COMPARISON OF CONVENTIONAL AND AUTOMATED METHODOLOGY FOR IDENTIFICATION AND CHARACTERIZATION OF LACTOBACILLI

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CHAPTER I

INTRODUCTION

As Americans continue to strive to live healthier lives much focus is placed on improving nutrition and health. In January 2000, the Department of Health and Human Services launched Healthy People 2010, a comprehensive, nationwide health promotion and disease prevention agenda. This initiative was developed upon two main goals – to increase quality and years of healthy life and to eliminate health disparities. One dietary change that can lend to the achievement of the Healthy People 2010 initiative is the increase in the consumption of probiotics.

Probiotics are defined as selected viable microorganisms used as dietary supplements having potential for improving health of man or animal following ingestion (Gilliland 2001). Some lactobacilli are well known as beneficial bacteria for use as probiotics, and also have a worldwide industrial use as starter cultures in the manufacturing of some fermented milk products. There are a variety of potential health benefits described in the literature resulting from the consumption of products containing live cultures of probiotics. There are specific characteristics that one must consider in developing a product with the goal of delivering health benefits through the consumption of probiotics. Research has shown that among strains within a specific species of lactobacilli significant variation in the ability to provide one or all of the potential health

benefits can be seen (Fortina and others 1998; Janda and Abbott 2002; Buckley and Roberts 2006). To add to this is the well documented existence of host specificity within the various species of lactobacilli (Morishita and others 1971; Kawai and Suegara 1977; Adlerberth and others 1996).

The importance of probiotics may become even more important as new roles are discovered. Antibiotics are powerful drugs that are used in treating many serious and life-threatening diseases. Although these drugs have saved millions of lives, they have also resulted in one of the primary concerns of modern medicine – antibiotic resistant bacterial strains. According to the National Institute of Allergy and Infectious Diseases (2008), more than 70 percent of the bacteria that cause hospital-acquired infections are resistant to at least one of the antibiotics most commonly used to treat them.

For the full potential of probiotics to be reached methods to appropriately identify and characterize specific strains based on metabolic activities as well as host specificity is a necessity.

The objective of this study was to perform a comparative analysis among various automated systems that include both identification and characterization tools in an effort to isolate markers that distinguish host specificity to better allow for the appropriate selection of a □viaries□s for use as treatments in modern medicine or as supplements for healthy living.

CHAPTER II

REVIEW OF LITERATURE

Lactobacillus acidophilus

In 1908 Eli Metchnikoff published a book titled "The Prolongation of Life" in which he described his beliefs that the complex microbial population in the colon was adversely affecting the host through what he called 'auto-intoxication'. He observed longevity in Bulgarian peasants, and suggested that this may be linked to their gut flora specifically to an elevated intake of milks containing lactic acid bacteria. This ignited the scientific community's interest into microorganisms that would later be labeled probiotics. Probiotic is a word that is literally interpreted to mean "for life" (FAO and WHO 2006). As defined previously, probiotics when used as dietary adjuncts demonstrated the potential for beneficial effects on human health and nutrition (Gilliland 2001; FAO and WHO 2006).

Lactobacillus acidophilus is one of the microorganisms on which much attention has been focused for a beneficial role in the intestinal tract thereby its potential impact on health. Lactobacilli are gram-positive, rod-shaped, catalase-negative bacteria (Buchanan and Gibbons 1974). They are also classified as facultatively anaerobic, nonsporulating, and acid-tolerant (Buchanan and Gibbons 1974). Lactobacilli have been found to inhabit various places of the human body to include: gastrointestinal tract, vagina, the skin, nasal

and conjunctive secretions, the ear, breast milk, and sperm (Walter and others 2000; Vásquez and others 2002). With significant research into their properties and role in immunological, digestive and respiratory functions, and their potential to alleviate infectious diseases in children and other high-risk groups, the popularity of probiotics in foods continue to grow.

Impact of Lactobacilli and Probiotics on Health

A great deal of research has been focused on the potential health benefits of consumption of probiotics since Metchnikoff's 1908 book was published. The importance of research that will allow for specific health claims of products containing probiotics is a priority for researchers interested in this area. Highlighted in this section are several of these potential health benefits which include improvement of lactose utilization, control of intestinal infections, reduction of serum cholesterol, immunomoduation activity, and anticarcinogenic activity.

Improvement of Lactose Utilization: Lactose maldigestors describe a subset of individuals that lack the ability to sufficiently digest lactose. This condition is due to a lack in enzyme activity in the small intestine, specifically β-galactosidase which hydrolyzes ingested lactose. Research has indicated that one possible solution for improving lactose digestion in individuals is through the consumption of probiotics such as *Lactobacillus acidophilus* (Kim and Gilliland 1983). It is thought that the presence of β-galactosidase inside the cells of *L. acidophilus* allows for this improved digestion. Further research into the mechanism of how *L. acidophilus* improves lactose digestion revealed that the presence of bile in the small intestines allows for increased cellular

permeability (Noh and Gilliland 1993). These authors explained that this allows for more lactose to be permitted into and hydrolyzed by the β-galactosidase inside the cells of *L. acidophilus* cells. Further validation of this claim of improved lactose digestion by *L. acidophilus* is provided by Montes and others (1995). Their research focused on the reduction of symptoms of lactose maldigestion in children. This research is significant when considering that a child's diet includes a substantial intake of lactose through milk consumption. This study utilized twenty children who were known to have lactose maldigestion. The children were provided one of the following on the morning of each day of the study: 1) 2% lowfat milk containing 11.6g of lactose; 2) 2% lowfat milk with 11.6g of lactose and 1g of freeze-dried concentrate of 10^{10} cells of *L. acidophilus* NCFM; or 3) 2% lowfat milk with 11.6g of lactose and 1g of freeze dried concentrate of 10^{8} cells of *L. lactis* and 10^{10} cells of *S. thermophilus*. These investigators concluded that adding *L. acidophilus* NCFM or the commercial yogurt starter cultures to milk just prior to consumption caused reduced symptoms of individuals known to suffer from lactose maldigestion.

Control of Intestinal Infections: There has been a great deal of research focused on the antagonistic effects that probiotics have on enteric pathogens. Various mechanisms that include competitive exclusion, production of organic acids, and other metabolites such as bacteriocins have been suggested by research as the means by which □viaries□s bacteria protect against intestinal infections (Marth and Steele 2001; Watkins and Miller 1983; Nowroozi and others 2004). Research demonstrated an antagonistic action of *L. acidophilus* NCFM toward the growth of *Staphylococcus aureus*, *Salmonella typhimurium*, and *Clostridium perfringens* in associative cultures grown in MRS broth

(Gilliland and Speck 1977b). Additionally, *L. acidophilus* 4962 showed inhibition toward *S. aureus, S. typhimurium*, and *Escherichia coli* when grown associatively in Milk-Thio medium (Gilliland and Speck 1977b). The research presented by Gilliland and Speck (1977b) utilized six different strains of *L. acidophilus* to demonstrate inhibitory effects. It was concluded that different strains of *L. acidophilus* exhibited different capabilities with regard to inhibitory activity, and that inhibition was caused by different factors other than just acid production.

Competitive exclusion by *L. acidophilus* was examined by Watkins and Miller (1983). These investigators showed that *L. acidophilus* when used as a prophylactic treatment significantly reduced mortality in gnotobiotic chicks when challenged with *S. typhimurium* and *S. aureus*. The data from this investigation demonstrated that the amount of *L. acidophilus* fed to the chicks caused a significant increase in the shedding of *L. acidophilus* which was associated with a significant reduction of shedding of *S. typhimurium* and *S. aureus*. The investigators concluded that the lactobacilli competitively displaced the enteric pathogens.

In addition to the competitive role that lactic acid bacteria may have in the digestive tract, research has also examined the role of their metabolites especially their production of bacteriocins. Gänzle and others (1999) examined the influence that bacteriocin curvacin A, produced by *L. curvatus* LTH 1174, had on the survival of *E. coli* and *Listeria innocua* in a dynamic model of the human stomach and small intestine. Curvacin A showed inhibitory effects toward both *E. coli* and *L. innocua*, and was not degraded in the gastric compartment. This investigation illustrated the potential for lactobacilli that are resistant to bile and capable of producing specific bacteriocins to be

useful in protecting against pathogens in the small intestines. More recently, a team of investigators attempted to determine a basis for selection of probiotics with the ability of protecting against intestinal pathogens by assessing the adhesive properties that included the ability to inhibit adhesion and/or to displace pathogens by various strains of *Lactobacillus* (Gueimonde and others 2006). A key observation made from this investigation was that great variability was seen among strains. Three strains of lactobacilli were tested: *L. casei* TMC 0409, *L. acidophilus* TMC 0356, and *L. rhamnosus* LA-2. All three strains were able to adhere to both intestinal mucus and to Caco-2 cells with *L. casei* having the greatest adhesion and *L. rhamnosus* having the least. The variability continued with regard to the inhibition of the adhesion of the enteric pathogens. None of the strains significantly inhibited the adhesion of the *E. coli* or the *Enterobacter sakazakii*. All three strains of lactobacilli significantly reduced adhesion of *Listeria monocytogenes*. *Salmonella typhimurium* adhesion was significantly reduced by *L. casei* and *L. acidophilus* but not by *L. rhamnosus*. The results regarding the ability of three strains of lactobacilli to displace the enteric pathogens included significant variation (15% and 68%). All three strains significantly displaced *L. monocytogenes, S. typhimurium, E. sakazakii*, and *Clostridium difficile*. The only cultures of lactobacilli that significantly displaced *E. coli* were the strains of *L. rhamnosus*. While there is a great deal of research demonstrating the ability of probiotics to control intestinal pathogens this publication summarizes the significance of the specificity within the various strains of probiotics and the need for detailed characterization.

Reduction of Serum Cholesterol: The medical field for many years has used cholesterol levels not only as an indicator of an individual's health and risk of heart disease but also of the health of the population as a whole. Arnett and others (2005) estimated that 13.2 million Americans are affected by coronary heart disease, the single largest cause of death in the United States. Trends of lower cholesterol levels were seen in the latter half of the 20th century; however, there is doubt as to whether this trend has continued into this century (Arnett and others 2005). With this knowledge it is no surprise the interest continues to grow in regard to the potential of preventing hypercholesterolemia through the consumption of probiotics. In 1974, Mann and Spoerry fed large amounts of milk fermented with a wild strain of *Lactobacillus* to the Maasai men and found this to decrease their serum cholesterol levels. Following this was a study published in 1975 by Harrison and Peat that looked at serum cholesterol levels of infants. Infant formula with added cells of *L. acidophilus* resulted in reduced serum cholesterol, whereas the infants receiving the infant formula without the *L. acidophilus* had higher serum cholesterol levels. These early publications ignited a tremendous interest in the potential that probiotics had in controlling serum cholesterol.

A study with the objective of obtaining new isolates of *L. acidophilus* for the purpose of assimilating cholesterol at significantly higher levels than commercially available strains was conducted (Buck and Gilliland 1994). This publication described significant strain to strain variation in the amount of cholesterol assimilated, the amount of bile salts deconjugated, and the bile tolerance of the strains. This research led to the identification of five new isolates that held significant potential for use as dietary adjuncts with the purpose of lowering serum cholesterol upon consumption.

Further research into the mechanism by which □viaries□s strains were capable of reducing serum cholesterol levels revealed the cholesterol was not metabolically degraded, but rather was recovered with the bacterial cells (Noh and others 1997). Other observations reported by this team of investigators included: 1) cells were more resistant to lysis by sonication if grown in the presence of cholesterol micelles and bile salts; 2) when comparing the uptake of cholesterol by cells in the presence of micelles made with unsaturated fatty acids versus saturated no difference was observed; and 3) the higher the concentration of Tween 80 in the growth medium the less cholesterol was taken up with the growth levels of the cultures remaining the same. The first observation led the investigators to conclude that the cell walls or membranes of the culture must be undergoing some type of alteration to lead to the increased resistance to lysis. Secondly, the type of fatty acid does not seem to influence the assimilation capability of the culture. Finally, the higher concentrations of Tween 80 may be adversely affecting the permeability of the cells consequently affecting the culture's capability of assimilating cholesterol.

In addition to assimilating the cholesterol, another possible mechanism of lowering serum cholesterol is the deconjugation of bile salts (Brashears and others 1998). They compared four strains of *Lactobacillus* to determine the mechanism by which cholesterol was removed from media. Two strains were *L. casei* and the other two strains were *L. acidophilus*. The investigation confirmed that the *L. acidophilus* removed the cholesterol from the media by means of assimilation as suggested in previous research. In comparison, the results suggested that the *L. casei* removal of cholesterol was mostly due to the coprecipitation of the cholesterol with the deconjugated bile salts. This paper

demonstrated once again the importance of selection of a □viaries□s for a specific activity. De Rodas and others (1996) reported data indicating that the hypocholesterolemic action of *L. acidophilus* was due to broth cholesterol assimilation and deconjugation. Pigs fed a selected strain of *L. acidophilus* not only exhibited reduced serum cholesterol levels but also reduced serum bile acids. The deconjugated bile acids are less well absorbed from the intestine this □viaries□s with the usual enterohepatic circulation of bile acids. The deconjugated ones are excreted in the feces.

All of this research has led to the design of clinical studies to examine the above described effects in humans. Anderson and Gilliland (1999) conducted two clinical trials that examined the effects of fermented milk on serum cholesterol in humans. The first trial compared the human isolate, *L. acidophilus* L1, to a swine isolate, *L. acidophilus* 43121. As would be suspected, the L1 strain significantly reduced the total serum and LDL cholesterol, while the 43121 strain had no statistical effect. It was the second trial that led to the quantitative conclusion that the fermented milk containing *L. acidophilus* L1 had the potential for lowering serum cholesterol by 3 to 4% in individuals considered to be hypercholesterolemic.

Immunomodulation Activity: The epithelial lining of the gastrointestinal tract offers a large surface area for the absorption of molecules and presents a barrier to an endless number of antigens that may pass through. The exclusion or elimination of foreign antigens is mediated by the gut immune system known as the gut-associated lymphoid tissue (GALT). There have been numerous publications reporting the effects of probiotics on the host's immune response. Perdigón and others (1995) reported that *L. casei* could stimulate production of secretory IgA and increase the activity of IgA, T

cells, and macrophages. Further studies into the effects on the specific immunity revealed increased levels of secretory IgA, IgA secreting cells, macrophages, CD4+, CD8+, and T-cells following oral consumption of *L. casei* (Alvarez and others 1998).

Isolauri and others (2000) conducted a study involving 27 infants with atopic eczema. The infants were either weaned from breast milk to a whey formula containing *Bifidobacterium lactis* or *Lactobacillus* GG or to a whey formula that was not supplemented with the □viaries□s strains. After 2 months of treatment, infants receiving the □viaries□s formula had a reduction in the extent, severity, and subjective symptoms as compared to the unsupplemented group. Additionally, reduction in serum CD4+ cells were seen in infants receiving the □viaries□s supplement. The serum CD4+ cells are elevated in diseases such as atopic eczema. Gill and others (2001) reported as much as 31% increase in phagocytic activity of monocytes and PMN cells, and as much as 102% increase in NK-cell tumouricidal activity following the oral administration of milk supplemented with *L. rhamnosus* HN001.

