Invited Review: The Scientific Basis of Lactobacillus acidophilus NCFM Functionality as a Probiotic

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ABSTRACT

Lactobacillus acidophilus NCFM is a probiotic strain available in conventional foods (milk, yogurt, and toddler formula) and dietary supplements. Its commercial availability in the United States since the mid-1970s is predicated on its safety, its amenability to commercial manipulation, and its biochemical and physiological attributes presumed to be important to human probiotic functionality. The strain has been characterized in vitro, in animal studies, and in humans. NCFM is the progenitor of the strain being used for complete chromosome sequencing and therefore will be a cornerstone strain for understanding the relationship between genetics and probiotic functionality. Both phenotypic and genotypic techniques have verified its taxonomic status as a type A1 L. acidophilus strain. It adheres to Caco-2 and mucus-secreting HT-29 cell culture systems, produces antimicrobial compounds, and is amenable to genetic manipulation and directed DNA introduction. NCFM survives gastrointestinal tract transit in both healthy and diseased populations. NCFM inhibits aberrant crypt formation in mutagenized rats, indicative of activity that could decrease the risk of colon cancer. A blend of probiotic strains containing NCFM decreased the incidence of pediatric diarrhea. NCFM led to a significant decrease in levels of toxic amines in the blood of dialysis patients with small bowel bacterial overgrowth. At adequate daily feeding levels, NCFM may facilitate lactose digestion in lactose-intolerant subjects. Further validation of the probiotic properties of NCFM in humans and clarification of its mechanisms of probiotic action are needed to better understand the role this strain might play in promoting human health.

(Key words: Lactobacillus acidophilus NCFM, probiotic, functional food)

INTRODUCTION

Probiotic functionality depends on the ability of a strain to confer health advantages on a host upon oral consumption of viable cells. Many different strains and species of lactobacilli (Table 1) and bifidobacteria have been used commercially as probiotics. Numerous mechanisms have been proposed for probiotic functionality and many clinical end targets have been measured (Sanders, 1999). However, it is difficult to assess the health effects that might be expected from probiotic consumption by the general population, since most research to date has focused on animal studies, biomarkers, or small human study group sizes. More extensive epidemiological evaluations will be necessary to better study these properties. Since such intervention trials are quite costly, it is important that strains be well characterized prior to this use. Concomitantly, extensive strain characterization is important prior to commercial development or use. This has led to research focused on probiotic strains of commercial interest. Lactobacillus acidophilus NCFM is one such strain (Figure 1).

Lactobacillus acidophilus NCFM was isolated and characterized in the food microbiology research laboratories at North Carolina State University (NCSU) in Raleigh by M. Speck and S. Gilliland and was subsequently studied there by T. Klaenhammer. The strain was isolated from a human source in the 1970s (Gilliland et al., 1975) and has since been the subject of research at NCSU and other institutions worldwide. This strain has been sold commercially (Rhodia, Inc., Madison, WI) for over 25 yr for use in the formulation of fluid milks and yogurts containing probiotic cultures, in dried dietary supplements, in toddler formula and in juice. (See Appendix for description of published strain designations for NCFM.)

This article will summarize research findings on NCFM.
Table 1. *Lactobacillus* species used as human probiotics.

<table>
<thead>
<tr>
<th>Species</th>
<th>Obligately homofermentative</th>
<th>Facultatively heterofermentative</th>
<th>Obligately heterofermentative</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>L. crispatus</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>L. amylovorus</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>L. gallinarum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. johnsonii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. delbrueckii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subsp. bulgaricus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. salivarius</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subsp. salivarius</td>
<td></td>
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</tr>
</tbody>
</table>

Table 2. Growth and fermentation characteristics of NCFM compared with the neotype *Lactobacillus acidophilus* strain (Gilliland and Speck, 1977b). +, growth; −, no growth.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NCFM</th>
<th><em>L. acidophilus</em> ATCC 4356 (Neotype)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth @ 15°C</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth @ 45°C</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ammonia from arginine</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cellobose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Salcin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 1. Electron micrograph of *Lactobacillus acidophilus* NCFM cells.

