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Demonstration of safety of probiotics — a review

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Abstract

Probiotics are commonly defined as viable microorganisms (bacteria or yeasts) that exhibit a beneficial effect on the health of the host when they are ingested. They are used in foods, especially in fermented dairy products, but also in pharmaceutical preparations. The development of new probiotic strains aims at more active beneficial organisms. In the case of novel microorganisms and modified organisms the question of their safety and the risk to benefit ratio have to be assessed. Lactic acid bacteria (LAB) in foods have a long history of safe use. Members of the genera *Lactococcus* and *Lactobacillus* are most commonly given generally-recognised-as-safe (GRAS) status whilst members of the genera *Streptococcus* and *Enterococcus* and some other genera of LAB contain some opportunistic pathogens. Lactic acid bacteria are intrinsically resistant to many antibiotics. In many cases resistances are not, however, transmissible, and the species are also sensitive to many clinically used antibiotics even in the case of a lactic acid bacteria- associated opportunistic infection. Therefore no particular safety concern is associated with intrinsic type of resistance. Plasmid-associated antibiotic resistance, which occasionally occurs, is another matter because of the possibility of the resistance spreading to other, more harmful species and genera. The transmissible enterococcal resistance against glycopeptide antibiotics (vancomycin and teicoplanin) is particularly noteworthy, as vancomycin is one of the last effective antibiotics left in the treatment of certain multidrug-resistant pathogens. New species and more specific strains of probiotic bacteria are constantly identified. Prior to incorporating new strains into products their efficacy should be carefully assessed, and a case by case evaluation as to whether they share the safety status of traditional food-grade organisms should be made. The current documentation of adverse effects in the literature is

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reviewed. Future recommendations for the safety of already existing and new probiotics will be given.

Keywords: Probiotics; Safety; Dairy products; Functional foods; Lactic acid bacteria; Antibiotic resistance

1. Introduction

Traditional dairy strains of lactic acid bacteria (LAB) have a long history of safe use. LAB including different species of *Lactobacillus* and *Enterococcus* have been consumed daily since humans started to use fermented milk as food. Probiotic species such as *Lactobacillus acidophilus* have been safely used for more than 70 years. However, the safety aspects have always to be considered and possible adverse effects should continuously be evaluated as illustrated by recent literature. Members of the genera *Lactococcus* and *Lactobacillus* are most commonly given the GRAS status whilst members of the genera *Streptococcus* and *Enterococcus* and some other genera of LAB contain some opportunistic pathogens (Table 1). The safety of probiotics has been considered in reviews and clinical reports which have drawn attention to isolated cases of human bacteraemia (Gasser, 1994; Aquirre and Collins, 1993; Saxelin et al., 1996a,b). Surveillance studies support the safety of commercial LAB (Saxelin et al., 1996a,b; Gasser, 1994; Adams and Marteau, 1995). Available data indicate that no harmful effects have been observed in controlled clinical studies with lactobacilli and bifidobacteria. Degradation of intestinal mucus has been used as the first marker of toxicity and in a recent study, specific commercial probiotic strains were shown to be inactive in mucosal degradation (Ruseler van Embden et al., 1994, 1995; Donohue et al., 1998). Traditional toxicity studies have been conducted with several strains of probiotic bacteria with no ill effects. New species and more specific strains of probiotic bacteria are constantly identified. It cannot be assumed that these novel probiotic organisms share the historical safety of tested or traditional strains (Salminen et al., 1998). Prior to incorporating them into products, new strains should be carefully assessed and tested for both safety and efficacy. If functional foods are to make an increasing impact on the European food market, it is necessary to ensure that the consumer receives accurate information and clear messages about the health benefits. The goal of the PROBDEMO project is to substantiate such information. Probiotic safety was discussed in the first PROBDEMO workshop on Probiotic safety and Selection criteria in November, 1996, in Helsinki. Before this LABIP (the lactic acid bacteria industrial platform) had held two workshops on probiotics The Safety of Lactic acid bacteria in November 1994 and Lactic acid bacteria as probiotics in November 1995. Also in the FAIR programme Functional Food Science in Europe the effects and safety of probiotics were

Table 1
Classification of probiotic organisms (Gasser, 1994; Donohue and Salminen, 1996a)

Organism	Infection potential
Lactobacillus	Mainly non-pathogens, some opportunistic infections (usually in immunocompromised patients)
Lactococcus	Mainly non-pathogens
Leuconostoc	Mainly non-pathogens, some isolated cases of infection
Streptococcus	Oral streptococci mainly non-pathogens (including *Streptococcus thermophilus*); some may cause opportunistic infections
Enterococcus	Some strains are opportunistic pathogens with haemolytic activity and antibiotic resistance
Bifidobacterium	Mainly non-pathogens, some isolated cases of human infection
Saccharomyces	Mainly non-pathogens, some isolated cases of human infection

assessed (Salminen et al., 1998). All of the workshops were sponsored by the European Commission, DG XII. The purpose of the meetings was to establish a clearly written consensus document on the safety and probiotic properties of LAB.

2. Studies on the safety of probiotics

Three approaches can be used to assess the safety of a probiotic strain: studies on the intrinsic properties of the strain, studies on the pharmacokinetics of the strain (survival, activity in the intestine, dose–response relationships, faecal and mucosal recovery) and studies searching for interactions between the strain and the host. The selection criteria for probiotics are illustrated in Fig. 1.

Some enzymatic properties such as excessive deconjugation of bile salts or degradation of mucus might be potentially detrimental (Donohue et al., 1998). Such properties can be studied in vitro (Donohue et al., 1998). Platelet aggregating properties (Korpela et al., 1997), and the enzymes which seem to favour cardiac valve colonisation (Pelletier et al., 1996) could also be studied in vitro. However, this does not seem necessary for the existing food and probiotic strains, as no infections have been reported for these strains.

The survival of the probiotics within the gastrointestinal tract, their translocation and colonisation properties, and the fate of their active components needs to be known to predict not only the positive effects but also the side-effects. The survival of ingested probiotics at different levels of the gastrointestinal tract differs between strains (Marteau et al., 1993; Pettersson et al., 1983). Some strains are rapidly killed in the stomach while others, such as strains of bifidobacteria or *L. acidophilus*, can pass through the entire gut at very high concentrations (Marteau et al., 1993). Milk as a vehicle appears to protect probiotics against gastric conditions (Saxelin, 1996).

The pharmacokinetics of probiotics can be measured in vivo using a faecal collection of intestinal intubation and colonic biopsy techniques (Saxelin, 1996; Johansson et al., 1993; Alander et al., 1997). The use of transit markers is very useful in determining the colonisation properties by comparing the pharmacokinetics of the probiotic and those of the marker (Marteau et al., 1993). Several in vitro models can help to predict the fate of ingested strains: they consist of simple models to test the sensitivity of the probiotic to acid or bile, and more sophisticated dynamic multicompartmental models simulating the dynamics of the transit and secretions in the gastrointestinal tract (Marteau et al., 1997a).