Herich and Levkut (2002) compiled a review of the research related to probiotics effect on the immune system of a host. The review suggested that with the broad spectrum of immunomodulating mechanisms, selection of specific lactobacilli strains to directionally modulate the host's system could hold potential for these strains to be used in treatments of infectious diseases, auto-immune diseases, and other immune disorders.

Anticarcinogenic Activity: Given the knowledge that many bacteria commonly found in the colon are capable of producing carcinogens and tumor promoters from various food components such as heterocyclic amines, polycyclic hydrocarbons, phenols, and fecapentaenes while others synthesize enzymes with genotoxic products the

anticarcinogenic activity of probiotics holds significant potential to exert control of these actions (Fuller and Perdigón 2003). Shahani and others (1983) reported that consuming □viaries□s fermented with *L. acidophilus*, *L. bulgaricus*, or a yogurt culture by male Swiss mice significantly inhibited tumor cell proliferation.

One hundred thirty-eight patients with superficial bladder cancer participated in a study in which they consumed *L. casei* (Aso and others 1995). Results confirmed observations of previous trials of an increase in the recurrence-free rate and the downgrading of recurrent tumors in the group receiving the oral preparation as compared to the placebo group. One mechanism for inhibiting tumor growth by *L. casei* was its ability to induce the production of cytokines, such as IFN-γ, IL-1β, and TNF-α (Matsuzaki 1998).

Lin and Chang (2000) reported data that showed *B. longum* and *L. acidophilus* possessed the capability of significantly reducing 4-nitroquinoline-N-oxide (4NQO). This compound is a known mutagen and carcinogen that causes DNA oxidative damage. Additionally, results from this study showed that both *B. longum* and *L. acidophilus* demonstrated antioxidative activity by inhibiting the peroxidation of linoleic acid. This antioxidative activity is relevant due to the role oxidative damage plays in cancer, emphysema, cirrhosis, atherosclerosis, and arthritis (Halliwell and Gutteridge 1984).

Commane and others (2005) published a review of the potential mechanism by which probiotics induce anticarcinogenic activity. These authors pointed out several times that it is very likely that the exact mechanism and the exact phase of carcinogenesis on which the anticarcinogenic activity is exerted is strain dependent. A summary of potential mechanisms listed in this review include anti-genotoxicity, inhibition of colonic

enzyme activity, control of growth of enteric pathogens, adhesion and interaction with colonic cells, modulation of the immune system, or production of metabolites. Continued research into a specific mechanism will be required to fully understand the capability of a □viaries□s strain's anticarcinogenic activity.

Lactobacilli use for Human Consumption

Assessing the efficacy of probiotics in humans requires that we understand that all □viaries□s strains are unique and different. Their properties and characteristics must be well defined, and studies on even closely related strains cannot be generalized to all strains of a given species (Gilliland and Speck 1977a; Lin and others 1991; Collins and others 1991; Commane and others 2005; Gueimonde and others 2006). Specific assessments of the properties of probiotics for use in foods begin with basics such as being capable of surviving passage through the digestive tract but also have the capability to proliferate in the gut (Gilliland 1979; Fernández and others 2003; Gueimonde and others 2006). This means they must be resistant to gastric juices and be able to grow in the presence of bile under conditions in the intestines, or be consumed in a food vehicle that allows them to survive passage through the stomach and exposure to bile (Gilliland 1979; Fernández and others 2003). It is the ability to remain viable at the target site and to be effective that should be verified for each potential □viaries□s strain. One recommendation established by the Joint FAO/WHO Expert Consultation on *Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria* held in 2001 was the refinement of *in vitro* tests to predict the ability of probiotics to function in humans. This recommendation was made based upon

the opinion that the currently available tests are not adequate to predict the functionality of □viaries□s microorganisms in the intestine. Although there are numerous □viaries□s products on the market for human consumption, many consumers are either skeptical or uneducated with regard to these products. Unfortunately, research has shown that many of the microorganisms included in these products may not be viable and most probably have not been exclusively selected for either a specific beneficial property or for their ability to survive the human gastrointestinal tract (Gilliland and Speck 1977b; Gilliland 1979; Schillinger 1999; Sandholm and Saarela 2003).

Host Specificity

As early as 1971, research has suggested that host specificity is exhibited by different strains of lactobacilli. Morishita and others (1971) identified a strain of *L. acidophilus* that had been isolated from a human intestinal tract which they was not able to implant into the intestinal track of a chick. Another study into the specificity of the adhesion of lactobacilli from rats, humans, swine and chickens was conducted by Kawai and Suegara (1977). They reported that only lactobacilli isolated from rats were able to attach to epithelial cells of the rat stomach, and only the strains isolated from chickens could adhere to crop epithelial cells (Kawai and Suegara 1977). More recently, Adlerberth and others (1996) performed a study to elucidate the mechanism by which *Lactobacillus plantarum* adhered to the human colonic carcinoma cell line HT-29. The HT-29 cells, *L. plantarum* in a concentration of 5×10^6 cells per ml, and Hanks' balanced salt solution were mixed for 30 minutes. The cells were then washed once and fixed in formalin for the number of bacteria attached to each of at least 40 cells to be counted via

interference-contrast microscopy. It was found that only some strains of *L. plantarum* could adhere to human intestinal cell lines at approximately 10 bacteria per cell utilizing various surface receptors.

There have been several studies that have refuted the host specificity of lactic acid bacteria, specifically lactobacilli (Rinkinen and others 2000; Nikoskelainen and others 2001; Rinkinen and others 2003). However, Pretzer and others (2005) provided more support of the potential for host specificity and the hope for a method that would reveal this specificity within a given bacterial strain. The goal for this work was to look into the identification of genetic factors involved in phenotypic traits such as adhesion ability. The resulting data from this experiment provided some understanding of the genetic background of □viaries□s adhesion and the potential role that genes such as the mannose adhesion-encoding gene of some strains of *L. plantarum* may have in the ability of probiotics to become established and persist in the intestinal tract. As pointed out in this paper, a great deal of variation of receptors (i.e. mannose receptors) within different host species is known to exist. The identification and understanding of the adhesion-encoding genes thereby becomes extremely significant to identifying host specificity traits of probiotics.

Identification and Characterization of Lactobacilli

The classification of lactic acid bacteria into different genera has been largely based on morphology, carbohydrate fermentation, growth at different temperatures, configuration of lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance as described in the *Bergey's Manual of Systematic Bacteriology*

(Kandler and Weiss 1986). Most genera form phylogenetically distinct groups, but for some, such as *Lactobacillus*, the phylogenetic clusters do not correlate with the current classification based on phenotypic characters.

In 1987, the ad hoc committee on reconciliation of approaches to bacterial systematics recommended that a distinct genospecies that cannot be differentiated from another genospecies on the basis of any known phenotypic property not be named until it can be differentiated by some phenotypic property (Wayne and others 1987). It is widely acknowledged that the taxonomy of the *Lactobacillus* genus has previously relied upon phenotypic heterogeneity (Andrighetto and others 1998). There has in the recent past been much controversy regarding the taxonomy of *Lactobacillus*. Andrighetto and others (1998) looked at 25 isolates from yogurt and cheeses with various identification techniques such as API 50 CHL profiles, species-specific DNA probes in dot-blot hybridization, amplification, and restriction analysis of the 16S rRNA gene (ARDRA), and by PCR using species-specific primers. The fermentation patterns observed identified strains into a classification that for some strains was completely different from the genotypic classification obtained from all of the other testing performed. The results of this study confirmed how complicated the science of identification of lactobacilli strains has become.

Schillinger (1999) cited numerous publications that described the significant changes and the confusion caused by such changes with the taxonomy of the *L. acidophilus* and the *L. casei* groups. The example he used from literature was the separation of the majority of strains in 1989 classified as *L. casei* into the new group of *L. paracasei*. However, seven years later the name *L. paracasei* was rejected and a new

name of *L. zeae* was introduced. This type of taxonomy reorganization can lead to incorrect labeling on manufactured products, and a body of literature that provided unintentional misinformation regarding specific characteristics of a particular strain (Shillinger 1999; Janda and Abbott 2002).

It is the demonstration of diversity both within phenotypic and genotypic properties of microbial species that has led to many difficulties within the field of bacterial taxonomy being highlighted in literature. The difficulties related to species designation which can be related to lactobacilli include fixed rules and cut-offs for genomic relatedness to the next species, over-classification of phenotypes of high interest to microbiology and medical fields, gene transfer between organisms, and the microbial species definition being developed from a limited sample of strains (Buckley and Roberts 2006). The species definition is currently based on characteristics from a very limited number of microorganisms that hold significance in communities such as the medical field. The system designed for these few organisms of significant interest has now been applied to the extremely diverse microbial field. It may be necessary to examine if the current system could better be improved to provide meaningful phylogenetic categorization to all microorganisms not just the few of high significance to certain fields.

Genotypic Identification and Characterization

New tools for classification and identification are currently replacing and/or complementing traditional phenotype-based methodologies. Genomic methods based on molecular techniques are starting to form the mainstay of strain typing. One of the major factors in driving the methodology in this direction for *Lactobacillus* is the small

variation in phenotypic characteristics seen between many of the currently described species (Dickson and others 2005). Modern taxonomic tools based on immunoassays, PCR techniques, and DNA hybridization methods are finding increasing applications (Le Jeune and others 1995; Castellanos and others 1996; Drake and others 1996). Ribosomal RNA sequencing and DNA-DNA hybridization studies have, according to some, improved our taxonomic knowledge on the relationships within lactobacilli (Collins and others 1991). While the ribosomal RNA sequencing has gained significant popularity some reported that back-up of a large database of rRNA sequences was necessary for species identification, and that rRNA sequence analysis may not be sufficient for very closely related species (Pot and others 1993). Polyphasic taxonomic approaches that are now recommended in the literature for identification of lactobacilli include a combination of methods such as gas chromatography, high-pressure liquid gas chromatography, soluble protein content, cell wall components, fermentation of carbohydrates, and DNA profile (Vandamme and others 1996; Janda and Abbott 2002). Similarly, a study was conducted to identify lactobacilli isolates from pigs based on SDS-PAGE of total soluble cell proteins and RAPD-PCR profiles (Du Toit and others 2003). Of the 24 strains tested 15 were identified as *L. buchneri* and 9 as *L. reuteri*. Carbohydrate fermentations profiles also were reported for various isolates from this study. The authors concluded that the fermentation profiles of the strains were unable to distinguish the two phenotypic or genotypic groups.

Identification to the species level is described as time-consuming and unreliable lending to the need for more rapid, accurate identification systems (Pot and others 1993; Dickson and others 2005; Moreira and others 2005). Genomic fingerprinting has been

reported as an efficient method for rapid analysis of these intra-strain relationships (Andrighetto and others 1998). A number of fingerprinting techniques describe simple procedures which make them very attractive for classification, however reproducibility of such procedures require highly standardized conditions. Some of these procedures require breaking the cells to obtain the crude DNA, and others are based on restriction endonuclease cleaving. While genomic techniques have made significant strides in describing the metabolic potential of different cultures (the ability to carry out a process), these techniques are incapable of predicting the phenotype (the performance of a process) (Tynkkynen and others 1999; Buckley and Roberts 2006). To further emphasize this point, genomic methods are not capable of determining what a particular organism will do in its natural environment. Genomic methods provide information regarding the presence of a gene and the capability of being expressed under laboratory conditions, but not the true activity of the strain in nature (Buckley and Roberts 2006). Such approaches do not indicate whether or not a specific gene is expressed phenotypically.

These genomic methods usually involve PCR methods or some variety of restriction enzyme analysis (Tynkkynen and others 1999). Tynkkynen and others (1999) compared the identification of strains of lactobacilli by the API 50CHL test to PFGE, RAPD analysis, and RiboPrinter. These authors provided a brief overview of each genomic method utilized in their study: randomly amplified polymorphic DNA (RAPD) analysis uses short arbitrary sequences as primers in PCR to amplify product patterns specific to a given species; pulsed-field gel electrophoresis (PFGE) separates large genomic fragments through the use of rare-cutting enzymes; and the RiboPrinter utilizes rRNA genes or their spacer regions as probes to hybridize with genomic restriction

fragments. One interesting observation made was the variation of identification between the various methods. The authors reported that when used for strain typing PFGE revealed 17 genotypes of the 24 strains studied, Ribotyping revealed 15 genotypes, and RAPD revealed 12 genotypes. The final conclusion of this study was that RAPD analysis, ribotyping, and PFGE all serve as a means for typing cultures, but are not capable of providing species specific information.

RIBOPRINTER: The RiboPrinter microbial characterization system is an automated technique that requires the processing of samples of pure culture. The claims of the manufacturer are that the system provides rapid, standardized, and accurate identification and characterization with the ability to track samples at the strain level. Once a pure sample of bacterial cells has been collected and loaded into the system the next step is lysis of those cells. Restriction enzymes are then used to cut the released DNA into fragments which are then separated by size through gel electrophoresis. They are then transferred to a membrane, where they are hybridized with a DNA probe and mixed with a chemiluminescent agent. A camera captures the light emission as image data, from which the system extracts the RiboPrinter pattern. This pattern is finally compared to other patterns in the system's database for characterization and identification. The results are then automatically printed into a report.

Ribotyping in some research has appeared to be more applicable for distinguishing species or strains which are not phylogenetically closely related. Zhong and others (1998) examined the efficacy of the use of ribotyping of cultures of *Lactobacillus* in an effort to characterize a number of isolates. While the concluding remarks of this paper indicates that species of *Lactobacillus* may possibly be separated

and identified on the basis of their ribotypes, the observation that these data profiles are insufficient in determining which DNA patterns correspond to specific □viaries□s properties was reported. Therefore, identification based on a ribotype could not be useful in determining the □viaries□s potential of strains of lactobacilli.

Phenotypic Identification and Characterization

There has been a recent move away from the conventional procedures using phenotypic (morphological, biochemical) assessments of culture identity, towards more sophisticated molecular procedures such as nucleic acid fingerprinting studies (Fuller and Perdigón 2003). Much controversy exists among research related to the relevance of the various testing methodologies used for phylogenetic, genotypic, and phenotypic identification of lactobacilli. Millière and others (1996) described atypical isolates of *Lactobacillus delbrueckii* ssp. *bulgaricus* from Pindidam (a Cameroonian fermented milk) that displayed genetic differences with regards to maltose and trehalose metabolism, but all physiological properties were typical of that species. Similarly, five isolates from artisanal hard cheeses were identified and characterized as three strains of *L. helveticus* and two *L. delbrueckii* ssp. *lactis* by protein profiles by SDS-PAGE and 16S rRNA gene sequence analysis (Hébert and others 2000). However, there was phenotypic variation seen in the fermentation patterns.