Taxonomy

The taxonomy of *L. acidophilus* has undergone significant revisions over the past decade, leading to the establishment of several new species (Klaenhammer and Russell, 1999; Klein et al., 1998). NCFM has been shown by several phenotypic (Gilliland and Speck, 1977b; Table 2) and genotypic (Kullen et al., 2000; Sanders et al., 1996) criteria to be a member of the type A1 *L. acidophilus* species. Hybridization with a species-specific oligonucleotide probe (5’TCTTTCGATGCATCCACA 3’; Pot et al., 1993) using slot blots provided further evidence that NCFM belongs to the type A1 *L. acidophilus* group (Sanders, et al., 1996). Sequencing of the 16S ribosomal RNA gene of NCFM confirmed its identity as *L. acidophilus* (Kullen et al., 2000). Fermentation and growth characteristics of NCFM are identical to the *L. acidophilus* neotype strain ATCC 4356 (Gilliland and Speck, 1977b; Table 2). It is a Gram-positive, homofermentative, catalase-negative rod. Its DNA has a 38.4% GC ratio. Fermentation results in 34% D- and 66% L-lactic acid. The percentage of D- and L-lactic acid of NCFM and other species of probiotic lactobacilli is shown in Table 3 (Girgis et al., 2000).

Studies using pulsed-field gel electrophoresis have shown that NCFM is highly related to the type *L. acidophilus* strain, ATCC 4356 (Figure 2). Band differences between strain 4356 and NCFM are apparent, although the genomic organization is predominantly the same. This technique shows restriction nuclease digestion patterns of total chromosomal DNA, and, as such, can discriminate between strains. However, small differences within the DNA sequence would not be detected by this technique unless they disrupt a restriction site used in the analysis.

Genetic Access

The ability to introduce homologous or heterologous DNA into commercial lactobacilli enables useful re-

Table 3. Percentage of lactic acid enantiomers formed by selected *Lactobacillus* strains. Lactic acid was measured using a commercial kit from Boehringer Mannheim (Indianapolis, IN; catalogue number 1112821).

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>% D-Lactic acid</th>
<th>% L-Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em></td>
<td>NCFM</td>
<td>33.8</td>
<td>66.2</td>
</tr>
<tr>
<td><em>L. acidophilus</em></td>
<td>ATCC 4356</td>
<td>21.5</td>
<td>78.5</td>
</tr>
<tr>
<td><em>L. amylovorus</em></td>
<td>ATCC 3620</td>
<td>56.3</td>
<td>43.7</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>ATCC 393</td>
<td>2.6</td>
<td>97.4</td>
</tr>
<tr>
<td><em>L. crispatus</em></td>
<td>ATCC 33820</td>
<td>56.4</td>
<td>43.6</td>
</tr>
<tr>
<td><em>L. delbrueckii</em></td>
<td>ATCC 4797</td>
<td>99.5</td>
<td>0.5</td>
</tr>
<tr>
<td><em>L. gallinarum</em></td>
<td>ATCC 33199</td>
<td>39.0</td>
<td>61.0</td>
</tr>
<tr>
<td><em>L. gasseri</em></td>
<td>ATCC 33323</td>
<td>59.1</td>
<td>40.9</td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td>NCK 936</td>
<td>40.5</td>
<td>59.5</td>
</tr>
<tr>
<td><em>L. johnsonii</em></td>
<td>ATCC 33200</td>
<td>54.6</td>
<td>45.4</td>
</tr>
</tbody>
</table>
search approaches, including strain improvement and investigation into the genetic linkage of important commercial traits. The lactobacilli have typically been refractory to high efficiency DNA transfer methods, especially transformation. *Lactobacillus acidophilus* NCFM was transformed with plasmid vectors with protocols developed by Luchansky et al. (1989, 1991) and Walker and Klaenhammer (1996), whereas transformation of the neotype strain, *L. acidophilus* ATCC 4356, resulted in significantly fewer or no transformants. Walker and Klaenhammer (1996) demonstrated reliable introduction of DNA into NCFM and other true *L. acidophilus* strains at frequencies of $2 \times 10^4$ chloramphenicol-resistant transformants/µg of pGK12 DNA. The physiological attributes of the NCFM strain enabling more efficient transformation are not known. However, NCFM appears to be better suited for genetic analysis than the type strain of *L. acidophilus*.

Genetic studies pave the way for future enhancement of the healthful properties of NCFM. A recent study by Kullen and Klaenhammer (1999) identified an acid-inducible operon (ATPase) in NCFM. This and similar systems in other bacteria are responsible for maintaining the acid tolerance under low pH conditions. Understanding the regulation of this operon could provide insight into the possible benefits of delivering probiotics in fermented foods and lead to improved survival under acid conditions. Other genetic studies have focused on understanding the mechanisms of bacteriocin production (Klaenhammer et al., 1992).

**Stability**

Stability of commercial probiotic strains is important to assure that stated levels of viable cells are delivered in probiotic products. Frozen culture concentrates of NCFM were tested for stability during frozen storage (Sanders et al., 1996). (In this publication, NCFM was coded as commercial strain LH1.) The results showed that concentrated cultures of NCFM lost only about a quarter of a log cycle in viability over 6 wk frozen storage at −20°C. Stability in a dried format at room temperature resulted in steadily decreasing viability until less than $10^2$ viable cells per gram were detected after 8 mo of storage (Crowell, 1998). The stability of NCFM in commercial products at room temperature has not been published.