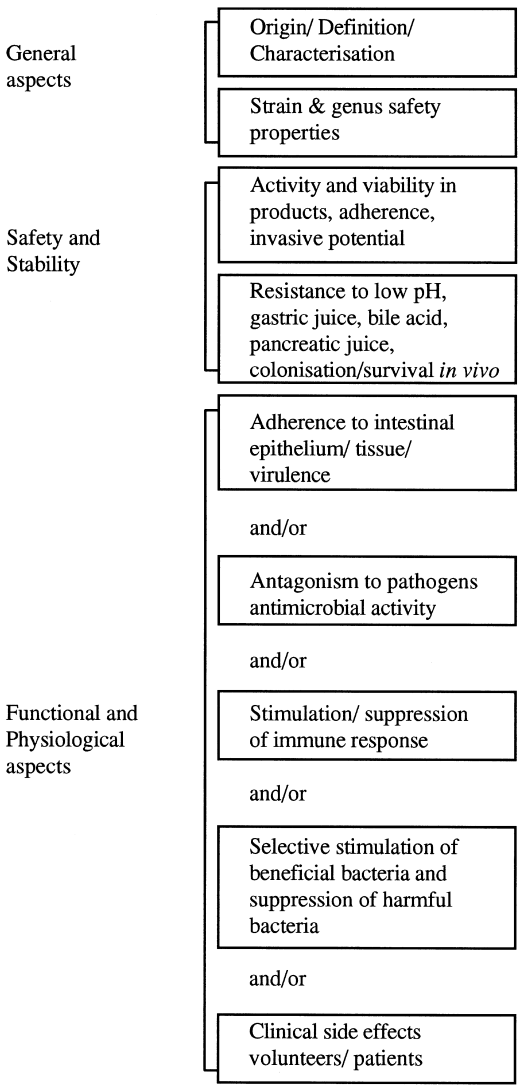

Fig. 1. Selection criteria for probiotics (Gasser, 1994; Donohue and Salminen, 1996a).

Illness related to microbiological agents in food is much more difficult to predict than illness caused by chemical agents (Tang et al., 1993). The concept of a minimum infective dose is very difficult because of

the large number of microbial and host factors involved and the high possibility for individual differences.

2.1. In vitro and animal studies

It is largely believed that probiotic strains should not invade host cells. The invasion capacity of a strain can be studied using cultured intestinal cells (Tang et al., 1993).

Animal models are of limited value in microbiological risk assessment (ILSI Europe, 1993). The high variability in species-specific responses does not allow the extrapolation of the results to humans. Pelletier et al. (1996) compared the virulence of dairy strains of lactobacilli to that of strains from patients with endocarditis using an experimental rabbit model. In this model a polyethylene catheter is inserted into the heart, to induce the formation of vegetation, and left in place for weeks. Strains of *L. rhamnosus* and *L. casei* adhered to the cardiac vegetation and left in place. In this model, strains of *L. rhamnosus* and *L. casei* adhered to the cardiac vegetations, and an inoculum size of 10^6 cfu/ml allowed discrimination of different adhesions between strains while an inoculum of 10^4 cfu/ml did not. However, all tested strains appeared to adhere to cardiac vegetations, at higher inoculum levels. The model has been designed for other purposes, the doses used are extremely high and unnatural and thus the relevance of the model should be validated and correlated with results from epidemiological surveillance studies. Additionally, in vitro models utilising endothelial cells or other cell systems may provide a more optimal and practical screening method.

Acute toxicity studies, using the same procedures as acute toxicity studies for chemicals were conducted for several strains of probiotics (Donohue et al., 1993; Donohue and Salminen, 1996a; Donohue et al., 1998). No acute toxicity was observed with any strain. Translocation of probiotics can be studied in animals, and can be increased using a lethal irradiation model simulating an immunocompromised state (for details see Dong et al., 1987). The specific role of probiotics in mucus degradation in vivo can be assessed using gnotobiotic animals (Ruseler van Embden et al., 1995). Immunological side-effects of probiotics can be assessed in animals (Blancuzzi et al., 1993; Okitsu-Negishi et al., 1996). All previously mentioned studies are very important in determining dose–response effects, and the role of intestinal lesions and immunosuppression. Information on the acute toxicity of probiotic bacteria and LAB indicate that it is not possible to reach oral dose levels related to death or dose levels with any harmful side-effects (Donohue and Salminen, 1996a; Donohue et al., 1993, 1998).

2.2. Studies in healthy volunteers and clinical trials

Extensive short-term clinical trials with healthy volunteers have demonstrated the safety of probiotics (Lidbeck et al., 1987, 1988; Orrhage et al., 1994; Saxelin, 1997, Table 2). Most studies mentioned that the probiotic did not induce more side-effects than the placebo or that it was well tolerated. In some studies, the presence (or absence) of gastrointestinal disorders was especially studied which seems rational since the first and probably only contact between probiotic products and the host occurs in the gastrointestinal tract (McFarland and Bernasconi, 1993; Wolf et al., 1995). Chronic ingestion of *L. johnsonii* LA 1 did not alter the jejunal permeability to proteins in healthy humans (Marteau et al., 1997b). In other cases, the safety of probiotics was studied using several biological parameters (Donohue and Salminen, 1996a,b).

2.2.1. Epidemiological surveillance

To date, the long history of safe use of probiotics remains the best proof of their safety. As the risk with each probiotic is assumed to be very low, the best approach in assessing it is probably to analyse it retrospectively in epidemiology studies and prospectively using pharmacovigilance methods.

The studies by Saxelin et al., 1996a,b) provide examples of epidemiological surveillance. These studies assessed whether the strains of lactobacilli involved in clinical infections were either identical to dairy strains or to other commercial strains. The value of such studies depends on its statistical power, i.e. on the number of cases studied, but epidemiological follow-up of introducing novel strains should be conducted for safety assessment.

Table 2
Safety studies and reported effects of current probiotic strains and yoghurt strains (Alander et al., 1997; Donohue and Salminen, 1996b)

Probiotic strain	Reported effect	Safety/studies In vitro/animal/human/studies
Lactobacillus acidophilus NFCO 1748	Treatment of constipation allevation of radiotherapy related diarrhoea, lowering of faecal enzymes	+
Lactobacillus casei Shirota	Balancing intestinal microflora, prevention of intestinal disturbances, treatment of superficial bladder cancer, lowering of faecal enzymes, immune enhancing, adjuvant	+
Lactobacillus GG (ACTT 53103)	Treatment of acute viral and bacterial diarrhoea in infants, prevention of antibiotics associated diarrhoea, immune enhancing, stabilisation of intestinal permeability	+
Lactobacillus acidophilus (*johnsonii*) LCI	Immune enhancing, vaccine, adjuvant, balancing intestinal microflora	+
Bifidobacterium bifidum	Prevention of rotavirus diarrhoea	+

2.3. Theoretical adverse effects of probiotics

Probiotics are living microorganisms. In theory, they may be responsible for four types of side-effects: systemic infections, risk of deleterious metabolic activities, risk of adjuvant side-effects and of immunomodulation, risk of gene transfer. Genetically modified probiotic organisms are currently not available for food use and their theoretical adverse effects are not considered.

