

Evaluation of Cheddar Cheese as a Food Carrier for Delivery of a Probiotic Strain to the Gastrointestinal Tract

G. GARDINER,^{*,1} C. STANTON,^{*,1} P. B. LYNCH,^{*,2}
J. K. COLLINS,^{†,3} G. FITZGERALD,^{†,3} and R. P. ROSS^{*,1,4}

^{*}Teagasc, Moorepark, Fermoy, County Cork, Ireland

[†]University College Cork, Ireland

ABSTRACT

Cheddar cheese was evaluated as a food carrier for the delivery of viable microorganisms of *Enterococcus faecium* (Fargo 688[®]; Quest Int., Naarden, The Netherlands) to the gastrointestinal tract. This strain had previously been shown to possess properties required of a probiotic microorganism including the ability to relieve irritable bowel syndrome. The strain was found to survive to high numbers in Cheddar cheese during ripening at 8°C for 15 mo (4×10^8 cfu/g) and in yogurt during storage at 4°C for 21 d (4×10^7 cfu/g). In an in vitro model system, Cheddar cheese was found to have a greater protective effect than yogurt upon exposure of the probiotic culture to porcine gastric juice at pH 2. Subsequently, a feeding trial involving 8 pigs per group was performed in which a rifampicin-resistant variant of the probiotic strain was fed for 21 d at a mean daily intake of 1.3×10^{10} cfu/d from Cheddar cheese or 3.7×10^9 cfu/d from yogurt. During the feeding period, Cheddar cheese yielded a significantly higher mean fecal probiotic count (2×10^6 cfu/g of feces) than did yogurt (5.2×10^5 cfu/g of feces). These data indicate that mature Cheddar cheese compares very favorably with fresh yogurt as a delivery system for viable probiotic microorganisms to the gastrointestinal tract.

(**Key words:** probiotic, Cheddar cheese, carrier, viable)

Abbreviation key: GIT = gastrointestinal tract, KAAA = kanamycin azide aesculin agar, RSM = reconstituted skim milk.

INTRODUCTION

Recent market studies have shown an increasing demand for functional foods containing live probiotic

cultures. In response to this growing demand for probiotic foods, the portfolio of products containing such health-promoting cultures is expanding. Although considerable marketing and research attention has focused on fermented milks and yogurts as food carriers for probiotic microorganisms (44), these products may not be optimal for the maintenance of high concentrations of some strains, as evidenced by poor viability (particularly of *Bifidobacterium* strains) in a range of commercial yogurts surveyed in both Europe and Australia (25, 30). Furthermore, it is now becoming evident that other foods offer potential for administration of these cultures [for review (44)]. For example, both *Bifidobacterium bifidum* and *Lactobacillus acidophilus* strains have been shown to survive at concentrations between 10^6 and 10^8 cfu/g or ml in ice cream (7, 21), frozen yogurt (22), and other frozen dairy desserts (27). In addition, various cheese varieties including Cheddar (10, 12), Gouda (19), cottage (4, 33), white-brined (14), caprine (18), and Crescenza (16) cheeses have been assessed as carriers for both probiotic *Lactobacillus* and *Bifidobacterium* strains.

For these products to be categorized as probiotic, it is important that the culture that is added at the time of manufacture remain viable at high concentrations during the relevant shelf-life or storage period. In general, the minimum concentration of probiotic microorganisms necessary to exert a beneficial effect remains unclear. However, a standard introduced by the Fermented Milks and Lactic Acid Bacteria Beverages Association in Japan stipulates that at least 10^7 viable bifidobacteria should exist per gram or milliliter of product to constitute a probiotic food (24), which is considerably higher than the therapeutic minimum dose of 10^5 viable cells/g or ml of product, which has otherwise been proposed (28).

Probiotics have most recently been defined as "living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (20). Health effects associated with the consumption of probiotic microorganisms that have been best substantiated include alleviation of lactose intolerance [for review, (29)], prevention or treatment of specific types of diarrhea (40), and stimula-

Received October 30, 1998.

Accepted February 22, 1999.

¹Dairy Products Research Center.

²Pig Production Department.

³Department of Microbiology.

⁴Corresponding author: Paul Ross, Teagasc, Dairy Products Research Center, Moorepark, Fermoy, County Cork, Ireland.

tion of the immune system [for review, (15)], whereas the role of lactic acid bacteria in areas such as the reduction of blood cholesterol and cancer prevention remains controversial. For probiotic microorganisms to exert these health benefits, large numbers of viable cells must survive passage through the harsh environment of the gastrointestinal tract (GIT), hence the need to select strains that are acid and bile tolerant and possess the capability to adhere to intestinal cells (8). In addition, the food product used to deliver the probiotic culture may influence the ability of the probiotic to survive in the GIT (42). Further criteria desirable for a probiotic strain include properties such as being of intestinal origin and safe for consumption and having clinically validated health effects (8, 39).

The present study investigates the efficacy of Cheddar cheese as a delivery system for probiotic microorganisms and uses a probiotic strain of *Enterococcus faecium* (Fargo 688[®]; Quest Int., Naarden, The Netherlands). This strain was chosen because it fulfills many of the probiotic criteria outlined above: being of intestinal origin, nonpathogenic, and bile and acid tolerant (23). Moreover, the probiotic efficacy of this strain has previously been demonstrated in clinical trials with 70% of irritable bowel syndrome patients experiencing an alleviation of symptoms following probiotic treatment (2). The present study compares Cheddar cheese and yogurt as systems for the delivery of this enterococcal probiotic strain, initially by means of an in vitro model system using extracted gastric juice and subsequently in an in vivo system by means of a pig-feeding trial. The data showed that 15-mo-old Cheddar cheese containing the probiotic strain delivered high concentrations of viable bacteria to the GIT as effectively as freshly prepared probiotic yogurt.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Fargo 688[®], a probiotic strain was identified as *Enterococcus faecium* by SDS-PAGE analysis of total cell protein (36). The strain was obtained in the form of a spray-dried powder that contained $>2 \times 10^{10}$ cfu/g. The *Enterococcus* strain was isolated from this powder with kanamycin azide aesculin agar (KAAA; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) as a selective medium and was stocked in LM17 (45; Difco Laboratories, Detroit, MI) containing 40% glycerol. A spontaneous rifampicin-resistant mutant of this strain (Fargo 688[®] Rif^r) was isolated by spread-plating 100 μ l (or 1 ml concentrated to 100 μ l)