The differences in genotypic and phenotypic characteristics have led to significant confusion with regards to the role of probiotics in various environments. One example found in the literature relates to the suspected associative relationship of consumption of *L. rhamnosus* and subsequent infections. Salminen and others (2002) reported that 11 of

26 isolates of *L. rhamnosus* from blood could not be distinguished from the □viaries□s *L. rhamnosus* GG using pulsed-field gel electrophoresis. Regardless, the authors stressed that no increase in incidence of *Lactobacillus* bacteremia could be demonstrated in relation to the increased use of the □viaries□s. Further support of the absence of a connection between □viaries□s consumption and an increased incidence of infections was reported by Ouwehand and others in 2004 that showed significant differences in phenotypic properties of isolates of *L. rhamnosus* from blood as compared to *L. rhamnosus* GG. Still yet, some authors continued to speculate on the possible connection between consumption of *L. rhamnosus* GG and subsequent bacterial infections. Land and others (2005) examined blood isolates that were reportedly indistinguishable by repetitive element sequence-based PCR DNA fingerprinting from the *L. rhamnosus* GG that had been administered to the patients as a treatment. More recently, Vancanneyt and others (2006) focused a study on the suspected role of *L. rhamnosus* as a commensal organism as compared to its role in clinical cases of disease. The conclusions of this study supported those of Salminen and others (2002) that the existence of evidence to support increased probability of infection due to consumption of probiotics did not exist.

API 50 CH**:** The API 50 CH as stated in the manufacturer's literature is a standardized system which contains 50 biochemical tests that provides a profile of a microorganism's ability to metabolize carbohydrates. The API 50 CH strips have microtubes containing the substrates that belong to different carbohydrate groups. The fermentation tests are performed by rehydrating the substrate in the microtube with a broth medium inoculated with a pure culture of *Lactobacillus.* During incubation, the fermentation and/or hydrolysis of a substrate is indicated by a color change in the

microtube. This color pattern can then be interpreted into a biochemical profile that can be compared to databases or published literature to determine the identification of the culture.

Numerous research publications have relied upon the API 50 CH system for microbial identification. The use of the API 50 CH profiles in determining the identification of cultures has proven to be reproducible so long as a specific temperature for a given species is maintained during testing (Nigatu and others 2000). This publication examined 49 different cultures, 45 of which were species of *Lactobacillus*. All but 11 strains showed significant variation among fermentation profiles when tested at 30°C and 37°C. Still a significant amount of skepticism remains related to this method as an identification tool. Several references on the reliability have been published stating that the identity of only about 30% of the strains of lactobacilli based on the API 50 CHL tests agreed with results from DNA-DNA hybridization (Dickson and others 2005; Moreira and others 2005).

BIOLOG: Biolog, Inc., has developed a patented technology to quickly characterize microbial isolates by examining their carbon source utilization profiles in a microtiter plate assay. As a microorganism begins to use the carbon sources in certain wells of the MicroPlate, it respires. This respiration process reduces a tetrazolium redox dye and those wells changes color to purple. The end result is a pattern of colored wells on the MicroPlate that is characteristic of that microorganism (somewhat of a "fingerprint"). A fiber optic reading instrument known as the MicroStation Reader will automatically read the bacterial pattern of the MicroPlate and feed this data into the MicroLog software. A search is conducted of the database and identification is provided

within seconds. The microbial identification involves five basic steps: Isolation of a pure culture on the Biolog media; Gram stain to determine testing protocol; Preparation of the inoculum to a specified cell density; Inoculation and incubation of the MicroPlate; and Reading of the MicroPlate to determine an identification.

VITEK: The VITEK Anaerobe Identification Card (ANI) is intended for rapid, computer assisted identification of medically important anaerobic and microaerophilic bacteria of human origin (bioMerieux, Anaerobe Identification Card for In Vitro Diagnostic Use, Material Packet). Listed as strains that may be accurately identified by this system under the intended use in the material packet are *Lactobacillus acidophilus, L. casei, L. catenaforme, L. cellobiosus, L. fermentum, L. jensenii,* and *L. minutis.* The ANI cards have thirty wells that contain 28 biochemical substrates. The results are based upon the ability of a pure culture to degrade the specific substrate in a given well which is detected by a variety of indicator systems. A pure culture must be established on very specific media so as to not suppress any glycolytic activity or reduce the test selectivity. Once the ANI card has been properly filled with the pure culture inoculum that is visually equivalent to a McFarland No. 3 standard, the card is incubated four hours at 35°C. The positive and negative wells are then recorded by visually comparing the wells to the VITEK hand held viewer. The results are then entered into the VITEK system where patterns are analyzed and an identification report is generated.

SHERLOCK MIDI MIS: Cellular fatty acid profiles have been utilized to identify bacterial strains. Research has shown that the fatty acid composition of a specific strain is very dependent upon factors such as growth temperature, pH, oxygen tension, growth phase, medium composition, salt concentration, and the age of cultures (Johnsson and

others 1995). The Sherlock Microbial Identification System (MIS) analyzes and identifies microorganisms isolated in pure culture on specified media. Sherlock relies on qualitative and quantitative fatty acid composition profiles. Fatty acids are extracted from unknown microorganisms by a five step process: Harvesting – removal of cells from media; Saponification – lysis of cells to liberate fatty acids from the cellular lipids; Methylation – formation of fatty acid methyl esters (FAMEs); Extraction – transfer of the FAMEs from the aqueous phase to the organic phase; and Base Wash – Aqueous wash of the organic extract prior to the chromatographic analysis. The fatty acids are automatically quantified and identified by the Sherlock software to provide a fatty acid profile. This profile is then compared to a specific library to determine the identity of the culture. For *Lactobacillus*, the Sherlock database used is called MOORE which requires anaerobic growth conditions at 35°C in PRAS PYG (PRAS Peptone Yeast Extract Broth with Glucose, Anaerobe Systems, Morgan Hill, California) broth. Following the library search, the computer prints the Composition Report which includes the peak naming, the library classification results, and the chromatogram.

Trends in Automated Identification Systems

A publication titled, "Bacterial Identification for Publication: When is Enough Enough?" highlighted some of the issues with the use of commercial systems for rapid identification (Janda and Abbott 2002). This publication explains that one major problem with these commercial systems is that they rely on databases. The accuracy of these databases is dependent upon the number of strains and the phenotypic diversity of strains used to develop them. Often times it is found that limited biotypes are utilized to develop

these databases, and very rarely is diversity in the geographical locations of the strains used in the database development properly regionalized. For species or strains that are encountered less often the databases may not be capable of accurately providing identification. This results in less reliable identification of strains. Additionally, these commercial systems using databases must take into consideration that between 1980 and 1996 there was a 238% increase in the number of bacterial names reported in literature, approximately 200 new names or combinations a year (Euzéby 1997). Another issue that lends to misidentification is the growing number of new strains being classified and identified based on very few numbers of samples. For instance, the percentage of newly identified species over the past decade that was based upon the analysis of one isolate was approximately 40% (Christensen and others 2001). Rosselló-Mora and Amann (2001) reported similar data recognizing that with the increase in automated molecular techniques such as the 16S rDNA sequencing, more species identification are reported based upon five or fewer strains and very limited differential biochemical characteristics.

The accuracy of these automated systems is dependent of a number of variables. Some of these variables include the specific organism tested, how current the database is, the thresholds of readers used, preparation of inoculum whether photometrically or visual, and growth condition requirements. Although these new systems offer expanded identification capabilities, shorter turnaround time, and automation specifically related to data management, there is sufficient room for improvement. While all of the potential related to these new systems is appealing to a degree, they still each have disadvantages that limit their utility in identifying cultures with specific industrial applicability.

Significance of Rapid, Accurate Identification and Classification of *Lactobacillus*

Although there are a significant number of □viaries□s products on the market for human consumption, there is a lot of skepticism regarding their beneficial effects. This skepticism is justly placed as research has shown that many of these products do not contain the microorganism that they claim to be present, they do not contain viable microorganisms, or there is no confirmation that the culture included was selected for the particular beneficial property claimed by the manufacturer. If the health claims of probiotics are to ever be substantiated, it is essential to establish which strains have been incorporated into a product, from what source they were isolated, and the mechanisms by which the health promoting benefit is exhibited. The biodiversity between strains within a particular species is suspected to have many of the answers to the potential use of probiotics as health promoting factors. The key is to develop scientific techniques that can rapidly identify these isolated strains and their species specificity with a high degree of accuracy, as well as provide significant data to support the mechanisms by which a particular strain is capable of eliciting a health promoting effect on the host.

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CHAPTER III

IDENTIFICATION OF *LACTOBACILLUS ACIDOPHILUS* AND *LACTOBACILLUS CASEI* BY CONVENTIONAL METHODOLOGY AND AUTOMATED MICROBIAL IDENTIFICATION SYSTEMS

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ABSTRACT

A comparative analysis of the conventional methodology with automated microbial identification and characterization systems for lactobacilli was conducted in this study. Thirty-two previously isolated cultures of lactobacilli were analyzed using various methodologies in an effort to rapidly identify and further classify their □viaries□s potential. The results obtained from the Vitek and the RiboPrinter systems were inconclusive and failed to provide reliable, reproducible identifications for these isolates. The Biolog and Sherlock MIDI MIS systems provided positive identifications of some of the isolates at a species level, and thereby offered potential for use as tools to identify and characterize isolates of lactobacilli. However, these two systems were unable to reliably provide the same species identification within a series of replications. In both systems it was observed that the top choices were more often switched in order from one replication to the next. The Sherlock MIDI MIS system appeared to offer the most potential for characterization of the strains given a positive identification was first provided by the system. Use of a larger pool and variety within strains in development of the databases or libraries for the automated systems, along with culture preparation techniques specific to lactobacilli would greatly enhance the applicability of some of these automated systems for use in appropriately identifying and characterizing the □viaries□s potential of isolates of lactobacilli.

INTRODUCTION

Probiotics have been defined as selected viable microorganisms used as dietary supplements having potential for improving health of man or animal following ingestion (Gilliland 2001). Many of the □viaries□s cultures have long been used worldwide in industry as starter cultures in the manufacturing of fermented dairy products. There are many potential health benefits described in the literature resulting from the consumption of products containing live cultures of probiotics, and a significant amount of research into these cultures and their beneficial role as health promoters has been conducted. More recently, the potential role that probiotics may offer the medical field related to antibiotic resistance or vaccine delivery systems has been recognized (Fuller and Perdigón 2003). Regardless of the targeted benefit, specific phenotypic characteristics should be considered in developing a product with the goal of delivering any health benefit through the consumption of probiotics.

There are many characteristics that must be determined prior to assessing the efficacy of the □viaries□s potential of a particular strain. The properties and characteristics of an individual strain should be well defined before making any claim as to an impact on human or animal health. Studies have shown that characteristics of even closely related strains cannot be generalized to all strains of that species (Gilliland and Speck 1977; Lin and others 1991; Collins and others 1991; Commane and others 2005; Gueimonde and others 2006). Therefore, if the full potential of probiotics is to be understood and

utilized, methods to appropriately identify and characterize these strains based on phenotypic characteristics as well as host specificity must be identified and accepted.

Lactobacillus acidophilus is one of the microorganisms with □viaries□s potential that has received the greatest amount of attention. However, a significant part in the difficulty of identification and characterization of this species lies in the controversy of the taxonomy of the genus *Lactobacillus*. For much of the past, the taxonomy of the genus of *Lactobacillus* has been based upon phenotypic properties (Andrighetto and others 1998). However more recently, new tools are replacing and/or complementing these traditional phenotype-based methodologies leading to a reorganization of this taxonomy. One factor used in support of this change in methodology for *Lactobacillus* is the small variation in phenotypic characteristics seen between many of the now described species (Dickson and others 2005). In contrast, these genomic techniques may describe only a potential ability of a culture to carry out a process through the presence of a gene, or the ability to express that gene in a laboratory. However, just the presence of a gene does not mean that gene will be expressed in nature (Tynkkynen and others 1999; Buckley and Roberts 2006).

The objective of this study was to compare various automated systems that consider both genotypic and phenotypic characteristics of cultures in an effort to distinguish host specificity and better allow for selection of a □viaries□s for targeted use.

MATERIAL AND METHODOLOGY

Culture Origin and Maintenance

Each of the cultures used in these experiments was obtained from the stock collection of the Food Microbiology Laboratory at Oklahoma State University. Included were 7 strains isolated from humans initially identified as *Lactobacillus acidophilus* and 7 isolated from humans initially identified as *L. casei.* Additional cultures initially identified as *L. acidophilus* included 8 isolated from swine, 4 isolated from rodents, 2 isolated from turkeys, 2 isolated from chickens, and 1 strain isolated from bovine. We also included the *L. acidophilus* type culture strain designated as 4356 from the American Type Culture Collection (ATCC) that our laboratory had previously obtained directly from ATCC.

Initially, testing of each strain by the same procedures used to assign the initial identification by the Food Microbiology Laboratory at Oklahoma State University was conducted. The fermentation patterns of each culture were determined using API 50 CH kits (Bio Mérieux, Bruxelles Belgium) according to the manufacturer's directions. Also, the catalase reaction, Gram stain reaction, and ability to grow or not grow at 15°C and at 45°C were recorded for each culture. Additionally, each culture was tested for ammonia production from arginine.

All of the cultures were maintained by weekly subculturing each into lactobacilli MRS broth (DeMan, Rogosa, and Sharpe, Difco Laboratories, Detroit Michigan) using a 1% inoculum followed by 18 hours of incubation at 37°C. Additionally, stock cultures of

each strain were maintained by monthly subculturing in MRS agar stabs (18 hours of incubation at 37°C). Between subculturings, all were stored at 5°C. Immediately prior to experimental use, the strains were subcultured three times in the MRS broth or a designated medium specified for that particular test method.

Automated Phenotypic Identification and Classification

Vitek

The Anaerobe Identification (ANI) cards (Vitek Systems, Hazelwood, Mo.) are molded plastic cards containing 28 wells of substrates for biochemical reaction determinations. The substrates detect specific bacterial glycosidases, aminopeptidases, phosphatases, and esterases. Also tested was the cultures' fermentation of various carbohydrates including glucose, trehalose, arabinose, raffinose, and xylose. The final tests of the card include triphenyl tetrazolium reduction, rapid arginine dihydrolase, and urease.

The sample preparation and procedure for analysis was conducted according to the manufacturer's directions. Two deviations from the manufacturer's directions were made. These included the use of MRS agar plates instead of blood agar plate and an incubation temperature of 37°C instead of 35°C. Both of the deviations were made in an effort to achieve sufficient growth of the isolates used in this study as the manufacturer's recommended agar plates and temperature combination did not result in sufficient growth.

Biolog

The Biolog Anaerobe (AN) Identification MicroPlate is 96-well plate with dehydrated panels containing tetrazolium violet, a buffered nutrient medium, and a different carbon source for each well except the control, which does not contain a carbon source. The 95 substrates contained in the Biolog AN MicroPlate include carbohydrates, carboxylic acids, amides, esters, amino acids, peptides, amines, alcohols, aromatic chemicals, halogenated chemicals, phosphorus- and sulfur-containing chemicals, and polymeric chemicals.

