Horizontal gene transfer of virulence determinants in selected bacterial foodborne pathogens

B.G. Kelly\textsuperscript{1*}, A. Vespermann\textsuperscript{2} and D.J. Bolton\textsuperscript{3}

\textsuperscript{1}School of Food Science and Environmental Health, Dublin Institute of Technology, Cathal Brugha Street, Dublin 1, Ireland.

\textsuperscript{2}Federal Institut for risk assessment, Thielallee 88-92, D-14195 Berlin, Germany

\textsuperscript{3}Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland

\textbf{Key words:} Horizontal gene transfer, \textit{E. coli} O157:H7, \textit{L. monocytogenes}, \textit{S. aureus}, \textit{Salmonella}.

\textsuperscript{*}Corresponding author. Tel.: +353-1-4027550; fax: +353-1-4024495

\textit{Email address:} bridget.kelly@dit.ie

\textbf{Abbreviations:} °C, degrees Celsius; DNA, deoxyribonucleic acid; EHEC, enterohemorrhagic \textit{E. coli}; HGT, horizontal gene transfer; Inc, incompatibility; MDR, multidrug resistant region; MRSA, methicillin resistant \textit{Staphylococcus aureus}; SaPI, \textit{Staphylococcus aureus} pathogenicity island; SCC, staphylococcal cassette chromosome; SGI, \textit{Salmonella} genomic island; STEC, shiga toxin-producing \textit{E. coli}; Stx, shiga toxin;
Abstract

This review describes horizontal gene transfer from a historical point of view, with descriptions of the first instances of the different bacterial transfer mechanisms: conjugation, transduction and transformation, as well as examples of some of the early acknowledged transfer events. Gene transfer from four selected foodborne pathogens: *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* are highlighted.
Introduction

Horizontal Gene Transfer (HGT) provides novel functions which may allow recipient organisms to proliferate in a previously unexploited niche, leading to diversification of natural populations (Virdi and Sachdeva, 2005). Previous reviews by Kelly et. al. (a, b, this issue) examined four foodborne pathogens: Escherichia coli, Listeria monocytogenes, Staphylococcus aureus and Salmonella spp and in respect to the virulence factors they contain as well as the role different mobile genetic elements play in the evolution of these organisms. This review gives a brief historical account of the different genetic transfer mechanisms in bacteria, how transfer events were first discovered and how they were first achieved in vitro. The transfer of genes from the same four foodborne bacterial pathogens is examined, and future transfer events are considered.

There is no doubt whatsoever about the importance of HGT in the architecture and constant evolution of bacterial species. In order for virulence genes to be transferred from a pathogenic species to a non-pathogenic recipient strain, and actually to be used in the original virulent capacity, there are a number of conditions that need to be fulfilled. Many barriers exist against the efficient transfer, uptake and stabilization of extraneous DNA. The ideal situation is where genes with a certain function, travel from donor to recipient by whatever means necessary, and end up expressing the same function in the recipient cell as in the original donor cell (Thomas and Nielsen, 2005).

The concept of horizontal gene transfer (HGT) was first supported by the transfer of virulence determinants between pneumococci in infected mice, a phenomenon
discovered by Griffith (1928), which later became known as transformation. Since then gene transfer mechanisms have been found to be ubiquitous in bacteria. As discussed in a previous review (Kelly et al., this issue), there are many ways that gene transfer occurs: transformation, transduction and conjugation. The transfer of DNA by vesicles as another method of gene transfer was discovered for bacteria and especially for *E. coli* O157:H7 recently (Kolling and Matthews, 1999, Yaron et al. 2000). The advent of genome sequencing technology and the completion of genome sequences from many bacteria has lead to comparisons between genomes. Phylogenetic comparison, where the similarity or dissimilarity of genomes are compared, and parametric comparison, where the total genome of an organism is examined and genes that appear to be atypical to the rest of the genome (by GC content for example) are two approaches that are used to determine whether horizontal gene transfer events have occurred in a genome (Lawrence and Ochman, 2002). From this sequencing data horizontal gene transfer events have found to be rife in most species examined thus far, an exception being *Buchnera aphidicola* which has not had any gene rearrangements or gene acquisitions in over 50 million years (Tamas et. al., 2002). From a laboratory perspective gene transfer has been demonstrated between a plethora of bacterial species and genera. Gene transfer has also been shown between bacteria to different kingdoms, as in the case of *Agrobacterium tumefaciens* and the transfer of the Ti plasmid to plants and yeast (Davison, 1999). The transfer of DNA from plants to bacteria has also been documented as in the case of a transgenic plant conferring kanamycin resistance to an *Acinetobacter* strain (Gebhard and Smalla, 1998).
Whether a particular gene is transferred successfully depends on the type of transfer, the relationship between the molecule being transferred with the type of transfer, and on other factors, such as the distribution of integrases, specific enzymes concerned with the integration of DNA (Eede et al., 2004). Genes acquired by horizontal transfer from any species can be deleterious, neutral or beneficial to the recipient strain. Genes that are deleterious are usually removed by selection, neutral genes could possibly be detained and genes that have a beneficial effect are selected for (Bolotin et al., 2004).

