Infectivity of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolates in a rat model of experimental endocarditis

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The potential pathogenicity of selected (potentially) probiotic and clinical isolates of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* was investigated in a rat model of experimental endocarditis. In addition, adhesion properties of the lactobacilli for fibrinogen, fibronectin, collagen and laminin, as well as the killing activity of the platelet-microbicidal proteins fibrinopeptide A (FP-A) and connective tissue activating peptide 3 (CTAP-3), were assessed. The 90 % infective dose (ID₉₀) of the *L. rhamnosus* endocarditis isolates varied between 10⁶ and 10⁷ c.f.u., whereas four of the six (potentially) probiotic *L. rhamnosus* isolates showed an ID₉₀ that was at least 10-fold higher (10⁸ c.f.u.) ($P < 0.001$). In contrast, the two other probiotic *L. rhamnosus* isolates exhibited an ID₉₀ (10⁶ and 10⁷ c.f.u.) comparable to the ID₉₀ of the clinical isolates of this species investigated ($P > 0.05$). Importantly, these two probiotic isolates shared the same fluorescent amplified fragment length polymorphism cluster type as the clinical isolate showing the lowest ID₉₀ (10⁶ c.f.u.). *L. paracasei* tended to have a lower infectivity than *L. rhamnosus* (ID₉₀ of 10⁷ to $\geq 10^8$ c.f.u.). All isolates had comparable bacterial counts in cardiac vegetations ($P > 0.05$). Except for one *L. paracasei* strain adhering to all substrates, all tested lactobacilli adhered only weakly or not at all. The platelet peptide FP-A did not show any microbicidal activity against the tested lactobacilli, whereas CTAP-3 killed the majority of the isolates. In general, these results indicate that probiotic lactobacilli display a lower infectivity in experimental endocarditis compared with true endocarditis pathogens. However, the difference in infectivity between *L. rhamnosus* endocarditis and (potentially) probiotic isolates could not be explained by differences in adherence or platelet microbicidal protein susceptibility. Other disease-promoting factors may exist in these organisms and warrant further investigation.

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INTRODUCTION

Some lactobacilli are natural commensals of the gastrointestinal tract, the oral cavity and the female urogenital tract. From artisanal manufacturing to a large industrial scale, *Lactobacillus* strains are used worldwide as starter cultures in the production of dairy products, fermented meat products, alcoholic beverages, sourdough and silage, but they are also one of the most commonly used organisms in probiotics for human consumption (Carr *et al.*, 2002). According to the Food and Agriculture

Organization of the United Nations (FAO) and the World Health Organization (WHO), a 'probiotic' is a live micro-organism that, when administered in adequate amounts, confers a health benefit to the host (WHO & FAO, 2001). Lactobacilli are generally recognized as safe and non-pathogenic in patients without impaired host defences and/or severe underlying disease (Alvarez-Olmos & Oberhelman, 2001; Borriello *et al.*, 2003; Husni *et al.*, 1997). In general, dairy strains of *Lactobacillus* have a long history of safe use (WHO & FAO, 2001). However, lactobacilli have also been implicated in a number of clinical infections, such as bacteraemia, endocarditis, pneumonia, septic arthritis and meningitis (Cannon *et al.*,

Abbreviations: FAFLP, fluorescent amplified fragment length polymorphism; ID₉₀, 90 % infective dose.

2005; Husni *et al.*, 1997; Salminen *et al.*, 2006). At least 73 cases of endocarditis associated with lactobacilli have been reported, in which *Lactobacillus casei* was the most commonly isolated species, followed by *Lactobacillus rhamnosus* and *Lactobacillus plantarum* (Cannon *et al.*, 2005). Due to the controversial taxonomic status of the species *L. casei*, it is likely that the majority of clinical *L. casei* isolates belong to *Lactobacillus paracasei* (Dellaglio *et al.*, 2002; Dicks *et al.*, 1996). Strikingly, some of the reported infections occurred in immunocompetent patients in association with daily consumption of (probiotic) yogurts (Avlami *et al.*, 2001; MacKay *et al.*, 1999; Rautio *et al.*, 1999; Ze-Ze *et al.*, 2004). Although the possible epidemiological link between clinical *Lactobacillus* isolates recovered from infected sites and *Lactobacillus* cultures isolated from (probiotic) dairy products remains controversial and difficult to assess (MacKay *et al.*, 1999; Presterl *et al.*, 2001; Rautio *et al.*, 1999; Salminen *et al.*, 2002; Soleman *et al.*, 2003), the increasing reports of *Lactobacillus*-associated infections raises questions concerning the safety of probiotic strains. However, this increase could also be due to an increased alertness for infections caused by *Lactobacillus*.