2.3.1. Infections

Only very few cases of fungemia, all of which occurred in patients with a catheter, have been reported in humans treated with the probiotic *Saccharomyces boulardii*, (Zunic et al., 1991), and no cases of infection have until now been traced back to LAB used in foods (Adams and Marteau, 1995). However, in one case of *L. fermentum* endocarditis, the role of a large daily consumption of milk and dairy products was discussed even though no connection with the illness could be proven and no proof of its use in dairy products in general has been shown (Gallemore et al., 1995). Rare cases of local or systemic infections including septicaemia and endocarditis due to lactobacilli, bifidobacteria or other lactic bacteria have been reported but the numbers are very low in comparison with other bacteremia cases (incidence of enterococci 5–15%, lactobacilli 0.1%, leuconostocs $<0.01\%$) (Aquirre and Collins, 1993; Gasser, 1994; Patel et al., 1994; Kalima et al., 1996; Saxelin et al., 1996a,b). Most LAB strains linked to clinical cases belong to the species *Enterococcus faecium* and *E. faecalis* but a few belong to *L. rhamnosus*, *L. casei* or *L. paracasei*, and *L. plantarum*, (Gasser, 1994; Adams and Marteau, 1995; Gasser, 1994). Some cases have also been observed other lactobacilli, such as *Lactobacillus acidophilus, Lactobacillus jensenii and Lactobacillus paracasei*, and with *Leuconostocs* (Aquirre and Collins, 1993, Gasser, 1994; Dhodapkar and Henry, 1996). Nearly all patients have had

serious underlying conditions which predisposed them to infection, particularly abnormal heart valves in the case of endocarditis, and the presence of a catheter in cases of septicaemia (Horwitch et al., 1995). Known risk factors for other opportunists such as extremes of age or pregnancy have not been identified as risk factors for LAB-associated infections (Adams and Marteau, 1995). In most cases of infection, the organism appeared to have come from the patient's own microflora. In order to assess the potential of lactobacilli to cause serious infections, the prevalence of bacteremia due to *Lactobacillus* species in Southern Finland during a 4- and a 6-year period was studied and the characteristics of the blood culture isolates and of dairy strains compared. In the first study, *Lactobacilli* were identified in 8 of 3317 blood culture isolates and none of the isolates corresponded to a dairy strain (Saxelin et al., 1996a). In the second study, 5192 bacteremia cases were reviewed with 12 infections caused by lactobacilli. None of these cases could be related to either commercial food, dairy or pharmaceutical strains (Saxelin et al., 1996b).

One may speculate that the existence of digestive lesions, or immunodeficiency might favour translocation of probiotics from the gut lumen. However, it must be emphasised that *S. boulardii* has been administered to patients with Crohn's disease, to suppress enteritis and AIDS, and that no case of infection was reported in the patients (McFarland and Bernasconi, 1993). In the few cases of fungemia due to *S. boulardii*, external contamination of the catheter was thought to be the route of entry. Similarly, *Lactobacillus* strains have been administered during clinical trials to premature children and to subjects with Crohn's disease or diarrhoeal diseases and no side-effects have been reported (Donohue and Salminen, 1996a,b).

2.3.2. Metabolic and enzymatic effects

When the small bowel is colonised with a large number of bacteria, those microorganisms present in high numbers may induce diarrhoea and intestinal lesions, especially through deconjugation and dehydroxylation of bile salts (Donohue et al., 1998). After ingestion of some probiotics, the concentrations of microorganisms passing through the small bowel may become as abundant as observed during pouchitis and small bowel bacterial overgrowth (Ruseler van Embden et al., 1994). A study performed in healthy humans with a terminal ileostomy demonstrated that *L. acidophilus* and *Bifidobacterium* spp. ingested with fermented dairy products could transform conjugated primary bile salts into non-toxic secondary bile salts in the small bowel (Marteau et al., 1995). As this biological effect was only minimal, although statistically significant, it should not be considered as a dangerous side-effect of the tested product. However, this study draws attention to the potential risk of excessive deconjugation or dehydroxylation of bile salts in the small bowel by probiotics. Studies are currently being undertaken to assess the effects of probiotics with high bile salt hydrolase activity (deconjugation). Monitoring of side-effects in these studies will be of importance, and if a positive effect is observed, the therapeutic window of the probiotic should be determined.

Excessive degradation of the intestinal mucus layer by probiotics may theoretically be detrimental. Some endogenous bacteria, including lactobacilli, and some strains of bacteroides have the ability to degrade mucus (Ruseler van Embden et al., 1989). Ruseler van Embden et al. (1995) studied the mucus degrading properties of three probiotic strains contained in fermented milks (*L. acidophilus*, *Bifidobacterium* sp., *L. rhamnosus* GG). No mucus degradation was observed in vitro or in gnotobiotic rats monoassociated with the test strains.

Australian researchers have reported that lactobacilli isolated from cases of infective endocarditis produce enzymes that may enable the breakdown of human glycoproteins, and the synthesis and lysis of fibrin clots (Oakley et al., 1995). These characteristics aid the colonisation and survival of bacteria associated with an endocarditis vegetation (Oakley et al., 1995). However, it remains unknown to date whether they enhance the infectious risk to a relevant extent, and whether they should be considered undesirable in probiotic strains.

2.3.3. Immunological effects

Oral administration of high doses of LAB did not induce immunological side-effects in mice (Sartor et al., 1988). However, the immunological side-effects have been observed in rats with systemic uptake of cell wall polymers from the intestinal lumen via colonic injury (McConnel et al., 1991), and during

small bowel bacterial overgrowth (Blancuzzi et al., 1993). To our knowledge, no immunological side-effect of any probiotic has been reported in man.

However when administered parenterally, bacterial cell wall components such as peptidoglycans from different gram-positive bacteria, including lactobacilli, can induce side-effects such as fever, arthritis, cardioangitis, hepatobiliary lesions or autoimmune diseases (Schwabb, 1993). These side-effects are mediated by cytokines, and it is now well demonstrated that cytokine secretion is elicited by some probiotics (Miettinen et al., 1996; Perdigon et al., 1991; Miettinen et al., 1996).

2.4. Reported probiotic related adverse effects

Until now, no case of clinical infection has been traced to ingested probiotic LAB (Adams and Marteau, 1995; Saxelin et al., 1996a,b). Three cases of fungemia during oral treatment with the yeast *Saccharomyces boulardii* have been reported, which all resolved during antifungal therapy (Pletinx et al., 1995). Two of them occurred in patients receiving intestinal decontamination with multiple antibiotics and all of them occurred in patients with a catheter. An extended contamination of the catheter was proposed. In one case, the role of an excessive dosage of *S. boulardii* (90 mg/kg/day instead of 50) was also discussed but increasing the daily dose may not be the reason for translocation (McFarland and Bernasconi, 1993; Elmer et al., 1996). Clinical diseases due to deleterious metabolic effects of probiotics seem not to be reported.

3. Occurrence of antibiotic resistance in lactic acid bacteria and bifidobacteria

3.1. Lactic acid bacteria

From the end of 1950 up to 1980 there were several studies on the antibiotic sensitivity and resistance of dairy starter bacteria (Whitehead and Lane, 1956; Marth and Ellickson, 1956; Richards and Kennedy, 1960; Feagan, 1962; Vakil and Shahani, 1969a,b; Cogan, 1972; Reinbold and Reddy, 1974; Sozzi and Smiley, 1980). This was triggered by the problems caused by antibiotic residues in milk causing starter failures in milk acidification. A wide variety of different techniques was used to evaluate the antibiotic sensitivity, and it is therefore difficult to compare data and make any definitive conclusions. Since dairy starter strains do not have any clinical significance there has not been any need to standardize the procedures even in later times. However, it appears that the thermophilic yoghurt starters are more sensitive to penicillin than the mesophilic cocci, while the opposite seems to be true with streptomycin (Cogan, 1972). Although some resistances appeared to be strain or species specific no pattern suitable for classification has emerged (Reinbold and Reddy, 1974).

Among the lactobacilli antibiotic resistance has been reported for strains isolated in pork and beef (Raccah et al., 1985; Ametrano-Vidal and Collins-Thopson, 1987), and especially from human and animal gastrointestinal tracts. Among 45 strains of *Lactobacillus fermentum* originating from human faeces most showed a low level penicillin resistance with minimal inhibitory concentrations (MIC) of 0.03–0.44 U/ml while two isolates had MICs of 17 and 13 U/ml. The penicillin resistance of these two strains could be eliminated by acriflavine treatment (Yokokura and Mutai, 1976). Resistance patterns among lactobacilli isolated from the human vagina were rather similar to those observed among industrial isolates, erythromycin resistance being a common phenomenon (Torriani et al., 1988).