**Gene transfer processes**

HGT processes were originally detected experimentally about 50 years ago. During the last two decades, conjugation, transformation and transduction have been identified in many bacterial species in a variety of bacterial habitats ranging from soils to biofilms to the gastrointestinal tract (de Vries and Wackernagel, 2004).

**Conjugation**

For conjugation, gene transfer mediated by cell-to-cell contact, the donor and recipient strains need to be in the same environment to allow for the creation of the conjugative machinery between the donor and recipient cells. The virulence genes in question need to be transferable, i.e. they need to exist on mobile genetic elements, segments of DNA encoding proteins important for the mediation of movement of DNA within genomes, as discussed previously (Kelly et. al., a, this issue). The transported DNA has to avoid the restriction nucleases of the recipient and these genes need to be recombined efficiently in order for effective future persistence. The recipient has the more active role that promotes the uptake of exogenous DNA in
transformation but with conjugative methods, the donor is the more active participant in the DNA transfer process (Thomas and Nielsen, 2005). The majority of bacterial gene transfer events in the environment are via conjugation. There are many types of conjugative events, which all have been shown to occur in natural conditions. Many conjugative plasmids and transposons have been shown to have a broad host range including most Gram-negative bacteria and even some Gram-positive bacteria. RK2 is an example of a plasmid capable of conjugal transfer between most gram negative bacteria (Thomas, 1981). This plasmid has been used to create plasmid vectors which have been transferred from Gram-negative *E. coli* to many Gram positive bacterial strains (Trieu-Cuot et al. 1987). Incompatibility group P (IncP) plasmids can transfer by conjugation into Gram-positive, Gram-negative bacteria and *Saccharomyces cerevisiae* (Thomson et al., 1993). Many diverse environments ranging from raw salmon cutting boards and the gut of soil micro-arthropods to *in planta* with citrus trees have been privy to horizontal gene transfer via conjugation mechanisms (Hoffmann et al., 1998, Davison, 1999, El Yacoubi et. al., 2007). Conjugal transfer of resistance plasmids was demonstrated *in vitro* in the 1970s by many groups including: Lakhotia et al (1972); Nivas et al.(1976), where R factors (plasmids which encode resistance determinants) were transferred between two Gram negative strains: *E. coli* and *Salmonella*. *In vitro* conjugative transfer between Gram-positive *Lactobacillus acidophilus* and *Lactobacillus reuteri* was proven by Vescovo et al. (1983). Droge et al. have extensively discussed the role conjugation plays in horizontal gene transfer with regards to biosafety, as this process is the most promiscuous in nature, (Droge et al., 1998).
Plasmids can be classified into what is known as incompatibility (Inc) groups, first introduced by Novick (1987). This Inc scheme is based on the introduction of a plasmid with an unknown Inc group, by conjugation or transformation, into a strain carrying a plasmid with a known Inc group. If the resident plasmid is eradicated from the progeny, then the unknown plasmid has the same Inc group as the resident plasmid. Plasmids with the same replication mechanisms are “incompatible”, and plasmids with different replication mechanisms are “compatible”. Therefore, two plasmids belonging to the same Inc group cannot proliferate in the same cell line (Carattoli et al., 2005). Plasmids with diverse Inc groups differ in host range of transfer, autonomous replication, pilus structure and size (Droge et al., 1998). This is important when considering the dissemination and evolution of plasmids conferring resistances or virulence determinants into strains which may have indigenous plasmids present.

