1327-1329.

23. Noh, D.O., S.H. Kim and S.E. Gilliland. 1997. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. J. Dairy Sci. 80:3107-3113.

24. Pekkanen, J., S. Linn, G. Heiss, C.M. Suchindran, A. Leon, B.M. Rifkind, and H.A. Tyroler. 1990. Ten-year mortality from cardiovascular disease in relation to cholesterol level among mean with and without pre-existing cardiovascular disease. N. Engl. J. Med. 322:1700-1707.

25. Sall, J. and A. Lehman. (eds). 1996. JMP Start Statistics: A guide to statistics and data analysis using JMP and JMPIN software. Duxbury Press. SAS Institute. Cary, N.C.

26. Schiff, E.R., N.C. Small, and J.M. Dietschy. 1972. Characterization of the kinetics of the passive and active transposrt mechanisms for bile acid absorption in the small intestine and colon of the rat. J. Clin. Inves. 51:1351-1362.

27. Sneath, P. H. A., N. S. Mair, and M. E. Sharp. (eds). 1986. *Bergey's Manual of Systemic Bacteriology*. 9th Ed. Williams and Wilkins Inc. Baltimore, MD.

28. Sutherland, I.W. 1972. *Bacterial Exocellular polysaccharides*. pp. 143-213. In A.H. Rose and D.W. Tempest (Eds.), Advances in Microbial Physiology. Academic Press. N.Y., New York.

29. Tannock, G.W., A. Tangerman, A. Van Scack., and M.A. McConnell. 1989. Deconjugation of bile acids by lactobacilli in the mouse small bowel. Appl. Environ. Microbiol. 60:3419-3420.

30. Thakur, C.P. and A.N. Jha. 1981. Influence of milk, yogurt and calcium on cholesterol-induced atherosclerosis in rabbits. Atherosclerosis. 39:211-215.

31. Uemura, J., T. Itoh, T. Kaneko, and K. Noda. 1998. Chemical characterization of exocellular polysaccharide from *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1. Milchwissenschaft. 53:443-446.

32. Vanderzant, C., and D.F. Splittstoesser. (eds). 1992. *Compendium of Methods for the Microbial Examination of Foods*, 3rd ed. Washington, D.C. American Public Health Association.

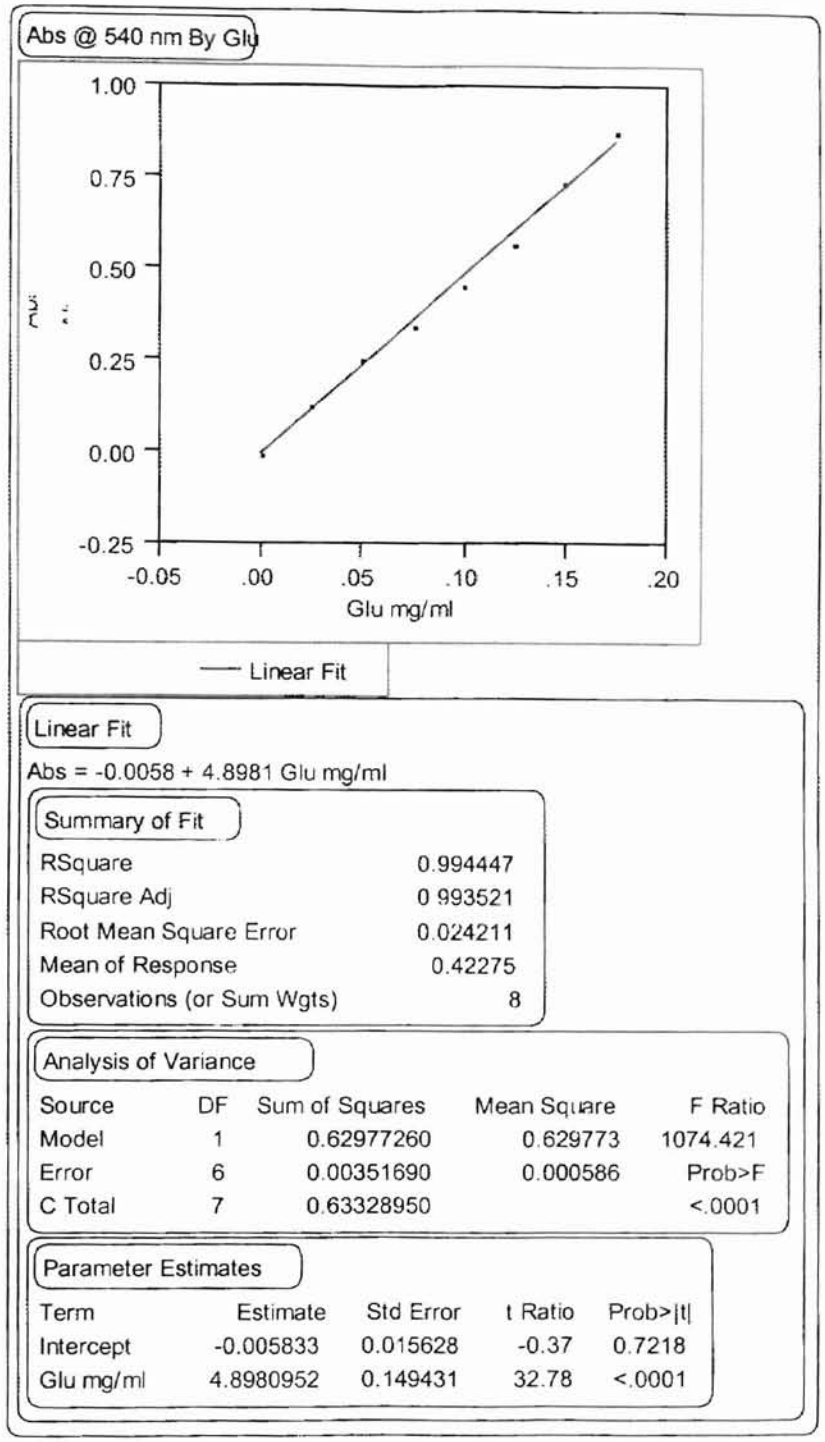
33. Walker, D.K. and S.E. Gilliland. 1993. Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. J. Dairy Sci. 76:956-961.

