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MINI-REVIEW

Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review

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Abstract Probiotics are live cultures, usually lactic acid bacteria, which are ingested to promote a healthy gastrointestinal tract. These organisms require certain traits to survive and compete in this niche, but these traits may be transferred to other microbiota in the gastrointestinal tract (GIT). Similarly, virulence factors from pathogens may be acquired by probiotic strains. Bacteria have developed a plethora of methods to transfer genetic material between strains, species and genera. In this review, the possible factors that may be exchanged and the methods of exchange are discussed.

Keywords Probiotics · Intestinal microbiota · Gene transfer

Introduction

The term "lactic acid bacteria" encompasses a large amount of heterogeneous bacteria that are inextricably linked to humans and animals. These organisms occur naturally in diverse environments, such as, the gastrointestinal, oral and respiratory tracts and in food-related environments, such as, milk-, meat-, plant products and wine. The human gastrointestinal tract (GIT) is an extremely complex environment that may be colonized by up to 10^{12} bacteria per gram of intestinal contents (Gueimonde and Salminen 2004). Although microorganisms colonize the gastrointestinal tract from the oral cavity to the rectum, most studies on the effect of probiotics have focussed on the tract from the stomach downwards, including the stomach, duodenum, jejunum, ileum, caecum and colon. The stomach and duodenum contain between 10^1 and 10^3 colony forming units per ml (CFU/ml), comprising predominantly lactobacilli, streptococci and yeasts, while the jejenum and ileum (10^4–10^8 CFU/ml) contain lactobacilli, Enterobacteriaceae, streptococci, *Bacteroides* spp., bifidobacteria and fusobacteria and the colon (10^{10}–10^{12} CFU/g) *Bacteroides* spp., bifidobacteria, streptococci, fusobacteria, Enterobacteriaceae, clostridia, *Veillonella* spp., lactobacilli, *Proteus* spp., staphylococci, *Pseudomonas* spp., yeasts and protozoa (Holzapfel et al. 1998). The colon microflora is dominated by strict anaerobic organisms (Holzapfel et al. 1998). Other organisms present in the human colon include methanogens such as *Methanobrevibacter smithii* and *Methanosphaera stadmaniae* (Brusa et al. 1993; Zhang et al. 2009). These organisms belong to the domain Archea and differ vastly from bacteria, and though they may have genetic elements that could contribute to horizontal gene exchange, they are not considered here.

Possible colonization of the oral cavity, pharynx and oesophagus by probiotics has largely been ignored, even though a large number of microorganisms colonize these areas in healthy humans (King et al. 2002; Lawson and Coyle 2010; Tian et al. 2010). Gene transfer may conceivably occur in all these areas if the probiotics and prevailing organisms are in contact long enough.

Although the composition of the intestinal microbiota may differ for every individual, microorganisms form a barrier between the host and the environment and play an essential role in protecting the host from pathogens and harmful food substances (Gueimonde and Salminen 2004).

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Within this niche, however, it is possible that genes may be transferred between different gut microbiota, gut microbiota and pathogens, or between pathogens, to adapt to an ever-changing environment. Of particular concern is the acquisition of antibiotic resistance genes and virulence traits by non-pathogenic organisms. Transfer of genes between bacteria is well documented, although research studies have focused on horizontal (or lateral) gene transfer (HGT) between pathogens, such as the spread of multidrug resistance (Ochman et al. 2000).

Since lactic acid bacteria (LAB) form a significant part of the microbiota of a healthy human intestinal tract, increasing use, and research into the health benefits of probiotics, defined as viable microbial food supplements beneficial to health, has lead to a vast amount of research information being available on this subject (Dicks and Botes 2010; Mattila-Sandholm et al. 1999; Salminen et al. 2004; Tamime 2005). These organisms, by nature of the use of probiotics, come into close contact with other intestinal bacteria, including pathogens, making the issue of HGT relevant.

Probiotics are usually lactobacilli, enterococci and/or bifidobacteria. Health benefits attributed to probiotics include antimicrobial activity, prevention of diarrhoea, improved lactose metabolism, anti-mutagenic and carcinogenic properties, reduced serum cholesterol and stimulation of the immune system (Shah 2007). An effective probiotic is need to adhere to epithelial cells, survive conditions such as low pH, pepsin and pancreatin activity and bile, and competitively exclude pathogens (Maragkoudakis et al. 2006; Vankerckhoven et al. 2008). An additional trait such as bacteriocin production is an added advantage. In addition to acquiring virulence traits from other organisms, however, the possibility exists that the very attributes that define a good probiotic may also be transferred to other organisms, including pathogens.