All samples were prepared and analyzed according to the Manufacturer's directions using the recommended Biolog Universal Anaerobe (BUA) agar plates.

Sherlock MIDI Microbial Identification System (MIS)

The Sherlock MIDI MIS analyzes and identifies microorganisms based on qualitative and quantitative fatty acid composition profiles. Fatty acids are extracted from the microorganisms by a five step process: harvesting; saponification; methylation; extraction of the fatty acid methyl esters (FAMEs); and a base wash.

For each isolate the manufacturer's recommendation for working with lactobacilli was followed. This included the use of the calibration standard provided by Sherlock that is analyzed after each of 10 sample runs to correct for any possible drift of retention time.

Automated Genotypic Identification and Classification

RiboPrinter

The RiboPrinter is a microbial characterization system that uses restriction enzymes to cut the DNA released from a pure culture into fragments which are then separated by size through gel electrophoresis. These fragments are transferred to a membrane, where they are hybridized with a DNA probe and mixed with a chemiluminescent agent. A camera is then able to capture the light emission as image data, from which the system extracts the RiboType or pattern. This pattern is compared to other patterns in the system's database for characterization and identification.

Each sample was prepared and analyzed on the RiboPrinter according to the procedures outlined for lactic acid bacteria by the manufacturer.

RESULTS AND DISCUSSION

The present study investigated the potential use of various automated systems for microbial identification and characterization as compared to conventional methodology for various isolates of lactobacilli previously identified as *L. acidophilus* and *L. casei*. Many literature reports have concluded that the properties and characteristics of a given strain must be well defined, and studies on even closely related strains cannot be generalized to all strains of that species in relation to their □viaries□s potential (Gilliland and Speck 1977; Lin and others 1991; Collins and others 1991; Commane and others 2005; Gueimonde and others 2006). With the growing interest in lactobacilli, an investigation into the applicability of the automated systems to reliably identify and characterize strains of lactobacilli from a variety of hosts is needed.

The observations obtained from all methodologies examined in this study support that analyses beyond these various systems are required to fully understand the □viaries□s potential of a given strain. One must carefully consider the development of the database or library if the intent is for its use in the identification of lactobacilli. Most of the automated systems in this study utilized databases or libraries for lactobacilli that lacked reproducibility or are not well documented thereby weakening the reliability of the identification by the systems. This insufficiency can be attributed somewhat to test based on phenotypic characteristics using databases designed with a phylogenic group of strains identified based on genomic characteristics. Additionally, the number of isolates, variety

of the isolates, and reliability of the source of the isolates used to build the database within each system significantly impacts the usefulness of the identification systems.

Another major generalization influencing the lack of reliability of some automated microbial identification systems is the established protocols with respect to culture medium, physiological age, and growth temperature or conditions to be used for the analyses. Data from this study supports the need for genus specific protocols. A majority of the systems have placed lactobacilli into an anaerobic database which dictates these preset culture preparation and handling conditions. The genus *Lactobacillus* is not made up of strict anaerobes, and therefore many do not grow well on some of the pre-selected media or in some of the pre-selected growth conditions in the given amount of incubation time. Any change to this predetermined protocol to better the resulting growth of strains of lactobacilli weakens the reliability of the identification and characterization obtained from the systems.

Confirmation of Initial Identification and Characterization

All strains had been isolated and identified in previous research projects. To verify these identifications, a series of 27 phenotypic tests were conducted to characterize the strains just as had been done initially on the isolates (Table 1). All 32 strains of lactobacilli were Gram positive rods, catalase negative, and tested negative for ammonia production from arginine. All of the strains previously identified as *L. acidophilus* were able to grow at 45°C but not at 15°C. Of the seven previously identified strains of *L. casei,* four (A17, M5, E5, and E10) were able to grow at 15°C but not at 45°C, and the final three (L19, M12, and N7) were able to grow both at 45°C and 15°C.

The carbohydrate fermentation patterns obtained from use of the API 50 CH kits was compared to the fermentation of specific carbohydrates as listed in *Bergey's Manual of Systematic Bacteriology* (Kandler and Weiss, 1986). The identification of these conventional phenotypic confirmation tests are summarized in Table 1. Of the 32 strains, 22 (68.75%) of the identifications were confirmed perfect matches to the respective species with 7 (21.88%) matching all but one fermentation result, and 3 (9.38%) matching all but two fermentation results. Any strain given a new classification or any reorganization to the taxonomy to the genus of *Lactobacillus* since 1986 may, of course, have a significant impact on these results.

Sherlock MIDI MIS

For the genus *Lactobacillus*, the Sherlock database used is called MOORE which includes strains of *L. acidophilus* and *L. casei.* Following the database search, the computer prints a Composition Report which includes peak naming, database classification results, and the sample's chromatogram. A summary of the results obtained using this system is summarized in Table 2. There were only 4 (12.5%) cultures for which this system gave the same species identification across all three replications. However, none of these 4 was identified by this system as being *L. acidophilus.* Overall, results from this system failed to be reproducible and reliable for the identification of the isolates of lactobacilli used in these experiments. For the isolates that gave a high similarity index (SIM) to a library match, it was not far enough from the SIM provided for the second match option to feel comfortable with the identification. Concerns that were noted during the analysis of these isolates include the grouping of lactobacilli into

the anaerobic database thereby requiring the use of a pre-selected anaerobic broth that did not provide maximum growth of the cultures.

While this system could not be used for confirmation of identity of these particular isolates the resulting data did show some potential for characterizing the strains. The software is capable of developing dendograms, 2-D plots, and histograms of the sample data. When using this cluster analysis software with results obtained from a single replication very distinct groupings and separations of isolates were seen. While the potential for strain separation or grouping may be useful, it can not be utilized reliably until the identification can be confirmed.

Biolog

The "metabolic fingerprints" read from these isolates were compared to the MicroLog AN database. This database was developed using over 70 species of lactic acid bacteria to include strains of *L. acidophilus* and *L. casei*. When the system captures a pattern from a MicroPlate it is matched to other patterns in the database through a method called Progressive ID (PID). This method identifies patterns by considering the progressive sequence in which the purple wells are formed. There are five identification types: species identification; genus identification; too few positives; too many borderlines; and no identification. For a good species identification, a similarity index of greater than 5.0, a distance value of lest than 5.0, and the distance values of the first and second choices separated by more than two is required.

The identification results of the 32 strains using this automated system are summarized in Table 3. There were only 5 (15.63%) of the cultures identified as the

same species for each of the three replications. However, none of these matched the original identification as *L. acidophilus or L. casei.* While other species identifications had the appropriate criteria to be considered a good identification a lack of reproducibility was demonstrated by this system. Many of the species identification did not have enough separation between option 1 and option 2 to allow for reproducibility. Often the top two options for identification would switch back and forth for a given isolate from one replication to the next. This observation supports how phenotypically close some of the species are within the genus of *Lactobacillus.* A concern with this automated identification system is that lactobacilli were grouped and analyzed as anaerobic microorganisms. Also, the database had the *L. acidophilus* split up into two separate groups as if the two groups were separate species.

RiboPrinter

The RiboPrinter upon completion of the image processing of the eight sample carrier generated patterns that were compared to libraries created by DuPont. The system automatically generated a Batch Information Report. This report contained: the instrument number and accession number; an events log to list any problems encountered during the run; starting date and time of the run; batch status; number of the samples or marker position; sample labels or identifiers entered by the operator; DuPont identification label to include the genus and species of the sample if available; characterization information such as the RiboGroup that most closely matches the sample or <None> if the system cannot match the sample to any patterns in the DuPont Identification Library; and the RiboPrint pattern for each sample.

All 32 isolates were tested using the RiboPrinter for one complete replication, 3 additional isolates have two complete replications, and 6 isolates that have data for three complete replications. Observations obtained from the RiboPrinter are summarized in Table 4. Of the 25 cultures previously identified as *L. acidophilus*, only six were identified as *L. acidophilus.* Repeated assays of 3 of these provided inconsistent results. None of those previously identified as *L. casei* were identified as *L. casei.* Overall, data for this set of isolates did not provide enough identifications or any reproducibility within the few identifications seen to justify completing replications 2 and 3 given the expense of running samples on this system. Further investigation is needed to fully understand the unreliability seen with identification of this set of isolates. One possible answer may be in a lack of consistency of the lysing of the cells from one sample to the next.

Vitek

After a comparison of the patterns to a library, the ANI computer program provides identification or several possible identifications for the cultures. The ANI database was designed using only 7 species of *Lactobacillus* which included *L. acidophilus* and *L. casei.* Additionally, an identification confidence level, probabilities, and comments are provided. Confidence levels for the identifications are expressed as 1. excellent, very good, or acceptable [this is an indication that the biopattern is either typical, had only minor biochemical discrepancies, or generally has no more than one major biochemical discrepancy compared to the first-choice organism]; 2. good confidence but marginal separation [GCMS – these may be typical biopatterns but are insufficient separation of the two or three listed species prevents a definitive

identification]; 3. questionable biopattern [biopattern resembles two species in the database, but the probability that the isolate may belong to a third taxon not listed by the program is too great to provide an identification]; 4. unidentified organism [the biochemical test pattern does not resemble any organism in the data base enough to provide an identification].

Summarized data collected from one completed replication of the 32 isolates using this system is presented in Table 5. There were 3 (9.38%) isolates that resulted in a very good or acceptable identification (of these 3, only one, *L. casei* A-17, agreed with the original identification); 7 (21.88%) that were good or GCMS; 3 (9.38%) that were questionable; and 19 (59.38%) yielded an identification confidence level of unidentified. No further replications were completed given the lack of identifications made with these isolates using this system. There are many concerns that developed with this automated system after the completion of the first replication. As an example, determination of a positive well is strictly subjective from one operator to the next. Secondly, there were only seven different species of *Lactobacillus* used to develop the database which does not seem to be an appropriate representation of different species known in this genus. However, the database did include *L. acidophilus* and *L. casei.* Finally, this system groups lactobacilli as anaerobics, and because of this the growth medium and temperature recommended did not allow for very much growth by any of the isolates used in this study.

In conclusion, both the Biolog and the Sherlock MIDI MIS systems hold promise in being used as rapid, identification and characterization systems for isolates of

lactobacilli. However, before automated systems such as these could be reliably used as a step in determining the identity of an isolate a procedure for sample preparation should be designed specifically for lactobacilli (including appropriate growth medium, temperature, conditions, and incubation times) and a new database developed. The database should then be based on a very large, very diverse set of isolates obtained from all types of host species using the sample preparation procedures specific for lactobacilli.

Table 1. Identification based on Conventional Phenotypic Characterization

Sample [1]	Identification [2]	Comments
L. acidophilus ATCC 4356	*L. acidophilus*	ID MATCH – Confirmed [3]
L. acidophilus L-1	*L. acidophilus*	ID MATCH – Except Mannitol[4]
L. acidophilus O-16	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus K-4	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus J-12	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus H-13	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus D-3	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus B-11	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus 381-IL-28	*L. acidophilus*	ID MATCH – Except Ribose
L. acidophilus L-23	*L. acidophilus*	ID MATCH – Except Mannitol
L. acidophilus A-4	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus GP2B	*L. acidophilus*	ID MATCH – Except Amygdalin
L. acidophilus GP3A	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus RP32	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus C2-5	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus D-1	*L. acidophilus*	ID MATCH – Except Amygdalin, Salicin
L. acidophilus A6	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus 6-L4	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus 6-S4	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus C2	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus T-4	*L. acidophilus*	ID MATCH – Confirmed

Table 1. Identification based on Conventional Phenotypic Characterization – continued

Sample [1]	Identification [2]	Comments
L. acidophilus Nfa-5	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus Nfa-8	*L. acidophilus*	ID MATCH – Confirmed
L. acidophilus PLb-3	*L. acidophilus*	ID MATCH – Except Mannitol, Ribose
L. acidophilus RAT-1	*L. acidophilus*	ID MATCH – Confirmed
L. casei E-5	*L. casei* ss *casei*	ID MATCH – Except Gluconate, Melibiose
L. casei E-10	*L. casei* ss *casei*	ID MATCH – Except Melibiose
L. casei M-5	*L. casei* ss *casei*	ID MATCH – Except Gluconate
L. casei M-12	*L. casei* ss *rhamnosus*	ID MATCH – Confirmed
L. casei L-19	*L. casei* ss *rhamnosus*	ID MATCH – Confirmed
L. casei A-17	*L. casei* ss *casei*	ID MATCH – Except Gluconate
L. casei N-7	*L. casei* ss *rhamnosus*	ID MATCH – Confirmed

[1] Except for *L. acidophilus* ATCC 4356 all isolates of lactobacilli use previously assigned strain designations.

[2] Identifications are based on carbohydrate fermentations listed in 1st Edition of *Bergey's Manual of Systematic Bacteriology*, 1986; Fermentation patterns determined using API 50 CH kits. All strains were catalase negative, Gram positive rods, and did not produce ammonia from arginine. All strains identified as *L. acidophilus* grew at 45˚C but not at 15˚C; Those identified as *L. casei* all grew at 15˚C; strains L19, M12, and N7 also grew at 45˚C.

[3] ID Match – Confirmed: The profile matched perfectly to identified strain based on fermentations as compared to *Bergey's Manual of Systematic Bacteriology.*

[4] ID Match – Except: The profile matched the identified strain characteristics except for the fermentation of the carbohydrate(s) listed.