Dutta and Devriese (1981a), (1981b) isolated several intestinal lactobacilli of animal origin (pigs, poultry and cattle) showing variable degrees of resistance against macrolide– lincosamide–streptogramin (MLS) antibiotics or growth promoters such as avoparcin, bacitracin or carbadox. They found that *Lactobacillus acidophilus* was consistently avoparcin-sensitive, and *Lactobacillus brevis* strains were susceptible to bacitracin. Bacitracin- resistant lactobacilli were prevalent in cattle and poultry, and carbadox-resistant isolates in pigs. As bacitracin in a growth promoter for cows and broilers and carbadox for swine, the results suggest that growth promoters positively select resistant strains. No plasmid analysis of the isolates was reported.

Several animal isolates of *Lactobacillus acidophilus* and *Lactobacillus reuteri* were tested for antibiotic resistance by Sarra et al. (1982). All 16 *Lactobacillus reuteri* strains were resistant to vancomycin and polymyxin B irrespective of their

source, while only four of the thirty *Lactobacillus acidophilus* strains were vancomycin resistant and seven chloramphenicol resistant. All but one of the chloramphenicol resistant isolates were derived from poultry.

Antibiotic resistance screening has shown that the spontaneous mutation rate to antibiotic resistance among lactobacilli can be quite high (in the order of $2 \cdot 10^5$, depending on the strain (Curragh and Collins, 1992), and this suggests a large strain specific variation to the antibiotic resistance patterns within the genus.

3.2. Bifidobacteria

Lim et al. (1993) assayed 37 natural bifidobacterial isolates against 18 different antibiotics. The inherent resistance of these organisms against neomycin, nalidixic acid and polymyxin B, already reported by Miller and Finegold (1967), was confirmed. Variable and strain dependent resistances were observed with the rest of the antibiotics tested. No genetic studies on the bifidobacterial antibiotic resistance appears to have been published.

3.3. Antibiotic resistance plasmids in lactobacilli

Irreversible loss of antibiotic resistance from a strain as a result of a treatment (i.e. exposure to acriflavin) known to eliminate plasmids, is an indication that the resistance is plasmid-linked. When the actual disappearance of a certain plasmid can be physically shown or, alternatively, the transfer of this plasmid to a sensitive strain makes the strain antibiotic resistant, the plasmid-linkage can be considered verified. The final proof is the localisation of antibiotic resistance gene(s) on the plasmid by means of cloning or hybridization.

Antibiotic resistance plasmids are of special interest from the safety point of view, because they may be conjugatively transferred to other strains, species, and even genera, including potential human or animal pathogens. While some of the resistances mentioned in Section 3.3 may well be plasmid encoded, direct evidence of antibiotic resistance plasmids has been obtained mainly in lactobacilli and enterococci.

Several antibiotic resistance plasmids from lactobacilli have been detected. Ishiwa and Iwata (1980) could indicate by curing experiments the plasmid-linkage of tetracycline and erythromycin resistances in *Lactobacillus fermentum* isolated from human faeces. Curing techniques have been applied by Vescovo et al. (1982) to study the strain dependent resistance to macrolides, tetracycline and chloramphenicol in *Lactobacillus acidophilus* and *Lactobacillus reuteri* of animal origin. Plasmid disappearance and loss of chloramphenicol resistance was observed by Morelli et al. (1983) in one strain of *Lactobacillus acidophilus* and two strains of *Lactobacillus reuteri* isolated from poultry.

Plasmid association has been suggested also in the variety of antibiotic resistances among the lactobacilli isolated from Nigerian fermented foods (Olukoya et al., 1993).

More detailed molecular analyses are available from four antibiotic resistance plasmids in lactobacilli. An erythromycin-resistance plasmid pLUL631 (Axelsson et al., 1988), encoding MLS-resistance of *erm*A-type, has been detected in *Lactobacillus reuteri* 1063 isolated from pig intestine. Another MLS-resistance plasmid, pLAR33a (of *erm*C-type) has been isolated from *Lactobacillus* sp. also of swine origin (Rinckel and Savage, 1990). A third example of MLS-resistance plasmids, this time isolated from a *Lactobacillus reuteri* derived from mouse faeces, is pGT633 coding for an *erm*C (or as the authors put it *erm*GT) type of resistance and being able to replicate in a number of Gram-positive hosts (Tannock et al., 1994).

A chloramphenicol resistance gene was localized on a plasmid of a *Lactobacillus plantarum* strain isolated from raw, ground pork (Ahn et al., 1992). The plasmid could either be electrotransformed into other hosts or conjugatively transferred to a number of other Gram-positive bacteria by the help of a wide host range, originally enterococcal (Clewell et al., 1974) erythromycin resistance plasmid pAMß1.

3.4. Vancomycin resistance of enterococci and in various genera of lactic acid bacteria

Vancomycin belongs to glycopeptide antibiotics, which inhibit the peptidoglycan synthesis. Peptidoglycan is an important structural component of the bacterial cell wall. Therefore Gram-positive bacteria are especially vulnerable to vancomycin treatment. Vancomycin acts by forming a complex with the

carboxyterminal D-alanine (D-ala) residue in the building blocks of the bacterial cell wall peptidoglycan (Reynolds, 1989). The complex formation prevents the transglycosylation reaction essential to the growth of nascent peptidoglycan polymer (Arthur et al., 1996).

Generally, the resistance against vancomycin is based on the modification of the peptidoglycan precursor with the replacement of the terminal D-alanine by D-lactate or in some cases by D-serine (Bugg et al., 1991). However, at least two different mechanisms to reach that end exist among bacteria, of which one is typical for enterococci displaying the transmissible vancomycin resistant phenotype. The other can be found in several species and genera of LAB (Arthur et al., 1996; Billot-Klein et al., 1994, Handwerger et al., 1994).

3.5. Enterococcal vancomycin resistance

Although enterococci are normal inhabitants of the gastrointestinal tract and are widely used both as human and as animal probiotics, concerns have been expressed about safety issues. Certain enterococcal strains have been encountered in clinical infections (Facklam, 1972; Shlaes et al., 1989). Conjugative antibiotic resistance plasmids — like pAMß1 mentioned previously — are common in enterococci, and the transfer of this plasmid has been shown to occur in vivo to an *Enterococcus faecalis* recipient strain in germ-free mice (Morelli et al., 1988). Especially the enterococcal conjugative vancomycin resistance has lately raised serious fears of the transmission of this trait to species and genera of clinical significance.

Enterococcal strains displaying a high level resistance to vancomycin have been isolated from clinical cases in the late 1980s (Uttley et al., 1988; Shlaes et al., 1989) and vancomycin resistant enterococci are nowadays commonly associated with nosocomial infections in hospital epidemics (Woodford et al., 1995; Leclercq and Curvalin, 1997). This resistance was found to be in vitro transferable, in addition to other enterococcal strains, also to other Gram-positive bacteria including *Listeria* (Leclercq et al., 1989) and *Staphylococcus aureus* (Noble et al., 1992). This finding is important and should be further studied, since vancomycin is one of the last antibiotics that are effective against multidrug-resistant staphylococci. In fact there has been a recent Japanese clinical case caused by vancomycin resistant staphylococci, although the origin of the resistance genes is not yet known [Dr. Jaana Vuopio-Varkila, National Public Health Institute, Helsinki, Finland, personal communication].