APPENDIX A

STANDARD CURVES

Figure 2

Anthrone Method - Calibration Curve



Linear Fit

Abs = -0.0058 + 4.8981 Glu mg/ml

Summary of Fit

RSquare	0.994447
RSquare Adj	0.993521
Root Mean Square Error	0.024211
Mean of Response	0.42275
Observations (or Sum Wgts)	8

Analysis of Variance

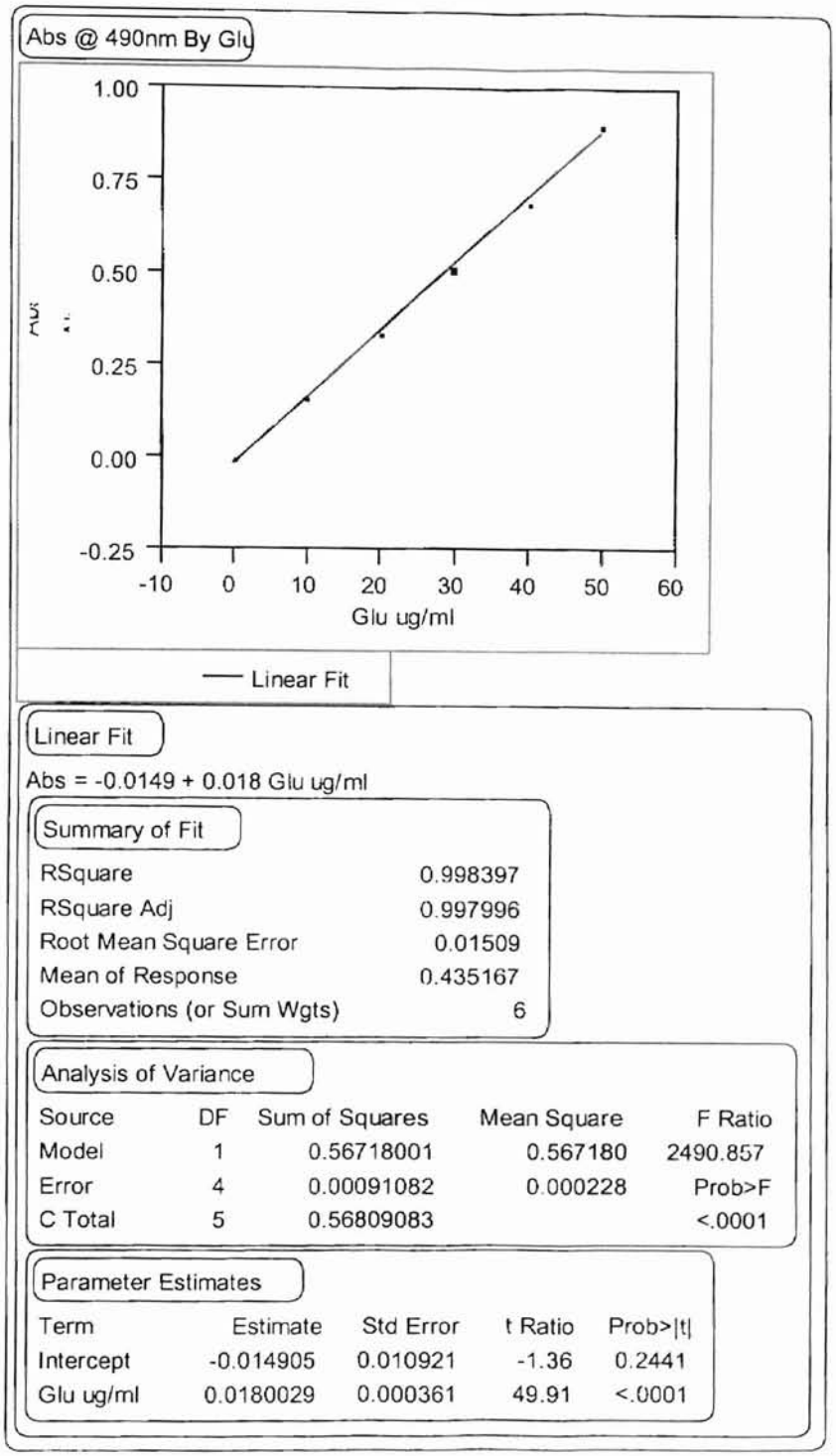
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.62977260	0.629773	1074.421
Error	6	0.00351690	0.000586	Prob>F
C Total	7	0.63328950		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.005833	0.015628	-0.37	0.7218
Glu mg/ml	4.8980952	0.149431	32.78	<.0001

Figure 3

Phenol Sulfuric Method - Calibration Curve



Linear Fit

Abs = -0.0149 + 0.018 Glu ug/ml

Summary of Fit

RSquare	0.998397
RSquare Adj	0.997996
Root Mean Square Error	0.01509
Mean of Response	0.435167
Observations (or Sum Wgts)	6