Table 2. Identification by Sherlock MIDI Microbial Identification System

Sample [1]	Identification [2]	Comments
L. acidophilus ATCC 4356	*L. jonhsonii, L. delbrueckii* ss *lactis, L. animalis*	Inconsistent Results
L. acidophilus L-1	*C. bifermentans, C. bifermentans, L.* P05	Non-Reproducible Results
L. acidophilus O-16	*L.* S01, *L. delbrueckii* ss *lactis, L. johnsonii*	Inconsistent Results
L. acidophilus K-4	*L. delbrueckii* ss *lactis, C. bifermentans, C. bifermentans*	Non-Reproducible Results
L. acidophilus J-12	*C. bifermentans, L. acidophilus, C. bifermentans*	Non-Reproducible Results
L. acidophilus H-13	*Enterococcus faecalis, E. faecalis, Eubacterium nodatum*	Non-Reproducible Results
L. acidophilus D-3	*L.* D12, *L. gallinarum, L. gallinarum*	Non-Reproducible Results
L. acidophilus B-11	*E. hallii, C. clostridioforme, C. Closstridioforme*	Non-Reproducible Results
L. acidophilus 381-IL-28	*Streptococcus oralis CFA gr 2, L. salivarius* ss *salivarius, L. salivarius* ss *salivarius*	Non-Reproducible Results
L. acidophilus L-23	No ID, *L. delbrueckii* ss *lactis, C. bifermentans*	Inconsistent Results
L. acidophilus A-4	*C. bifermentans, L. delbrueckii* ss *lactis, L. delbrueckii* ss *lactis*	Non-Reproducible Results

Table 2. Identification by Sherlock MIDI Microbial Identification System – continued

Sample [1]	Identification [2]	Comments
L. acidophilus GP2B	*L. delbrueckii* ss *lactis, C. bifermentans, E. faecalis*	Inconsistent Results
L. acidophilus GP3A	*C. bifermentans, C. bifermentans, L. delbrueckii* ss *lactis*	Non-Reproducible Results
L. acidophilus RP32	*C. bifermentans, C. bifermentans, C. bifermentans*	A species ID Confirmed
L. acidophilus C2-5	*L.* S01, *L.* S01, *L johnsonii*	Non-Reproducible Results
L. acidophilus D-1	*L. amylovorus CFA gr 2, C. bifermentans, C. bifermentans*	Non-Reproducible Results
L. acidophilus A6	*L. delbrueckii* ss *lactis, C. bifermentans, C. bifermentans*	Non-Reproducible Results
L. acidophilus 6-L4	*Streptococcus SM4,* No ID, *C. bifermentans*	Inconsistent Results
L. acidophilus 6-S4	*Streptococcus* ss *SM2, C. bifermentans, L. acidophilus*	Inconsistent Results
L. acidophilus C2	*L. vaginalis, L. vaginalis, L. vaginalis*	A species ID Confirmed

Table 2. Identification by Sherlock MIDI Microbial Identification System – continued

Sample [1]	Identification [2]	Comments
L. acidophilus T-4	*L. vaginalis, L. vaginalis, L. vaginalis*	A species ID Confirmed
L. acidophilus Nfa-5	*L. delbrueckii* ss *lactis, C. sordellii, C. sordellii*	Non-Reproducible Results
L. acidophilus Nfa-8	*C. sordellii, C. sordellii, L. hamsteri*	Non-Reproducible Results
L. acidophilus PLb-3	*L. mali, L. plantarum, Lactococcus lactis*	Inconsistent Results
L. acidophilus RAT-1	*L. vaginalis, Actinomyces israelii, L. vaginalis*	Non-Reproducible Results
L. casei E-5	*Lactococcus lactis, Lactococcus lactis, L. mali*	Non-Reproducible Results
L. casei E-10	*L. mali, L. mali, L. mali*	A species ID Confirmed
L. casei M-5	*Lactococcus lactis, Lactococcus lactis, E. faecalis*	Non-Reproducible Results
L. casei M-12	*E. faecalis, Lactococcus lactis, L. delbrueckii* ss *bulgaricus*	Inconsistent Results
L. casei L-19	*Lactococcus lactis,* No ID, *L. mali*	Inconsistent Results

Table 2. Identification by Sherlock MIDI Microbial Identification System – continued

Sample [1]	Identification [2]	Comments
L. casei A-17	*E. faecalis, Lactococcus lactis, E. faecalis*	Non-Reproducible Results
L. casei N-7	*E. faecalis, Lactococcus lactis, Lactococcus lactis*	Non-Reproducible Results

[1] Except for *L. acidophilus* ATCC 4356 all isolates of lactobacilli have previously assigned strain designations.

[2] Identifications are based on three replications of all strains using procedures according to manufacturer for use of the Sherlock MIDI MIS for identifying lactobacilli.

[3] If all three replications provided identical identifications a Species was confirmed; If two of the three replication results were the same identification then Non-reproducible results is noted; If all three replication results were different then Inconsistent results is noted; if two of the three results are No ID then Unreliable results are noted.

Table 3. Identification by the Biolog Microlog Automated System

Sample [1]	Identification [2]	Comments
L. acidophilus ATCC 4356	No ID, *L. acidophilus* BGA, No ID	Unreliable Results
L. acidophilus L-1	*Lactobacillus, L. crispatus, L. crispatus*	Non-reproducible Results
L. acidophilus O-16	*L. hamsteri, L. hamsteri, L. gasseri*	Non-reproducible Results
L. acidophilus K-4	*L. hamsteri, Lactobacillus, L. hamsteri*	Non-reproducible Results
L. acidophilus J-12	*L. gasseri, L. gasseri, L. gasseri*	A species ID Confirmed
L. acidophilus H-13	*Bifidobacterium, B. pullorum, B. merycicum*	Non-reproducible Results
L. acidophilus D-3	No ID, *L. gasseri, L. gasseri*	Non-reproducible Results
L. acidophilus B-11	*L. catenaformis,* No ID, *L. catenaformis*	Non-reproducible Results
L. acidophilus 381-IL-28	*Clostridium tertium, L. murinus/paracasei* ss *tolerans,* No ID	Inconsistent Results
L. acidophilus L-23	*L. crispatus, L. crispatus, L. crispatus*	A species ID Confirmed
L. acidophilus A-4	*L. crispatus, Lactobacillus, L. hamsteri*	Inconsistent Results

Table 3. Identification by the Biolog Microlog Automated System – continued

Sample [1]	Identification [2]	Comments
L. acidophilus GP2B	*L. delbrueckii* ss *lactis, L. hamsteri, L. hamsteri*	Non-reproducible Results
L. acidophilus GP3A	*L. hamsteri, L. crispatus, L. delbrueckii* ss *delbrueckii*	Inconsistent Results
L. acidophilus RP32	*Lactobacillus, Lactobacillus, Lactobacillus*	Genus ID Confirmed
L. acidophilus C2-5	*Lactobacillus, L. crispatus,* No ID	Inconsistent Results
L. acidophilus D-1	*L. crispatus, L. amylovorus, L. hamsteri*	Inconsistent Results
L. acidophilus A6	*Lactobacillus, L. hamsteri, L. hamsteri*	Non-reproducible Results
L. acidophilus 6-L4	*Weissella viridescens, L. crispatus, W. viridescens*	Non-reproducible Results
L. acidophilus 6-S4	*W. viridescens, W. viridescens, L. delbrueckii* ss *delbrueckii*	Non-reproducible Results
L. acidophilus C2	*Propionibacterium propionicus* BGA, *P. propionicus* BGA, P. propionicus *BGA*	A species ID Confirmed

Table 3. Identification by the Biolog Microlog Automated System – continued

Sample [1]	Identification [2]	Comments[3]
L. acidophilus T-4	*L. crispatus, P. propionicus* BGA, *L. crispatus*	Non-reproducible Results
L. acidophilus Nfa-5	*L. delbrueckii* ss *lactis, L. delbrueckii* ss *lactis, L. hamsteri*	Non-reproducible Results
L. acidophilus Nfa-8	*L. hamsteri, L. hamsteri, L. hamsteri*	A species ID Confirmed
L. acidophilus PLb-3	No ID, *L. murinus/paracasei* ss *tolerans, L. murinus/paracasei* ss *tolerans*	Non-reproducible Results
L. acidophilus RAT-1	*P. propionicus* BGA, *L. delbrueckii* ss *lactis, L. hamsteri*	Inconsistent Results
L. casei E-5	*L. hamsteri, L. delbrueckii* ss *lactis, L. hamsteri*	Non-reproducible Results
L. casei E-10	*Lactobacillus, L. hamsteri, L. delbrueckii* ss *lactis*	Inconsistent Results
L. casei M-5	*L. casei, L. rhamnosus, L. rhamnosus*	Non-reproducible Results
L. casei M-12	*L. plantarum, L. rhamnosus, L. plantarum*	Non-reproducible Results

Table 3. Identification by the Biolog Microlog Automated System – continued

Sample [1]	Identification [2]	Comments
L. casei L-19	*L. plantarum, L. casei, L. casei*	Non-reproducible Results
L. casei A-17	*L. paracasei* ss *paracasei, L. rhamnosus, L. paracasei* ss *paracasei*	Non-reproducible Results
L. casei N-7	*L. rhamnosus, L. rhamnosus, L. rhamnosus*	A species ID Confirmed

[1] Except for *L. acidophilus* ATCC 4356 all isolates of lactobacilli have previously assigned strain designations.

[2] Identifications are results from three replications of each strain using procedures according to manufacturer for use of the Biolog Microlog Automated System for identifying lactobacilli; Identifications are expressed as No ID, a Genus ID, or a Species ID.

[3] If all three replications provided identical identifications a Species/Genus ID was confirmed; If two of the three replication results were the same identification then Non-reproducible results is noted; If all three replication results were different then Inconsistent results is noted; if two of the three results are No ID then Unreliable results are noted.

Table 4. Identification by the RiboPrinter

Sample [1]	Identification [2]	Comments[3]
L. acidophilus ATCC 4356	*L. acidophilus,* No ID, No ID	Inconsistent Results
L. acidophilus L-1	No ID, No ID, No ID	
L. acidophilus O-16	*L. acidophilus,* No ID, *L. gasseri*	Inconsistent Results
L. acidophilus K-4	*L. acidophilus,* No ID, *L. acidophilus*	Inconsistent Results
L. acidophilus J-12	*P. pseudoalcaligenes,* No ID, No ID	Inconsistent Results
L. acidophilus H-13	No ID, No ID, No ID	
L. acidophilus D-3	No ID, No ID	
L. acidophilus B-11	No ID, No ID	
L. acidophilus 381-IL-28	*L. animalis*	
L. acidophilus L-23	No ID	
L. acidophilus A-4	No ID	
L. acidophilus GP2B	No ID	
L. acidophilus GP3A	*L. acidophilus*	
L. acidophilus RP32	*Vibrio cholerae*	
L. acidophilus C2-5	*L. acidophilus*	
L. acidophilus D-1	No ID	
L. acidophilus A6	No ID	
L. acidophilus 6-L4	No ID	
L. acidophilus 6-S4	*L. acidophilus*	

Table 4. Identification based on the use of the RiboPrinter – continued

Sample [1]	Identification [2]	Comments
L. acidophilus C2	No ID	
L. acidophilus T-4	No ID	
L. acidophilus Nfa-5	No ID	
L. acidophilus Nfa-8	No ID	
L. acidophilus PLb-3	No ID	
L. acidophilus RAT-1	*V. cholerae*	
L. casei E-5	No ID, No ID	
L. casei E-10	No ID	
L. casei M-5	No ID	
L. casei M-12	No ID	
L. casei L-19	No ID	
L. casei A-17	No ID	
L. casei N-7	No ID	

[1] Except for *L. acidophilus* ATCC 4356 all isolates of lactobacilli use previously assigned strain designations.

[2] Identifications are based on three replications for 6 strains, 2 replications for 3 strains, and one replication for all other strains using procedure according to manufacturer for use of RiboPrinter in identifying lactobacilli.

[3] No comments were provided for samples that gave no identification on any run of that sample; for samples ran in replications none of the results were consistent to allow for a confirmed identification.

Table 5. Identification by the Vitek Automated System

Sample [1]	Identification [2]	Comments[3]
L. acidophilus ATCC 4356	*Actinomyces odontolyticus*	GCMS
L. acidophilus L-1	*A. odontolyticus*	Unidentified
L. acidophilus O-16	*L. acidophilus*	Questionable
L. acidophilus K-4	*A. odontolyticus*	GCMS
L. acidophilus J-12	*A. odontolyticus*	Unidentified
L. acidophilus H-13	*A. odontolyticus*	Unidentified
L. acidophilus D-3	*A. odontolyticus*	GCMS
L. acidophilus B-11	*Clostridium perfringens*	Unidentified
L. acidophilus 381-IL-28	*C. perfringens*	Unidentified
L. acidophilus L-23	*A. odontolyticus*	Good
L. acidophilus A-4	*A. odontolyticus*	GCMS
L. acidophilus GP2B	*A. odontolyticus*	Unidentified
L. acidophilus GP3A	*A. odontolyticus*	GCMS
L. acidophilus RP32	*A. odontolyticus*	GCMS
L. acidophilus C2-5	*A. odontolyticus*	Unidentified
L. acidophilus D-1	*A. odontolyticus*	Unidentified
L. acidophilus A6	*A. odontolyticus*	Unidentified
L. acidophilus 6-L4	*A. odontolyticus*	Acceptable
L. acidophilus 6-S4	*C. perfringens*	Unidentified
L. acidophilus C2	*C. perfringens*	Unidentified
L. acidophilus T-4	*A. odontolyticus*	Unidentified

Table 5. Identification by the Vitek Automated System – continued

Sample [1]	Identification [2]	Comments
L. acidophilus Nfa-5	*A. odontolyticus*	Unidentified
L. acidophilus Nfa-8	*Bifidobacterium* species	Unidentified
L. acidophilus PLb-3	*A. naeslundii*	Unidentified
L. acidophilus RAT-1	*A. odontolyticus*	Unidentified
L. casei E-5	*C. perfringens*	Unidentified
L. casei E-10	*C. perfringens*	Unidentified
L. casei M-5	*L. acidophilus*	Questionable
L. casei M-12	*L. acidophilus*	Very Good
L. casei L-19	*L. acidophilus*	Questionable
L. casei A-17	*L. casei*	Acceptable
L. casei N-7	*C. perfringens*	Unidentified

[1] Except for *L. acidophilus* ATCC 4356 all isolates of lactobacilli use previously assigned strain designations.

[2] Identifications are based on one replication using procedure according to manufacturer for use of Vitek in identifying lactobacilli.

[3] Comments are reflective of the confidence level provided by the Vitek System: excellent, very good, or acceptable; good confidence but marginal separation [GCMS]; questionable; or unidentified. Confidence levels are based on probabilities of a match generated by the database search.

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APPENDIX A

ORIGIN OF CULTURES USED IN THESE EXPERIMENTS

Table 6. Species origin of the *Lactobacillus* cultures used in these experiments

Culture	Species origin of isolates
L. acidophilus ATCC 4356	Human
L. acidophilus L-1	Human
L. acidophilus O-16	Human
L. acidophilus K-4	Human
L. acidophilus J-12	Human
L. acidophilus H-13	Human
L. acidophilus D-3	Human
L. acidophilus B-11	Human
L. acidophilus 381-IL-28	Cattle
L. acidophilus L-23	Pig
L. acidophilus A-4	Pig
L. acidophilus GP2B	Pig
L. acidophilus GP3A	Pig
L. acidophilus RP32	Pig
L. acidophilus C2-5	Pig
L. acidophilus D-1	Pig
L. acidophilus A-6	Pig
L. acidophilus 6-L4	Chicken
L. acidophilus 6-S4	Chicken
L. acidophilus C-2	Turkey
L. acidophilus T-4	Turkey
L. acidophilus Nfa-5	Rodent
L. acidophilus Nfa-8	Rodent
L. acidophilus PLb-3	Rodent
L. acidophilus RAT-1	Rodent
L. casei E-5	Human
L. casei E-10	Human
L. casei M-5	Human
L. casei M-12	Human
L. casei L-19	Human
L. casei A-17	Human
L. casei N-7	Human

APPENDIX B

COLLECTION OF RAW DATA FROM THE IDENTITY OF CULTURES BY API 50 CH KIT

Table 7. Confirmation of identity of cultures of *Lactobacillus acidophilus* by API 50 CH

Test[1]	La[2]	La-4356[3]	La-L1	La-O16	La-K4	La-J12
Amygdalin	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-
Cellubiose	+	+	+	+	+	+
Esculin	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Gluconate	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Mannitol	-	-	+	-	-	-
Mannose	+	+	+	+	+	+
Melezitose	-	-	-	-	-	-
Melibiose	+/-	-	+	+	-	-
Raffinose	+/-	+	+	-	+	-
Rhamnose	-	-	-	-	-	-
Ribose	-	-	-	-	-	-
Salicin	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Trehalose	+/-	+	+	+	+	+
Xylose	-	-	-	-	-	-

[1]All cultures were Gram + rods; catalase negative; negative for ammonia production from arginine; grew at 45˚C; and did not grow at 15˚C

[2]La=*Lactobacillus acidophilus;* reactions as listed in the 1st Edition of *Bergey's Manual of Systematic Bacteriology*, 1986.