of an overnight culture on LM17 agar containing 500 μ g/ml of rifampicin (Sigma Chemical Co., Poole, Dorset, United Kingdom). Following incubation at 37°C for 72 h, the colonies that had grown were selected, confirmed as identical to the parent strain by the randomly amplified polymorphic DNA (RAPD) method (12), and stocked as described above. Both Fargo 688[®] and Fargo 688[®] Rif^r were routinely cultured in LM17 broth at 37°C. *Lactococcus lactis* ssp. *cremoris* strains 227 and 223, obtained from Chr. Hansen's Laboratories (Little Island, Cork, Ireland) in the form of freeze-dried pellets, were used as starters for cheesemaking. These were grown overnight at 21°C in 10% (wt/vol) reconstituted skim milk (RSM) that had been previously heat-treated (90°C for 30 min). A starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (CH1; Chr. Hansen's Laboratories) was used for yogurt making and grown in 10% RSM at 45°C until the pH was reduced to 4.5 (~4 h).

Cheddar Cheese Manufacture and Bacteriological Analyses

Pilot-scale Cheddar cheeses (450 L of cheese milk) were manufactured as previously described (12) with the addition of 1.5% inoculum of the mixed strain starter culture to each vat. One vat was a control, to which starter culture only was added, and two experimental vats contained the probiotic strain as an adjunct to the starter culture. The Fargo 688[®] probiotic powder (containing 2×10^{10} cfu/g) was added at 0.01% (wt/vol) to one experimental vat, and the second was inoculated at 2% with Fargo 688[®] Rif^r (used for subsequent selective enumeration in feeding trials) grown overnight in 10% (wt/vol) RSM. Cheeses were ripened at 8°C for 9 to 15 mo and aseptically sampled in duplicate for bacteriological analysis at intervals during this period. Cheddar cheese samples were emulsified in sterile 2% (wt/vol) trisodium citrate and diluted in maximum recovery diluent (Oxoid Ltd.), and appropriate dilutions were pour-plated. Probiotic cultures were enumerated in the cheeses by plating on KAAA following overnight incubation at 37°C (Fargo 688[®]), or on LM17 agar containing 500 μ g/ml of rifampicin after incubation for 3 d at 37°C (Fargo 688[®] Rif^r). Viability of lactobacilli and starter lactococci in the cheeses during ripening was determined on LM17 agar following 3 d of incubation at 30°C and on *Lactobacillus* selective agar (38; Becton Dickinson, Cockeysville, MD, USA) following 5 d of incubation at 30°C under anaerobic conditions (anaerobic jars with 'Anaerocult A' gas packs; Merck, Darmstadt, Germany), respectively.

Yogurt Manufacture and Bacteriological Analyses

Yogurt was manufactured on a laboratory scale as follows: homogenized pasteurized milk fortified with 4% (wt/vol) skim milk powder and 1% (wt/vol) sucrose (Sigma Chemical Co.) was heat treated at 90°C for 30 min, cooled to 43°C, and a 1.5% inoculum of CH1 starter culture was added. The control yogurt contained starter culture only, whereas probiotic yogurts contained an additional 1% inoculum of either Fargo 688® or Fargo 688® Rif^r grown overnight in 10% (wt/vol) RSM supplemented with 1.5% Bios 2000 (Visby, Tonder, Denmark). Inoculated milks were dispensed into plastic cartons in 100-ml volumes, incubated at 43°C, and the fermentation was terminated at pH 4.5, at which point the yogurt was cooled on ice and stored at 4°C for up to 22 d. At regular intervals during this storage period, viability of both bacteria in the yogurt was determined by plating serial dilutions of Fargo 688® on KAAA and incubating overnight at 37°C and of Fargo 688® Rif^r on LM17 agar containing 500 µg/ml of rifampicin and incubating for 3 d at 37°C.

Gastric Juice Studies

Gastric contents collected from 13 porcine stomachs (obtained from a local slaughter plant), which ranged in pH from 2.3 to 4.0, were pooled and filtered through glass wool. Porcine gastric juice was obtained by centrifugation twice at 13,000 × *g* for 30 min and filtration (Whatman no. 113 filter; Whatman International Ltd., Maidstone, Kent, United Kingdom). The gastric juice was then checked for sterility by plating on M17 agar containing 1% glucose and incubating at 37°C for 3 d. The pH was adjusted to 2 using 1*N* HCl, and the gastric juice was stored at -20°C until use. The protective effect of Cheddar cheese or yogurt on the probiotic culture upon exposure to gastric juice at pH 2 was then investigated as follows. A number of sterile test tubes were prepared, each containing 10 ml of porcine gastric juice at pH 2. To achieve a final probiotic cell concentration of approximately 10⁸ cfu/ml, either 5 g of grated Cheddar cheese or 5 ml of yogurt, both containing the enterococcal probiotic strain, were added to each test tube, and the contents were mixed well. All test tubes were incubated in a shaking water bath at 37°C, and probiotic survival was determined at 0, 5, 30, 60, 90, and 120 min by removing one tube at each time point. For the yogurt-gastric juice mixture, 10 ml were immediately neutralized at the appropriate time point, by adding 0.1*N* NaOH, and further diluted (1 in 10) with maximum recovery diluent. Subsequent dilutions were performed, and probiotic survival was de-

termined on KAAA pour plates, which were incubated overnight at 37°C. At each time point, probiotic viability in the Cheddar cheese-gastric juice mixture was determined by neutralizing the entire mixture, further diluting 1 in 10 with 2% trisodium citrate and homogenizing in a stomacher (Lab-Blender 400; Seward Medical, London, United Kingdom). Subsequent dilutions were performed in diluent, and the enterococcal probiotic strain was enumerated as described earlier. To determine the ability of the probiotic cells alone to survive in gastric juice at pH 2, cells were harvested from an overnight culture in LM17, washed in phosphate buffer at pH 6.5, and resuspended in gastric juice to achieve a final cell concentration of approximately 10⁸ cfu/ml. Viable probiotic cells were enumerated at 0, 2.5, 5.5, 7.5, 10, and 12.5 min intervals by neutralizing 10 ml of the inoculated gastric juice, diluting, and pour-plating as previously described. Subsequently, the survival of washed probiotic cells in gastric juice pH 4.74 and 3.65 (adjusted using 1*N* NaOH) was evaluated as just described. These pH values were chosen as representative of those pH values to which 10-ml gastric juice, pH 2, increases upon addition of 5 g of Cheddar cheese or 5 ml of yogurt, respectively.