Evidence of horizontal gene transfer between lactic acid bacteria and enteric bacteria, and between different lactic acid bacteria has been reported (Bolotin et al. 2004b). Comparative genomic analysis of *Lactococcus lactis* suggests a fairly recent horizontal gene transfer event between lactococci and Gram-negative enterobacteria of the gene *ycdB*, the function of which is unknown. Several factors support this theory: The G + C content of the gene is closer to that of lactococci than enterobacteria. *ycdB* does not occur in species phylogenetically closer related to enteric bacteria. In lactococci, streptococci and enterococci, there is conservation of the gene order upstream of *ycdB*, which is only found in very closely related species of enterobacteria. Conserved gene order indicates a common ancestor, while the absence thereof points to lateral gene transfer (Bolotin et al. 2004b).

Within the lactic acid bacteria, the "*Lactobacillus acidophilus* group" consists of several phylogenetically closely related lactobacilli. These include *Lactobacillus delbrueckii* subsp. *bulgaricus*, which is extensively used for yogurt production, and *Lactobacillus acidophilus* and *Lactobacillus johnsonii*, isolated from the human gut. Based on protein phylogenetic analysis, Nicolas et al. (2007) concluded that while extensive genetic exchange took place between *L acidophilus* and *L. johnsonii*, little or no transfer took place between *L. delbrueckii* subsp. *bulgaricus* and *L. johnsonii*. Nicolas et al. (2007) suggested that the environment where these organisms occur played a role in the horizontal gene transfer events.

Gene transfer capability

Lactic acid bacteria are masters in adaptation. This is reflected in the relatively small size of their genomes, 1.7–3.3 Mbp, containing a variety of genes between 1,600 and 3,000, indicating that adaptation has involved both gene loss and acquisition (Horvath et al. 2009; Makarova and Koonin 2007). Comparative genome sequence analysis of lactic acid bacteria has provided some insight into the historical development of these genomes (Makarova and Koonin 2007; Makarova et al. 2006; Pfeiler and Klaenhammer 2007). These organisms have adapted to grow in nutrient rich environments.

Natural gene transfer usually occurs through the uptake of naked DNA (transformation), viruses (transduction), or plasmids (conjugation; Sorek et al. 2007). The existence of these gene transfer systems has long been established and was recently reviewed (Thomas and Nielsen 2005). Of these mechanisms, conjugation is thought to be prevalent in the transfer of antibiotic gene transfers (Mathur and Singh 2005). Comparative genomics suggests that the organization of genomes of various organisms in the gut is such that they may adapt to an environment (Morelli et al. 2004).

Whole plasmids or segments of genomic DNA may be taken up by transformation. Many human pathogenic bacteria, such as species of *Campylobacter*, *Haemophilus*, *Helicobacter*, *Neisseria*, *Pseudomonas*, *Staphylococcus* and *Streptococcus*, are naturally transformable (Thomas and Nielsen 2005).

Transduction occurs when bacterial DNA is inadvertently included into the DNA of bacteriophages during replication, causing the transfer of foreign DNA to other bacterial genomes in subsequent bacterial infections (Sørensen et al. 2005). While bacteriophages occur in abundance and can horizontally transfer genes by incorporation into host genomes, they usually have a limited host range (Sørensen et al. 2005). Numerous lactic acid

bacteria have been determined to harbour bacteriophages (Yasmin et al. 2010) and those that occur in *Lactococcus* spp. in particular have been studied intensively because of their deleterious effects on dairy fermentations (Forde and Fitzgerald 1999). Bacteriophages often carry virulence factors (Sørensen et al. 2005).

Conjugative transfer of DNA is mediated by cell-to-cell contact, mating-pair formation and DNA transfer through conjugative pili, a process that usually results in the acquisition of a plasmid (Sørensen et al. 2005; Thomas and Nielsen 2005). In lactic acid bacteria, this method of DNA transfer has been observed most often. In enterococci, conjugative transfer systems may be activated through pheromone production by plasmid-free recipient strains to induce a mating response by donor cells harbouring members of specific families of conjugative plasmids (Clewell et al. 2002). This response initiates the activation of membrane proteins that promote aggregation of donors and recipients (Thomas and Nielsen 2005). Other bacteria such as *Staphylococcus aureus* produce similar peptides that may induce enterococci to aggregate (Flannagan and Clewell 2002). This observation indicates that pheromone-inducible plasmids, which are highly transmissible, may facilitate the spread of virulence traits between different species of enterococci, and between enterococci and pathogens (Flannagan and Clewell 2002).

A significant percentage of lateral gene transfer can be attributed to mobile genetic elements, such as, plasmids, insertion sequences, transposons and introns (Ochman et al. 2000). Since these elements are widespread in lactic acid bacteria, these organisms have the ability to exchange genetic information between strains of the same species, different species, or different genera (Morelli et al. 2004).

Plasmids give bacteria the ability to acquire traits that are competitively advantageous in specific niches, for example antibiotic resistance and the ability to use particular carbon sources (Sørensen et al. 2005). Successful plasmid acquisition and stability depend on various factors such as copy number and host specificity. Genes encoding numerous virulence factors occur on plasmids: In *Enterococcus faecalis*, the *bee* operon encoding pili is located on a conjugative plasmid (Hendrickx et al. 2009). In *Lactococcus lactis*, several important traits such as lactose catabolism, bacteriocin production, proteolytic systems and bacteriophage resistance are plasmid encoded. These characteristics may be horizontally transferred for competitive advantage (Siezen et al. 2005).