Two types of conjugative genetic systems coding for vancomycin resistance exist in enterococci (Fig. 2). The *vanA* system is inducible and conveys a high level resistance to vancomycin and teicoplanin, while the so-called *vanB* system causes an inducible resistance to variable levels of vancomycin only (Arthur et al., 1996).

The *vanA* system has been characterized from a nonconjugative transposon (or a mobile gene bloc) Tn*1546* (Arthur et al., 1993, 1996). It consists of nine genes, seven of which are involved in the production of vancomycin resistant phenotype. The key enzymes are a ligase (VanA) and a dehydrogenase (VanH). The former causes the linkage of D-lactate instead of D-alanine as the terminal residue in the peptidoglycan precursor, and the latter the reduction of pyruvate to D-lactate. In addition, there is a two-component induction system (VanR–VanS) regulating the synthesis of VanA, VanH and VanX (VanX is a dipeptidase eliminating the terminal D-alanine). The additional vancomycin resistance genes are *vanY* encoding a carboxypeptidase with similar functions to VanX and *vanZ* causing teicoplanin resistance by an unknown mechanism.

The *vanB* system is very similar to *vanA* (Evers and Courvalin, 1996; Arthur et al., 1996). The principal differences are in the regulatory systems and in the structure of VanY-type carboxypeptidase. In *vanB* system the *vanZ* teicoplanin resistance gene

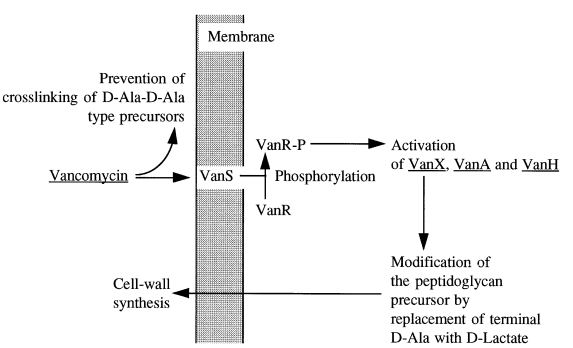

Fig. 2. Mechanism of enterococcal vancomycin resistance (Donohue et al., 1998; Donohue and Salminen, 1996a).

is lacking, instead there is a gene, *vanW,* with an unknown function. The *vanA* system is typically located on large plasmids or, in some cases, on chromosomal elements, while the *vanB* gene clusters seem to be exclusively chromosomally located. Both types are conjugatively transmissible to recipients representing different strains, species and even genera. In some motile enterococcal species (*E. gallinarum, E. casseliflavus, E. flavescens*) there exists also a third type of vancomycin resistance, v*anC* (Evers et al., 1996; Arthur et al., 1996). In this system the terminal D-alanine is replaced by D-serine. The genetic bases of *vanC* type of resistance has not been elucidated to the same extent as the *vanA* and *vanB* systems.

3.6. Vancomycin resistance in certain other lactic acid bacterial genera

The presence of peptidoglycan precursors terminating with D-lactate instead of D-alanine has been demonstrated in lactobacilli, pediococci and leuconostocs intrinsically resistant to high levels of vancomycin (Handwerger et al., 1994; Billot-Klein et al., 1994). The ligases responsible to this modification are, however, on the molecular level only very distantly related to enterococcal VanA or VanB ligases (Elisha and Courvalin, 1995; Evers et al., 1996) and no induction of this type of resistance has been observed (Hugyens, 1995). There has been, so far, no indication that this vancomycin resistance would represent an inducible, transmissible genetic system similar to those observed in enterococci.

3.7. Vancomycin resistance in probiotic strains of lactobacilli

As vancomycin resistance is an intrinsic property of many LAB it is to be expected that probiotic strains could also have this phenotype. Relatively few studies have been carried out on this issue.

The results of the extensive studies performed by Tynkkynen et al., 1998 on a probiotic lactobacillus *Lactobacillus rhamnosus* GG (*Lactobacillus* GG) can be summarized as follows:

Lactobacillus GG neither transferred its vancomycin resistance to sensitive recipients nor received antibiotic resistances from enterococcal donors in conjugation experiments. In molecular biological examinations no sequences resembling the enterococcal vancomycin-resistance genes could be detected in *Lactobacillus* GG. In conclusion, there is so far no indication that the vancomycin resistance in *Lactobacillus* GG is related to the transmissible enterococcal types of resistance.

4. Conclusions

An enormous amount of money and energy can be spent in assessing the risk of each probiotic strain. A low risk may be accepted, but the risk to benefit ratio needs to be clearly established. This requires relevant information on the efficacy and safety of the products. When novel strains, species and genera are selected for probiotic use the current safety assessment procedures described in the EU novel foods directive need to be carefully followed (Huggett and Conzelman, 1997).

LAB display a wide range both of natural and antibiotic sensitivity and resistance. In most cases antibiotic resistance is not of the transmissible type, but represents an intrinsic species or genus specific characteristic of the organism. These cases are not likely to constitute any safety concern. LAB and related organisms (with the exception of enterococci) are seldom associated with infections, and even in those cases there are plenty of safe antibiotics available to which they are sensitive. This applies also to species and genera intrinsically resistant to vancomycin.

Although plasmid-linked antibiotic resistances are not very common among LAB they do occur, and safety implications should be taken into consideration. Strains harbouring resistance plasmids should not be used either as human or animal probiotics. Checking the ability of a proposed probiotic strain to act as a donor of conjugative antibiotic resistance genes may be a prudent precaution in some instances (and in particular in the case of animal feeding, where the use of antibiotics as growth promoters apparently creates selective advantage for spreading of the resistance factors).

The enterococcal strains are normal inhabitants of the gastrointestinal tract and are present in many traditional fermented foods without any apparent risk. However, the indiscriminate use of antibiotics in human and veterinary medicine as well as animal

growth promoters have created a situation where the spread of multidrug resistances by enterococcal carriers is possible, as indicated by Morelli et al., 1988. The enterococcal transmissible vancomycin resistance poses an important issue, both in the treatment of enterococcal infections, and in the apprehended case where the resistance could be transferred into multidrug-resistant staphylococci. However, no evidence of this occurring in clinical cases has been demonstrated so far. It is self-evident that no vancomycin resistant enterococci should be used as either human or animal probiotics. In animal feeding, where growth promoters like avoparcin may be used, even the vancomycin sensitive strains should have exceptionally well documented positive effects to counteract the small risk that they may receive the resistance from some donor. Finally, it should be noted that the concern of transferable antibiotic resistance is a result of a major problem related to the abuse of antibiotics in general.

4.1. Recommendations and conclusions for safety of probiotic cultures for traditional or novel foods

The EU regulation covers novel food and food ingredients which have not hitherto been used for human consumption to a significant degree within the EU. In probiotics, the major difference is that we have to consider whether foods or food ingredients consisting of or containing microorganisms are novel. Genetically modified organisms are by definition always novel. For other microbes the safety assessment is based on the decision of the novel status. This includes the phenotypic and genotypic characterisation and the occurrence of such microbes in foods as well as previous history of use. Thus, further criteria are then defined if a 'novel' status is given. In case of *Lactobacillus* GG and *Lactobacillus johnsonii* the UK Advisory Committee on Novel Foods and Ingredients has assessed the strains and concluded that they are not novel. All other current probiotics have a longer history of use within the EU. Our suggestions offer one way of assessing them and ensuring the safety of current probiotic bacteria.

(1) The producer that markets the food has the ultimate responsibility for supplying a safe food. Probiotic foods should be as safe as other foods.

(2) When the probiotic food turns out to be a novel food it henceforth will be subject to the appropriate legal approval (EU directive for novel foods).