Feeding Trial

A total of 32 crossbred pigs (12 males and 20 females), weaned at ~27 d of age, were ranked by weight within sex, and three blocks of four males per block and five blocks of four females per block formed. Blocks represented pigs of the same sex and of similar weight, and within each block pigs were allocated at random to four groups (as outlined later) for a randomized block design. Each pig was housed individually with control pigs housed in isolation from probiotic pigs to prevent cross-contamination. In addition to Cheddar cheese or yogurt, as outlined later, all pigs received commercial creep feed ('Startrite 90'; SCA Ltd., Naas, Co. Kildare, Ireland) prepared without antibiotics. The feeding trial consisted of three consecutive periods: adjustment period (4 d), probiotic administration period (21 d), and postadministration period (10 d). During the administration period, 100 g of 15-mo-ripened Cheddar cheese containing 4.9 × 10⁸ cfu/g of Fargo 688® Rif^r was provided to one group (n = 8) of pigs daily, while another group (n = 8) received 100 ml of yogurt (stored for a maximum of 1 wk) containing 3.7 × 10⁷ cfu/ml of the same microorganism. In addition, two corresponding control groups (n = 8) received either 100 g of cheese or 100 ml of yogurt without probiotic culture. Fecal samples were collected twice weekly, stored at 4°C, and ana-

lyzed within 24 h. Previous studies had shown no difference in bacterial counts between fresh and stored samples. Fecal samples were homogenized in maximum recovery diluent as 10-fold dilutions, further diluted, and appropriate dilutions were pour-plated. The probiotic strain (Fargo 688[®] Rif^r) was enumerated on KAAA containing 500 $\mu\text{g/ml}$ of rifampicin as a selective agent and 100 $\mu\text{g/ml}$ pimaricin (Merck) to inhibit yeasts and molds after incubation for 2 d at 37°C. In addition, coliform bacteria were enumerated on violet red bile agar (VRBA; Oxoid Ltd.) following incubation at 37°C for 24 h. Additional parameters monitored throughout the trial included creep feed intake and body weight gain. Statistical analyses (ANOVA to compare treatments and regression analysis to investigate the relationship between intake and probiotic excretion) were performed using Genstat (13).

RESULTS AND DISCUSSION

Cheddar cheese has previously been shown to support the survival of high concentrations (10^7 to 10^8 cfu/g) of some probiotic microorganisms during the ripening period (10, 12, 44), thereby indicating its potential as a probiotic dairy product. The present study evaluates Cheddar cheese as a delivery system for a probiotic microorganism *E. faecium* Fargo 688[®]. For the purposes of this evaluation, Cheddar cheese harboring this probiotic strain was compared with yogurt containing the same microorganism, initially in vitro and subsequently in vivo.

Manufacture of Probiotic Cheddar Cheese and Yogurt

The enterococcal probiotic strain was incorporated during manufacture as a starter adjunct into both Cheddar cheese and yogurt, and its survival over time in both products was monitored. This probiotic strain survived in yogurt during storage for 21 d at 4°C at concentrations between 3.1 and 4.6×10^7 cfu/ml (Figure 1a) and in Cheddar cheese during 9 mo (250 d) of ripening at concentrations of 1.1 to 3.9×10^8 cfu/g, having grown from an initial (d 1) concentration of 6.9×10^7 cfu/g (Figure 1b). Similarly, a Rif^r variant of the probiotic strain maintained viability in yogurt during the 22-d storage period and in Cheddar cheese during 15.5 mo (430 d) of ripening and was present at concentrations of 4.2×10^7 cfu/ml and 1.7×10^8 cfu/g, respectively, in the stored products (Figure 1a and b). The shelf-life of each of the products investigated here differs considerably; yogurt, a fresh product, is generally consumed within days or weeks of manufacture, whereas Cheddar cheese is ripened for a considerable period (at least 6 mo).

Survival of Probiotic Strain in Porcine Gastric Juice

Among the desirable properties recommended for a probiotic microorganism is the ability to tolerate acidic conditions such as those encountered in the stomach so that bacterial viability is maintained during gastric transit (8, 39). Apart from the intrinsic acid resistance of the probiotic strain, survival during passage through the stomach is also influenced by the nature of the food carrier used for delivery of the probiotic. Therefore, the ability of the enterococcal probiotic cells to resist gastric transit was investigated in yogurt or Cheddar cheese by means of a simulation in vitro, whereby the viability of the strain (alone or from Cheddar cheese or yogurt) was tested in porcine gastric juice, pH 2. Porcine gastric juice was used because subsequent feeding trials were performed in pigs, and a pH value of 2 was chosen to simulate conditions in the porcine stomach during fasting (11). The results demonstrated that viable probiotic counts were reduced from 1.4×10^7 to 8.8×10^5 cfu/ml after incubation for 5.5 min in gastric juice, pH 2, and after only 8 min, no viable probiotic cells were detectable (Figure 2). In a parallel experiment in which the probiotic strain was added in Cheddar cheese to gastric juice, pH 2, no loss of viability was observed; concentrations of 4.6 to 6.6×10^7 cfu/ml were maintained over a 2-h period (Figure 2). Similarly, when probiotic yogurt was added to gastric juice, only a 10-fold reduction in probiotic numbers (from 1×10^7 to 1×10^6 cfu/ml of gastric juice) was observed over the 2-h exposure period (Figure 2). These data show that food carriers such as Cheddar cheese or yogurt greatly enhance the survival of this strain in gastric juice, which is most likely due to the buffering capacity of the food product. Other studies have shown a similar buffering effect; for example, addition of milk to gastric juice has been found to significantly increase the pH and enhance survival of *Lactobacillus* species in this environment (9), and Charteris et al. (6) have shown improved gastric transit tolerance for some *Lactobacillus* and *Bifidobacterium* species in simulated gastric juice upon milk protein addition. Also, Pettersson et al. (34) have demonstrated improved survival of *L. acidophilus* in gastric juice when the pH was increased from 3.8 to 6.8 with milk. Furthermore, the data presented in this study indicate that Cheddar cheese may be a more effective food carrier for the probiotic strain than yogurt. This result may be accounted for by the greater buffering capacity of Cheddar cheese; addition of 5 g of cheese to 10 ml of gastric juice increased the pH from 2 to 4.74, whereas 5 ml yogurt increased the pH to only 3.65.