Infective endocarditis is a lethal condition if not treated aggressively with antibiotics and optionally combined with surgery (Moreillon & Que, 2004). Pathogens associated with infective endocarditis possess surface adhesins, such as fibrinogen and fibronectin-binding proteins, which mediate attachment to cardiac vegetations, and are involved in valve colonization and infection (Moreillon *et al.*, 2002). For instance, recombinant lactococci expressing staphylococcal adhesins were found to increase their infectivity by more than 100-fold in experimental endocarditis (Que *et al.*, 2001). Furthermore, lactobacilli, including *L. rhamnosus* strains isolated from infective endocarditis, have been shown to possess some characteristics that could be associated with the initial stages of colonization of endocarditis vegetations, such as binding to fibronectin, fibrinogen and collagen (Harty *et al.*, 1993; Schillinger *et al.*, 2005). Interestingly, the probiotic strain *L. rhamnosus* GG has also been shown to display a moderate infectivity in a rabbit model of endocarditis (Asahara *et al.*, 2003). Platelet microbicidal proteins, present in the α -granules of platelets secreted at sites of endovascular damage, are able to kill pathogens on the vegetations. In this regard, it has been suggested that bacterial strains resistant to killing by antimicrobial peptides from human platelets may be more potent inducers of infective endocarditis compared with their susceptible counterparts (Fowler *et al.*, 2000).

The aim of the present study was to evaluate the potential pathogenicity of different probiotic isolates of *L. rhamnosus* and *L. paracasei* in a rat model of experimental endocarditis. This model was chosen for two main reasons. First, it simulates the pathophysiology of infective endocarditis in humans (Carbon, 1993; Moreillon & Que, 2004). Similar to human infection, three main events occur: (i) a pre-existing valve lesion (induced by catheterization) becomes

colonized within minutes during transient bacteraemia (intravenous bacterial inoculation), which involves valve tissue and bacterial factors; (ii) bacterial persistence and growth within the cardiac lesions; and (iii) dissemination of septic emboli to distant organs. Secondly, several reviews summarizing clinical cases of *Lactobacillus* infections (Aguirre & Collins, 1993; Cannon *et al.*, 2005; Gasser, 1994; Husni *et al.*, 1997) concluded that *Lactobacillus* was most commonly associated with endocarditis and bacteraemia, and that the species *L. casei* and *L. rhamnosus* were the most commonly isolated species. Both are also frequently used in probiotic preparations, although it should be noted that most isolates of *L. casei* reported in the literature in fact belong to *L. paracasei* due to the controversial taxonomic status of the former species (Dellaglio *et al.*, 2002; Dicks *et al.*, 1996). Using this model, the infectivity of probiotic *Lactobacillus* isolates was determined in comparison with *Lactobacillus* isolates from patients with infective endocarditis.

METHODS

Bacterial isolates and growth conditions. A total of ten *L. rhamnosus* and seven *L. paracasei* isolates were tested in this study. All of the isolates originated from the PROSAFE strain collection (Vankerckhoven *et al.*, 2004). The isolates were selected based on their genotype determined by fluorescent amplified fragment length polymorphism (FAFLP) and PFGE fingerprinting (Vancanneyt *et al.*, 2006), and their identity was confirmed by protein profiling and FAFLP analysis (Huys *et al.*, 2006). Descriptive details of the 17 isolates studied are listed in Table 1. Isolates were routinely grown on MRS agar or in MRS broth (Difco Laboratories) at 37 °C.