(3) When a strain has a long history of safe use, it will be safe as a probiotic strain and will not result in a novel food.

(4) The best test for food safety is a well documented history of safe human consumption. Thus, when a strain belongs to a species for which no strains are known that are pathogenic and for which other strains have been described that have a long history of safe use, it is likely to be safe as a probiotic strain and will not result in a novel food.

(5) When a strain belongs to a species for which no pathogenic strains are known but which does not have a history of safe use, it may be safe as a probiotic strain but should be regarded as a novel food and hence should be treated as such.

(6) When a new strain belongs to a species for which pathogenic strains are known, it will result in a novel food.

(7) Proper state of the art taxonomy is required to describe a probiotic strain. Today this includes DNA–DNA hybridization and rRNA sequence determination. This reasoning specifically applies to mutants of a probiotic strain.

(8) In line with recommendation (1), strains that carry transferable antibiotic resistance genes, i.e. genes encoding proteins that inactivate antibiotics, should not be marketed.

(9) Strains that have not been properly taxonomically described using the approaches as indicated above under (7) should not be marketed. Strains should also be deposited in an internationally recognised culture collection.

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References

Adams, M.R., Marteau, P., 1995. On the safety of lactic acid bacteria from food. Intern. J. Food Microbiol. 27, 263–264.

Aguirre, M., Collins, M.D., 1993. Lactic acid bacteria and human clinical infection. J. Appl. Bacteriol. 75, 95–107.

Ahn, C., Collins-Thompson, D., Duncan, C., Stiles, M.E., 1992. Mobilization and location of the genetic determinant of chloramphenicol resistance from *Lactobacillus plantarum* caTC2R. Plasmid 27, 169–176.

Alander, M., Korpela, R., Saxelin, M., Vilpponen-Salmela, T., Mattila-Sandholm, T., von Wright, A., 1997. Recovery of *Lactobacillus rhamnosus* GG from human colonic biopsies. Lett. Appl. Microbiol. 24, 361–364.

Ametrano-Vidal, C., Collins-Thopson, D.L., 1987. Resistance and sensitivity of meat lactic acid bacteria to antibiotics. J. Food Protect. 50, 737–740.

Arthur, M., Molinas, C., Depardieu, F., Courvalin, P., 1993. Characterization of TN*1546*, a Tn*3* related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J. Bacteriol. 175, 117–127.

Arthur, M., Reynolds, P., Courvalin, P., 1996. Glycopeptide resistance in enterococci. Trends Microbiol. 4, 401–407.

Axelsson, L.T., Ahrne, S.I.S., Andersson, M.C., Stahl, S.R., 1988. Identification and cloning of a plasmid encoded erythromycin resistance from *Lactobacillus reuteri*. Plasmid 20, 171–174.

Billot-Klein, D., Gutman, L., Sablé, S., Guittet, E., van Heijenoort, J., 1994. Modification of peptidoglycan precursors is a common feature of the low-level vancomycin-resistant VanB-type enterococcus D366 and of the naturally glycopeptide-resistant species *Lactobacillus casei*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides* and *Enterococcus gallinarum*. J. Bacteriol. 176, 2398–2405.

Blancuzzi, V., Roberts, E.D., Wilson, D., Fryer, L.R., O'Byme, E.M., DiPasquale, G., 1993. Pathogenesis of *Lactobacillus casei* induced polyarthritis in Lewis rats: 1. Time related changes in histopathologicalhisotpahological scores and hematology, Agents Actions. 39, special conference, C183–185.

Bugg, T.D.H., Wright, G.D., Dutka-Malen, S., Arthur, M., Courvalin, P., Walsh, C.T., 1991. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of an depsipieptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. Biochemistry 30, 10408–10415.

Clewell, D., Yagi, Y., Dunny, G., Schults, S., 1974. Characterization of three plasmid DNA molecules from *Streptococcus faecalis*. Identification of a plasmid determining erythromycin resistance. J. Bacteriol. 117, 283–289.

Cogan, T.M., 1972. Susceptibility of cheese and yoghurt starters to dihydrostreptomycin, chlortetracycline and oxytetracycline. Aust. J. Dairy Technol. 17, 182–183.

Curragh, H.J., Collins, M.A., 1992. High levels of spontaneous drug resistance in *Lactobacillus*. J. Appl. Bacteriol. 73, 31–36.

Dhodapkar, K.M., Henry, N.K., 1996. *Leuconostoc* bacteremia in an infant with short-gut syndrome: case report and literature review. Mayo Clin Proc. 71, 1171–1174.

Dong, M.Y., Chang, T.W., Gorbach, S.L., 1987. Effects of feeding *Lactobacillus* GG on lethal irradiation in mice. Diag. Microbiol. Infect. Dis. 7, 1–7.

Donohue D.C., Deighton M., Ahokas J.T., Salminen S., 1993. Toxicity of lactic acid bacteria. In: Saminen S., von Wright A., (Eds.), Lactic Acid Bacteria. Marcel Dekker, New York, pp. 307–313.

Donohue, D.C., Salminen, S., 1996. Safety assessment of probiotic bacteria. Asia Pac. J. Clin. Nutr. 5, 25–28.

Donohue, D.C., Salminen, S., 1996. Safety of *Lactobacillus* GG (ATCC 53103). Nutr. Today 31, 12s–15s.

Donohue, D., Salminen, S., Marteau, P., 1998. Safety of probiotic bacteria. In: Salminen, S., von Wright, A. (Eds.), Lactic Acid Bacteria. Marcel Dekker, New York, pp. 369–384.

Dutta, G.N., Devriese, L.A., 1981. Sensitivity and resistance to growth promoting agents in animal lactobacilli. J. Appl. Bacteriol. 51, 283–288.

Dutta, G.N., Devriese, L.A., 1981. Degradation of macrolide–lincosamide–streptogramin antibiotics by *Lactobacillus* strains from animals. Ann. Microbiol. (Inst. Pasteur). 132A, 51–57.

Elisha, B.G., Courvalin, P., 1995. Analysis of genes encoding D-alanine: D-alanine ligase related enzymes in *Leuconostoc mesenteroides* and *Lactobacillus* spp. Gene 152, 79–83.

Elmer, G.W., Surawicz, C.M., McFarland, L.V., 1996. Biotherapeutic agents, A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. J. Am. Med. Assoc. 275, 870–876.

Evers, S., Casadewall, B., Charles, M., Dutka-Malen, S., Galimand, M., Courvalin, P., 1996. Evolution of structure and substrate specificity in D-alanine:D-alanine ligases and related enzymes. J. Mol. Evol. 42, 706–712.

Evers, S., Courvalin, P., 1996. Regulation of VanB-type vancomycin resistance gene expression by the $VanS_B$–$VanR_B$ two-component regulatory system in *Enterococcus faecalis* V583. J. Bacteriol. 178, 1302–1309.

Facklam, R.R., 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. 23, 1131–1139.

Feagan, G.T., 1962. Sensitivity of cheese starters to dihydrostreptomycin, chlortetracycline and oxytetracycline. Aust. J. Dairy Technol. 17, 182–183.

Gallemore, G.H., Mohon, R.T., Ferguson, D.A., 1995. *Lactobacillus fermentum* endocarditis involving a native mitral valve. J. Terun. Med. Assoc. 88, 306–308.

Gasser, F., 1994. Safety of lactic acid bacteria and their occurrence in human clinical infections. Bull. Inst. Pasteur. 92, 45–67.