Mahillon and Chandler (1998) defined insertion sequences (IS) as segments of DNA smaller than 2.5 kb that are capable of inserting at multiple sites in a target molecule. These segments of DNA are phenotypically cryptic and have a simple genetic organization. These elements can be as short as 600–700 bp, encoding a transposase. In the genomes of bacteria, the presence of several closely related IS elements facilitate recombination events that may also include sections of unrelated DNA (Thomas and Nielsen 2005).

Several mobile elements have been found in lactobacilli, including IS*L2* in *Lactobacillus helveticus*, IS*L3* in *Lactobacillus delbrueckii*, IS*1223* in *Lctobacillus johnsonii*, IS*1163* and IS*1520* in *Lactobacillus sakei* and IS*Lp11* in *Lactobacillus plantarum* (Nicoloff and Bringel 2003). DNA sequence comparisons of various LAB indicated that during cheese manufacturing it was possible for insertion elements to be horizontally transferred, most likely through conjugation (Guédon et al. 1998).

Conjugative transposons are widespread in lactic acid bacteria and have been found to confer resistance to tetracycline, erythromycin, chloramphenicol and kanamycin, code for nisin production and sucrose fermentation (Mathur and Singh 2005). These genetic elements have a large host range, vary in size from 16 to 70 kb, and may be inserted into plasmids or chromosomal genes in one or multiple copies (Mathur and Singh 2005). The role that conjugative transposons play in the spread of antibiotic resistance is indicated by similar resistance genes present in diverse bacterial species (Scott 2002). In a review by Burrus and Waldor (2004), conjugative transposons are termed integrative and conjugative elements (ICEs), since they are self-transmissible mobile genetic elements and spread by conjugative transfer to new hosts. The authors also proposed that ICEs promote the mobilization of genomic islands.

In 2000, Mojica et al. described the occurrence of clustered regularly interspaced short palindromic repeats (CRISPRs) found in a wide diversity of prokaryotes. These elements are short palindromic sequences 24–40 bp in length that contain highly reserved inner and terminal inverted repeats of up to 11 bp and are usually adjacent to CRISPR-associated (*cas*) genes (Godde and Bickerton 2006; Horvath et al. 2009). More than one CRISPR locus may occur in a particular genome, and wide variation occurs in the CRISPR loci of strains of the same species (Bolotin et al. 2004a; Godde and Bickerton 2006). CRISPRs provide acquired resistance to phages (Horvath et al. 2009).

Integrons are immobile elements, but they contain gene cassettes that may be mobilized (Fluit and Schmitz 1999). The most common gene cassettes that occur on integrons are those that encode antibiotic resistance.

Pathogenicity islands are gene clusters with more than one virulence gene that are absent in non-pathogenic bacteria. These DNA fragments may occupy relatively large genome regions, which often differ in its G + C content from the host. These islands are frequently associated with mobile genetic elements and usually flanked by direct

repeats (Schmidt and Hensel 2004). A 154 kb pathogenicity island has been identified in *E. faecalis* containing genes encoding cytolysin, enterococcus surface protein, several unknown genes and mobile genetic elements including a transposase (Schmidt and Hensel 2004; Shankar et al. 2004).

Evidence of potential in vivo horizontal gene transfer

Over the last 30 years, a vast number of publications have reported the in vitro transfer ability of various plasmids, transposons and other mobile genetic elements inter- and intraspecies and is beyond the scope of this review. The focus of this review will be on in vivo transfer where either donors or recipients are lactic acid bacteria, including probiotics.

Numerous cases of in vivo horizontal gene transfer studies, primarily in the gut of gnotobiotic mice, have been reported. In terms of virulence, in vivo studies mostly reported transfer of antibiotic resistance, including resistance to vancomycin, tetracycline and erythromycin. In vivo studies by Bourgeois-Nicolaos et al. (2006) indicated that vancomycin A (vanA) resistance could be transferred more readily from animals to humans by *Enterococcus faecium* than by *E. faecalis*. Moubareck et al. (2003) also reported the transfer of vanA from porcine to human enterococci in the gastrointestinal tract of mice. Mater et al. (2005) reported that the vanA gene cluster could be transferred between enterococci without selective pressure. In a later study by the same authors (Mater et al. 2008), the transfer of vanA resistance from *E. faecium* to a commercially available probiotic strain of *L. acidophilus* was achieved in vivo.