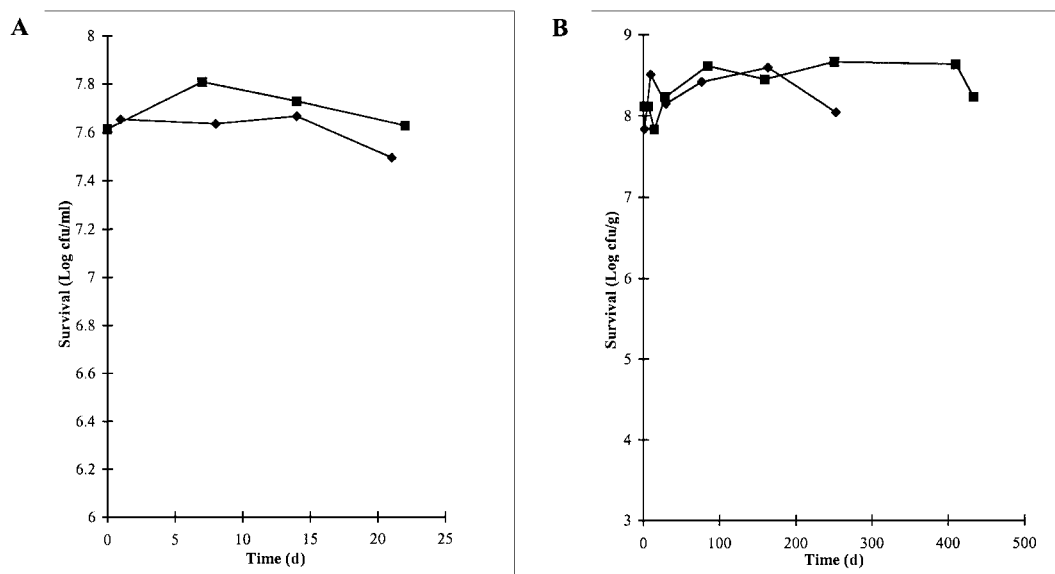


Figure 1. Survival of Fargo 688® (◆) and Fargo 688® Rifr (■) (Quest Int., Naarden, The Netherlands) in yogurt during storage at 4°C for 21 d (A) and in Cheddar cheese during ripening at 8°C for 250 to 430 d (9 to 15 mo) (B). Day 0 represents the day of manufacture.

To investigate whether probiotic protection in these food environments was solely due to buffering capacity of the food, the effect of gastric juice at pH 4.74 and 3.65 on the probiotic strain was determined. The probiotic strain remained viable at concentrations between 1.4 and 2.4×10^7 cfu/ml of gastric juice for 2 h at pH 4.74 (Figure 3), yielding a survival pattern similar to that observed when probiotic Cheddar cheese was added to gastric juice, pH 2 (Figure 2). However, when the probiotic strain was exposed to gastric juice, pH 3.65, a 1 million-fold reduction in cell numbers was observed after 1 h with viable counts declining from 1.6×10^7 to 2×10^1 cfu/ml of gastric juice, during this period (Figure 3). Thus, gastric juice, pH 3.65, was found to have a more lethal effect on the probiotic cells alone than did gastric juice, pH 2, when the probiotic strain was added in yogurt (1 million-fold decline in 1 h compared with a 10-fold decline in 2 h), although the final pH of the yogurt-gastric juice mixture was also 3.65. These data suggest that there are factors other than buffering capacity, possibly including properties such as the presence of protective extracellular polysaccharide, which may increase the survival of the probiotic strain when exposed to gastric juice, pH 2. Cheddar cheese may also possess protective characteristics apart from buffering capacity, such as the dense matrix and high fat content, which may have contributed to the enhanced survival of the probiotic microorganisms upon exposure to gastric juice. Indeed, a previous study has shown that *Bifidobacterium pseudolongum*, when pro-

tected by microencapsulation, survives a simulated gastric environment in larger numbers than when unprotected (37).

Pig-Feeding Studies

Although we have shown that the enterococcal probiotic carried in Cheddar cheese survives incubation with gastric juice in vitro, survival in the GIT can only be accurately determined in vivo. We have previously shown (44) that Cheddar cheese is as effective as yogurt for delivery of viable probiotic lactobacilli to the porcine small intestine. In the present study, fecal probiotic concentrations were examined to compare the ability of Cheddar cheese and yogurt to carry viable probiotic enterococci through the porcine GIT. The pig provides a suitable model system because the GIT is physiologically and anatomically similar to that of humans (31). To trace the administered probiotic strain, it was necessary to use an antibiotic (rifampicin)-resistant variant (Fargo 688® Rifr), which could be selectively enumerated using antibiotic-containing medium. During the 21-d administration period (d 0 to 20, for which d 0 represents the first day of probiotic administration), this probiotic strain was ingested by one group of pigs ($n = 8$) in Cheddar cheese at a mean daily intake of 1.3×10^{10} cfu/d, while a second group ($n = 8$) received 3.7×10^9 cfu/d in yogurt. In addition to the probiotic-fed groups, two corresponding (cheese and yogurt) control groups ($n = 8$) received no probiotic, and at no