Transformation

Many bacterial species are naturally transformable, i.e. they have the natural ability to take up naked DNA from the environment. In 1994 Lorenz and Wackernagel (1994) listed 44 bacterial strains as naturally transformable; this list was updated by de Vries and Wackernagel in 2004 to make almost 90 species described as naturally transformable to date. This does not include the transformation abilities of the many unculturable bacteria that exist in a range of environments. These naturally transformable strains exist in many different niches and have many different physiologies. Transformation has been shown to occur in many natural ecosystems ranging from soil microcosms to river or spring water. The level of transformability and competence varies between strains of a species, and therefore the fraction of
transformable bacteria present is more than likely underestimated (de Vries and
Wackernagel, 2004). The exogenous DNA must have certain features in order to be
successfully integrated into the host genome. Some naturally competent bacteria such
as *Bacillus subtilis* and *Acinetobacter* take up exogenous DNA from any source with
the same efficiency, whereas bacteria such as *Neisseria gonorrhoeae* and
*Haemophilus influenzae* take up DNA preferentially from their own or related species.
The size of the DNA associating with the cell is important, with high molecular
weight DNA being the best at effecting transformation. The DNA may be modified by
restriction enzymes if it originates from a genetically distant source from the recipient
organism. Effective integration of the extraneous DNA occurs by homologous
recombination and the more divergent the sequences, the less frequent the
homologous recombination events (de Vries and Wackernagel, 2004). Bacterial
species which are not naturally transformable need to be made competent by artificial
means. This was first achieved chemically by treating bacteria to ice-cold solutions of
CaCl$_2$ and heating the DNA to 37°C or 42°C (Mandel and Higa, 1970, Cohen, 1972).
The present day methods for making bacterial cells competent are variations based on
these original techniques, variations which include the use of: divalent cations;
different buffers; harvesting the cells at different stages of the growth cycle; changing
the extent and temperature of heat shock; to name but a few (Sambrook and Russell,
2001). Cells can also be made competent by physical means such as electroporation,
where the cells are exposed to an electrical charge destabilizing the cell membranes
and inducing the formation of transient membrane pores through which DNA
molecules can pass. Originally, this method was used for eukaryotic cells, but was
subsequently adapted for transforming *E. coli* and other bacteria by plasmids (Dower
et al., 1988, Miller, 1988). This method is thought of as the easiest, most efficient and
most reproducible method for transformation of bacterial cells with DNA (Sambrook and Russell, 2001). In transformation, where exogenous DNA is taken up directly from the environment, integrated and expressed under natural growth conditions, DNA must firstly remain intact by avoiding degradation from nucleases in the environment; it must be actively taken up by competent bacteria and then successfully recombined into the host genome to ensure efficient persistence in future generations (Thomas and Nielsen, 2005).

**Transduction**

Transduction, bacteriophage-mediated gene transfer, is a specific HGT process with a limited host range, described as a significant factor in the evolution of bacteria (Brabban et al., 2005). Many bacterial species contain a number of prophages that encode various virulence factors: *E. coli* O157:H7 contains Stx1 and Stx2 prophages, which confer the bacterium with Shiga-toxin producing capabilities (Wick et al., 2005); bacteriophage-encoded functions enabling *Salmonella* to invade tissues and avoid immune responses are encoded by bacteriophages (Boyd and Brussow, 2002); and Staphylococcal Enterotoxin A (SEA) and Staphylococcal Enterotoxin E (SEE) of *S. aureus* are associated with a temperate bacteriophage (Balaban and Rasooly, 2000; Novick et al., 2001). The barriers to transduction are similar to the barriers mentioned for conjugation and transformation. The very specific nature of the bacteriophage interaction also serves as a barrier for efficient HGT between different bacterial species.

Bacteriophages (phages) are common in the environment, and can transfer genes by a generalized or a specialized transduction process. Transduction in the environment
was thought to be unlikely due to the highly specific nature of the host range of bacteriophages. Some bacteriophages can exist as lysogens, where the phage DNA integrates with the host DNA and lies dormant as a part of the host genome until induced back to the infective lytic cycle by some environmental signal. These lysogenic phages are protected from degradation by the host cell in which they reside. The DNA protection offered by the phage protein coat in lytic phages also confers relative stability from environmental degradation. Phages have been known as mediators of environmental gene exchange and they also play an integral role in the evolution of new food-borne pathogens (Brabban et al., 2005). Transduction was originally discovered in 1952 by Zinder and Lederberg (Zinder and Lederberg, 1952), while working on transformation in *Salmonella* strains. Bacteriophage λ was used as a cloning vehicle in transduction in the 1970s by a variety of researchers (Sambrook and Russell, 2001), and since then a great deal of λ and non-λ derived phage vectors have been described and used in molecular biology for the orchestrated transfer of genes from strain to strain.