NCFM showed excellent stability in milk inoculated with about $10^7$ cfu/ml. After 21 d at 4°C or 10°C, levels of NCFM were essentially unchanged (Sanders et al., 1996). NCFM was also stable in commercially produced fermented dairy products (Iturriria et al., 1999). After 52 d of storage at 4°C, NCFM counts in strawberry yogurt fell only from $1.2 \times 10^7$ cfu/g to $8.7 \times 10^6$ cfu/g and in plain yogurt from $2.4 \times 10^7$ cfu/g to $1.5 \times 10^7$ cfu/g, reflecting minimal decreases in numbers. However, comparative counts of NCFM on MRS agar, with and without 0.15% bile, revealed that about 50% of the population was injured after 52 d of storage in plain yogurt. In cottage cheese, counts fell from $6 \times 10^4$ cfu/g to $3 \times 10^4$ cfu/g, a 50% drop in viable cells after 37 d at 4°C. The losses reflected here indicate that NCFM is suitably stable in commercial dairy products.

Stability is dependent on many factors, including growth conditions and storage conditions (relative humidity, oxygen content, stabilizers, and temperature). The importance of growth conditions, including the presence of calcium, in the production of stable frozen...
Antimicrobial Activity

Antimicrobial activity is thought to be an important means for probiotic bacteria to competitively exclude or inhibit activities of harmful or pathogenic intestinal microbes. Antimicrobial compounds produced by probiotic bacteria include organic acids (lactic and acetic acid), hydrogen peroxide (in environments in which oxygen is present), diacetyl, β-hydroxypropionaldehyde (produced by *Lactobacillus reuteri*) or bactericidal or bacteriostatic peptides and proteins (DeVuyst and Vandamme, 1994). The ability to inhibit a range of microbes (pathogenic, spoilage, and other lactic acid bacteria) in laboratory assays and cocultivation experiments has been demonstrated repeatedly with many members of lactic acid bacteria. Figure 3 shows bacteriocin activity produced by *L. acidophilus* NCFM against another *Lactobacillus* strain, *Lactobacillus delbrueckii* subsp. *lactis* ATCC 4797 (Barefoot and Klaenhammer, 1983). What is less clear, however, is the role that inhibitory activity plays in vivo. A reduction in fecal pH and an increase in short chain fatty acids has been correlated with higher fecal counts of lactobacilli and bifidobacteria in some feeding studies, suggesting that probiotic growth and concomitant production of fermentative metabolites in vivo have a measurable, physiological influence. The role of bacteriocins in vivo is, however, less clear. Bacteriocins are proteinaceous compounds produced by bacteria that exhibit a bactericidal or bacteriostatic activity against susceptible bacteria. In vitro studies have shown that the majority of strains of *Lactobacillus* frequently (but not always), produce bacteriocins that kill closely related species. The roles and in vivo activities of *Lactobacillus* bacteriocins remain to be demonstrated.

Antagonistic activity was produced by NCFM against foodborne disease agents, *Staphylococcus aureus*, *Salmonella typhimurium*, enteropathogenic *Escherichia coli*, and *Clostridium perfringens* (Gilliland and Speck, 1977a). This study documented an 80 to 90% inhibition of these pathogens in broth culture under laboratory conditions. Inhibition resulted from organic acids, hydrogen peroxide, and perhaps other antimicrobial products.

*L. acidophilus* NCFM was found to produce a bacteriocin, designated lactacin B (Barefoot and Klaenhammer, 1983). The biochemical and genetic properties of this bacteriocin were characterized in detail (Barefoot and Klaenhammer, 1983; Barefoot et al., 1994; Klaenhammer et al., 1992; Nettles, 1992). In vitro tests of inhibition indicated a range of activity only against other *Lactobacillus* strains and *Enterococcus faecalis*, not against pathogens (Barefoot and Klaenhammer, 1983). The molecular mass of this bacteriocin was determined to be 6500 and its sensitivity to proteinase K and pronase confirmed its proteinaceous nature. It is stable to heat (121°C, 3 min, pH 5), cold (−20°C), and chemical chaotropic agents (β-mercaptoethanol, 8 M urea, and 1% SDS). Properties of this bacteriocin have been reviewed extensively (Barefoot et al., 1994; Klaenhammer et al., 1992).