Experimental endocarditis. Sterile aortic vegetations were produced in female Wistar rats (180–200 g) by insertion of a polyethylene catheter across the aortic valve, as described previously (Heraief *et al.*, 1982; Moreillon *et al.*, 1995). Twenty-four hours after catheterization, rats were injected intravenously with 0.5 ml saline containing increasing numbers of organisms (10^4 – 10^8 c.f.u.), allowing a precise titration of the infectivity of each of the tested organisms. Bacterial inocula were prepared from liquid cultures incubated at 37 °C for 18 h. The cells were washed in saline and diluted to the chosen inoculum size in the same solute. The concentration of viable cells was determined by enumeration of plated serial dilutions of the inoculum on MRS agar. Groups of 7 to 11 animals per strain and per inoculum were used. Animals were sacrificed 72 h after bacterial challenge. The heart and infected valves were dissected under sterile conditions after which vegetations were weighed, homogenized, serially diluted and plated on to MRS. Colony counts were performed after 48 h incubation at 37 °C. The minimal infective dose infecting $\geq 90\%$ of aortic vegetations [90% infective dose (ID₉₀)], was determined for each of the isolates. Control stains, including *Staphylococcus aureus* Newman, *Streptococcus gordonii* Challis and mutants of *Lactococcus lactis* 1363, with or without expression of fibronectin-binding protein A (FnbpA), were used as positive and negative standards of infectivity (Moreillon *et al.*, 1995; Que *et al.*, 2001; Stutzmann Meier *et al.*, 2001). Bacterial densities were expressed as log₁₀ c.f.u. (g tissue)⁻¹. The incidences of valve infection were compared using Fisher's exact test. Differences between median bacterial densities in infected tissues were analysed using a Mann–Whitney U-test. A value of $P < 0.05$ was considered to be statistically significant.

Table 1. List of strain isolates studied

PRSF no.	Other strain no.	Species	Depositor*	Category†	Source, geographical origin, year of isolation	AFLP cluster‡	Reference
PRSF-L373	A77/87; LMG 23553	<i>L. rhamnosus</i>	D. Harty	E	Blood (endocarditis)	II	Harty <i>et al.</i> (1993)
PRSF-L235	CCUG 28641	<i>L. rhamnosus</i>	CCUG	E	Heart valve, Sweden, 1991	I	
PRSF-L393	CJ61; LMG 23327	<i>L. rhamnosus</i>	CHUV	E	Blood (endocarditis)	III	
PRSF-L358	L-1198; LMG 23576	<i>L. rhamnosus</i>	A. Avlami	E	Blood (endocarditis)	VII	Avlami <i>et al.</i> (2001)
PRSF-L336		<i>L. rhamnosus</i>	D46	P	Faeces, USA, 1986	I	
PRSF-L175	Gorbach-Goldin (GG); LMG 18243	<i>L. rhamnosus</i>	BCCM/LMG	P	Faeces, USA	I	
PRSF-L397	LB21	<i>L. rhamnosus</i>	D21	N	Faeces, Sweden, 1999	II	
PRSF-L303		<i>L. rhamnosus</i>	D10	P	Dairy product, Canada, 1981	IV	
PRSF-L376		<i>L. rhamnosus</i>	D10	P	Non-human, France, 1998	VII	
PRSF-L477		<i>L. rhamnosus</i>		R	Faeces (human)	VII	
PRSF-L292	YS8866441	<i>L. paracasei</i>	D. Harty	E	Blood (endocarditis)	I	Harty <i>et al.</i> (1993)
PRSF-L288	A22/73	<i>L. paracasei</i>	D. Harty	E	Blood (endocarditis)	IX	Harty <i>et al.</i> (1993)
PRSF-L404	MR191	<i>L. paracasei</i>	D22	P	Dairy product, France	II	
PRSF-L302		<i>L. paracasei</i>	D04	P	Faeces (human), Italy, 1977	IV	
PRSF-L343		<i>L. paracasei</i>	D10	P	Dairy product, Canada, 1994	IX	
PRSF-L360		<i>L. paracasei</i>	D01	P	Faeces (human), Italy, 1998	X	
PRSF-L101		<i>L. paracasei</i>	CFPL	F	Faeces, France, 1998	X	

*BCCM/LMG, BCCM/LMG Bacteria Collection, Ghent University; CCUG, Culture Collection University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden; CHUV, Centre de Collection de type Microbien, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; CFPL, Université Lille, Lille, France; Dx, potential (probiotic) strain received from company x.

†E, Human endocarditis strain; F, human faecal strain; P, strain used commercially as a probiotic; R, research strain under investigation as a potential probiotic strain; N, nutritional isolate from an industrial starter.