**Transfer of genes from selected donor organisms**

The capacity of bacteria to transfer virulence determinants should be a source of concern for the food industry. Transfer of virulence genes from a pathogenic bacterial strain to a non-pathogenic strain may result in the emergence of new foodborne pathogens, and widespread contamination of the food chain. The occurrence of gene transfer in four foodborne pathogens *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus* and *Salmonella* species will be discussed in this section in
order to gain insight into the potential problems that may arise in the future. Some of the transfer events that occur in each bacterium are outlined in table 1.

Escherichia coli

Resistance to tetracycline, sulphonamides, ampicillin and streptomycin are commonly observed in *E. coli* isolates. During the last few years, resistance to clinically relevant, front-line antimicrobials such as fluoroquinolones, extended-spectrum \(\beta\)-lactams (including extended-spectrum cephalosporins) has been emerging among *E. coli* strains (Schroeder et al., 2002). The isolation of antimicrobial-resistant *E. coli* from the intestinal flora of healthy humans and animals, in addition to data which demonstrates that *E. coli* readily transfers plasmids to strains within species or between shiga toxin-producing *E. coli* (STEC, also known as EHEC, enterohemorrhagic *E. coli*) (Johnson et al., 1994) and to strains of different genera such as *Hafnia alvei* (Zhao et al., 2001), indicates that commensal *E. coli* strains are an important reservoir of transferable antimicrobial resistance genes (Singh et al., 2005). With isolates of *Salmonella enterica* serovar Typhi, *E. coli* and *Klebsiella pneumoniae* recovered from stool samples it was shown that *E. coli*, *K. pneumoniae*, and S. Typhi were all conjugally proficient; antibiotic resistance was transferred to S. Typhi at high frequencies. Resistance markers were transferred in to *E. coli* K-12 at a frequency of approximately \(7 \times 10^{-3}\). Similarly, *E. coli* K-12 (containing the R factors from *K. pneumoniae*) was able to transfer resistance to clinical isolates of *K. pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* at a frequency of approximately \(3 \times 10^{-3}\) (Schwalbe et al., 1990).
Shiga toxin production is almost invariably associated with lamboid stx phages. Phages are considered as highly mobile genetic elements which play a profound role in horizontal gene transfer and the emergence of new STEC pathotypes (Muniesa, 2000). The wide distribution of Stx1 and Stx2 variants in different bacteria indicates that these phages possess the ability of transmitting stx genes throughout the Enterobacteriaceae, although, several studies reported a limited host range for individual stx phages (Saunders et al., 2001). Stx1- toxin producing strains have been found among Shigella dysenteriae type I, Shigella sonnei (Strauch et al. 2001), widely distributed in > 100 serotypes of E. coli (Karch et al. 1999) and occasionally in other Enterobacteriaceae such as Citrobacter freundii (Schmidt et al., 1993, Tschäpe et al., 1995), and Enterobacter cloacae (Paton and Paton, 1996). Stx phages can be transmitted between different bacteria in vivo and in vitro. Extra-intestinal transmission of phages is also possible. The presence of infectious shiga toxin phages could be observed in sewage and in faecally contaminated rivers. Phages or lysogenic strains harbouring Stx2 phages might be the natural reservoir of Stx2 genes and lysogenization could be the main cause of the emergence of STEC strains. Lysogenization/conversion processes could take place in food and water and probably inside the human and animal gut. Ingestion of Stx2 phages has been shown to result in the conversion of non-Stx2- E. coli strains present inside the gut to Stx2-producing E. coli strains thus producing new pathogenic strains (Muniesa and Jofre, 2004). Further studies on the ecology and physiology of Stx phages and their hosts are needed to get a better understanding of the mechanisms and evolutionary forces that extend the genetic spectrum of E. coli and other bacterial pathogens (Creuzburg et al., 2005). The key role bacteriophage-mediated gene transfer has been discussed by Brabban et al (2005), where they found that Stx-encoding bacteriophage continues to mediate the
transfer of virulence genes within the *E. coli* family and beyond and may be a critical factor in the evolution of pathogens and the emergence of new pathogens.

