Probiotic Enterococcus faecium Strain Is a Possible Recipient of the vanA Gene Cluster

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The characteristics of Enterococcus faecium have led to concern regarding the safety of probiotics that contain this bacterium. The results of an in vitro filter mating assay indicate that a probiotic E. faecium strain might be a potential recipient of vancomycin resistance genes.

Several studies have suggested that probiotics have beneficial health effects in humans and animals, although few well-performed clinical trials have been done to evaluate these effects [1, 2]. A probiotic that contains Enterococcus faecium (SF68 strain) has been proposed elsewhere to be clinically effective in the prevention of antibiotic-associated diarrhea [3] and in the treatment of diarrhea in children [4]. However, in another study, no antidiarrheal property of E. faecium (SF68) was found [5]. A fermented milk product that contains E. faecium (Gaio; MD Foods) has been sold in Denmark and in the United Kingdom for several years. The manufacturer claims the product has a hypocholesterolemic effect on individuals [6]; however, 2 long-term studies, which lasted 12 weeks and 6 months, respectively, failed to show any reduction in cholesterol levels in individuals who received Gaio yogurt, compared with that in control subjects at the end of treatment [7, 8].

Although enterococci generally have low pathogenicity, they increasingly are a cause of nosocomial infection in the United States and Europe, especially in immunocompromised patients. This might be explained in part by the intrinsic tolerance of these enterococci against several antimicrobial agents and to harsh conditions, and their tendency to gain antibiotic resistance [9].

The nature of E. faecium has led to discussions regarding the safety of using E. faecium as a food supplement. In a recent study of the ecological effects of the fermented milk product Gaio on the normal microflora of healthy Danish and Swedish volunteers who were or were not receiving simultaneous administration of vancomycin, no emergence of or increase in the number of vancomycin-resistant enterococci could be detected in any of the 40 volunteers after 10 days of intake. However, none of the Swedish volunteers and only 1 of the Danish volunteers harbored detectable levels of vancomycin-resistant enterococci when they entered the study [10]. The possibility that the probiotic strain can acquire transferable vancomycin resistance has yet to be elucidated.

In the present study, an in vitro conjugation assay done by use of a filter paper mating method was performed according to the method described by Murray and Hodel-Christian [11]. The recipient strain was the probiotic strain of E. faecium from the aforementioned Gaio product with induced resistance to streptomycin and rifampicin (strept' rif'). Two different vanA-positive E. faecium strains, type strain CCUG 36804 (Culture Collection, University of Göteborg, Sweden) and clinical strain SMI 141 (isolated in 1995 at the Swedish Institute for Infectious Disease Control, Stockholm, Sweden), were used as donor strains. Filters were incubated on blood agar plates at 37°C for 24 h. Recipient, donor, and transconjugant strains were isolated quantitatively on selective agar media that contained the appropriate antimicrobial agents.

Both donor strains transferred the vanA gene cluster to the recipient strain during the conjugation assay. The rates of conjugation were $4.0 \times 10^{-6}$ cfu per recipient, for CCUG 36804, and $1.8 \times 10^{-7}$ cfu per recipient for SMI 141. The conjugation event was confirmed by PCR, as were the species type and vanA carriage status of the donors, by use of E. faecium–specific primers and vanA gene-specific primers [12], as shown in figure 1. The recipient, donor, and transconjugant strains were further characterized by means of pulsed-field gel electrophoresis. This analysis showed that the parent probiotic strain (E. faecium), the recipient strain (E. faecium strept' rif'), and the transconjugant strains had identical DNA banding patterns and that this differed markedly from the pulsed-field gel electrophoresis patterns of the donor strains, indicating true conjugation events (figure 2).

The results of the present study address the issue of the possible health risk of eating foods that contain enterococci. Previous studies have shown that transfer of vanA genes is possible in the gastrointestinal tracts of germ-free mice [13]. The authors of a study of gene transfer in the avian gastrointestinal tract, claim that in vitro methods, such as forced filter mating and liquid...
intake of high concentrations of viable Enterococcus faecium, which may facilitate dissemination and proliferation of resistance genes.

In conclusion, the probiotic Enterococcus faecium strain is a possible recipient of the vanA gene cluster under in vitro conditions. Therefore, the risk for in vivo conjugation cannot be ruled out and should be considered in evaluations of the safety of enterococcal probiotics.

References