Table 8. Confirmation of identity of cultures of *Lactobacillus acidophilus* by API 50 CH

Test[1]	La[2]	La-H13	La-D3	La-B11	La-381IL28	La-L23
Amygdalin	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-
Cellubiose	+	+	+	+	+	+
Esculin	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Gluconate	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Mannitol	-	-	-	-	-	+
Mannose	+	+	+	+	+	+
Melezitose	-	-	-	-	-	-
Melibiose	+/-	+	-	-	+	+
Raffinose	+/-	+	-	+	+	+
Rhamnose	-	-	-	-	-	-
Ribose	-	-	-	-	+	-
Salicin	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Trehalose	+/-	+	+	-	+	+
Xylose	-	-	-	-	-	-

[1]All cultures were Gram + rods; catalase negative; negative for ammonia production from arginine; grew at 45˚C; and did not grow at 15˚C

[2]La=*Lactobacillus acidophilus;* reactions as listed in the 1st Edition of *Bergey's Manual of Systematic Bacteriology*, 1986.

Table 9. Confirmation of identity of cultures of *Lactobacillus acidophilus* by API 50 CH

Test[1]	La[2]	La-A4	La-GP2B	La-GP3A	La-RP32	La-C25
Amygdalin	+	+	-	+	+	+
Arabinose	-	-	-	-	-	-
Cellubiose	+	+	+	+	+	+
Esculin	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Gluconate	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-
Mannose	+	+	+	+	+	+
Melezitose	-	-	-	-	-	-
Melibiose	+/-	+	+	+	-	+
Raffinose	+/-	+	+	+	+	+
Rhamnose	-	-	-	-	-	-
Ribose	-	-	-	-	-	-
Salicin	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Trehalose	+/-	+	-	-	+	+
Xylose	-	-	-	-	-	-

[1]All cultures were Gram + rods; catalase negative; negative for ammonia production from arginine; grew at 45˚C; and did not grow at 15˚C

[2]La=*Lactobacillus acidophilus;* reactions as listed in the 1st Edition of *Bergey's Manual of Systematic Bacteriology*, 1986.

Table 10. Confirmation of identity of cultures of *Lactobacillus acidophilus* by API 50 CH

Test[1]	La[2]	La-D1	La-A6	La-6L4	La-6S4	La-C2
Amygdalin	+	-	+	+	+	+
Arabinose	-	-	-	-	-	-
Cellubiose	+	+	+	+	+	+
Esculin	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Gluconate	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-
Mannose	+	+	+	+	+	+
Melezitose	-	-	-	-	-	-
Melibiose	+/-	+	-	+	+	+
Raffinose	+/-	+	+	+	+	+
Rhamnose	-	-	-	-	-	-
Ribose	-	-	-	-	-	-
Salicin	+	-	+	+	+	+
Sorbitol	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Trehalose	+/-	-	+	-	-	-
Xylose	-	-	-	-	-	-

[1]All cultures were Gram + rods; catalase negative; negative for ammonia production from arginine; grew at 45˚C; and did not grow at 15˚C

[2]La=*Lactobacillus acidophilus;* reactions as listed in the 1st Edition of *Bergey's Manual of Systematic Bacteriology*, 1986.

Table 11. Confirmation of identity of cultures of *Lactobacillus acidophilus* by API 50 CH

Test[1]	La[2]	La-T4	La-Nfa5	La-Nfa8	La-PLb3	La-RAT1
Amygdalin	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-
Cellubiose	+	+	+	+	+	+
Esculin	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Gluconate	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Mannitol	-	-	-	-	+	-
Mannose	+	+	+	+	+	+
Melezitose	-	-	-	-	-	-
Melibiose	+/-	+	-	-	+	-
Raffinose	+/-	+	+	+	+	+
Rhamnose	-	-	-	-	-	-
Ribose	-	-	-	-	+	-
Salicin	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Trehalose	+/-	-	-	-	+	+
Xylose	-	-	-	-	-	-

[1]All cultures were Gram + rods; catalase negative; negative for ammonia production from arginine; grew at 45˚C; and did not grow at 15˚C

[2]La=*Lactobacillus acidophilus;* reactions as listed in the 1st Edition of *Bergey's Manual of Systematic Bacteriology*, 1986.

Table 12. Confirmation of identity of cultures of *Lactobacillus casei* ss *casei* by API 50 CH

Test[1]	Lcc[2]	Lcc-E5	Lcc-E10	Lcc-M5	Lcc-A17
Amygdalin	+	+	+	+	+
Arabinose	-	-	-	-	-
Cellubiose	+	+	+	+	+
Esculin	+	+	+	+	+
Fructose	+	+	+	+	+
Galactose	+	+	+	+	+
Gluconate	+	-	+	-	-
Glucose	+	+	+	+	+
Lactose	+/-	+	-	+	+
Maltose	+	+	+	+	+
Mannitol	+	+	+	+	+
Mannose	+	+	+	+	+
Melezitose	+	+	+	+	+
Melibiose	-	+	+	-	-
Raffinose	-	-	-	-	-
Rhamnose	-	-	-	-	-
Ribose	+	+	+	+	+
Salicin	+	+	+	+	+
Sorbitol	+	+	+	+	+
Sucrose	+	+	+	+	+
Trehalose	+	+	+	+	+
Xylose	-	-	-	-	-

[1]All cultures were Gram + rods; catalase negative; negative for ammonia production from arginine; grew at 15˚C; and did not grow at 45˚C

[2]Lcc=*Lactobacillus casei* ss *casei;* reactions as listed in the 1st Edition of *Bergey's Manual of Systematic Bacteriology*, 1986.

Table 13. Confirmation of identity of cultures of *Lactobacillus casei* ss *rhamnosus* by API 50 CH

Test[1]	Lcr[2]	Lcr-M12	Lcr-L19	Lcr-N7
Amygdalin	+	+	+	+
Arabinose	+/-	-	-	-
Cellubiose	+	+	+	+
Esculin	+	+	+	+
Fructose	+	+	+	+
Galactose	+	+	+	+
Gluconate	+	+	+	+
Glucose	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+
Mannitol	+	+	+	+
Mannose	+	+	+	+
Melezitose	+	+	+	+
Melibiose	-	-	-	-
Raffinose	-	-	-	-
Rhamnose	+	+	+	+
Ribose	+	+	+	+
Salicin	+	+	+	+
Sorbitol	+	+	+	+
Sucrose	+	+	+	+
Trehalose	+	+	+	+
Xylose	-	-	-	-

[1]All cultures were Gram + rods; catalase negative; negative for ammonia production from arginine; grew at 15˚C and at 45˚C

[2]Lcr=*Lactobacillus casei* ss *rhamnosus;* reactions as listed in the 1st Edition of *Bergey's Manual of Systematic Bacteriology*, 1986.

APPENDIX C

COLLECTION OF RAW DATA FROM THE SHERLOCK MIDI MIS

Table 14. Collection of raw data from replication #1 showing identification of various lactobacilli by Sherlock MIDI MIS

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus ATCC 4356	*L. jonhsonii*	0.556	*L. animalis*	0.438	*Clostridium bifermentans*	0.438
L. acidophilus L-1	*C. bifermentans*	0.289	*L. gallinarum*	0.288	*L. acidophilus*	0.280
L. acidophilus O-16	*Lactobacillus S01*	0.345	*C. bifermentans*	0.336	*L. johnsonii*	0.329
L. acidophilus K-4	*L. delbrueckii* ss *lactis*	0.550	*C. bifermentans*	0.502	*L. acidophilus*	0.429
L. acidophilus J-12	*C. bifermentans*	0.554	*L. S01*	0.490	*L. johnsonii*	0.480
L. acidophilus H-13	*E. faecalis*	0.350	*C. clostridioforme*	0.278	*L. bifermentans*	0.253
L. acidophilus D-3	*Lactobacillus* D12	0.345	*G. morbillorum CFA gr. 2*	0.336	*L. gallinarum*	0.310
L. acidophilus B-11	*E. hallii*	0.436	*E. saburreum*	0.345	*C. clostridioforme*	0.340
L. acidophilus 381-IL-28	*Streptococcus oralis CFA gr 2*	0.290	*L. salivarius ss salivarus*	0.260	*L. plantarum*	0.211
L. acidophilus L-23	NO ID	0.000				
L. acidophilus A-4	*C. bifermentans*	0.499	*L. acidophilus*	0.375	*L. gasseri*	0.312

Table 14. Collection of raw data from replication #1 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus GP2B	*L. delbrueckii* ss *lactis*	0.490	*C. bifermentans*	0.403	*L. acidophilus*	0.342
L. acidophilus GP3A	*C. bifermentans*	0.401	*L. acidophilus*	0.351	*L. S01*	0.326
L. acidophilus RP32	*C. bifermentans*	0.539	*L. paracasei* ss *paracasei*	0.391	*L. S01*	0.354
L. acidophilus C2-5	*L. S01*	0.527	*C. bifermentans*	0.502	*L. acidophilus*	0.465
L. acidophilus D-1	*L. amylovorus CFA gr 2*	0.321	*C. bifermentans*	0.318	*L. acidophilus*	0.309
L. acidophilus A-6	*L. delbrueckii* ss *lactis*	0.527	*C. bifermentans*	0.492	*L. acidophilus*	0.384
L. acidophilus 6-L4	*Streptococcus SM4*	0.784	*Streptococcus SM2*	0.737	*G. morbillorum CFA gr 3*	0.686
L. acidophilus 6-S4	*S. SM2*	0.741	*S. SM4*	0.712	*G. morbillorum CFA gr 3*	0.668
L. acidophilus C-2	*L. vaginalis*	0.687	*L. sharpeae*	0.529	*L. kefir*	0.455
L. acidophilus T-4	*L. vaginalis*	0.696	*L. sharpeae*	0.590	*L. kefir*	0.516

Table 14. Collection of raw data from replication #1 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus Nfa-5	*L. delbrueckii ss lactis*	0.393	*L. hamsteri*	0.382	*E. faecium*	0.340
L. acidophilus Nfa-8	*C. sordellii*	0.420	*L. curvatus*	0.401	*C. bifermentans*	0.368
L. acidophilus PLb-3	*L. mali*	0.147	*L. reuteri*	0.062	*S. oralis CFA gr 2*	0.058
L. acidophilus RAT-1	*L. vaginalis*	0.650	*L. sharpeae*	0.512	*L. kefir*	0.379
L. casei E-5	*Lactococcus lactis*	0.259	*L. mali*	0.115		
L. casei E-10	*L. mali*	0.240	*Lactococcus lactis*	0.093		
L. casei M-5	*Lactococcus lactis*	0.427				
L. casei M-12	*E. faecalis*	0.149	*Lactococcus lactis*	0.148	*L. agilis*	0.091
L. casei L-19	*Lactococcus lactis*	0.382				

Table 14. Collection of raw data from replication #1 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. casei A-17	*E. faecalis*	0.255	*Lactococcus lactis*	0.174	*L. agilis*	0.086
L. casei N-7	*E. faecalis*	0.186	*L. delbrueckii ss lactis*	0.085	*L. mali*	0.059

[1] Opt1 = The result of the database search for the best matches based on associated similarity indices as identified as option 1. Best match is Opt1, next best match is Opt2, and the third best database match is Opt3.

[2] SIM = Similarity Index is a numerical value which expresses how closely the fatty acid composition of the sample compares with the mean fatty acid composition of the strains used to create the library entry listed as the match to that SIM.

Table 15. Collection of raw data from replication #2 showing identification of various lactobacilli by Sherlock MIDI MIS

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus ATCC 4356	*L. delbrueckii* ss. *Lactis*	0.554	*L. confusus*	0.470	*C. bifermentans*	0.460
L. acidophilus L-1	*C. bifermentans*	0.332	*L. acidophilus*	0.298	*L. gallinarum*	0.294
L. acidophilus O-16	*L. delbrueckii ss. Lactis*	0.406	*L. sharpeae*	0.296	*C. bifermentans*	0.295
L. acidophilus K-4	*C. bifermentans*	0.204	*L. oris*	0.082		
L. acidophilus J-12	*L. acidophilus*	0.793	*C. bifermentans*	0.723	*L. delbrueckii* ss *lactis*	0.722
L. acidophilus H-13	*Enterococcus faecalis*	0.343	*C. clostridioforme*	0.267	*L. bifermentans*	0.253
L. acidophilus D-3	*L. gallinarum*	0.338	*C. bifermentans*	0.311	*L. acidophilus*	0.300
L. acidophilus B-11	*C. clostridioforme*	0.337	*E. eligens*	0.184	*E. saburreum*	0.175
L. acidophilus 381-IL-28	*L. salivarius ss salivarius*	0.323	*S. oralis CFA gr 2*	0.315	*L. salivarius ss salivarius*	0.289
L. acidophilus L-23	*L. delbrueckii ss lactis*	0.289	*C. bifermentans*	0.273	*L. acidophilus*	0.255
L. acidophilus A-4	*L. delbrueckii ss lactis*	0.488	*C. bifermentans*	0.439	*L. acidophilus*	0.373

Table 15. Collection of raw data from replication #2 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus GP2B	*C. bifermentans*	0.314	*L. acidophilus*	0.310	*L. gasseri*	0.297
L. acidophilus GP3A	*C. bifermentans*	0.361	*L. acidophilus*	0.345	*L. johnsonii*	0.339
L. acidophilus RP32	*C. bifermentans*	0.480	*L. delbrueckii* ss *lactis*	0.427	*L. S01*	0.422
L. acidophilus C2-5	*L. S01*	0.477	*L. johnsonii*	0.472	*C. bifermentans*	0.465
L. acidophilus D-1	*C. bifermentans*	0.339	*L. acidophilus*	0.319	*L. gasseri*	0.284
L. acidophilus A-6	*C. bifermentans*	0.340	*L. acidophilus*	0.214	*L. paracasei* ss *paracasei*	0.198
L. acidophilus 6-L4	*No Identification*	0.000				
L. acidophilus 6-S4	*C. bifermentans*	0.391	*L. acidophilus*	0.368	*L. delbrueckii ss lactis*	0.345
L. acidophilus C-2	*L. vaginalis*	0.686	*L. sharpeae*	0.513	*L. kefir*	0.446
L. acidophilus T-4	*L. vaginalis*	0.637	*L. kefir*	0.539	*A. israelii*	0.523

Table 15. Collection of raw data from replication #2 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus Nfa-5	*C. sordellii*	0.564	*C. bifermentans*	0.554	*L. curvatus*	0.542
L. acidophilus Nfa-8	*C. sordellii*	0.424	*L. curvatus*	0.404	*C. bifermentans*	0.372
L. acidophilus PLb-3	*L. plantarum*	0.709	*L. fermentum*	0.569	*L. reuteri*	0.500
L. acidophilus RAT-1	*A. israelii*	0.618	*L. vaginalis*	0.438	*Bifidobacterium D02A*	0.413
L. casei E-5	*Lactococcus lactis*	0.231	*L. reuteri*	0.024		
L. casei E-10	*L. mali*	0.308	*Lactococcus lactis*	0.187		
L. casei M-5	*Lactococcus lactis*	0.432				
L. casei M-12	*Lactococcus lactis*	0.157	*E. faecalis*	0.148	*L. agilis*	0.093
L. casei L-19	*NO Identification*	0.000				

Table 15. Collection of raw data from replication #2 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. casei A-17	*Lactococcus lactis*	0.178	*E. faecalis*	0.071	*L. mali*	0.068
L. casei N-7	*Lactococcus lactis*	0.302	*E. faecalis*	0.106		

[1] Opt1 = The result of the database search for the best matches based on associated similarity indices as identified as option 1. Best match is Opt1, next best match is Opt2, and the third best database match is Opt3.