Doucet-Populaire et al. (1992) observed the transfer of the conjugative plasmid pAT191 from *E. faecalis* to *Escherichia coli* in mice, conferring kanamycin resistance. Variable results were obtained by other authors who also demonstrated transfer of conjugative plasmids between *L. lactis* and *E. faecalis* (Gruzza et al. 1993, 1994; Igimi et al. 1996). Jacobsen et al. (2007) proved that tetracycline and erythromycin resistance could be transferred from *L. plantarum* to *E. faecalis* in the gastrointestinal tracts of gnotobiotic rats. Morelli et al. (1988) found that transfer of antibiotic resistance genes between two enteric lactic acid bacteria was not affected by the presence of subtherapeutic levels of antibiotic in the diet or water. Licht et al. (2002) used a streptomycin-treated mini-pig to determine the transfer of the pheromone-inducible pCF10 plasmid between strains of *E. faecalis*. Their results also indicated that this plasmid could be transferred even in the absence of selective pressure, with subsequent persistence of the plasmid in colonized strains.

The transfer of other virulence factors has also been researched. Huycke et al. (1992) observed the transfer of pheromone-responsive plasmids, which often carry genes encoding cytolysin and other virulence genes between strains of *E. faecalis* in the GIT of Syrian hamsters. Hirt et al. (2002) concluded that *E. faecalis* strains carrying the pCF10 plasmid showed increased virulence in a rabbit endocarditis model.

Launay et al. (2006) reported the transfer of transposon Tn1549 containing the vancomycin B2 operon from *Clostridium symbiosum* to *E. faecium* and *E. faecalis* in the GIT of gnotobiotic mice. Similarly, the transfer of conjugative transposon Tn1545 with a tetracycline resistance gene from *E. faecalis* to *Listeria monocytogenes* was observed (Doucet-Populaire et al. 1991). Bahl et al. (2004) studied the in vivo transfer of Tn916 conferring tetracycline resistance amongst strains of *E. faecalis*. While transfer took place, some tetracycline-sensitive strains also persisted in the intestines of gnotobiotic rats.

Coburn et al. (2007) observed the transfer of a 150 kb pathogenicity island between two *E. faecalis* strains in the mouse gastrointestinal tract.

Pathogenicity

Pathogenicity, or virulence, has been extensively reviewed by several authors (Arnold et al. 2007; de Sousa 2003; Finlay and Falkow 1997; Jett et al. 1994). Although most bacteria are harmless, the interaction between a host and a bacterium may in certain instances result in the deterioration of the health of the host, to varying degrees. Pathogenic bacteria may be either primary or opportunistic and have specialized traits to be able to compete for colonization of host tissue and cause disease. In addition, several virulence factors have been identified that distinguish a pathogen from non-virulent organisms (de Sousa 2003). Bacteria may develop spontaneous variation in virulence due to small genomic changes, either within a nucleotide sequence, redistribution of sections of DNA within the genome, or by acquiring DNA from other organisms (de Sousa 2003). These changes may be accelerated when an organism is exposed to stress, either by the host defence system or by the environmental stress outside the host (Arnold et al. 2007).

Although lactic acid bacteria are generally regarded as safe and do not usually cause disease, isolated cases of infections have been reported in patients receiving antibiotic treatment or that were severely immune compromised (Bernardeau et al. 2008; Vesterlund et al. 2007). In these patients, endocarditis, bacteraemia and localized infections, such as abdominal abscesses, pulmonary infections and peritonitis, are the most common reported diseases caused by *Lactobacillus* spp. (Slover 2008).

Enterococci, mostly *E. faecalis* and *E. faecium*, are predominant organisms in the human intestinal tract. These two species, together with *Enterococcus durans*, are frequently isolated from human faeces (Franz and Holzapfel 2004). Although enterococci are widely distributed in food products, used as starter cultures and are potentially good probiotics, these organisms are opportunistic pathogens that frequently cause nosocomial infections and have intrinsic low-level resistance to several antimicrobial products, including beta-lactams, clindamycin, lincomycin, aminoglycosides and tetracycline (Franz and Holzapfel 2004; Murray 1990). Several virulence factors have been identified in enterococci (Eaton and Gasson 2001; Franz and Holzapfel 2004).

Factors involved in virulence

Acquired antibiotic resistance

Antibiotic-resistant bacteria occur in high numbers in animals and humans regularly treated with antibiotics (Teuber et al. 1999). While antibiotic resistance in itself is not pathogenic, the emergence of multiple resistant bacteria and the possibility that genes encoding antibiotic resistance may be transferred between starter cultures, probiotics, commensal bacteria and pathogens in the gut, are of great concerns (Ammor et al. 2007; Salyers et al. 2004; Zhou et al. 2005). Lactobacilli, pediococci and leuconostocs have a high intrinsic resistance to vancomycin (Mathur and Singh 2005). Danielsen and Wind (2003) reported natural resistance by some lactobacilli to antibiotics, such as, bacitracin, fucidic acid, cefoxitin, ciprofloxacin, kanamycin, metronidazole, nitrofurantoin, norfloxacin, streptomycin, sulphadiazine, teicoplanin and trimethroprim/sulphamethoxazole. While antibiotic resistance occurs amongst species of LAB, clear-cut patterns have not been determined (Ammor et al. 2007). Great variation in intrinsic antibiotic resistance exists between different species of *Lactobacillus* (Ammor et al. 2007; Danielsen and Wind 2003). Intrinsic resistance to antibiotics is not horizontally transferable, but a natural trait of a particular organism (Mathur and Singh 2005). The focus in this review will be on acquired antibiotic resistance. Jacobsen et al. (2007) proved that tetracycline and erythromycin resistance plasmids from *L. plantarum* could be horizontally transferred to an *E. faecalis* strain in the gastrointestinal tract of rats. In the context of HGT, it has been demonstrated that enterococci may acquire transferable resistance to vancomycin, chloramphenicol, erythromycin and clindamycin (Murray 1990).