Horwitch, C.A., Furseth, H.A., Larson, A.M., Jones, T.L., Olliffe, J.F., Spach, D.H., 1995. Lactobacillemia in three patients with AIDS. Clin. Infect. Dis. 21, 1460–1462.

Handwerger, S., Pucci, M.J., Volk, K.J., Liu, J., Lee, M.S., 1994. Vancomycin-resistant *Leuconostoc mesenteroides* and *Lactobacillus casei* synthesize cytoplasmic peptidoglycan precursors that terminate in lactate. J. Bacteriol. 176, 260–264.

Huggett, A., Conzelman, C., 1997. EU regulation on novel foods: consequences for the food industry. Trends Food Sci. Technol. 8, 133–139.

Hugyens, F., 1995. Vancomycin resistance is not inducible in *Leuconostoc, Pediococcus* and *Enterococcus faecalis* A256. S. African J. Sci. 91, 94–98.

ILSI Europe, 1993. A scientific basis for regulations on pathogenic microorganisms in foods. Summary of a workshop held in May 1993 and organised by the Scientific Committee on Microbiology, ILSI Press.

Ishiwa, H., Iwata, M., 1980. Drug resistance plasmids in *Lactobacillus fermentum*. J. Gen Appl. Microbiol. 26, 71–74.

Johansson, M.L., Molin, G., Jeppson, B., Nobaek, S., Ahrné, S., Bengmark, S., 1993. Administration of different *Lactobacillus* strains in fermented oatmeal soup. In vivo colonization of human intestinal mucosa and effect on the indigenous flora. Appl. Environ. Microbiol. 59, 15–20.

Kalima, P., Masterton, R.G., Roddie, P.H., Thomas, A.E., 1996. *Lactobacillus rhamnosus* infection in a child following bone marrow transplant. J. Infect. 32, 165–167.

Korpela, R., Moilanen, E., Saxelin, M., Vapaasalo, H., 1997. *Lactobacillus rhamnosus* GG (ATCC 53013) and platelet aggregation in vitro. Int. J. Food Microbiol. 37, 83–86.

Leclercq, R., Curvalin, P., 1997. Resistance to glycopeptides in enterococci. Clin. Infect. Dis. 24, 545–556.

Leclercq, R., Derlot, E., Weber, M., Duval, J., Courvalin, P., 1989. Transferable vancomycin and teicoplanin resistance in *Enterococcus faecium*. Antimicr. Agent Chemother. 33, 10–15.

Lidbeck, A., Gustafsson, J-Å., Nord, C.E., 1987. Impact of *Lactobacillus acidophilus* supplements on the human oropharyngeal and intestinal microflora. Scand. J. Infect Dis. 19, 531–537.

Lidbeck, A., Edlund, C., Gustafsson, J-Å., Kager, L., Nord, C.E., 1988. Impact of Lactobacillus acidophilus on the normal intestinal microflora after administration of two antimicrobial agents. Infection 16, 329–336.

Lim, K.S., Huh, C.S., Baek, Y.J., 1993. Antimicrobial susceptibility of bifidobacteria. J. Dairy Sci. 76, 2168–2174.

Marteau, P., Pochart, P., Bouhnik, Y., Rambaud, J.C., 1993. Fate and effects of some transiting microorganisms in the human gastrointestinal tract. World Rev. Nutr. Diet. 74, 1–21.

Marteau, P., Gerhardt, M.F., Myara, A., Bouvier, E., Trivin, F., Rambaud, J.C., 1995. Bifidobacteria and lactobacilli ingested in fermented dairy products can metabolize bile salts in the human small intestine. Microbiol. Ecol. Health Dis. 8, 151–157.

Marteau, P., Minekus, M., Havenaar, R., Huis in't Veld, J., 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and effects of bile. J. Dairy Sci. 80, 1031–1037.

Marteau, P., Vaerman, J.P., Dehenin, J.P., Bord, S., Brassart, D., Pochart, P., Desjeux, J.F., Rambaud, J.C., 1997. Effect of intrajejunal perfusion and chronic ingestion of *Lactobacillus acidophilus* strain La1 on serum concentrations and jejunal secretions of immunoglobulins and serum proteins in healthy humans. Gastroenterol. Clin. Biol. 21, 293–308.

Marth, E.H., Ellickson, B.E., 1956. Problems created by the presence of antibiotics in milk products — a review. J. Milk Food Technol. 22, 266–272.

McConnel, M.A., Mercer, A.A., Tannock, G.W., 1991. Transfer of plasmid pAMß1 between members of the normal microflora inhabiting the murine digestive tract and modification of the plasmid in a *Lactobacillus reuteri* host. Microb. Ecol. Health Dis. 4, 343–355.

McFarland, L., Bernasconi, P., 1993. *Saccharomyces boulardii*: a review of an innovative biotherapeutic agent. Microbial Ecol. Health Dis. 6, 157–171.

Miettinen, M., Vuopio-Varkila, J., Varkila, K., 1996. Production of human necrosis factor α, interleukin 6, and interleukin 10 is induced by lactic acid bacteria. Infect Immunol. 64, 5403–5405.

Miller, L.G., Finegold, S.M., 1967. Antibacterial sensitivity of *Bifidobacterium* (*Lactobacillus bifidus*). J. Bacteriol. 93, 125–130.

Morelli, L., Sarra, P.G., Bottazzi, V., 1988. In vivo transfer of pAMβ1 from *Lactobacillus reuteri* to *Enterococcus faecalis*. J. Appl. Bacteriol. 65, 371–375.

Morelli, L., Vescovo, M., Bottazzi, V., 1983. Identification of chloramphenicol resistance plasmids in *Lactobacillus reuteri* and *Lactobacillus acidophilus*. Int. J. Microbiol. 1, 1–5.

Noble, W., Virani, Z., Cree, R.G.A., 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS Lett Microbiol. 93, 195–198.

Oakley, H.J., Harty, D.W.S., Knox, K.W., 1995. Enzyme production by lactobacilli and the potential link with infective endocarditis. J. Appl. Bacteriol. 78, 142–148.

Okitsu-Negishi, S., Nakano, I., Suzuki, K., Hashira, S., Abe, T., Yoshino, K., 1996. The induction of cardiangitis by *Lactobacillus casei* cell wall in mice I. The cytokine production from murine macrophages by *Lactobacillus casei* cell wall extracts. Clin. Immunol. Immunopathol. 78, 30–40.

Olukoya, D.K., Ebigwei, S.I., Adebawo, O.O., Osiyemi, F.O., 1993. Plasmid profiles and antibiotic susceptibility patterns of *Lactobacillus* isolated from fermented foods in Nigeria. Food Microbiol. 10, 279–285.

Orrhage, K., Brismar, B., Nord, C.E., 1994. Effect of supplements with *Bifidobacterium longum* and *Lactobacillus acidophilus* on the intestinal microbiota during administration of clindamycin. Microb. Ecol. Health Dis. 7, 17–25.

Patel, R., Cockerill, F.R., Porayko, M.K., Osmon, D.R., Ilstrup, D.M., Kenting, M.R., 1994. Lactobacillemia in liver transplant patients. Clin. Infect. Dis. 18, 207–212.

Pelletier, C., Bouley, C., Bourliov, P. and Carbon, C., 1996. Evaluation of safety properties of *Lactobacillus* strains by using an experimental model of endocarditis in rabbit. Abstract, SOMED Meeting, Paris, 1996.

Perdigon, G., De Jorrat, M.E.B., De Petrino, S.F., De Budeguer, M.V., 1991. Effect of oral administration of *Lactobacillus casei* on various biological functions of the host. Food Agric Immunol. 3, 93–102.