Analysis of Variance

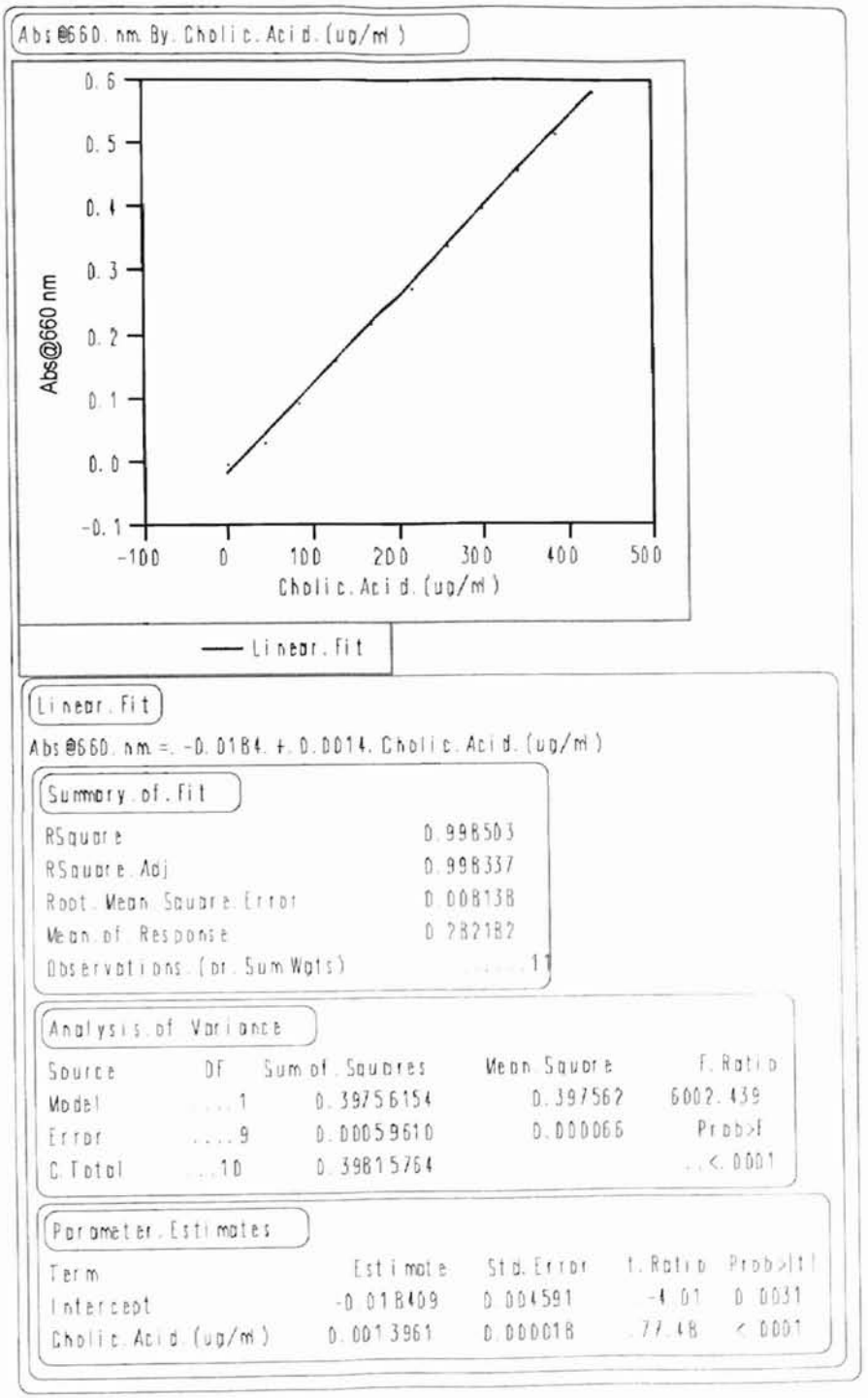
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.56718001	0.567180	2490.857
Error	4	0.00091082	0.000228	Prob>F
C Total	5	0.56809083		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.014905	0.010921	-1.36	0.2441
Glu ug/ml	0.0180029	0.000361	49.91	<.0001

Figure 4

Cholic/Glycocholic Acid Assay - Calibration Curve



Method for measurement of cholic acid from Walker, D.K. and S.E Gilliland. 1993. J. Dairy Science. 76:956-961 adapted from Irvin et al. 1944. J. Biol. Chem. 153:439-455.

1. Adjust 20ml of broth culture to pH 7.0 and bring to 25ml using distilled water.
2. Centrifuge 10 min @ 12000xg at 0°C.
3. Remove 15ml of the supernatant and adjust to pH 1.0 with 10 N HCl.
4. Adjust to 24 ml using distilled water.
5. Extract 3 ml portions with 9ml Ethyl Acetate.
6. Evaporate 3ml of Ethyl Acetate layer to dryness under flow of nitrogen gas @60°C.
7. Disslove residue in 1 ml of 0.01 N NaOH.
8. Add 6 ml of 16 N sulfuric acid.
9. Add 1 ml of 1% furfuraldehyde.
10. Heat for 13 min @ 65°C.
11. Cool to room temperature.
12. Add 5 ml glacial acetic acid and mix
13. Read Abs at 660nm against a reagent blank.
14. Calculate the cholic acid present in the original culture.

APPENDIX B

CAPSULE AND GRAM STAINING

TABLE 4

Results of capsule stains for strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*

Culture[1]	Capsule [2]
Lb-18	+
Lb-10442	+
Lb-OSU5	+
Lb-OSU4	+
St-143	+
St-OSU1	+
St-OSU2	+
St-OSU3	+

[1] Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus.*

[2] Capsular material was identified through negative staining techniques by the presence of unstained areas surrounding the bacterial cell walls. The india ink method was used as described in *MELE in Microbiology*, 5th Ed., Johnston, G.B., 1992. Bellwether Press Division, Edina, MN.

TABLE 5

Results of Gram stains for strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*

Culture[1]	Gram reaction [2]
Lb-18	Gram + rods
Lb-10442	Gram + rods
Lb-OSU5	Gram + rods
Lb-OSU4	Gram + rods
St-143	Gram + cocci
St-OSU1	Gram + cocci
St-OSU2	Gram + cocci
St-OSU3	Gram + cocci

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.
[2]Observed results of Gram stains.