Enterococci are often the causative agents of infections in hospitalized patients and nosocomial bloodstream infections (Vankerckhoven et al. 2008). In enterococci, six vancomycin resistance types have been identified phenotypically and genotypically, and their mechanisms of action have been extensively reviewed by Courvalin (2006). VanC is an intrinsic characteristic of *Enterococcus gallinarium* and *Enterococcus casseliflavus–Enterococcus flavescens*, while VanA, VanB, VanD, VanE and VanG can be acquired. The operons encoding VanA and VanB may be located on a plasmid or on the chromosome, while the operons encoding VanC, VanD, VanE and VanG are located on the chromosome. VanA and VanB acquired resistance has been linked with the mobile elements Tn1546 (VanA) and Tn1547 or Tn1549 (VanB; Teuber et al. 1999).

In lactic acid bacteria, scientific evidence points to conjugation as the common method of antibiotic resistance transfer of mobile genetic elements such as plasmids, transposons and integrons.

Virulence factors

A few virulence factors have been reported to date for *Lactobacillus* spp. and *Bifidobacterium* spp., both of which are used as probiotics. A possible virulence trait in lactobacilli may be the ability to aggregate human platelets, which have been found in strains of *Lactobacillus rhamnosus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus oris*, *L. plantarum* and *Lactobacillus salivarius* (Harty et al. 1994). Several virulence factors that may be involved in the infection of a host with pathogenic strains have been recognized (Eaton and Gasson 2001; Franz and Holzapfel 2004). Virulence factors may either be colonization factors, such as, adhesions that promote the adhesion of bacteria to the host cell, invasions, which promote invasion of epithelial cells, evasions, which are substances that evade the host immune system such as phagocytosis, the complement system or antibodies, and siderophores, which are produced when iron concentrations are low (de Sousa 2003). Several enterococcal cell wall-anchored surface proteins are implicated in enterococcal pathogenesis, including aggregation substance, enterococcal surface protein, collagen-binding components and pili (Hendrickx et al. 2009). In addition, secreted products produce or promote the formation of lesions in the host, such as exotoxins, which are either active in the cytoplasmic membrane, alter membrane permeability or act enzymatically in the cell, endotoxins, such as lipopolysaccharides from Gram-negative organisms, hydrolytic enzymes such as proteases and hyaluronidase, super antigens, which interact with lymphocyte receptors, and antigens that induce autoimmune diseases (de Sousa 2003; Jett et al. 1994). The genetic determinants of a variety of these factors have been determined:

1. The toxin cytolysin

Cytolysin is an exotoxin with a bifunctional bacteriocin and haemolytic effect (Haas et al. 2002). This toxin, related to lantibiotics and produced by some strains of *E. faecalis*, causes the invading organism to evade the host immune system (Franz and Holzapfel 2004). This enzyme lyses human, rabbit and horse erythrocytes (Chow et al. 1993) and inhibits Gram-positive but not Gram-negative bacteria (Jett et al. 1994). Cytolysin may either be chromosomally encoded within a pathogenicity island, or by pheromone-responsive plasmids such as pAD1 (Chow et al. 1993; Ike et al. 1984; Shankar et al. 2004,). Eight genes—*cylL*$_L$, *cylL*$_s$, *cylM*, *cylB*, *cylA* and *cylI* transcribed as one operon, and *cylR1*, *cylR2,* transcribed as a second operon—encode products necessary for cytolysin production (Shankar et al. 2004). *cylL*$_L$ and *cylL*$_s$ encode two precursors that are posttranslationally modified by CylM. After posttranslational modification, both interact with CylB, an ATP-binding cassette transporter. After secretion, CylL$_L$ and CylL$_S$ are further modified by CylA, a protease which removes six amino acids from both subunits to form the active toxin units CylL$_L$″ and CylL$_S$″ (Shankar et al. 2004). CylI is an immunity protein. The products encoded by *cylR1* and *cylR2* are involved with repression of the cytolysin genes. No obvious DNA sequence similarities to enterococcal *cylL*$_L$ and *cylL*$_S$ were observed with BLAST analysis.

2. Enterococcal LPxTG surface proteins

These proteins and their genetic determinants were recently extensively reviewed by Hendrickx et al. (2009) and includes aggregation substance (AS), *Enterococcus s*urface protein (Esp), adhesins and other adhesive molecules such as *Enterococcus* endocarditis antigen and pili.