[2] SIM = Similarity Index is a numerical value which expresses how closely the fatty acid composition of the sample compares with the mean fatty acid composition of the strains used to create the library entry listed as the match to that SIM.

Table 16. Collection of raw data from replication #3 showing identification of various lactobacilli by Sherlock MIDI MIS

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus ATCC 4356	*L. animalis*	0.505	*L. johnsonii*	0.497	*C. bifermentans*	0.438
L. acidophilus L-1	*Lactobacillus P05*	0.720	*Streptococcus SM2*	0.613	*Gemella morbillorum CFA gr.3*	0.560
L. acidophilus O-16	*L. johnsonii*	0.371	*C. bifermentans*	0.350	*L. acidophilus*	0.306
L. acidophilus K-4	*C. bifermentans*	0.514	*L. delbrueckii* ss *lactis*	0.383	*L. acidophilus*	0.317
L. acidophilus J-12	*C. bifermentans*	0.591	*L. S01*	0.542	*L. acidophilus*	0.498
L. acidophilus H-13	*Eubacterium nodatum*	0.309	*E. S01*	0.273	*E. rectale II*	0.207
L. acidophilus D-3	*L. gallinarum*	0.339	*C. bifermentans*	0.308	*G. morbillorum CFA gr. 4*	0.299
L. acidophilus B-11	*C. clostridioforme*	0.304	*E. saburreum*	0.200	*E. nodatum*	0.189
L. acidophilus 381-IL-28	*L. salivarius ss salivarius*	0.229	*S. oralis CFA gr 2*	0.185	*L. rhamnosus*	0.166
L. acidophilus L-23	*C. bifermentans*	0.333	*L. acidophilus*	0.319	*L. gasseri*	0.269
L. acidophilus A-4	*L. delbrueckii* ss *lactis*	0.374	*C. bifermentans*	0.359	*L. acidophilus*	0.339

Table 16. Collection of raw data from replication #3 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus GP2B	*E. faecium*	0.141	*C. botulinum* type *A2*	0.120	*C. botulinum* type *B*	0.050
L. acidophilus GP3A	*L. delbrueckii ss lactis*	0.387	*L. sharpeae*	0.306	*C. bifermentans*	0.303
L. acidophilus RP32	*C. bifermentans*	0.353	*L. acidophilus*	0.340	*L. johnsonii*	0.325
L. acidophilus C2-5	*L. johnsonii*	0.386	*E. faecium*	0.366	*L. S01*	0.364
L. acidophilus D-1	*C. bifermentans*	0.395	*L. acidophilus*	0.361	*L. gasseri*	0.312
L. acidophilus A-6	*C. bifermentans*	0.344	*L. acidophilus*	0.279	*L. johnsonii*	0.239
L. acidophilus 6-L4	*C. bifermentans*	0.315	*L. acidophilus*	0.298	*L. gasseri*	0.264
L. acidophilus 6-S4	*L. acidophilus*	0.392	*C. bifermentans*	0.389	*L. delbrueckii ss lactis*	0.377
L. acidophilus C-2	*L. vaginalis*	0.703	*Actinomyces israelii*	0.593	*L. kefir*	0.520
L. acidophilus T-4	*L. vaginalis*	0.691	*L. sharpeae*	0.588	*L. kefir*	0.514

Table 16. Collection of raw data from replication #3 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. acidophilus Nfa-5	*C. sordellii*	0.570	*C. bifermentans*	0.554	*L. curvatus*	0.515
L. acidophilus Nfa-8	*L. hamsteri*	0.429	*L. delbrueckii ss lactis*	0.382	*E. faecium*	0.371
L. acidophilus PLb-3	*Lactococcus lactis*	0.338	*L. plantarum*	0.275	*L. reuteri*	0.218
L. acidophilus RAT-1	*L. vaginalis*	0.655	*L. sharpeae*	0.518	*L. kefir*	0.386
L. casei E-5	*L. mali*	0.231	*Lactococcus lactis*	0.129		
L. casei E-10	*L. mali*	0.214	*Lactococcus lactis*	0.193		
L. casei M-5	*E. faecalis*	0.136	*E. durans*	0.098	*L. mali*	0.053
L. casei M-12	*L. delbrueckii* ss *bulgaricus*	0.123	*E. faecalis*	0.089	*L. mali*	0.063
L. casei L-19	*L. mali*	0.138	*E. faecalis*	0.126	*L. agilis*	0.055

Table 16. Collection of raw data from replication #3 showing identification of various lactobacilli by Sherlock MIDI MIS – continued

Sample	Opt1[1]	SIM[2]	Opt2	SIM	Opt3	SIM
L. casei A-17	*E. faecalis*	0.235	*L. mali*	0.205	*Lactococcus lactis*	0.152
L. casei N-7	*Lactococcus lactis*	0.313	*E. faecalis*	0.105		

[1] Opt1 = The result of the database search for the best matches based on associated similarity indices as identified as option 1. Best match is Opt1, next best match is Opt2, and the third best database match is Opt3.

[2] SIM = Similarity Index is a numerical value which expresses how closely the fatty acid composition of the sample compares with the mean fatty acid composition of the strains used to create the library entry listed as the match to that SIM.

APPENDIX D

REAGENT PREPARATION FOR SHERLOCK MIDI MIS

Reagent 1 – Saponification Reagent

- Add 45 grams of sodium hydroxide to 150 mL of methanol and 150 mL of deionized distilled water
- Stir until pellets are dissolved

Reagent 2 – Methylation Reagent

Reagent 2a:

- Slowly add 325 mL of 6.00N hydrochloric acid to 275 mL of methanol while stirring

Reagent 2b:

- Slowly add 325 mL of 50% sulfuric acid to 275 mL of methanol while stirring

Reagent 3 – Extraction Solvent

- Add 200 mL of methyl-tert-butyl ether to 200 mL of hexane and stir

Reagent 4 – Base Wash

- Add 5.4 grams of sodium hydroxide to 450 mL of deionized distilled water
- Add 120 grams of sodium chloride to solution
- Stir until pellets are dissolved

Reagents were prepared fresh each month. They were stored at room temperature in bottles with Teflon-lined caps.

APPENDIX E

COLLECTION OF RAW DATA FROM THE BIOLOG

Table 17 . Collection of raw data from replication #1 showing identification of various lactobacilli using the Biolog Microlog Automated System

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus ATCC 4356	NO ID	--	--	--	--	--
L. acidophilus L-1	Genus	*Lactobacillus delbrueckii* ss *delbrueckii*	--	0.30	5.76	*L. crispatus*
L. acidophilus O-16	Species	*L. hamsteri*	79	0.62	3.31	*L. gasseri*
L. acidophilus K-4	Species	*L. hamsteri*	100	0.87	1.89	*L. delbrueckii* ss *lactis*
L. acidophilus J-12	Species	*L. gasseri*	100	0.80	2.99	*L. hamsteri*
L. acidophilus H-13	Species	*Bifidobacterium merycicum*	89	0.60	4.92	*B. catenulatum*
L. acidophilus D-3	NO ID	--	--	--	--	--
L. acidophilus B-11	Species	*L. catenaformis*	94	0.66	4.54	*B. asteroides*
L. acidophilus 381-IL-28	Species	*Clostridium tertium*	99	0.61	5.85	*L. murinus/paracasei ss tolerans*
L. acidophilus L-23	Species	*L. crispatus*	99	0.71	4.36	*Weissella viridescens*

Table 17. Collection of raw data from replication #1 showing identification of various lactobacilli using the Biolog Microlog Automated System – continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus A-4	Species	*L. crispatus*	88	0.55	5.78	*L. hamsteri*
L. acidophilus GP2B	Species	*L. delbrueckii* ss *lactis*	98	0.54	7.01	*L. crispatus*
L. acidophilus GP3A	Species	*L. hamsteri*	93	0.67	4.31	*L. crispatus*
L. acidophilus RP32	Genus	*L. acidophilus* BGB	--	0.44	7.00	*L. hamsteri*
L. acidophilus C2-5	Genus	*L. crispatus*	--	0.49	7.54	*L. fermentum*
L. acidophilus D-1	Species	*L. crispatus*	99	0.86	2.01	*L. hamsteri*
L. acidophilus A-6	Genus	*L. hamsteri*	--	0.49	6.93	*L. crispatus*
L. acidophilus 6-L4	Species	*W. viridescens*	95	0.86	1.45	*Eubacterium hallii*
L. acidophilus 6-S4	Species	*W. viridescens*	91	0.73	3.00	*L. sanfranciscensis*
L. acidophilus C-2	Species	*Propionibacterium propionicus* BGA	92	0.69	3.88	*L. crispatus*

Table 17. Collection of raw data from replication #1 showing identification of various lactobacilli using the Biolog Microlog Automated System – continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus T-4	Species	*L. crispatus*	99	0.71	4.39	*Actinomyces naeslundii / viscosus*
L. acidophilus Nfa-5	Species	*L. delbrueckii* ss *lactis*	83	0.61	4.00	*L. hamsteri*
L. acidophilus Nfa-8	Species	*L. hamsteri*	71	0.52	4.15	*L. delbrueckii ss lactis*
L. acidophilus PLb-3	NO ID	--	--	--	--	--
L. acidophilus RAT-1	Species	*P. propionicus BGA*	97	0.85	1.89	*E. hallii*
L. casei E-5	Species	*L. hamsteri*	99	0.53	7.37	*L. rhamnosus*
L. casei E-10	Genus	*L. bifermentans*	--	0.48	5.84	*L. delbrueckii* ss *lactis*
L. casei M-5	Species	*L. casei*	98	0.55	7.00	*L. rhamnosus*

Table 17. Collection of raw data from replication #1 showing identification of various lactobacilli using the Biolog Microlog Automated System – continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. casei M-12	Species	*L. plantarum*	99	0.59	6.23	*L. alimentarius*
L. casei L-19	Species	*L. plantarum*	98	0.64	5.45	*Pediococcus urinaeequi*
L. casei A-17	Species	*L. paracasei* ss *paracasei*	100	0.61	6.00	*L. rhamnosus*
L. casei N-7	Species	*L. rhamnosus*	99	0.67	4.92	*L. hamsteri*

[1] Identification type describes to what degree an identification was made: no identification; to the genus level; or to the species level.

[2] Opt1 indicates the best match as option 1 for identification; where as the next best match is listed under Opt2.

[3] PROB allows you to compare the Biolog IDs to other systems that use this type of calculation.

[4] SIM is a similarity index that indicates how good the sample results match the database pattern of the organism listed as OPT1. (SIM = 1.0 is a perfect match; a positive ID must be based on SIM > 0.5)

[5] DIST is the distance or number of mismatches between the sample results and the database pattern for the organism listed as OPT1. (a positive ID must be based on DIST < 5.0 with the DIST of the first and second choices being more than two distance points apart)

Table 18. Collection of raw data from replication #2 showing identification of various lactobacilli using the Biolog Microlog Automated System

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus ATCC 4356	Species	*L. acidophilus* BGA	74	0.60	3.03	*L. hamsteri*
L. acidophilus L-1	Species	*L. crispatus*	79	0.51	5.41	*W. viridescens*
L. acidophilus O-16	Species	*L. hamsteri*	81	0.62	3.63	*L. gasseri*
L. acidophilus K-4	Genus	*L. hamsteri*	--	0.45	2.52	*L. delbrueckii* ss *lactis*
L. acidophilus J-12	Species	*L. gasseri*	82	0.69	2.31	*L. delbrueckii* ss *lactis*
L. acidophilus H-13	Species	*B. pullorum*	88	0.64	4.24	*L. murinus/paracasei* ss *lactis*
L. acidophilus D-3	Species	*L. gasseri*	100	0.86	2.03	*L. hamsteri*
L. acidophilus B-11	NO ID	--	--	--	--	--
L. acidophilus 381-IL-28	Species	*L. murinus/paracasei ss tolerans*	100	0.62	5.80	*L. viaries ss araffinosus*
L. acidophilus L-23	Species	*L. crispatus*	95	0.52	7.22	*L. amylovorus*

Table 18. Collection of raw data from replication #2 showing identification of various lactobacilli using the Biolog Microlog Automated System – continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus A-4	Genus	*L. delbrueckii ss lactis*	--	0.41	7.31	*L. acidophilus BGB*
L. acidophilus GP2B	Species	*L. hamsteri*	88	0.60	4.82	*L. crispatus*
L. acidophilus GP3A	Species	*L. crispatus*	100	0.78	3.37	*W. viridescens*
L. acidophilus RP32	Genus	*L. hamsteri*	--	0.32	7.10	*L. delbrueckii* ss *lactis*
L. acidophilus C2-5	Species	*L. crispatus*	98	0.63	5.57	*L. hamsteri*
L. acidophilus D-1	Species	*L. amylovorus*	84	0.54	5.59	*L. crispatus*
L. acidophilus A-6	Species	*L. hamsteri*	100	0.80	2.96	*L. delbrueckii* ss *lactis*
L. acidophilus 6-L4	Species	*P. propionicus* BGA	99	0.86	2.06	*L. crispatus*
L. acidophilus 6-S4	Species	*P. propionicus* BGA	99	0.83	2.40	*E. hallii*
L. acidophilus C-2	Species	*L. crispatus*	99	0.70	4.49	*L. acidophilus*
L. acidophilus T-4	Species	*W. viridescens*	74	0.56	3.62	*L. sanfranciscensis*

Table 18. Collection of raw data from replication #2 showing identification of various lactobacilli using the Biolog Microlog Automated System – continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus Nfa-5	Species	*L. delbrueckii ss lactis*	95	0.60	5.71	*L. hamsteri*
L. acidophilus Nfa-8	Species	*L. hamsteri*	95	0.64	4.97	*L. delbrueckii ss lactis*
L. acidophilus PLb-3	Species	*L. murinus/paracasei ss tolerans*	100	0.59	6.41	*B. suis*
L. acidophilus RAT-1	Species	*L. delbrueckii ss lactis*	92	0.54	6.46	*L. gasseri*
L. casei E-5	Species	*L. delbrueckii* ss *lactis*	97	0.67	4.61	*W. halotolerans / hellenica*
L. casei E-10	Species	*L. hamsteri*	97	0.65	4.94	*L. delbrueckii* ss *lactis*
L. casei M-5	Species	*L. rhamnosus*	100	0.79	3.09	*L. casei*
L. casei M-12	Species	*L. rhamnosus*	100	0.55	7.16	*L. hamsteri*
L. casei L-19	Species	*L. casei*	98	0.66	5.00	*L. rhamnosus*

Table 18. Collection of raw data from replication #2 showing identification of various lactobacilli using the Biolog Microlog Automated System – continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. casei A-17	Species	*L. rhamnosus*	100	0.68	4.91	*L. hamsteri*
L. casei N-7	Species	*L. rhamnosus*	98	0.69	4.58	*L. casei*

[1] Identification type describes to what degree an identification was made: no identification; to the genus level; or to the species level.