Morabito et al. (2002) isolated STEC *E. coli* strains from humans, cattle, and food. Strains belonging to serogroups O26, O111, and O157 were examined for susceptibility to several antimicrobial drugs. Integrons (class 1) were found more frequently in strains belonging to serogroups O111 and O26 than in the O157 isolates. DNA sequence analysis showed that most of the integrons contained the *aadA1* gene cassette conferring resistance to streptomycin/spectinomycin alone or associated with the *drfA1* gene cassette conferring resistance to trimethoprim. In a O157:H7 strain, one integron carried the *aadA2* and *dfxA12* gene cassettes, conferring resistance to streptomycin/spectinomycin and trimethoprim, and the open reading frame F (OrfF) encoding unknown functions. Most of the integrons were carried by *Tn21* derivative transposons and were transferable by conjugation to an *E. coli* K-12 strain. Singh et al. (2005) reported that the transfer of integrons (located on plasmids) by conjugation between strains of *E. coli* resulted in transfer of antimicrobial-resistant phenotypes for ampicillin, chloramphenicol, cefalothin, gentamicin, tetracycline, trimethoprim, sulfamethoxazole and streptomycin and facilitated the emergence and dissemination of antimicrobial resistance among STEC in humans and food animals. Horizontal transfer of nonconjugative plasmids has been shown in biofilms between mixed *E. coli* strains by Maeda et al. (2006), with transformation being the most likely mechanism of DNA uptake. This may prove important in the transfer and dissemination of plasmid-encoded virulence determinants in natural environments, and also raises questions as to whether this type of DNA transfer is possible between other bacterial species.
Listeria monocytogenes

*L. monocytogenes* is the only one of the eight species of *Listeria* classified as pathogenic for humans, causing a highly fatal illness known as listeriosis (Vazquez-Boland et al., 2001). However, in rare cases another species, *Listeria ivanovii*, which is considered specific to ruminants, is pathogenic to humans (Snapir et al., 2006). Foodborne transmission is the main route of acquiring listeriosis. Immunocompromised individuals such as: the elderly, newborns, and people with HIV are more susceptible to the condition than individuals in the fullness of health.

Many strains of *L. monocytogenes* have demonstrated resistance to various antibiotics, with the first multiresistant strain isolated from France in 1988 (Poyart-Salmeron et al., 1990). Conjugal transfer of various plasmids from *Enterococcus* and *Streptococcus* to *Listeria* and between species of *Listeria* has been described in many reviews (Charpentier and Courvalin, 1999). Further evidence of transfer events comes from Bertrand et al. (2005). Sequence analysis of tetracycline resistant determinant *tet(M)* from a variety of *L. monocytogenes* strains infers that the acquisition of this resistant gene has been from successive transfers between other Gram-positive organisms. Generalized transduction, where any gene within a donor organism can be transferred to a recipient strain by lytic or temperate bacteriophage, has been demonstrated in *L. monocytogenes* by Hodgson et al. (2000), suggesting another method for HGT events in *Listeria*. 
Transfer of the tetracycline resistance gene, tet(M), from a food source of *L. monocytogenes* to an *Enterococcus faecalis* strain has been shown by Bertrand et al. (2005). The *L. monocytogenes* strain was found not to contain a detectable plasmid, but does possess a member of the transposon Tn916-Tn1545 family. This may indicate that the transfer of tet(M) involved movement of a conjugative transposon element. A study by Zhang et al. (2007) also showed transfer of tet(M) from *L. monocytogenes*, previously isolated from retail foods, to *E. faecalis*.

Transfer of any other virulence determinant from *L. monocytogenes* has not been documented to date, but Johnson et. al. (2004) found an atypical strain of *L. innocua* that contained genes from the *Listeria* pathogenicity island-1 (LIPI-1), usually found in *L. monocytogenes*. One explanation the authors put forward for this anomalous strain is horizontal gene transfer from *L. monocytogenes* to *L. innocua*. However, a later study by Volokov et al. (2007) deemed it highly unlikely that horizontal gene transfer occurred between the *L. monocytogenes* and the atypical *L. innocua* strain, but that the *L. innocua* strain evolved from an ancestral *L. monocytogenes* strain.