Aggregation substance is a plasmid-encoded pheromone-inducible multifunctional LPxTG surface protein produced by *E. faecalis*. Proteins with the motif LPxTG (Leu-Pro-x (any aa)-Thr-Gly) is a class of cell wall-anchored surface proteins in Gram-positive bacteria that span the cell wall to display functional domains to the surrounding environment (Hendrickx et al. 2009; Isenmann, et al. 2000; Navarre and Schneewind 1999). Three conjugative plasmids, pPD1, pCF10 and pAD1, containing the AS protein encoding genes *asp1*, *asc10* and *asa1*, respectively, have been characterized. These proteins share high amino acid similarities. Expression of the AS protein enables close contact between cells for conjugation and subsequent transfer of virulence plasmids (Hendrickx et al. 2009). The aggregation substance AS may also play a role in translocation of enterococci into epithelial cells (Franz and Holzapfel 2004; Hendrickx et al. 2009; Mundy et al. 2000). Chow et al. (1993) reported an increase in morbidity of rabbits with endocarditis if the causative enterococcal agent expressed both cytolysin and the aggregation substance as opposed to strains that only expressed one of the traits.

*Enterococcus s*urface protein is produced by strains of *E. faecalis* and *E. faecium*. Most of the organisms that produce this protein have been isolated from infection-derived isolates, whereas none isolated from food produce the protein, thus suggesting a role in pathogenicity (Franz and Holzapfel 2004). The protein is chromosomally encoded with a theoretical mass of 202 kDa (Franz and Holzapfel 2004; Shankar et al. 1999). The *esp* gene is usually contained within a pathogenicity island which is often harboured by clinical *E. faecium* and *E. faecalis* strains (Hendrickx et al. 2009). Esp is a cell wall-anchored protein characterized by its ability to form biofilms and may therefore be implicated in enterococcal infections that are biofilm associated, such as, endocarditis, urinary tract-, ocular-, root canal- and implant device-related infections (Heikens et al. 2007; Hendrickx et al. 2009). Heikens et al. (2009) recently reported that Esp did not significantly increase adhesion to intestinal epithelial cells.

MSCRAMMs is a family of surface proteins amongst the host of proteins that pathogenic bacteria have evolved to adhere to and invade host tissues and to evade defence systems. MSCRAMMs share characteristics, such as, an N-terminal signal peptide, an A-domain consisting of folds similar to immunoglobulin, B-domains with more than one repeat and a C-terminal cell wall anchor (Sillanpää et al. 2008, 2009a, b).

Enterococcus faecium and *E. faecalis* produce adhesins Ace and Acm, respectively, which binds to collagen. Ace have been determined to be expressed during human infections, while the gene for Acm is widespread amongst *E. faecium*, although only expressed by clinical isolates, indicating a link with virulence (Franz and Holzapfel 2004). Ace and Acm share some similarity in protein sequence (Hall et al. 2007). Sillanpää et al. (2008, 2009a, b) have identified numerous predicted MSCRAMMs in the genome of *E. faecalis* and *E. faecium*, based on sequence analysis.

Expression of the adhesin-like endocarditis antigens produced by *E. faecalis* has been shown to be induced by *E. faecalis* growth in serum (Franz and Holzapfel 2004). Teng et al. (2003) identified an antigen, SagA, which was essential for *E. faecium* growth, and also bound to fibrinogen, collagens, fibronectin and laminin. Genome sequencing of *Leuconostoc kimchii*, a lactic acid bacterium isolated from Korean vegetable products, indicated the presence of a similar protein to endocarditis antigen found in enterococci, suggesting that the genes encoding this factor may be widely spread.

Pili play an important role in initial adherence to host tissues, followed by colonization of mucosal surfaces

(Hendrickx et al. 2009). Pili are localized cell-surface proteinaceous filaments, and the genes encoding enterococcal pili are arranged in operons with at least one sortase gene. Two pilin gene clusters, the biofilm enhancer pili operon (*bee*) and the endocarditis- and biofilm-associated pili operon (*ebp*), have been described for *E. faecalis*. Four pilin gene clusters have been found in *E. faecium*, and their exact role in pathogenicity is the focus of ongoing research (Hendrickx et al. 2009).

Genome sequencing of *L. rhamnosus* GG has revealed the presence of two pilin gene clusters, *spaCBA* and *spaFED*. The *spaFED* gene cluster was also found in *L. rhamnosus* LC705. No homology was found between these clusters and other bacterial pilin genes, although some similarity was found in the protein sequences of pilin proteins found in *E. faecalis* and *E. faecium* (Kankainen et al. 2009). *spaCBA,* only found in the probiotic *L. rhamnosus* GG, was determined to encode mucus binding pili.