APPENDIX C

IDENTITY OF CULTURES OF *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* AND *STREPTOCOCCUS SALIVARIUS* SUBSP. *THERMOPHILUS*

TABLE 6

Identity of cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus*

Test[1]	Lb[2]	Lb-18	Lb-10442	Lb-OSU5	Lb-OSU4
Amygdalin	-	-	-	-	-
Arabinose	-	-	-	-	-
Cellobiose	-	-	-	-	-
Esculin	-	-	-	-	-
Fructose	+	+	+	+	+
Galactose	-	-	-	-	-
Glucose	+	+	+	+	+
Gluconate	-	-	-	-	-
Lactose	+	+	+	+	+
Maltose	-	-	-	-	-
Mannitol	-	-	-	-	-
Mannose	-	+	+	+	+
Melezitose	-	-	-	-	-
Melibiose	-	-	-	-	-
Raffinose	-	-	-	-	-
Rhamnose	-	-	-	-	-
Ribose	-	-	-	-	-
Salicin	-	-	-	-	-
Sorbitol	-	-	-	-	-
Sucrose	-	-	-	-	-
Trehalose	-	-	-	-	-
Xylose	-	-	-	-	-

[1]All cultures are Gram + rods that grow at 45° C.

[2]Lb=*Lactobacillus delbrueckii* subsp *bulgaricus*; reactions as listed in the 9th Edition of Bergey's Manual of Systematic Bacteriology.

TABLE 7

Identity of cultures of *Streptococcus salivarius* subsp. *thermophilus*

Test[1]	St[2]	St-143	St-OSU1	St-OSU2	St-OSU3
Arabinose	-	-	-	-	-
Fructose	+	+	-	-	+/-
Galactose	+/-	+/-	-	-	+
Glucose	+	+	+	+	+
Glycerol	-	-	-	-	-
Inulin	-	-	-	-	-
Lactose	+	+	+	+	+
Maltose	+/-	-	-	-	-
Mannitol	-	-	-	-	-
Mannose	+	+	-	-	-
Rhamnose	-	-	-	-	-
Salicin	-	-	-	-	-
Sorbitol	-	-	-	-	-
Sucrose	+	+	+	+	+
Xylose	-	-	-	-	-

[1]All cultures are Gram + cocci that grow at 45° C.

[2]St=*Streptococcus salivarius* subsp. *thermophilus*; reactions as listed in the 9th Edition of Bergey's Manual of Systematic Bacteriology.

APPENDIX D

RAW DATA
FROM
SCREENING EXPERIMENTS

TABLE 8

Capsular Exocellular Polysaccharide concentration (μg/ml) of 8 hour cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in milk at 45° C.

	μg/ml [2]			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	384	365	446	398
Lb-10442	457	463	423	448
Lb-OSU5	527	472	356	451
Lb-OSU4	559	416	439	471
St-143	477	390	449	439
St-OSU1	696	407	330	478
St-OSU2	627	492	396	505
St-OSU3	924	947	699	857

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

[2]EPS (μg/ml) values are expressed as glucose concentration of the cellular fraction of the cultures. Glucose concentrations were determined through the use of the anthrone method.

TABLE 9

Culture pH of 8 hour cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in milk at 45° C for capsular EPS determination.

	pH		
Culture[1]	Rep 1	Rep 2	Rep 3
Lb-18	4.55	4.85	4.90
Lb-10442	4.50	4.95	4.89
Lb-OSU5	5.16	4.79	5.00
Lb-OSU4	4.69	4.85	4.84
St-143	5.08	5.17	5.31
St-OSU1	4.78	4.82	4.84
St-OSU2	4.33	4.76	4.61
St-OSU3	4.43	4.40	4.52

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

TABLE 10

Plate Counts on MRS Agar (with 4% Lactose) of 8 hour cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in milk at 45° C for capsular EPS determination.

	Log_{10} cfu/ml			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	7.82	7.64	7.80	7.76
Lb-10442	8.41	7.83	7.88	8.11
Lb-OSU5	8.60	8.36	8.34	8.45
Lb-OSU4	8.57	8.60	8.59	8.59
St-143	8.08	7.94	7.90	7.98
St-OSU1	8.94	9.26	9.04	9.11
St-OSU2	9.08	8.93	8.87	8.97
St-OSU3	9.26	9.34	9.38	9.32

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

TABLE 11

Capsular Exocellular Polysaccharide concentration ($\mu g/10^7$ cfu) of 8 hour cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in milk at 45° C.