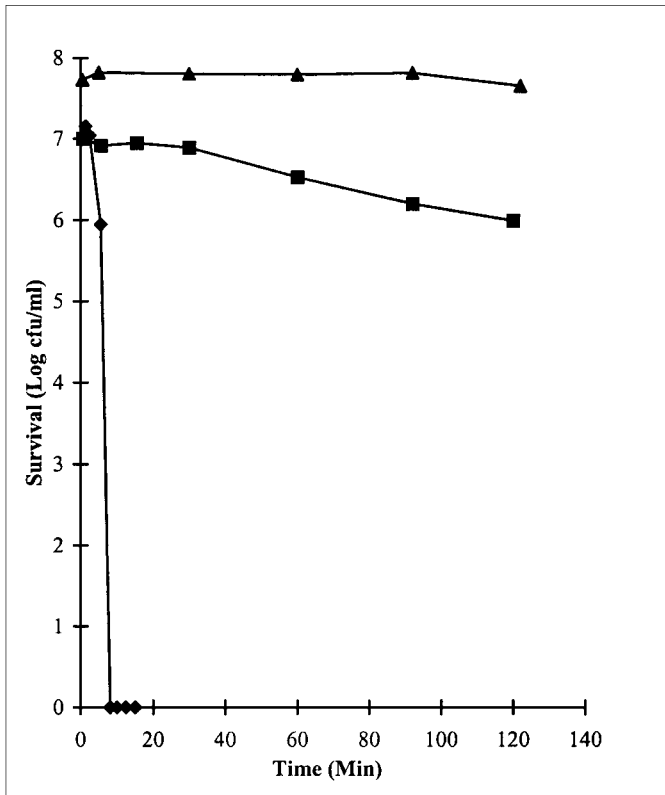


Figure 2. Survival of Fargo 688® (Quest Int., Naarden, The Netherlands) washed cells (◆) in yogurt (■) or in Cheddar cheese (▲) in porcine gastric juice, pH 2, over time.

time during the study were these pigs found to excrete rifampicin-resistant enterococci. Prior to administration of the first probiotic feed on d 0, this strain was not detected in the feces of any pig. However, all probiotic-fed pigs excreted the organism by d 3 and continued to do so during the 21-d administration period (Figure 4). Although probiotic intake remained relatively constant throughout the administration period, initial (d 3) concentrations of probiotic excretion in both the cheese-fed and yogurt-fed groups were 10-fold higher than were subsequent excretion concentrations (Figure 4). This result may be due to an inability of the probiotic strain to colonize the GIT upon initial administration. More importantly, during the probiotic feeding period of 21 d, Cheddar cheese yielded a significantly ($P < 0.05$) higher mean fecal probiotic count (2×10^6 cfu/g) than did yogurt (5.2×10^5 cfu/g) (Figure 4). The mean fecal recovery of the probiotic strain in both cheese-fed and yogurt-fed groups declined to 3.9×10^1 and 9.6 cfu/g of feces, respectively, 8 d after probiotic feeding ended (Figure 4). At this time, the probiotic microorganism could still be detected in 62% of

cheese-fed pigs and 57% of yogurt-fed pigs. The data suggest that administration of the probiotic strain in mature Cheddar cheese resulted in significantly higher mean concentrations of daily probiotic excretion than did freshly prepared yogurt, suggesting that Cheddar cheese is more effective than yogurt as a delivery system for viable probiotic organisms to the porcine GIT, even though the product was aged for 15 mo.

Fecal recovery of ingested probiotic strains has previously been investigated in pigs (26, 46) as well as in humans (1, 5, 17, 43). Although probiotic bacteria have been administered in many different forms, including dried preparations (41), capsules (43), and fermented milks (5, 17), there are few reports concerning the effect of the carrier used for probiotic delivery. Saxelin et al. (42) compared fecal recovery of *Lactobacillus* GG in humans, following oral administration either in a fermented milk or as an enterocoated tablet, and found no significant difference between these carriers. However, it was concluded that both the enterocoated tablet and fermented milk offered more effective means of

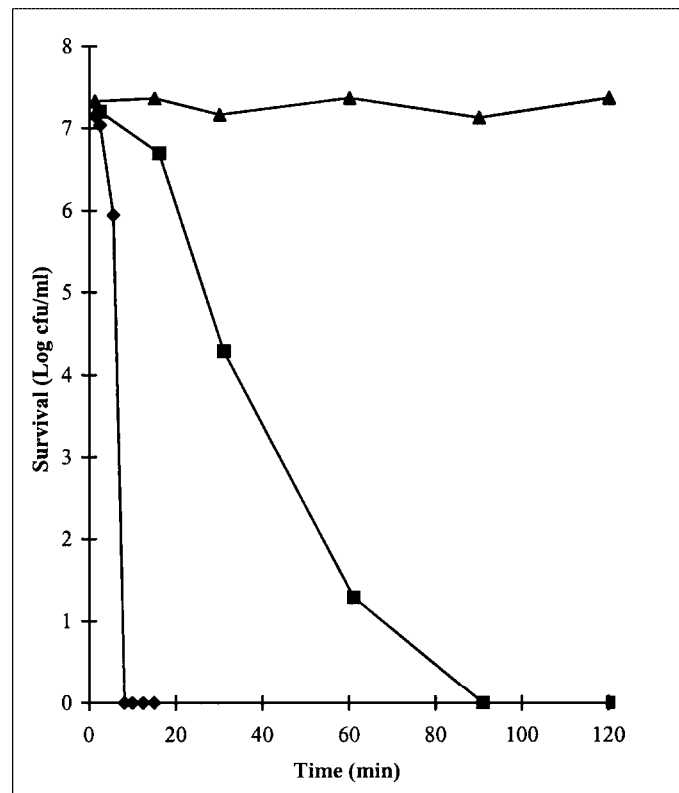


Figure 3. Survival of Fargo 688® (Quest Int., Naarden, The Netherlands) washed cells in porcine gastric juice at pH 2 (◆), 3.65 (■), or 4.74 (▲) over time.

administering this strain than did a freeze-dried powder form previously used (41). Although this study established that the vehicle used for delivery of a probiotic strain is an important consideration, the present study is novel in that it compares 15-mo-ripened Cheddar cheese and freshly prepared yogurt as food carriers for delivery of viable probiotic microorganisms to the GIT.