‡Vancanneyt *et al.*, 2006.

Adherence to immobilized matrix proteins. Adhesion to extracellular matrix proteins was examined as described previously (Entenza *et al.*, 2005). Serial dilutions of fibrinogen, fibronectin, collagen type IX and laminin (Sigma), all of human origin, were used to coat 96-well polystyrene plates (Maxisorp; Nunc International). The lactobacilli were grown to stationary phase in MRS broth at 37 °C. A sample of 50 µl bacterial cells, corresponding to 10⁹ c.f.u. ml⁻¹, was added to each well and incubated for 2 h at 37 °C. The absorbance values (A₅₇₀) were determined using a Multiscan ELISA reader. Each batch of assays included control strains with known protein-binding levels as well as blank cells; the latter were coated with PBS + 2 % BSA only. Mutants of *Lactococcus lactis* 1363 expressing either *S. aureus* surface adhesin clumping factor A (Que *et al.*, 2001), FnbpA (Que *et al.*, 2001) or collagen-binding protein were used as positive control strains for fibrinogen, fibronectin and collagen, respectively. *S. aureus* Phillips was used as a positive control for laminin. Strain *Lactococcus lactis* PIL253 (Que *et al.*, 2001) was used as a negative control strain for all substrates. According to the absorbance readings of the control strains at the highest concentration, isolates were classified as adherent (A₅₇₀ > 0.3), weakly adherent (0.1 ≤ A₅₇₀ ≤ 0.3), or non-adherent (A₅₇₀ < 0.1) (Styriak *et al.*, 2003). Each experiment was repeated on two or more independent occasions.

The statistical significance of the difference in adhesion characteristics of the tested lactobacilli was analysed by an unpaired *t*-test. A value of *P* < 0.05 was considered to be statistically significant.

Killing activity of platelet microbicidal proteins. The bactericidal action of two platelet microbicidal proteins (both manufactured by Eurogentec), fibrinopeptide A (FP-A) (Tang *et al.*, 2002) and connective tissue activating peptide 3 (CTAP-3) (Krijgsveld *et al.*, 2000),

in which the cationic character was reinforced by an isoleucine to lysine substitution and by a C-terminal amidation in order to increase antimicrobial activity (Brogden, 2005; Bulet *et al.*, 2004), was determined in 96-well plates. The lactobacilli were grown in MRS broth at 37 °C overnight. Bacterial suspensions were transferred in 2-(*N*-morpholino)ethanesulfonic acid (MES) at pH 5.5 or in PBS + 0.06 % (w/v) tryptone soy broth at pH 7.0 for assays of FP-A (Tang *et al.*, 2002) and CTAP-3 (Krijgsveld *et al.*, 2000), respectively. An inoculum of 10⁵–10⁶ c.f.u. ml⁻¹ was added to microtitre wells containing 500 µM FP-A or CTAP-3 (final well volume 100 µl). Colony counts were performed after 2 h incubation at 37 °C. Strains *Lactococcus lactis* 1363 and *S. aureus* Cowan were used as positive and negative control strains, respectively. Each assay batch also contained bacteria in MES or PBS + 0.06 % (w/v) tryptone soy broth without peptide. Assays were performed on two independent occasions. The platelet microbicidal proteins were considered to have a killing effect when a 2–3 log decrease in c.f.u. was observed.

The statistical significance of the difference in microbicidal activity of FP-A and CTAP-3 against the tested lactobacilli was analysed by an unpaired *t*-test. A value of *P* < 0.05 was considered to be statistically significant.

RESULTS

Infectivity of control strains

The ID₉₀ values of *S. aureus* Newman and *Streptococcus gordonii* were 10⁴ and 10⁵ c.f.u., respectively (Fig. 1). These

relatively low inocula correspond to the high infectivity of true endocarditis pathogens (Moreillon *et al.*, 1995; Stutzmann Meier *et al.*, 2001). In contrast, the ID₉₀ of the FnbpA-negative *Lactococcus lactis* strain was 100- to 1000-fold (10^7 c.f.u.) higher compared with those of the *S. aureus* and *Streptococcus gordonii* control strains. However, a mutant of the same *Lactococcus* strain expressing the staphylococcal virulence factor FnbpA exhibited a decreased ID₉₀ of 10^6 c.f.u., thus demonstrating the capability of FnbpA to confer infectivity to lactococci.