	$\mu g/10^7$ cfu			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	58.2	82.9	70.8	70.6
Lb-10442	17.6	68.1	56.4	47.4
Lb-OSU5	13.2	20.5	16.2	16.6
Lb-OSU4	15.1	10.4	11.3	12.3
St-143	39.7	44.4	56.8	47.0
St-OSU1	8.0	2.3	3.0	4.4
St-OSU2	5.2	5.8	5.4	5.5
St-OSU3	5.1	4.3	2.9	4.1

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

TABLE 12

Free Exocellular Polysaccharide concentration (μg/ml) of 8 hour cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in milk at 45° C.

	μg/ml [2]			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	44.7	36.3	51.1	44.0
Lb-10442	21.2	16.2	28.2	21.9
Lb-OSU5	13.1	5.8	9.6	9.5
Lb-OSU4	7.5	34.1	6.5	16.0
St-143	5.6	5.1	6.8	5.8
St-OSU1	67.8	58.3	44.7	56.9
St-OSU2	11.7	5.6	5.9	7.7
St-OSU3	15.6	10.4	6.1	10.7

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

[2]EPS (μg/ml glucose) values were determined through the use of the phenol-sulfuric method.

TABLE 13

Culture pH of 8 hour cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in milk at 45° C for free EPS determination.

	pH		
Culture[1]	Rep 1	Rep 2	Rep 3
Lb-18	4.56	4.60	4.57
Lb-10442	4.71	4.57	4.57
Lb-OSU5	5.05	5.09	4.62
Lb-OSU4	4.70	4.61	4.37
St-143	4.99	5.36	4.92
St-OSU1	4.64	4.81	4.70
St-OSU2	4.45	4.61	4.47
St-OSU3	4.42	4.48	4.54

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

Table 14

Comparison of amounts (mg/ml) of free exocellular polysaccharide produced by strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* grown in 10% NFDM.

Culture[1]	EPS[2,3]
St-OSU1	56.9[a]
Lb-18	44.0[a]
Lb-10442	21.9[b]
Lb-OSU4	16.0[bc]
St-OSU3	10.7[bc]
Lb-OSU5	9.5[bc]
St-OSU2	7.7[c]
St-143	5.8[c]

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.
[2] EPS is expressed as glucose mg/ml in 8 hour cultures grown at 45° C.
[3]Values are the means of three replications. Means with no common superscript letters differ significantly ($P<0.05$).

APPENDIX E

RAW DATA
FROM
BINDING EXPERIMENTS

TABLE 15

Capsular Exocellular Polysaccharide concentration (μg/ml) of 8 hour cultures of selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in MRS broth with 4% lactose at 45° C.

	μg/ml[2]			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	95	72	84	84
Lb-10442	167	167	171	168
St-OSU1	92	79	78	83
St-OSU3	79	50	51	60
St-143	96	73	81	83
Control	7	6	6	6

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

[2]EPS (μg/ml) values are expressed as glucose concentration of the cellular fraction of the cultures. Glucose concentrations were determined through the use of the phenol-sulfuric method.

TABLE 16

Plate Counts on MRS Agar (with 4% Lactose) of 8 hour cultures of selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in MRS broth at 45° C for capsular EPS determination.

	Log_{10} cfu/ml			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	7.82	8.36	8.40	8.19
Lb-10442	8.74	8.75	8.95	8.81
St-OSU1	8.57	8.28	8.59	8.48
St-OSU3	8.08	8.14	8.11	8.11
St-143	8.15	8.08	8.30	8.18
Contol[2]	0.00	0.00	0.00	0.00

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.
[2]Uninnoculated control.

TABLE 17

Binding of cholic acid by capsular EPS associated with the cells of selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in MRSL at 45° C.

	μg/ml cholic acid[3]			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	357	405	379	380
Lb-10442	327	370	357	352
St-OSU1	366	422	405	398
St-OSU3	392	431	413	411
St-143	388	431	409	409
Contol[2]	392	435	418	415

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus.*

[2]Uninnoculated control.

[3]μg/ml of cholic acid in medium after 2 hour incubation with cells of each strain. Cells were removed by centrifugation.