Fecal coliforms were also examined in the present study because these microorganisms are recognized as undesirable, particularly in pigs in which fecal coliform concentrations have been observed to increase in scouring animals (32). In the present study, there was no evidence that probiotic administration influenced fecal coliform concentrations in pigs (Figure 5). This phenomenon is demonstrated by the fact that mean levels for the entire duration of the trial (i.e., both during and after probiotic administration) were not found to be significantly different ($P > 0.05$) between pigs fed probiotic cheese or yogurt (1.4×10^7 and 1.1×10^7 cfu/g of feces, respectively) and

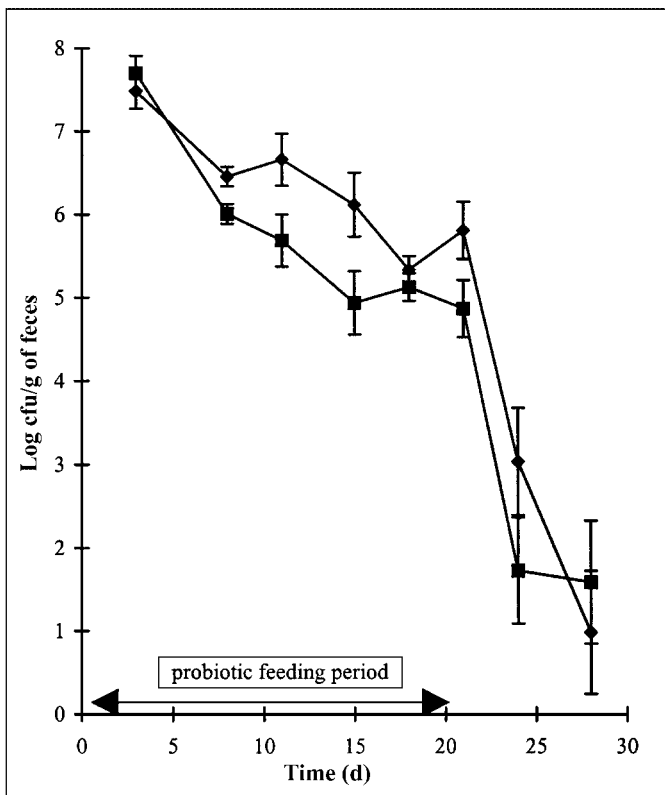


Figure 4. Excretion of the probiotic strain Fargo 688® (Quest Int., Naarden, The Netherlands) Rif^R in pigs fed this strain in Cheddar cheese (\blacklozenge) or yogurt (\blacksquare) from d 0 to 20, where d 0 represents the first day of probiotic administration. Values are means for 8 pigs with standard errors of the mean indicated by vertical bars.

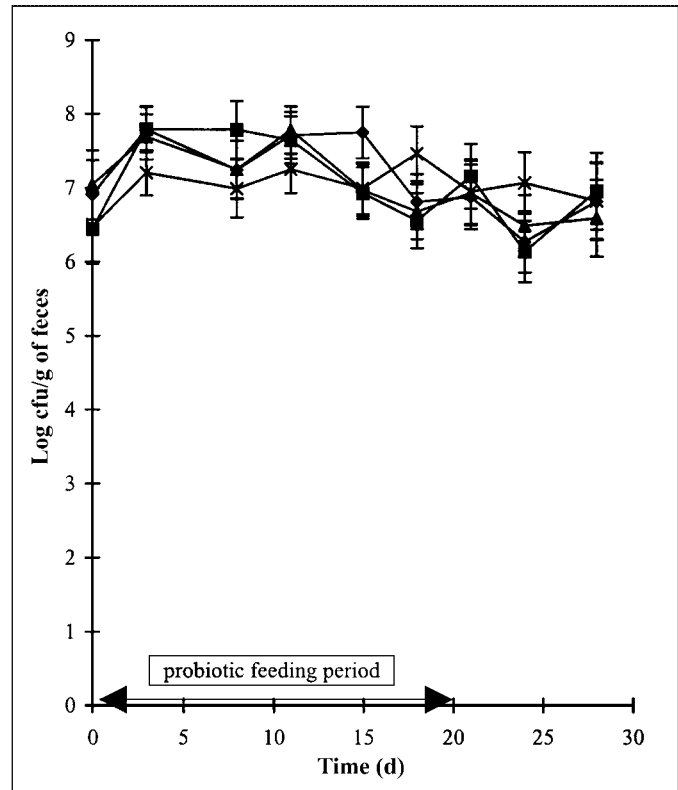


Figure 5. Presence of coliform in feces of pigs fed Fargo 688® (Quest Int., Naarden, The Netherlands) Rif^R -containing Cheddar cheese (\blacklozenge) or yogurt (\blacktriangle) compared with pigs fed control Cheddar cheese (\blacksquare) or yogurt (X). Probiotic administration was between d 0 and 20, where d 0 represents the first day of probiotic administration. Values are means for 8 pigs with standard errors of the mean indicated by vertical bars.

pigs fed control cheese or yogurt (1.1×10^7 and 1.1×10^7 cfu/g of feces, respectively) (Figure 5). Nevertheless, probiotic cultures have previously been shown to possess the ability to reduce intestinal coliform concentrations, perhaps by a competitive exclusion mechanism. Muralidhara et al. (32) reported that feeding an *L. lactis* strain to pigs suppressed coliform numbers, whereas other studies have described how lactic acid bacteria and acidophilus milk also reduced coliform numbers in pigs and humans (3, 46). However, a study on humans conducted by Pettersson et al. (35) showed that administration of *L. acidophilus* NCDO 1748 had no effect on coliform counts of ileostomy contents.