Adherence

Adherence and colonization are attributes considered to be important for probiotic functionality, particularly in roles in which intestinal enterocytes are stimulated. Intimate, extended association with the intestinal mucosa is likely to require adherence of the bacterium with the epithelial cells, and without it, the probiotic may not influence some important physiological parameters. Unfortunately, in vitro studies on adherence are often not predictive of the in vivo situation, since conditions in vitro are so vastly different than in vivo. Furthermore, even strains isolated from human sources are allochthonous or nonnative to consumers, and, as such, should not be expected to behave as native strains do. Gastrointestinal (GI) tract colonization by autochthonous strains is mature and reasonably stable from early childhood (Tannock, 1999). Results from probiotic feeding studies suggest that exogenous strains, even those demonstrating adhering capability in vitro and sup-

**Figure 3.** Zones of inhibition produced by NCFM bacteriocin against *Lactobacillus delbrueckii* subsp. *lactis* ATCC 4797. This bacteriocin also inhibited strains of *Lactobacillus bulgaricus*, *Enterococcus faecalis*, *Lactobacillus fermentum* and *Lactococcus lactis* (Barefoot and Klaenhammer, 1983).
plied in great number, generally do not persist (Alander et al., 1999; Bouhnik, et al., 1992). Although some adherence may occur, it does not appear to be at high efficiency or accompanied by prolific colonization, as populations of probiotics steadily drop to undetectable levels in feces when consumption has ceased. Studies employing biopsies of intestinal tissues are rare, but are necessary to confirm adherence and retention at key locations in the GI tract.

Nevertheless, the adhering capability to intestinal cells in tissue culture is a commonly assessed attribute for probiotic strains, and NCFM has been included in such studies. *L. acidophilus* NCFM was shown to adhere to human fetal intestinal cells and Caco-2 cells, and the level of attachment was enhanced in the presence of calcium ions (Greene and Klaenhammer, 1994; Kleeman and Klaenhammer, 1982). The mucus-secreting intestinal cell line, HT-29, was thought to more closely mimic in vivo conditions, providing an improvement over the nonsecreting intestinal cell lines. Later evidence from cell culture experiments suggested that NCFM adheres through a protein-mediated mechanism, although other probiotic lactobacilli may use carbohydrate moieties as well. Compared with other lactobacilli assayed, this adherence was high and fairly stable at physiologically relevant pH values between 6 and 8 (Greene and Klaenhammer, 1994). Using scanning electron microscopy, Hood and Zottola (1987) showed that NCFM did not appear to have an external polysaccharide layer, an observation that may be important to cell surface binding capability. The adherence of NCFM compared with other lactobacilli is summarized in Table 4. These results provide in vitro evidence that NCFM can adhere to human cells, but the fate of NCFM inside the human intestine has not been assessed.

**Cholesterol**

The reduction of total cholesterol or low-density lipoproteins found in human plasma is thought to lower the risk of coronary heart disease. Probiotic cultures have been suggested to play a role in reduction of these blood lipids, although research to date is equivocal (Taylor and Williams, 1998). Studies on *L. acidophilus* NCFM (Gilliland et al., 1985; Gilliland and Walker, 1990) suggested its ability to remove cholesterol from a laboratory growth medium. NCFM was reported to take up cholesterol in the presence of bile and in the absence of oxygen, both conditions present in the intestinal tract. Evaluation of other *L. acidophilus* strains showed that cholesterol assimilation was strain-dependent. The significance of these in vitro studies cannot, however, be determined without human studies that evaluate serum cholesterol levels in NCFM consumers. Cholesterol-assimilating and isogenic, nonassimilating strains must be compared in a clinical trial to assess the importance of this attribute.

Thompson et al. (1982) fed 250 ml of Sweet Acidophilus milk daily to 12 healthy subjects for a 9-wk study period, as part of a wider study on the effects of different dairy products on serum cholesterol. Sweet Acidophilus is a registered trademark of the North Carolina Dairy Foundation (NCSU, Raleigh, NC) and is used to describe low fat milk supplemented with $2 \times 10^6$ cfu/ml NCFM cells. The test product was consumed for 3 wk of this time. The authors concluded that among Sweet Acidophilus milk, yogurt, and buttermilk, none had a significant effect on serum cholesterol. The levels of viable *L. acidophilus* NCFM were not reported in the study, although typical Sweet Acidophilus milk formulation levels would have provided $-2 \times 10^6$ cfu of viable *L. acidophilus* per day in this study. It would be worthwhile to determine whether feeding humans higher levels of NCFM would have a different effect.

**Gastrointestinal Tract Influence**

An important attribute for certain probiotic bacteria functions is survival and growth in the intestinal tract. Once in the GI tract, probiotic bacteria have the opportunity to influence the populations and activities of different intestinal bacteria. Therefore, demonstrating that a probiotic can survive GI transit and influence GI tract flora is important for establishing probiotic characteristics.