[2] Opt1 indicates the best match as option 1 for identification; where as the next best match is listed under Opt2.

[3] PROB allows you to compare the Biolog IDs to other systems that use this type of calculation.

[4] SIM is a similarity index that indicates how good the sample results match the database pattern of the organism listed as Opt1. (SIM = 1.0 is a perfect match; a positive ID must be based on SIM > 0.5)

[5] DIST is the distance or number of mismatches between the sample results and the database pattern for the organism listed as Opt1. (a positive ID must be based on DIST < 5.0 with the DIST of the first and second choices being more than two distance points apart)

Table19. Collection of raw data from replication #3showing identification of various lactobacilli using the Biolog Microlog Automated System

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus ATCC 4356	NO ID	--	--	--	--	--
L. acidophilus L-1	Species	*L. crispatus*	100	0.74	3.85	*L. vaginalis*
L. acidophilus O-16	Species	*L. gasseri*	99	0.59	6.34	*L. alimentarius*
L. acidophilus K-4	Species	*L. hamsteri*	70	0.54	3.43	*L. delbrueckii* ss *lactis*
L. acidophilus J-12	Species	*L. gasseri*	89	0.64	4.27	*L. hamsteri*
L. acidophilus H-13	Species	*B. meycicum*	80	0.54	5.05	*B. pullorum*
L. acidophilus D-3	Species	*L. gasseri*	86	0.74	2.00	*L. hamsteri*
L. acidophilus B-11	Species	*L. catenaformis*	97	0.61	5.80	*B. asteroides*
L. acidophilus 381-IL-28	NO ID	--	--	--	--	--
L. acidophilus L-23	Species	*L. crispatus*	91	0.50	7.08	*L. helveticus*
L. acidophilus A-4	Species	*L. hamsteri*	84	0.61	4.04	*L. acidophilus BGA*

Table19. Collection of raw data from replication #3 showing identification of various lactobacilli using the Biolog Microlog Automated System - continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus GP2B	Species	*L. hamsteri*	86	0.65	3.70	*L. delbrueckii* ss *lactis*
L. acidophilus GP3A	Species	*L. delbrueckii* ss *lactis*	97	0.59	6.00	*L. crispatus*
L. acidophilus RP32	Genus	*L. hamsteri*	--	0.36	7.63	*L. acidophilus* BGB
L. acidophilus C2-5	NO ID	--	--	--	--	--
L. acidophilus D-1	Species	*L. hamsteri*	62	0.50	3.05	*L. crispatus*
L. acidophilus A-6	Species	*L. hamsteri*	95	0.73	3.49	*L. acidophilus BGA*
L. acidophilus 6-L4	Species	*W. viridescens*	72	0.60	2.65	*L. delbrueckii* ss *delbrueckii*
L. acidophilus 6-S4	Species	*L. delbrueckii* ss *delbrueckii*	99	0.66	5.06	*L. sanfranciscens*
L. acidophilus C-2	Species	*P. propionicus* BGA	100	0.73	4.04	*E. hallii*
L. acidophilus T-4	Species	*L. crispatus*	100	0.70	4.61	*L. delbrueckii* ss *delbrueckii*

Table 19. Collection of raw data from replication #3 showing identification of various lactobacilli using the Biolog Microlog Automated System - continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. acidophilus NFa-5	Species	*L. hamsteri*	89	0.59	5.28	*L. delbrueckii ss lactis*
L. acidophilus NFa-8	Species	*L. hamsteri*	96	0.57	6.32	*L. delbrueckii ss lactis*
L. acidophilus PLb-3	NO ID	--	--	--	--	--
L. acidophilus RAT-1	Species	*P. propionicus BGA*	100	0.65	5.26	*C. tyrobutyricum*
L. casei E-5	Species	*L. hamsteri*	88	0.56	5.55	*L. rhamnosus*
L. casei E-10	Species	*L. delbrueckii* ss *lactis*	99	0.77	3.30	*W. minor*
L. casei M-5	Species	*L. rhamnosus*	100	0.69	4.71	*L. paracasei* ss *paracasei*
L. casei M-12	Species	*L. plantarum*	99	0.59	6.23	*L. alimentarius*
L. casei L-19	Species	*L. casei*	81	0.50	6.00	*L. paracasei* ss *paracasei*

Table19. Collection of raw data from replication #3 showing identification of various lactobacilli using the Biolog Microlog Automated System - continued

Sample	ID TYPE[1]	Opt1[2]	PROB[3]	SIM[4]	DIST[5]	Opt2
L. casei A-17	Species	*L. paracasei* ss *paracasei*	100	0.61	6.00	*L. rhamnosus*
L. casei N-7	Species	*L. rhamnosus*	100	0.79	3.12	*L. casei*

[1] Identification type describes to what degree an identification was made: no identification; to the genus level; or to the species level.

[2] Opt1 indicates the best match as option 1 for identification; where as the next best match is listed under Opt2.

[3] PROB allows you to compare the Biolog IDs to other systems that use this type of calculation.

[4] SIM is a similarity index that indicates how good the sample results match the database pattern of the organism listed as Opt1. (SIM = 1.0 is a perfect match; a positive ID must be based on SIM > 0.5)

[5] DIST is the distance or number of mismatches between the sample results and the database pattern for the organism listed as Opt1. (a positive ID must be based on DIST < 5.0 with the DIST of the first and second choices being more than two distance points apart)

APPENDIX F

COLLECTION OF RAW DATA FROM THE RIBOPRINTER

Table 20. Collection of raw data from replications #1, #2, and #3 showing identification of various lactobacilli by the RiboPrinter

Sample	REP1 [1]	REP2 [2]	REP3
L. acidophilus ATCC 4356	*Lactobacillus acidophilus*	NO ID	NO ID
L. acidophilus L-1	NO ID	NO ID	NO ID
L. acidophilus O-16	*Lactobacillus acidophilus*	NO ID	*Lactobacillus gasseri*
L. acidophilus K-4	*Lactobacillus acidophilus*	NO ID	*Lactobacillus acidophilus*
L. acidophilus J-12	*Pseudomonas pseudoalcaligenes*	NO ID	NO ID
L. acidophilus H-13	NO ID	NO ID	NO ID
L. acidophilus D-3	NO ID	NO ID	
L. acidophilus B-11	NO ID	NO ID	
L. acidophilus 381-IL-28	*Lactobacillus animalis*		
L. acidophilus L-23	NO ID		
L. acidophilus A-4	NO ID		
L. acidophilus GP2B	NO ID		
L. acidophilus GP3A	*Lactobacillus acidophilus*		
L. acidophilus RP32	*Vibrio cholerae*		
L. acidophilus C2-5	*Lactobacillus acidophilus*		
L. acidophilus D-1	NO ID		
L. acidophilus A6	NO ID		
L. acidophilus 6-L4	NO ID		
L. acidophilus 6-S4	*Lactobacillus acidophilus*		
L. acidophilus C2	NO ID		
L. acidophilus T-4	NO ID		

Table 20. Collection of raw data from replications #1, #2, and #3 showing identification of various lactobacilli by the RiboPrinter - continued

Sample	REP1 [1]	REP2 [2]	REP3
L. acidophilus NFa-5	NO ID		
L. acidophilus NFa-8	NO ID		
L. acidophilus PLb-3	NO ID		
L. acidophilus RAT-1	*Vibrio cholerae*		
L. casei E-5	NO ID	NO ID	
L. casei E-10	NO ID		
L. casei M-5	NO ID		
L. casei M-12	NO ID		
L. casei L-19	NO ID		
L. casei A-17	NO ID		
L. casei N-7	NO ID		

[1]Replication 1 for each sample on the RiboPrinter was completed.

[2] Replications 2 and 3 were only completed for a subset of the samples as the results were not useful, and the testing is expensive.

APPENDIX G

COLLECTION OF RAW DATA FROM THE VITEK

Table 21. Collection of raw data from replication #1 showing identification of various lactobacilli by Vitek

Sample	Opt1[1]	Prob[2]	Opt2	Prob	Opt3	Prob	Confidence Level[3]
L. acidophilus ATCC 4356	*Actinomyces odontolyticus*	70	*Lacotabacillus acidophilus*	27	*Actinomyces israelii*	1	GCMS
L. acidophilus L-1	*A. odontolyticus*		*Clostridium perfringens*				Unidentified
L. acidophilus O-16	*L. acidophilus*						Questionable
L. acidophilus K-4	*A. odontolyticus*	70	*L. acidophilus*	27	*A. israelii*	1	GCMS
L. acidophilus J-12	*A. odontolyticus*		*C. perfringens*		*Bifidobacterium species*		Unidentified
L. acidophilus H-13	*A. odontolyticus*		*Bifidobacterium species*		*C. histolyticum*		Unidentified
L. acidophilus D-3	*A. odontolyticus*	70	*L. acidophilus*	27	*A. israelii*	1	GCMS
L. acidophilus B-11	*C. perfringens*		*Bifidobacterium species*				Unidentified

Table 21. Collection of raw data from replication #1 showing identification of various lactobacilli by Vitek – continued

Sample	Opt1[1]	Prob[2]	Opt2	Prob	Opt3	Prob	Confidence Level[3]
L. acidophilus 381-IL-28	*C. perfringens*						Unidentified
L. acidophilus L-23	*A. odontolyticus*	70	*L. acidophilus*	27	*A. israelii*	1	Good
L. acidophilus A-4	*A. odontolyticus*	70	*L. acidophilus*	27	*A. israelii*	1	GCMS
L. acidophilus GP2B	*A. odontolyticus*		*C. perfringens*				Unidentified
L. acidophilus GP3A	*A. odontolyticus*	78	*L. acidophilus*	19	*A. israelii*	1	GCMS
L. acidophilus RP32	*A. odontolyticus*	71	*L. acidophilus*	28	*A. israelii*	<1	GCMS
L. acidophilus C2-5	*A. odontolyticus*		*C. perfringens*				Unidentified
L. acidophilus D-1	*A. odontolyticus*		*C. perfringens*				Unidentified
L. acidophilus A-6	*A. odontolyticus*		*C. perfringens*				Unidentified
L. acidophilus 6-L4	*A. odontolyticus*	99					Acceptable
L. acidophilus 6-S4	*C. perfringens*		*Bifidobacterium species*				Unidentified

Table 21. Collection of raw data from replication #1 showing identification of various lactobacilli by Vitek – continued

Sample	Opt1[1]	Prob[2]	Opt2	Prob	Opt3	Prob	Confidence Level[3]
L. acidophilus C-2	*C. perfringens*		*Bifidobacterium species*		*A. odontolyticus*		Unidentified
L. acidophilus T-4	*A. odontolyticus*		*C. perfringens*				Unidentified
L. acidophilus NFa-5	*A. odontolyticus*		*Bifidobacterium species*				Unidentified
L. acidophilus NFa-8	*Bifidobacterium species*		*C. perfringens*				Unidentified
L. acidophilus PLb-3	*A. naeslundii*		*C. tertium*		*L. jensenii*		Unidentified
L. acidophilus RAT-1	*A. odontolyticus*		*C. perfringens*				Unidentified
L. casei E-5	*C. perfringens*						Unidentified
L. casei E-10	*C. perfringens*						Unidentified
L. casei M-5	*L. acidophilus*		*C. perfringens*		*L. casei*		Questionable

Table 21. Collection of raw data from replication #1 showing identification of various lactobacilli by Vitek - continued

Sample	Opt1[1]	Prob[2]	Opt2	Prob	Opt3	Prob	Confidence Level[3]
L. casei M-12	*L. acidophilus*	99					Very Good
L. casei L-19	*L. acidophilus*		*C. perfringens*		*L. casei*		Questionable
L. casei A-17	*L. casei*	98	*L. acidophilus*	1	*L. jensenii*	<1	Acceptable
L. casei N-7	*C. perfringens*		*Bifidobacterium species*				Unidentified

[1]The Vitek offers several possible identifications for an organism: Opt1 is option 1 for the best possible match; Opt2 is the next best option for identification; and Opt3 is the third best option for identification.

[2] Prob is a % probability that is generated for each option to indicate the probability that a positive identification can be associated with that option.

[3]Confidence level for the identifications is expressed as: excellent, very good, or acceptable; good confidence but marginal separation [GCMS]; questionable; or unidentified.

VITA

Trenna Diann Blagden

Candidate for the Degree of

Doctor of Philosophy

Thesis: COMPARISON OF CONVENTIONAL AND AUTOMATED METHODOLOGY FOR IDENTIFICATION AND CHARACTERIZATION OF LACTOBACILLI

Major Field: Food Science

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Scope and Method of Study: Numerous potential health benefits of consumption of products containing probiotics have been highlighted in research for many years. More recently, the prospective role probiotics may offer in response to antibiotic resistance and other medical issues has become a topic of interest. Methods that appropriately identify and characterize lactobacilli to fully understand the potential of probiotic strains based on metabolic activities as well as host specificity is a necessity. Attention is now focused on comparing various automated systems and conventional methodology for identification and characterization capable of distinguishing host specificity and selection of a probiotic strain for use as treatments or answers in modern medicine or as supplements for healthy living.

Findings and Conclusions: Thirty-two previously isolated cultures of lactobacilli were analyzed using various methodologies in an effort to rapidly identify and further classify their probiotic potential. The results obtained from the Vitek and the RiboPrinter systems were inconclusive and failed to provide reliable, reproducible identifications for these isolates. The Biolog and Sherlock MIDI MIS systems demonstrated potential for use as tools to identify and characterize isolates of lactobacilli. The Biology and Sherlock MIDI MIS systems provided positive identifications of the isolates at a species level. However, these two systems were unable to reliably provide the same species identification within a series of replications. In both systems it was observed that the top choices were more often switched in order from one replication to the next. The Sherlock MIDI MIS system appeared to offer the most potential for characterization of the strains given a positive identification was made by the system. Use of a larger pool and variety within strains in development of the databases or libraries for the automated systems, along with culture preparation techniques specific to lactobacilli would greatly enhance the applicability of some of these automated systems for use in appropriately identifying and characterizing the probiotic potential of isolates of lactobacilli.

ADVISER'S APPROVAL: Dr. Stanley E. Gilliland