Infectivity of endocarditis and probiotic lactobacilli

As depicted in Fig. 1, the ID₉₀ of the *L. rhamnosus* endocarditis isolates varied between 10^6 and 10^7 c.f.u., i.e. in a range situated between the ID₉₀ of true endocarditis pathogens (10^4 – 10^5 c.f.u.) and that of the FnbpA-negative *Lactococcus lactis* strain (10^7 c.f.u.). On contrast, two probiotic isolates (PRSF-L303 and PRSF-L376), one nutritional isolate (PRSF-L397) and one probiotic research isolate (PRSF-L477) of *L. rhamnosus* exhibited ID₉₀ values that were at least 10-fold higher (10^8 c.f.u.) than those of the clinical *L. rhamnosus* isolates ($P < 0.001$) and of the FnbpA-negative *Lactococcus lactis* strain ($P = 0.003$). The remaining two probiotic *L. rhamnosus* isolates (PRSF-L175 and PRSF-L336) demonstrated an ID₉₀ in line with the lower range (10^6 – 10^7 c.f.u.) of the clinical *L. rhamnosus* isolates ($P > 0.05$). Importantly, these two isolates shared the same FAFLP cluster (type I) as the clinical *L. rhamnosus* isolate (PRSF-L235) showing the lowest ID₉₀ (10^6 c.f.u.).

Although the densities of lactobacilli in the vegetations were much lower than those provoked by *S. aureus* and *Streptococcus gordonii* [7 – $9 \log_{10}$ c.f.u. (g tissue)⁻¹] ($P < 0.05$), the range of the mean bacterial densities in infected vegetations of each tested inoculum were similar for probiotic [3.9 – $6.3 \log_{10}$ c.f.u. (g tissue)⁻¹] and endocarditis isolates [5.7 – $6.8 \log_{10}$ c.f.u. (g tissue)⁻¹] ($P > 0.05$). These results indicated that, once the infection has been established, no difference in the evolution of the infection for either organism was observed.

L. paracasei tended to have a lower infectivity than *L. rhamnosus* in the rat model used, with an ID₉₀ of 10^7 to $\geq 10^8$ c.f.u. ml⁻¹ for endocarditis as well as probiotic and faecal isolates (Fig. 1). As with *L. rhamnosus*, the mean bacterial densities in infected vegetations were remarkably lower than those provoked by *S. aureus* and *Streptococcus gordonii*, but were similar for probiotic [4.7 – $5.9 \log_{10}$ c.f.u. (g tissue)⁻¹] and endocarditis isolates [5.6 – $6.1 \log_{10}$ c.f.u. (g tissue)⁻¹] ($P > 0.05$).

To investigate these findings further in terms of endocarditis-promoting determinants, adherence to extracellular matrix proteins and resistance to killing by platelet microbicidal proteins was assayed.

Adhesion to immobilized host matrix proteins

Adhesion of lactobacilli to the extracellular matrix proteins fibrinogen, fibronectin, collagen IX and laminin was investigated. The A₅₇₀ values for fibrinogen, fibronectin, collagen and laminin of *L. rhamnosus* and *L. paracasei*

Fig. 1. Infectivity of *Lactobacillus* isolates. E, Human endocarditis strain; F, human faecal strain; P, strain used commercially as a probiotic; R, research strain under investigation as a potential probiotic; N, nutritional isolate from industrial starter.

isolates are shown in Fig. 2. Except for the faecal *L. paracasei* strain PRSF-L101, which was weakly adherent to fibrinogen ($A_{570}=0.12\text{--}0.28$) but exhibited adherence to fibronectin ($A_{570}=0.55\text{--}1.20$), collagen ($A_{570}=0.25\text{--}0.60$) and laminin ($A_{570}=0.37\text{--}0.59$), all tested *Lactobacillus* isolates adhered only weakly or not at all ($0.1 < A_{570} < 0.3$). No statistically significant differences were observed between endocarditis and probiotic isolates in adhesion to fibrinogen, fibronectin, collagen and laminin ($P > 0.05$).