Although the main focus of the present study was to evaluate food carriers for probiotic administration, the effect of probiotic feeding on pig performance was also investigated. Probiotic treatment has previously been shown to stimulate growth and improve feed efficiency in pigs (32, 46). However, in the present study, no significant effects ($P > 0.05$) on body weight

TABLE 1. Performance of pigs that were fed the *Enterococcus faecium* probiotic strain of Fargo 688® Rif^r (Quest Int., Naarden, The Netherlands) in Cheddar cheese or yogurt compared to that of control pigs.¹

	SED ²	Probiotic		Control	
		Cheddar cheese (n = 8)	Yogurt (n = 8)	Cheddar cheese (n = 8)	Yogurt (n = 8)
Initial (d 0) BW, kg	0.07	7.98	7.96	7.99	7.99
BW at d 30, kg	1.19	25.75	26.31	25.75	27.5
BW gain, kg	1.22	17.77	18.35	17.76	19.51
Feed conversion efficiency ³	0.04	1.05	1.06	1.06	1.02

¹Probiotic administration was from d 0 to 20, where d 0 represents the first day of probiotic administration.

²SED = Standard error of the difference.

³Measured as kilogram of dry matter intake (including creep feed and cheese or yogurt) per kilogram of BW gain.

gain or feed conversion efficiency were observed for pigs receiving the enterococcal probiotic strain in either Cheddar cheese or yogurt compared with control animals (Table 1). In addition, the serum IgG response to the administered probiotic strain was measured by flow cytometry for a subset of the population of pigs. A positive response was found in pigs fed the probiotic strain, but no response was detected in the control pigs (data not shown). Probiotic microorganisms have previously been shown to stimulate or modulate the immune system [for review see (15)]. However, the mechanisms involved are, as yet, not fully understood.

CONCLUSIONS

The results of the present study demonstrate that the probiotic *E. faecium* strain Fargo 688® survives to high cell numbers in Cheddar cheese and yogurt during the shelf-life or ripening period. In vitro studies designed to simulate gastric transit demonstrated that Cheddar cheese has a greater buffering effect and afforded greater protection to the probiotic strain upon exposure to gastric juice when compared with yogurt, even though the Cheddar cheese was at least 14 mo older. Subsequent pig-feeding studies yielded higher fecal concentrations of the probiotic strain when ingested from 15-mo-old cheese compared with fresh yogurt. These data suggest that Cheddar cheese is at least as effective as, if not superior to, yogurt for delivery of viable probiotic microorganisms to the porcine GIT, even though the cheese product was some 15 mo old.

ACKNOWLEDGMENTS

The technical assistance of Sean Tobin, Dermot Davis, Finbar Drinan, Eddie Mulholland, and Tom

Cullinan is gratefully acknowledged. We thank Dan Benson and Frederic Lejard (Quest International) for helpful discussions and Dermot Harrington (Teagasc headquarters) for statistical analyses. This work was supported by the European Research and Development Fund. G. Gardiner was supported by a Teagasc Walsh Fellowship.

REFERENCES

- Ahrne, S., M. L. Johansson, and G. Molin. 1995. Intestinal passage of *Lactobacillus rhamnosus* DSM 6594 after oral administration in fermented milk. *Neth. Milk Dairy J.* 49: 201-206.
- Allen, W. D., M. A. Linggood, and P. Porter, inventors. 1996. Probiotic. Unilever NV, assignee. Eur. Pat. No. 0,508,701,B1.
- Ayelo, A. D., I. A. Angelo, and K. M. Shanani. 1980. Effect of ingesting *Lactobacillus acidophilus* milk upon fecal flora and enzyme activity in humans. *Milchwissenschaft* 35:730-733.
- Blanchette, L., D. Roy, G. Belanger, and S. F. Gauthier. 1996. Production of cottage cheese using dressing fermented by bifidobacteria. *J. Dairy Sci.* 79:8-15.
- Bouhnik, Y., P. Pochart, P. Marteau, G. Arlet, I. Goderel, and J. C. Rambaud. 1992. Fecal recovery in humans of viable *Bifidobacterium* sp. ingested in fermented milk. *Gastroenterology* 102:875-878.
- Charteris, W. P., P. M. Kelly, L. Morelli, and J. K. Collins. 1998. Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract. *J. Appl. Microbiol.* 84:759-768.
- Christiansen, P. S., D. Edelsten, J. R. Kristiansen, and E. W. Nielsen. 1996. Some properties of ice cream containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. *Milchwissenschaft* 51:502-504.
- Collins, J. K., and G. Thornton. 1998. Selection of probiotic strains for human applications. *Int. Dairy J.* 8:487-490.
- Conway, P. L., S. L. Gorbach, and B. R. Goldin. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J. Dairy Sci.* 70:1-12.
- Dinakar, P., and V. V. Mistry. 1994. Growth and viability of *Bifidobacterium bifidum* in Cheddar cheese. *J. Dairy Sci.* 77: 2854-2864.
- Easter, R. A. 1988. Acidification of diets for pigs. Pages 61-71 in *Recent Advances in Animal Nutrition*. D.J.A. Cole and W. Haresign, ed. Butterworths, London, United Kingdom.
- Gardiner, G., R. P. Ross, J. K. Collins, G. Fitzgerald, and C. Stanton. 1998. Development of a probiotic Cheddar cheese con-