3. Gelatinase

Gelatinase is a protease involved in the hydrolysis of substrates, such as, gelatin, casein, collagen, haemoglobin and small bioactive proteins such as *E. faecalis* sex pheromone-related peptides (Archimbaud et al. 2002; Jett et al. 1994; Makinen et al. 1989). This extracellular zinkmetalloendopeptidase was first characterized more than 40 years ago, but its contribution towards virulence of a particular isolate has not been established, although the protein is often expressed by clinical *E. faecalis* isolates (Bleiweis and Zimmerman 1964; Casas and Zimmerman 1969; Grutter and Zimmerman 1955; Shugart and Beck 1964). Gelatinase production is usually associated with enterococci from clinical samples, but has also been detected in enterococci isolated from dairy and meat products (Lopes et al. 2006).

Studies on 29 clinical strains of *E. faecalis* isolated from patients with endocarditis (10) or bacteremia (10) or from stools of healthy volunteers (9; Archimbaud et al. 2002) indicated that gelatinase was produced by isolates from both ill and healthy groups, and in itself was not essential for pathogenesis. Although the virulent effect of gelatinase has not been conclusively demonstrated, gelatinase production may be correlated with the presence of the cytolysin gene (Archimbaud et al. 2002), gentamicin resistance (Dupont et al. 1998) and collagen binding in root canal infections (Hubble et al. 2003), indicating a possible role in pathogenesis. *gelE* is often found in clinical *E. faecalis* strains but Dupre et al. (2003) could not detect the *gelE* gene in 32 clinical *E. faecium* strains, as opposed to 11 of 15 strains of *E. faecalis*. Similar observations were reported by Elsner et al. (2000) and Kanemitsu et al. (2001). In contrast, gelatinase activity and the *gelE* gene were detected in the species *E. faecalis, E. faecium, E. durans* and *E. hirae*, isolated from raw ewe's milk and cheese by Lopes et al. (2006), *E. faecalis, E. faecium and E. casseliflavus* isolated from house flies (Macovei and Zurek 2006). Joyanes et al. (2000) found that gelatinase played no role in adhesion of *E. faecalis* or *E. faecium* to surfaces such as urinary catheters.

The gene encoding gelatinase, *gelE*, was sequenced by Su et al. (1991). In *E. faecalis*, *gelE* is not plasmid encoded, while expression of GelE is regulated by the *fsr* locus (Arias et al. 2007; Dupont et al. 1998; Qin et al. 2001). Genotypic and phenotypic investigations by Creti et al. (2004) indicated that although *gelE* was present in both clinical and commensal isolates, it was not always expressed. Their results also showed that gelatinase was more often produced by invasive than non-invasive *E. faecalis* strains. BLAST analysis indicates that, to date, *gelE* only occurs in enterococci.

4. Hyaluronidase

The role of hyaluronidase in infection has been extensively reviewed (Girish and Kemparaju 2007; Hynes and Walton 2000). Hyaluronidase facilitates the spread of bacteria and toxins through the host tissue by causing tissue damage (Kayaoglu and Orstavik 2004). This enzyme (also called a spreading factor) degrades hyaluronan to disaccharides, which may be transported and metabolized intracellularly to supply pathogens with nutrients (Hynes and Walton 2000; Pecharki et al. 2008; Starr and Engleberg 2006). Microbial hyaluronidase production is linked to enterococcal virulence primarily because the enzyme is linked to pathogenicity through enzymatic degradation of host tissue in other organisms (Franz and Holzapfel 2004; Girish and Kemparaju 2007; Jett et al. 1994). The enzyme is produced by several Gram-positive bacteria, such as, *Streptococcus* spp, *Staphylococcus* spp., *Peptostreptococcus* spp., *Propionibacterium* spp. and *Clostridium* spp. Several of these species may cause mucosal or skin infections (Hynes and Walton 2000, Kayaoglu and Orstavik 2004, Pecharki et al. 2008). Rice et al. (2003) reported the sequence of a 1,659 bp ORF, designated hyl_{Efm}, from *E. faecium* that was homologous to hyaluronidase genes previously reported, but no functional virulence effect was determined. In clinical strains of *E. faecium*, the hyl_{Efm} gene was carried on large conjugative plasmids (Arias et al. 2009).

5. Extracellular superoxide and sex pheromone

To combat infections, phagocytes produce superoxide (O_2^-) and other reactive oxygen species (ROS) such as H_2O_2 and hydoxyl (Hassett and Cohen 1989). Huycke et al. (1996) assessed the frequency of extracellular superoxide production. Rates of superoxide production produced by

E. faecalis and *E. faecium* strains isolated from bacteremia and endocarditis infections suggested an association between extracellular superoxide production and invasiveness of the organism (Huycke et al. 1996, Mundy et al. 2000). Superoxide production is induced by sex pheromones, which also induce the secretion of lysosomal enzymes (Franz and Holzapfel 2004). The production of a sex pheromone was first described by Dunny et al. (1978) who characterized a substance initially described as a clumping-inducing agent because it caused donor cells to aggregate. This substance was subsequently redefined as a sex pheromone since it is produced by recipient cells to facilitate the transfer of certain conjugative plasmids by donor cells in *E. faecalis*. One such plasmid, the conjugative tetracycline resistance pCF10, has been extensively characterized (Dunny et al. 1985; Tortorello and Dunny 1985; Tortorello et al. 1986). Sex pheromones are therefore also considered to be virulence factors (Eaton and Gasson 2001). BLAST analysis of the DNA sequence for pheromone-responsive conjugative vancomycin resistance plasmids (Genbank accession number AB247327) isolated from *E. faecalis* (Lim et al. 2006) indicated that substantial sections of this plasmid occurred in organisms other than enterococci.