Gilliland et al. (1978) demonstrated that levels of lactobacilli increase significantly in the feces of human subjects consuming nonfermented milk containing NCFM. Because the technology was not available at that time to specifically track the NCFM strain, GI survival of NCFM was deduced from increases in total fecal lactobacilli.

More recently, Crowell (1998) studied the ability to recover NCFM from both healthy and ill individuals consuming $2 \times 10^{10}$ NCFM daily. NCFM could be isolated from the feces of healthy subjects and from jejunal contents and brushings of chronic kidney failure patients fed NCFM in capsules. (Capsules were coated with hydroxypropylmethyl cellulose phthalate to enhance NCFM survival through the stomach.) To specifically identify NCFM among indigenous lactobacilli, isolates of lactobacilli were randomly chosen and subjected to pulsed field gel electrophoresis. Chromosomal DNA patterns specific to NCFM were identified among those isolates. NCFM was not recovered from fecal samples from any of five subjects after NCFM feeding had been stopped for 2 wk (Figure 4). This suggests that to achieve probiotic functionality, NCFM should be re-
### Table 4. Comparison of adherence properties of NCFM and other probiotic lactobacilli. +, adherence observed; −, no adherence; HT29, indicates HT29 or HT29-MTX cells.

<table>
<thead>
<tr>
<th>Lactobacillus species and strain</th>
<th>Cell line used in adherence assay</th>
<th>Factors involved in adherence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HITH s0074</td>
<td>Caco-2</td>
<td>HT29</td>
</tr>
<tr>
<td><em>L. acidophilus</em> NCFM</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. acidophilus</em> LA</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. acidophilus</em> LB</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. acidophilus</em> LA1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. crispatus</em> BG2FO4</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. gasseri</em> ADH</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. plantarum</em> 299 &amp; 299V</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. delbrueckii</em> 1489</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 4.** Pulsed-field gel electrophoresis patterns of chromosomal DNA from lactobacilli isolated from healthy humans fed $2 \times 10^{10}$ cfu/day NCFM (Crowell, 1998). A, prefeeding; B, during feeding; C, 2 wk postfeeding. Arrows indicate pattern generated by NCFM.
Lactose Intolerance

The consumption of lactose by certain people can result in intestinal discomfort due to the colonic fermentation of lactose that passes through the small intestine undigested. Fermented dairy products have been repeatedly shown to enhance tolerance to lactose compared with unfermented products or lactose alone (Shah, 1993). This observation has been attributed in part to the ability of lactic acid bacteria to serve as a source of lactase in the small intestine, contributing to the digestion of lactose in the lactase-deficient person. Studies have suggested that, in general, the yogurt starter cultures, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (organisms that do not survive intestinal passage) are better at decreasing breath hydrogen production and symptoms of lactose maldigestion than lactobacilli of intestinal origin, even when the studies controlled for cell count (Lin et al., 1991, 1998). A few studies of probiotic lactobacilli have been shown to improve lactose digestion or symptoms in lactase-deficient individuals (Kim and Gilliland, 1983; Lin et al. 1998; Montes et al., 1995; Mustapha et al., 1997).

Total cellular lactase levels are not always predictive of a strain’s ability to aid lactose digestion in vivo (Mustapha et al., 1997). This may be because strains differ in their ability to make lactase available in the small intestine (lactase stability, lactose transport, and lactase release) or due to the nature of cell lysis and lactase assay. Bile sensitivity and acid tolerance seem to be attributes important to lactase availability in vivo (Mustapha et al., 1997).

Several in vitro evaluations of lactase levels of NCFM have been reported. Lactase activity on a colony forming unit-basis was reported to be 4.43 μg of α-nitrophenol × 10⁻⁷/cfu per minute for NCFM (Sanders et al., 1996), a level that was high among all probiotic lactobacilli tested. Levels for the other probiotic lactobacilli in the study ranged from <0.01 to 4.73 μg of α-nitrophenol × 10⁻⁷/cfu per minute. However, compared to levels observed for all *S. thermophilus* strains (which ranged from 11.8 to 189 μg of α-nitrophenol × 10⁻⁷/cfu per minute), levels of lactase in probiotic lactobacilli were low. Hughes and Hoover (1995), in a paper comparing NCFM to some bifidobacteria strains, showed that the lactase activity of NCFM was roughly equivalent to *Bifidobacterium bifidum, Bifidobacterium breve,* and *Bifidobacterium longum* strains examined. Nielsen and Gilliland (1992) conducted some basic experiments to purify and characterize the lactase enzyme of NCFM, providing biochemical information on the enzyme’s size, conditions for optimum activity, and stability to refrigerated storage. These data demonstrate the active lactase present in NCFM.