- taining human-derived *Lactobacillus paracasei* strains. Appl. Environ. Microbiol. 64:2192-2199.
- 13 Genstat 5 Reference Manual. Release 3. 1994. Clarendon Press, Oxford, United Kingdom.
 - 14 Ghoddusi, H. B., and R. K. Robinson. 1996. The test of time. Dairy Ind. Int. 61:25-28.
 - 15 Gill, H. 1998. Stimulation of the immune system by lactic cultures. Int. Dairy J. 8, 535-544.
 - 16 Gobetti, M., A. Corsetti, E. Smacchi, A. Zocchetti, and M. De Angelis. 1998. Production of Crescenza cheese by incorporation of bifidobacteria. J. Dairy Sci. 81:37-47.
 - 17 Goldin, B. R., S. L. Gorbach, M. Saxelin, S. Barakat, L. Gualtieri, and S. Salminen. 1992. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. Dig. Dis. Sci. 37: 121-128.
 - 18 Gomes, A.M.P., and F. X. Malcata. 1998. Development of a probiotic cheese manufactured from goat milk: response surface analysis via technological manipulation. J. Dairy Sci. 81: 1492-1507.
 - 19 Gomes, A.M.P., F. X. Malcata, F.A.M. Klaver, and H. G. Grande. 1995. Incorporation and survival of *Bifidobacterium* sp. strain Bo and *Lactobacillus acidophilus* strain Ki in a cheese product. Neth. Milk Dairy J. 49:71-95.
 - 20 Guarner, F., and G. J. Schaafsma. 1998. Probiotics. Int. J. Food Microbiol. 39:237-238.
 - 21 Hekmat, S., and D. J. McMahon. 1992. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. J. Dairy Sci. 75:1415-1422.
 - 22 Holcomb, J. E., J. F. Frank, and J. U. McGregor. 1991. Viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in soft-serve frozen yogurt. Cult. Dairy Prod. J. 30:4-5.
 - 23 Hunter, J. O., A. J. Lee, M.E.J. Barratt, M. A. Linggood, and J. A. Blades. 1996. *Enterococcus faecium* strain PR88—an effective probiotic. Gut 38(Suppl. 1):62.(Abstr.)
 - 24 Ishibashi, N., and S. Shimamura. 1993. Bifidobacteria: research and development in Japan. Food Technol. 46:126-135.
 - 25 Iwana, H., H. Masuda, T. Fujisawa, H. Suzuki, and T. Mitsouka. 1993. Isolation of *Bifidobacterium* spp. in commercial yoghurts sold in Europe. Bifidobacteria and Microflora 12: 39-45.
 - 26 Jonsson, E. 1986. Persistence of a *Lactobacillus* strain in the gut of sucking piglets and its influence on performance and health. Swed. J. Agric. Res. 16:43-47.
 - 27 Laroia, S., and J. H. Martin. 1991. Effect of pH on survival of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in frozen fermented dairy desserts. Cult. Dairy Prod. J. 26:13-21.
 - 28 Lee, Y. K., and S. Salminen. 1995. The coming of age of probiotics. Trends Food Sci. Technol. 6:241-245.
 - 29 Marteau, P., T. Vesa, and J. C. Rambaud. 1997. Lactose maldigestion. Pages 65-88 in Probiotics 2: Applications and Practical Aspects. R. Fuller, ed. Chapman and Hall, London, United Kingdom.
 - 30 Micanel, N., I. N. Haynes, and M. J. Playne. 1997. Viability of probiotic cultures in commercial Australian yogurts. Aust. J. Dairy Technol. 52:24-27.
 - 31 Moughan, P. J., M. J. Birtles, P. D. Cranwell, W. C. Smith, and M. Pedraza. 1992. The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. World Rev. Nutr. Diet. 67:40-113.
 - 32 Muralidhara, K. S., G. G. Sheggeby, P. R. Elliker, D. C. England, and W. E. Sandine. 1977. Effect of feeding lactobacilli on the coliform and *Lactobacillus* flora of intestinal tissue and feces from piglets. J. Food Prot. 40:288-295.
 - 33 O'Riordan, K., and G. F. Fitzgerald. 1998. Evaluation of bifidobacteria for the production of antimicrobial compounds and assessment of performance in cottage cheese at refrigeration temperature. J. Appl. Microbiol. 85:103-114.
 - 34 Pettersson, L., W. Graf, L. Alm, S. Lindwall, and A. Stromberg. 1983. Survival of *Lactobacillus acidophilus* NCDO 1748 in the human gastrointestinal tract. 1. Incubation with gastric juice in vitro. Symp. Swed. Nutr. Found. XV:123-125.
 - 35 Pettersson, L., W. Graf, and U. Sewelin. 1983. Survival of *Lactobacillus acidophilus* NCDO 1748 in the human gastrointestinal tract 2. Ability to pass the stomach and intestine in vivo. Symp. Swed. Nutr. Found. XV:127-130.
 - 36 Pot, B., C. Hertel, W. Ludwig, P. Descheemaeker, K. Kersters, and K. H. Schleifer. 1993. Identification and classification of *Lactobacillus acidophilus*, *L. gasseri*, and *L. johnsonii* strains by SDS-PAGE and rRNA-targeted oligonucleotide probe hybridization. J. Gen. Microbiol. 139:513-517.
 - 37 Rao, A. V., N. Shiwarnarain, and I. Maharaj. 1989. Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices. Can. Inst. Food Sci. Technol. J. 22: 345-349.
 - 38 Rogosa, M., J. A. Mitchell, and R. T. Wiseman. 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. 62:132-133.
 - 39 Salminen, S., E. Isolauri, and E. Salminen. 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. Antonie van Leeuwenhoek 70: 347-358.
 - 40 Salminen, S., A. G. Ouwehand, and E. Isolauri. 1998. Clinical applications of probiotic bacteria. Int. Dairy J. 8:563-572.
 - 41 Saxelin, M., S. Elo, S. Salminen, and H. Vapaatalo. 1991. Dose response colonisation of faeces after administration of *Lactobacillus casei* strain GG. Microb. Ecol. Health Dis. 4:209-214.
 - 42 Saxelin, M., M. Ahokas, and S. Salminen. 1993. Dose response on the faecal colonisation of *Lactobacillus* strain GG administered in two different formulations. Microb. Ecol. Health Dis. 6:119-122.
 - 43 Saxelin, M., T. Pessi, and S. Salminen. 1995. Fecal recovery following oral administration of *Lactobacillus* strain GG (ATCC 53103) in gelatine capsules to healthy volunteers. Int. J. Food Microbiol. 25:199-203.
 - 44 Stanton, C., G. Gardiner, P. B. Lynch, J. K. Collins, G. Fitzgerald, and R. P. Ross. 1998. Probiotic Cheese. Int. Dairy J. 8: 491-496.
 - 45 Terzaghi, B. E., and W. E. Sandine. 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. 29: 807-813.
 - 46 Tortuero, F., J. Rioperez, E. Fernandez, and M. L. Rodriguez. 1995. Response of piglets to oral administration of lactic acid bacteria. J. Food Prot. 58:1369-1374.