TABLE 18

Percent of cholic acid bound by capsular EPS associated with the cells of selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in MRSL at 45° C.

	% cholic acid bound[3]			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	8.4	6.9	9.3	8.2
Lb-10442	16.8	15.1	14.1	15.3
St-OSU1	6.6	3.1	2.9	4.2
St-OSU3	0.0	0.7	1.2	0.6
St-143	1.1	1.3	2.1	1.5
Contol[2]	0.0	0.0	0.0	0.0

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus.*

[2]Uninnoculated control.

[3]% bound= (μg/ml cholic in control – μg/ml cholic in sample)/(μg/ml cholic in contol)x100.

TABLE 19

Binding of cholic acid (μg cholic acid/mg EPS) by capsular EPS associated with the cells of selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in MRSL at 45° C.

	μg cholic acid/mg EPS			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	383	461	512	452
Lb-10442	439	439	383	422
St-OSU1	310	189	172	224
St-OSU3	0	65	108	56
St-143	52	86	116	86
Contol[2]	0	0	0	0

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.
[2]Uninnoculated control.

TABLE 20

Binding of glycocholic acid by capsular EPS associated with the cells of selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in MRSL at 45° C.

	μg/ml glycocholic acid[3]			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	478	492	600	522
Lb-10442	478	507	570	517
St-OSU1	468	473	575	507
St-OSU3	478	478	590	517
St-143	478	473	580	512
Contol[2]	473	482	580	512

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

[2]Uninnoculated control.

[3]μg/ml of glycocholic acid in the spent broth after 2 hour incubation withh agitation with cells of each strain. Cells were removed by centrifugation.

TABLE 21

Percent of glycocholic acid bound by capsular EPS associated with the cells of selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in MRSL at 45° C.

	% glycocholic acid bound[3]			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	-1.0	-1.7	-2.9	-1.9
Lb-10442	-1.5	-4.9	2.1	-1.4
St-OSU1	1.2	1.7	1.1	1.3
St-OSU3	-0.9	0.8	-1.4	-0.5
St-143	-1.4	1.5	0.6	0.2
Contol[2]	0.0	0.0	0.0	0.0

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.

[2]Uninnoculated control.

[3]% bound= (μg/ml glycocholic in control – μg/ml glycocholic in sample)/(μg/ml glycocholic in contol)x100.

TABLE 22

Binding of glycocholic acid (μg glycocholic acid/mg EPS) by capsular EPS associated with the cells of selected strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* grown in MRSL at 45° C.

	μg glycocholic acid/mg EPS			
Culture[1]	Rep 1	Rep 2	Rep 3	Average
Lb-18	-59	-98	-224	-126
Lb-10442	-49	-151	78	-39
St-OSU1	68	112	93	93
St-OSU3	-59	63	-176	-5
St-143	-73	117	44	29
Contol[2]	0	0	0	0

[1]Lb = *Lactobacillus bulgaricus*; St = *Streptococcus thermophilus*.
[2]Uninnoculated control.

VITA

Riley Pigeon

Candidate for the Degree of

Master of Science

Thesis: EXOCELLULAR POLYSACCHARIDES FROM *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* AND *STREPTOCOCCUS SALIVARIUS* SUBSP. *THERMOPHILUS*

Major Field: Animal Science

Biographical:

Personal Data: Born in Siloam Springs, Arkansas on March 6, 1975, the son of James and Ruth Pigeon. Married July 16, 1994 to Teryl Tye. Son, Trevor, born April 10, 1997. Son, Trenton, born August 18, 1999.

Education: Graduated from Kansas High School, Kansas, Oklahoma in May 1993 with honors as valedictorian; received Bachelor of Science degree in Animal Science with an emphasis in Food Science from Oklahoma State University, Stillwater, Oklahoma in May 1997. Completed the requirements for the Master of Science degree with a major in Animal Science at Oklahoma State University, Stillwater, Oklahoma in May, 2000.

Experience: Raised on a farm in Kansas, Oklahoma; employed as a farm laborer during the summers. Employed as an undergraduate laboratory assistant by the Animal Science Department at Oklahoma State University, Stillwater, Oklahoma from 1993-1997. Employed as a Graduate Research Assistant 1997-1998 by the Animal Science Department at Oklahoma State University. Employed as a Food Technologist by McKee Foods Corporation in Gentry, Arkansas, 1998-present.

Professional Memberships: Institute of Food Technologists.