In rats fed unfermented milk containing NCFM and a water control, no temporal changes in lactase activity in the stomach, small intestine, cecum, or colon were observed, although a diet supplemented with yogurt did improve lactase levels in the small intestine only (Rao et al., 1991). This indicates that this milk may not be the most advantageous formulation for targeting improvement of lactose digestion. Both the choice of lactic bacterium (yogurt cultures compared with *L. acidophilus*) and formulation levels (~10⁸ cfu/g in yogurt compared to 2 × 10⁶ cfu/g in unfermented, culture-
containing milk) could be optimized for the desired physiological effect.

Several studies have examined the effect of dairy products containing NCFM on lactose intolerance in human subjects. Newcomer et al. (1983) tested NCFM added to milk at fairly low levels (2 × 10^9/ml) to aid the digestion of lactose-deficient subjects, but showed no improvement, a result also demonstrated by McDonough et al. (1987) and Payne et al. (1981) using NCFM in Sweet Acidophilus milk. In contrast, NCFM included at high levels in milk was found to reduce lactose intolerance symptoms (bloating, diarrhea, gas) in 9 of 10 lactose-maldigesting children (Montes et al., 1995). Similarly, Kim and Gilliland (1983) found that NCFM improved lactose utilization in human subjects, although other studies did not confirm this finding (Lin et al., 1991; McDonough et al., 1987; Newcomer et al., 1983; Savaiano et al., 1984).

In interpreting these results, it is important to note that adequate levels of the culture must be consumed for a positive effect. Some products with L. acidophilus contain levels of the culture insufficient to help with lactose digestion. These studies show that not all uses of NCFM resulted in an improved digestion of lactose. However, given in adequately high levels, some symptom relief and improved digestion of lactose may occur in lactose-maldigesters when they consume NCFM. Presumably, these effects result from the bacterium’s ability to metabolize lactose during digestion and transit through the GI tract.

Small Bowel Bacterial Overgrowth

The influence of NCFM on microflora-associated symptoms experienced by dialysis patients with chronic kidney disease has been studied (Simenhoff et al., 1996). As a result of their disease, these patients suffer from disruption of the environment of their small intestine, leading to an abnormally high level of bacterial growth in this organ. This condition is known as small bowel bacterial overgrowth. The small bowel normally harbors relatively few microbes. Although colonization of the distal ileum reflects that of the cecum, the rapid transit time and high bile concentrations generally limit small intestinal colonization. Small bowel bacterial overgrowth can be diagnosed directly by confirmation of elevated microbe counts in small intestinal samples, or indirectly by determining a significant increase in breath hydrogen 45 min after lactulose ingestion, indicating bacterial fermentation in the small bowel. (Colonic fermentation of this substrate occurs much later.) Bacterial overgrowth is accompanied by production of toxic metabolites, including nitrosodimethylamines and dimethylamines. These compounds can be measured in the blood of the patients.

Blinded, placebo-controlled studies demonstrated that feeding freeze-dried preparations of NCFM lowered the levels of dimethylamine and nitrosodimethylamines in the blood of these patients (Dunn et al., 1998a, 1998b; Simenhoff et al., 1996). In addition, the nutritional status of the patients improved, as evidenced by increased calorie consumption and weight gain (Dunn et al., 1998b). No adverse effects were observed with NCFM treatment. These studies suggest that NCFM inhibits the populations or activities of dimethylamine- and nitrosodimethylamine-producing overgrowth bacteria, and can positively influence colonization of the small bowel.

Anti-carcinogenicity

Studies have been conducted in animal and human systems to evaluate the control of negative activities of human intestinal flora. Animal experiments, which evaluate the levels or time-course of mitogen-induced tumor development or indices of tumor development are important since this type of evaluation can never be conducted directly in humans. Activities of microbial enzymes, which are thought to play a role in the conversion of procarcinogens to carcinogens, have been measured in feces. These enzymes include β-glucuronidase, nitroreductase, and azoreductase. Studies conducted on rats consuming a meat-based diet showed a lower incidence of colon cancer after a 20-wk experimental period, suggesting that the NCFM supplement increased the latency period for colon cancer in experimental rats (Goldin and Gorbach, 1980). Administration of NCFM along with antibiotics was shown to decrease colon tumors in rats (Goldin and Gorbach, 1984c). Goldin and Gorbach (1984a) also tested the effect of feeding NCFM on the production of free amines in the feces of rats consuming a meat-based diet. They found that NCFM-fed rats had a significantly lower level of free amines. Using human subjects, Goldin and Gorbach (1984b) found that daily consumption of milk containing NCFM resulted in a 2- to 4-fold reduction in the activity of these three fecal enzymes. Similar results were found in another human study (Goldin and Gorbach, 1984c) and also in rats (Goldin et al., 1980).