Killing activity of platelet microbicidal proteins

The microbicidal activity of two platelet proteins, FP-A and CTAP-3, was assayed. FP-A, which is anionic (Tang *et al.*, 2002), did not show activity against any of the *Lactobacillus* isolates (Fig. 3a). In sharp contrast, peptide CTAP-3, in which the cationic character was reinforced (see Methods), was highly effective and killed the majority of the tested isolates (Fig. 3b). No statistically significant differences were seen between endocarditis and probiotic isolates in susceptibility to these platelet microbicidal proteins ($P > 0.05$).

DISCUSSION

The present results indicate that probiotic, nutritional and probiotic research isolates of *Lactobacillus* are less able to cause experimental endocarditis than isolates commonly responsible for human endocarditis (Glauser *et al.*, 1983;

Moreillon *et al.*, 1995). Indeed, it required a 100- to 1000-fold greater number of lactobacilli than *S. aureus* or *Streptococcus gordonii* to infect $\geq 90\%$ of the animals. Thus, the overall low infectivity of probiotics corresponded more to that of the FnbpA-negative *Lactococcus lactis* strain (Que *et al.*, 2001) than to that of true human pathogens.

However, when comparing *L. rhamnosus* isolates of clinical and probiotic origins, differences in infectivity were observed. Clinical *L. rhamnosus* isolates were 10 to 100 times less infective than *S. aureus* and *Streptococcus gordonii*, whereas the infectivity of probiotic *L. rhamnosus* isolates was an additional one $\log_{10}$ lower than that of clinical *L. rhamnosus*. These results suggest the possible existence of a virulence factor present in the clinical isolates, but absent in (potentially) probiotic or nutritional isolates of *L. rhamnosus*. In addition, the fact that these isolates have been implicated in endocarditis in humans could be related to the presence of specific host factors, as *Lactobacillus* endocarditis has been described as occurring mostly, but not exclusively, in patients with severe underlying disease or in immunocompromised patients. Importantly, the two most infective probiotic *L. rhamnosus* isolates, PRSF-L175 (=LMG 18243=*L. rhamnosus* GG) and PRSF-L336, in the experimental endocarditis model (ID_{90} of $10^6\text{--}10^7$ c.f.u.) could be differentiated by PFGE fingerprinting but belonged to the same FAFLP cluster (type I) as the most infective human endocarditis *L. rhamnosus* isolate, PRSF-L235 (Vancanneyt *et al.*, 2006). Previously, Asahara *et al.* (2003) reported that the widely used probiotic strain *L.*

Fig. 2. Bacterial adherence of *Lactobacillus* isolates to increasing concentrations of immobilized fibrinogen (a), fibronectin (b), collagen (c) and laminin (d). Non-adherent isolates ($A_{570} < 0.1$) are not shown. Data are expressed as the mean $\pm$ SD of two to three independent experiments. E, Human endocarditis strain; F, human faecal strain; P, strain used commercially as a probiotic; R, research strain under investigation as a potential probiotic; ClfA, mutant of *Lactococcus lactis* 1363 expressing *S. aureus* surface adhesin clumping factor A; CNA, mutant of *Lactococcus lactis* 1363 expressing collagen-binding protein.

Fig. 3. Microbicidal activity of FP-A (a) and CTAP-3 reinforced in its cationic nature (b). White bars, unexposed bacterial cultures; black bars, cultures exposed to 500 μ M FP-A (a) or CTAP-3 (b).

rhamnosus GG displayed a moderate infectivity in experimental endocarditis in rabbits. Moreover, isolates of *L. rhamnosus* that were indistinguishable from *L. rhamnosus* GG by PFGE or repetitive sequence-based PCR have been implicated in a few cases of human infection such as endocarditis and bacteraemia (Land *et al.*, 2005; Rautio *et al.*, 1999; Salminen *et al.*, 2002). Although clinical isolates were found in all FAFLP clusters, FAFLP cluster type I might represent a specific genotype that is possibly more prone to adapt and eventually infect its animal host compared with other members of *L. rhamnosus*.

According to the findings of Cannon *et al.* (2005), *L. casei* was the most commonly isolated species in endocarditis cases associated with lactobacilli, but due to the controversial taxonomic status of the species *L. casei*, it is likely that the majority of clinical isolates belonged to *L. paracasei* (Dellaglio *et al.*, 2002; Dicks *et al.*, 1996). In the present study, *L. paracasei* isolates were less infective in a rat endocarditis model than *L. rhamnosus*, independent of their origin (endocarditis, probiotic or faecal), which might be explained by the predominance of *Lactobacillus acidophilus* and *L. casei* in the gastrointestinal tract, although few studies have been performed using reliable identification techniques (Fingold *et al.*, 1983; Mikelsaar & Mändar, 1993).