Pettersson, L., Graf, W., Sewelin, U., 1983. Survival of *L. acidophilus* NCDO 1748 in the human gastrointestinal tract. 2. Ability to pass the stomach and intestine in vivo. In: Hallgren B. (Ed.), Nutrition and the Intestinal Flora XV, Symp. Swed. Nutr. Found, Uppsala, Almqvist & Wiksell, pp. 127–130.

Pletinx, M., Legein, J., Vandenplas, Y., 1995. Fungemia with *SaccharomycesSaccharomyce boulardii* in a 1-year old girl with protracted diarrhoea. J. Ped. Gastroenterol. Nutr. 21, 113–115.

Raccah, M., Kovac, S.L., Mayer, C.M., 1985. Susceptibility of meat lactic acid bacteria to antibiotics. Food Microbiol. 2, 271–275.

Reinbold, G.W., Reddy, M.S., 1974. Sensitivity or resistance of dairy starter and associated microorganisms to selected antibiotics. J. Milk Technol. 37, 517–521.

Reynolds, P.E., 1989. Structure, biochemistry and mechanism of

action of glycopeptide antibiotics. Eur. J. Clin. Microbial. Infect. Dis. 8, 943–950.

Richards, R.J., Kennedy, H.E., 1960. Antibiotic resistance of starter cultures belonging to streptococcus. J. Dairy Sci. 43, 852–853.

Rinckel, L.A., Savage, D.C., 1990. Characterization of plasmids and plasmid-borne macrolide resistance from *Lactobacillus* sp. strain 100-33. Plasmid 23, 119–125.

Ruseler van Embden, J.G.H., van der Helm, R., van Licshour, L.C.M., 1989. Degradation of intestinal glycoproteins by *Bacteroides vulgatus*. FEMS Microbiol. Lett. 58, 37–42.

Ruseler van Embden, J.G., Schouten, W.R., van Lieshout, L.M., 1994. Pouchitis: result of microbial imbalance. Gut 35 (5), 658–664.

Ruseler van-van Embden, J.G.H., van Lieshout, L.M.C., Gosselink, M.J., Marteau, P., 1995. Inability of *Lactobacillus casei* strain GG, *L. acidophilus*, and *Bifidobacterium bifidum* to degrade intestinal mucus glycoproteins: clearing the way for mucosa-protective therapy. Scand. J. Gastroenterol. 30, 675–680.

Salminen, S., Bouley, C., Boutron-Ruault, M.-C., Cummings, J.H., Franck, A., Gibson, G.R., Isolauri, E., Moreau, M.C., Roberfroid, M., Rowland, I., 1998. Functional Food Science and Gastrointestinal Physiology and Function. Br. J. Nutr. 80 (Suppl. 1), S147–S171.

Sarra, P.G., Vescovo, M., Morelli, L., Cabras, M., 1982. Antibiotic resistance in *L. acidophilus* and *L. reuteri* from animal gut. Ann. Microbiol. Enzymol. 32, 71–76.

Sartor, R.B., Bond, T.M., Schwabb, J.H., 1988. Systemic uptake and intestinal inflammatory effects of luminal bacterial cell wall polymers in rats with acute colonic injury. Infect. Immunol. 56, 2101–2108.

Saxelin, M., 1997. *Lactobacillus* GG — a human probiotic strain with thorough clinical documentation. Food Rev. Int. 13, 293–313.

Saxelin, M., 1996. Colonization of the human gastrointestinal tract by probiotic bacteria. Nutr. Today 31, 5S–8S.

Saxelin, M., Chuang, N.H., Chassy, B., Rautelin, H., Mäkelä, P.H., Salminen, S., Gorbach, S.L., 1996. Lactobacilli and bacteremia in Southern Finland, 1989–1992. Clin. Infect. Dis. 22, 564–566.

Saxelin, M., Rautelin, H., Salminen, S., Mäkelä, P.H., 1996. Safety of commercial products with viable *Lactobacillus* strains. Inf. Dis. Clin. Pract. 5, 331–335.

Schwabb, J.H., 1993. Phlogistic properties of peptido–glycan–polysaccharide polymers from cell walls of pathogenic and normal-flora bacteria which colonise humans. Infect. Immun. 61, 4535–4539.

Shlaes, D.M., Bouvet, A., Devine, C., Shlaes, J.H., Al-Obeid, S., Williamson, R., 1989. Inducible, transferable resistance to vancomycin in *Enterococcus faecalis* A256. Antimicrob. Agents Chemother. 33, 198–203.

Sozzi, T., Smiley, M.B., 1980. Antibiotic resistance of yoghurt starter cultures *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Appl. Environ. Microbiol. 40, 862–865.

Tang, P., Foubister, V., Pucciarelli, M.G., Finlay, B.B., 1993. Methods to study bacterial invasion. J. Microbiol. Methods 18, 227–240.

Tannock, G.W., Kuchansky, J.B., Miller, L., Connel, H., Thode-Andersen, S., Mercer, A.A., Klaenhammer, T.R., 1994. Molecular characterization of a plasmid-borne (pGT633) erythromycin resistance determinant (*ermGT*) from *Lactobacillus reuteri* 100-63. Plasmid 31, 60–71.

Torriani, S., Sottili, U., Giorg, A., Vescovo, M., Dellaglio, F., 1988. Plasmid DNA and Antibiotic susceptibility in *Lactobacillus* strains from human vagina. Microbiol.-Aliments-Nutr. 6, 63–68.

Tynkkynen, S., Singh, K.V., Varmanen, P., 1998. Vancomycin resistance factor in *Lactobacillus rhamnosus* GG is not related to enterococcal vancomycin resistance 8van)genes. Int. J. Food Microbiol. 41, 195–204.

Uttley, A.H., Collins, C.H., Naidoo, J., George, R.C., 1988. Vancomycin-resistant enterococci. Lancet i, 57–78.

Vakil, J.R., Shahani, K.M., 1969. Carbohydrate metabolism of lactic acid cultures. II. Different pathway of lactose metabolism of *S. lactis* and their sensitivity to antibiotics. J. Dairy Sci. 52, 162–168.

Vakil, J.R., Shahani, K.M., 1969. Carbohydrate metabolism of lactic acid cultures III. Glycolytic enzymes of *Streptococcus lactis* and their sensitivity to antibiotics. Can. J. Microbiol. 15, 753–759.

Vescovo, M., Morelli, L., Bottazzi, V., 1982. Drug resistance plasmids in *Lactobacillus reuteri* and *Lactobacillus acidophilus*. Int. J. Microbiol. 1, 1–5.

Whitehead, H.R., Lane, D.J., 1956. The influence of penicillin on the manufacture and ripening of cheddar cheese. J. Dairy Res. 23, 355–360.

Wolf, B.W., Garleb, K.A., Ataya, D.G., Casas, I.A., 1995. Safety and tolerance of *Lactobacillus reuteri* in healthy adult male subjects. Microbiol. Ecol. Health Dis. 8, 41–50.

Woodford, N., Johnson, A.P., Morrison, D., Speller, D.C.E., 1995. Current perspectives on glycopeptide resistance. Clin. Microbiol. Rev. 8, 585–615.

Zunic, P., Lacotte, J., Pegoix, M., Buteux, G., Leroy, G., Mosquett, B., Molin, M., Fongérnie, Á., 1991. *Saccharomyces boulardii*. Therapie 46, 497–501.

Yokokura, T., Mutai, M., 1976. Penicillin resistance and its elimination by treatment with acriflavine in *Lactobacillus fermentum*. Jap. J. Microbiol. 20, 241–242.