More recently, a blinded, placebo-controlled study was conducted to evaluate the effect of NCFM on abnormal lesions in the colon of mutagenized rats (Rao et al., 1999). This study found a 29 and 39% inhibition of these aberrant colonic crypts in rats fed freeze-dried NCFM at 2 and 4% of their diet, respectively.

Taken together, these results support a positive role of NCFM in reducing potentially harmful microbial activities in the intestine.
Immune Function

It has been postulated that probiotic bacteria may mediate antipathogenic and anticarcinogenic activities through their ability to positively influence the mucosal and systemic immune responses. NCFM has been tested for this ability in two animal systems. Wagner et al. (1997a) demonstrated that NCFM elicited antibody- and cell-mediated responses to *Candida albicans* in immunodeficient mice, which was thought to play a role in decreasing the severity of candidiasis. The levels of serum IgG, IgA, and IgM were greater in euthymic immunocompromised mice. Interestingly, the *Bifidobacterium* strain used in this study increased serum IgG, IgA, and IgM levels in athymic and euthymic mice, while treatment with the *L. reuteri* strain resulted in early death of all athymic mice. Strain-specific responses are evident in the study of this model system.

Yogurt prepared with a yogurt culture (DPL ABY-2C, Rhodia, Inc.) containing NCFM, *S. thermophilus*, *L. bulgaricus*, and *Bifidobacterium infantis* was tested for its effect on the mucosal and systemic IgA and IgG response of mice immunized orally with cholera toxin (Tejada-Simon et al., 1999). Intestinal and serum IgA specific for cholera toxin were higher in mice fed the yogurt prepared with *S. thermophilus*, *L. bulgaricus*, *B. infantis*, and NCFM than in mice fed skim milk or mice fed yogurt made with only *S. thermophilus* and *L. bulgaricus*. These results suggest that a combined culture including NCFM may increase immune response to oral antigens, although the specific role of NCFM, if any, in these results was not determined in these studies.

Urogenital Applications

Urogenital infections are widespread in women. These infections are frequently caused by *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*, which originate in the intestinal tract. The use of ingested or intravaginal applications of probiotic bacteria to control the incidence of these infections has been explored. NCFM was tested using several laboratory assays for traits thought to be useful in helping prevent urinary and vaginal tract infections (Reid, 2000). NCFM was suggested to produce a biosurfactant that inhibited by over 90% the adhesion of *Enterococcus faecalis* 1131 to polystyrene. NCFM was also shown to adhere to uroepithelial and vaginal epithelial cells collected from urine of healthy premenopausal women during midmenstrual cycle. Furthermore, preincubation of NCFM with these same cells with subsequent exposure to three uropathogens (*E. coli* Hu734, *K. pneumoniae*, and *P. aeruginosa* AK1) showed that NCFM competitively excluded these pathogens, with inhibitions of 30, 11, and 30%, respectively. Because hydrogen peroxide production may play a role in competitive exclusion of these urogenital pathogens, levels were assessed for NCFM. NCFM was found to produce H$_2$O$_2$, but at a low level relative to varying levels found among different strains (Figure 5). NCFM characteristics found through these in vivo tests suggest its potential application in the control of urogenital infections.

Diarrhea

The specific effect of NCFM as a single strain on diarrhea has not been studied. However, NCFM was included as part of a probiotic blend tested on pediatric populations in Mexico. Initially, tolerance to the probiotic blend (containing *L. acidophilus* NCFM, *L. reuteri*, and *B. infantis* BB1) was tested in a placebo-controlled study of 72 subjects 12 to 36 mo of age randomized into four groups (Ruiz-Palacios et al., 1996a). The groups received either placebo, or formula providing a dose of 10$^5$, 10$^8$, or 10$^{10}$ cfu of probiotic culture daily. No adverse reactions were detected over the 3-wk feeding period. This initial evaluation was followed by investigation into the effect of probiotic-supplemented formula on the prevention of community-acquired diarrhea (Ruiz-Palacios, et al., 1996b). In one study, children aged 12 to 36 mo were randomized into two groups, one receiving a beverage containing the probiotic blend described above at 10$^{10}$ cfu/d (119 subjects completed study) and the other receiving a control (120 subjects). Both lactobacilli were provided in the beverage at equal levels, whereas the *Bifidobacterium* strain was present at approximately 1 log cycle lower levels. Intake, tolerance, stool...
pattern, and fecal counts of lactobacilli were monitored. A higher portion of children fed the probiotic were free of diarrhea (90/119) than the control group (77/120), although no differences were seen in severity of diarrhea. Seven cases of diarrhea were caused by rotavirus in the control group, whereas only two cases were diagnosed as such among the probiotic group. In another study, a 16-wk feeding study was conducted with children 12 to 32 mo of age to determine the effect of two different probiotic preparations on diarrhea (Guerrero et al., 1996). In this study, three groups were defined as follows: control (132 subjects), probiotic beverage with L. reuteri, L. acidophilus NCFM, and B. infantis BBI (130 subjects) and probiotic beverage with a different strain of L. acidophilus and Bifidobacterium (133 subjects). The probiotic drink containing L. reuteri, L. acidophilus NCFM, and B. infantis BBI significantly decreased the incidence of diarrhea. Reductions in diarrhea were reduced in both probiotic beverage groups. These studies demonstrate that L. acidophilus NCFM is well tolerated among pediatric populations even in high doses. However, the role of NCFM in reducing the risk of diarrhea in young children cannot be specifically determined from these studies.