The tested *Lactobacillus* isolates demonstrated a weak or negligible ability to bind extracellular matrix proteins such as fibrinogen, fibronectin, collagen and laminin in adhesion assays. In contrast, the faecal isolate *L. paracasei* PRSF-L101 adhered strongly to fibronectin but showed no increased infectivity in the endocarditis model. The fact that this faecal strain, which adhered strongly to fibronectin but weakly to fibrinogen, was not more prone to cause endocarditis compared with the human isolates might suggest that the first step in developing endocarditis depends primarily on the ability of the bacteria to bind to fibrinogen rather than to fibronectin, which has also been demonstrated for *Lactococcus lactis* (Que *et al.*, 2005). The current findings thus correlate with the lack of evidence of potential endocarditis-promoting determinants such as surface adhesins mediating adherence to cardiac vegetations in lactobacilli.

It has been shown that platelet microbicidal proteins may contribute to the eradication of susceptible bacteria from cardiac vegetations (Dankert *et al.*, 1995; Yeaman, 1997). However, in the current study, the different infectivity of clinical and probiotic *L. rhamnosus* isolates could not be explained by the activity of the platelet microbicidal proteins FP-A and CTAP-3. FP-A was unable to kill any of the

tested micro-organisms. In contrast, CTAP-3 exerted a broader spectrum of activity, with antimicrobial activity against the majority of the lactobacilli tested. However, the observed activity was irrespective of the origin of the isolates. The difference in microbicidal activity between both peptides could be explained by the reinforced cationic nature (isoleucine to lysine substitution and C-terminal amidation) of the CTAP-3 peptide used, which probably increases its antimicrobial activity. In agreement with the findings of Krijgsvelde *et al.* (2000), we did not detect any bactericidal activity against selected *Lactobacillus* isolates using the similar, albeit at higher concentrations, non-cationic reinforced CTAP-3 peptide (data not shown). Possibly, these findings might also explain the lack of bactericidal activity of CTAP-3 in other studies (Tang *et al.*, 2002).

Taking the observed differences in infectivity of the tested lactobacilli in the animal model into consideration, the presence of other disease-promoting factors such as the production of pro-coagulant and/or fibrinolytic enzymes enabling colonization and survival of lactobacilli in an endocarditis vegetation (Oakey *et al.*, 1995) cannot be excluded.

New *Lactobacillus* strains with health-promoting properties are increasingly being introduced on to the probiotic market. However, although lactobacilli have traditionally been recognized as safe and food-grade organisms, it cannot automatically be assumed that new potential probiotics share the same history as strains with a long history of use in food fermentations. Moreover, rare cases of sepsis, bacteraemia and endocarditis (Cannon *et al.*, 2005; Husni *et al.*, 1997; Salminen *et al.*, 2006) indicate the clinical relevance of assessing the safety of new potentially probiotic cultures on an individual basis. The current paper provides an indication of which organisms might warrant further investigation and surveillance.

Notwithstanding the fact that intraspecies and interspecies differences were demonstrated for the tested isolates, our findings indicate that probiotic *Lactobacillus* isolates exhibited a low level of virulence in the rat model of experimental endocarditis. Except for members of FAFLP cluster I, (potentially) probiotic and nutritional *L. rhamnosus* isolates appeared to be less infective than clinical *L. rhamnosus* isolates, and *L. paracasei* isolates were poorly infective, irrespective of their origin. Nevertheless, this difference in infectivity between endocarditis and probiotic *L. rhamnosus* isolates could not be explained by differences in adherence or by platelet microbicidal protein susceptibility. Presumably, other disease-promoting factors might exist in these organisms and this hypothesis certainly warrants further investigation. In our experience, the experimental endocarditis model proved to be highly useful as a potential screening method to assess the safety of bacterial strains intended for probiotic use belonging to species that may possess the ability to provoke this severe pathology. Also, the clinical relevance of the results of the

experimental model should be evaluated further by comparing them with epidemiological data.

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