Transferable traits of probiotics

Bacteriocins

Bacteriocins are ribosomally synthesized peptides produced by microorganisms that usually exhibit antimicrobial activity against organisms closely related to the producing bacteria (Van Reenen et al. 2003). In addition to the requirements necessary for an organism to be a good probiotic, bacteriocin production may be an added advantage. Bacteriocin genes may be on the chromosome or on plasmids. The bacteriocin pediocin PA-1 is produced by several different species such as *Pediococcus acidilactici*, *Pediococcus parvulus*, *Pediococcus pentosaceus*, *L. plantarum* and *Bacillus coagulans* (Bennik et al. 1997; Le Marrec et al. 2000; Mora et al. 2000; Miller et al. 2005; Todorov and Dicks 2009). The operon encoding this bacteriocin is usually found on a plasmid suggesting some kind of transfer, although little homology occurs between the rest of the plasmid DNA sequence or the proteins encoded. These proteins often encode mobilizable elements.

Resistance to bile

One of the criteria for probiotics is to have resistance to bile for survival in the gastrointestinal tract, and several bile salt hydrolases have been identified (Begley et al. 2006). Bile is produced in the liver, concentrated in the gall bladder and released into the duodenum, where it aids fat digestion by emulsifying and solubilizing lipids. It is a powerful antimicrobial substance and consists mainly of bile acids, cholesterol and phospholipids (Begley et al. 2005, 2006). Primary bile acids are produced in the liver from cholesterol. Secondary bile acids are produced in the intestine through modification of primary bile acids by bacterial enzymes (Begley et al. 2005). The enzyme bile salt hydrolase (BSH) detoxifies bile by deconjugating bile acids.

Bile salt hydrolase activity has been identified in bacterial species of several genera associated with the gastrointestinal tract, including *Lactobacillus*, *Enterococcus, Bifidobacterium*, *Clostridium* and *Bacteroides.* The enzyme is also produced by the opportunistic pathogen *E. faecalis* and other pathogens such as *L. monocytogenes* and *Xanthomonas maltophilia.*

Several authors have speculated on the role of horizontal gene transfer in regard to BSH (Begley et al. 2006; Elkins et al. 2001; McAuliffe et al. 2005). By comparing genomes, Dussurget et al. (2002) found a BSH in *L. monocytogenes* that was absent from the genome of *Listeria innocua*, although the flanking regions were similar, and which showed 67% similarity to the amino acid sequence of a *L. plantarum* BSH. *Listeria monocytogenes* shares similar environments to lactobacilli, such as the intestine and food. Tanaka et al. (2000) found 37 to 48% identity between the BSH amino acid sequence of *Bifidobacterium longum* SBT2928 and the BSH of several lactobacilli, and 36% identity to *C. perfringens* BSH. Some probiotic strains have multiple BSHs (Begley et al. 2006). *Lactobacillus acidophilus* NCFM expresses two BSA encoded by the genes *bshA* and *bshB*. These proteins have different amino acid sequences and substrate specificities and show similarity to BSH enzymes from other lactobacilli (McAuliffe et al. 2005). *Lactobacillus plantarum* WCFS1 expresses several different BSHs (Bron et al. 2006).

Conclusions

Horizontal gene transfer allows an organism to effectively compete in a new environment. Since microbial genomes stay more or less constant in size, microorganisms must acquire and lose DNA by retaining those genes whose functions prove useful (Lawrence 1999). In an ever-changing environment such as the gastrointestinal tract, one may speculate that the introduction of new organisms such as probiotics may eventually lead to the acquisition or loss of specific functions. Fortunately, universal trends indicate that interspecies recombination decreases exponentially with sequence divergence (Majewski et al. 2000).

Studies by Maisonneuve et al. (2001) and Duval-Iflah et al. (1998) on the effect of fermented milk on transconjugants in the gastrointestinal tract of mice have indicated that diet may influence the transfer of genetic material and subsequent persistence of transconjugants. Despite the necessity for microbial genomes to adapt and maintain themselves in an environment where they are challenged by horizontal gene transfer, these genomes remain stable (Tønjum et al. 2004). In addition, many horizontally transferred genes are likely to have negative effects on the recipient chromosome, causing eventual death and loss of the deleterious mutations within the population (Thomas and Nielsen 2005).

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