Competitive Colonization

The ability of NCFM to protect two different strains of immunodeficient mice from Candida infection was evaluated by Wagner et al. (1997a). The oral and anal cavities of these mice were inoculated with NCFM and then exposed to Candida albicans. Mice not colonized with NCFM showed high levels of Candida throughout the GI tract. Lower levels of Candida were present in mice associated with NCFM. In addition, significantly lower levels of lethality were seen at both 4 wk and 8- to 12-wk assessment periods. Mice of one strain associated with NCFM showed no mortality, whereas mice from this same strain exposed to Candida without probiotic showed 50 and 100% mortality at these time periods, respectively. These results clearly show the protective effect that NCFM can have against systemic infections.

Safety

In an immunodeficient mouse model system, Wagner et al. (1997b) evaluated the tolerance of neonatal and adult mice to colonization by NCFM. Neither neonatal nor adult mice suffered any mortality when colonized with high levels of NCFM (10^6 to 10^7 cfu/g of feces in neonates and 10^8 to 10^10 cfu/g of feces in adults). Although L. reuteri did not translocate, some mortality of neonatal mice was observed with this bacterium, suggesting the need to proceed cautiously when using high doses of this strain in neonatal, immunocompromised hosts. Furthermore, production of serum IgM and IgG was induced by NCFM in one strain of immunocompromised mice, supporting a potential role for this bacterium in immunomodulation activities.

CONCLUSIONS

In conclusion, a significant body of research aimed at understanding L. acidophilus NCFM at microbiological, genetic, and clinical levels has been conducted over the past 25 yr. These studies have provided insight into the probiotic functionality of this strain. Furthermore, this strain has been used successfully in commercial applications, with a minimum of technological hurdles. Confirmation of probiotic functionality will, however, require well-controlled clinical evaluations aimed at appropriate target populations and clinical end points.

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**APPENDIX**

Multiple strain designations appear in the literature for NCFM or for single colony isolates of the NCFM parent culture. The designations NCFM, N2, NCK56, NCK45, N2 and RL8K are essentially identical strains. The parent NCFM culture carried the NCSU laboratory designation RL8K and was composed of rough and smooth variants. These variants were designated RL8K-R (bile sensitive) and RL8K-S (bile resistant) upon isolation from the RL8K culture (Klaenhammer and Kleeman, 1981). N2 is a smooth, bile-resistant isolate from NCFM selected by scientists at Marschall Products (now Rhodia Inc.) as a bile-resistant colony, and as this pure culture, is used as the seed for all commercial production runs of NCFM. The mixed parent culture has not been used commercially or for research studies since before1975. NCK56 is a Klaenhammer laboratory designation for N2, and NCK45 is a Klaenhammer laboratory designation for the parent culture NCFM comprised of rough and smooth colony variants NCFM. These different isolates cannot be differentiated genetically by pulsed field gel electrophoresis (Walker and Klaenhammer, 1996), a technique which provides an electrophoretic pattern of restriction enzyme digested chromosomal DNA. A purified isolate of the RL8K-S culture, designated ATCC700396, is undergoing chromosomal DNA sequencing (Cano and Wiloughby, 1999; Klaenhammer, 1998). In some papers involving NCFM, the strain is not identified or NCFM
may be present only as one strain in a combination of several lactic acid bacteria. However, the source of the culture used is generally provided, indicating that the strain used was *L. acidophilus* from Marschall, Miles, Rhône-Poulenc or Rhodia. These different company names reflect changes in business structure or ownership of the company marketing NCFM, but not in marketing rights. In some cases, commercial cultures provided to a study were coded to keep the identity of specific strains confidential. In studies conducted by Simenhoff and colleagues on small bowel bacterial overgrowth in chronic kidney failure patients, *L. acidophilus* NCFM was abbreviated LBA. Any confusion over use of NCFM in publications reviewed for this paper was clarified by personal communications with company representatives and researchers. NCFM is a registered trademark of the North Carolina Dairy Foundation.