*Staphylococcus aureus*

*S. aureus* contains several pathogenicity islands, which contain virulence determinants and toxin genes. There are seven pathogenicity islands described based on particular characteristics of their structure in genome regions (Gill et al., 2005). Phage related integrase genes are present on these pathogenicity islands, suggesting that they are integrated and excised in a method similar to prophages. SaPI, known as the prototype pathogenicity island, is mobilizable with the assistance of the transducing phages (Novick et al., 2001, Novick, 2003). Transfer of toxin genes by phage conversion or
lysogenic bacteriophage is a central means in virulent strain evolution (Ito, 2003). It is thought that all the related pathogenicity islands are mobilizable in the same way as SaPI1, but this has not been proven to date. Úbeda et al. (2003) reported that the bovine specific pathogenicity island SalPIbov2 is mobile without the assistance of helper phage. It has also been shown by Maiques et. al. (2007) that SalPIbov2 can be induced to replicate by different staphylococcal phages. Antibiotics have been shown to induce the SOS response in *S. aureus* resulting in a cascade reaction which promotes the induction of horizontal transfer of pathogenicity island SapIbov1 (Úbeda et al., 2005). Recently, it was demonstrated that SOS response activation by β-lactam antibiotics really stimulates *S. aureus* pathogenicity island transfer (Maiques et al., 2006). Prophage mobilization was also shown to occur by inducing the SOS response with ciprofloxacin (Cirz et al., 2007). Some of the Staphylococcal enterotoxins, responsible for food poisoning, reside on pathogenicity islands thus they may be transferred to strains without certain enterotoxins. When using SOS response-inducing antibiotics, prudence should be exercised, not just only because of the promotion of the spread of antibiotic resistance genes, but also because of the promotion of the induction of pathogenicity islands containing virulence determinants. Through genome comparisons Gill et al. (2005) hypothesised a continuing evolution of virulence and resistance determinants in *S. aureus*, and a transition of *S. epidermidis* from a commensal organism to an opportunistic pathogen via the acquisition of extra virulence factors (Gill et al., 2005).

**Salmonella**

*Salmonella* species contain a wide variety of mobile genetic elements from pathogenicity islands to conjugative transposons, as previously described (Kelly et al.,
b, this issue). Pathogenicity islands contain clusters of functionally related genes necessary for virulence in *Salmonella*. Ten *Salmonella* pathogenicity islands (SPIs) (Bishop *et al.*, 2005) and other regions known as “islets” containing only a few virulence genes (van Asten and van Dijk, 2005) have been found in *Salmonella* species to date. Many of the more prominent *Salmonella* virulence determinants are associated with mobile genetic elements and in many cases have been shown to transfer both *in vivo* and *in vitro*.

It was originally thought that the antibiotic resistant *Salmonella enterica* serovar Typhimurium DT104 was spread clonally in Europe and the United States (Cloeckaert and Schwarz, 2001). But then it was found that *Salmonella* SGI-1, the genomic island which contains the multiple resistance region was present in a wide range of serovars by Levings *et al.* (2005). This element is present on a 13 kb integron which was shown to be transferable. Doublet *et al.* (2005) reported that SGI-1 could be transferred by conjugation between *Salmonella* strains that were SGI-1 negative and to *E. coli*, with the help of plasmid R55, a helper Inc C plasmid. The mobility of this resistance entity more than likely contributes to spread of antibiotic resistance genes between *S. enterica* serovars. Therefore, the dissemination of *Salmonella* genomic island-1(SGI-1), containing the multidrug resistance region (MDR) by horizontal gene transfer, has been proven by the discovery of this element in different serovars of *S. enterica* besides *S. Typhimurium* DT104 and *S. Enteritidis* (Velge *et al.*, 2005). Ahmed *et al.* (2007) reported that the Gram-negative bacterium *Proteus mirabilis* contained a variant of SGI-1 which seemed to be integrated at a chromosomal site different to the *attB* site in *Salmonella* spp. Inspired by this finding, Doublet *et al.* (2007) scanned the sequenced genome for SGI-1 homologous integration sites in P.
mirabilis and found one gene with 70% identity to the thdF integration site (attB) in *Salmonella*. Moreover, they scanned many bacteria other than *Salmonella* and *E. coli*, including *Shigella* spp, *Legionella pneumophila* and *Klebsiella pneumoniae* and found potential attB sites.

*Salmonella* strains harbour many different temperate bacteriophages, belonging mainly to the P22 family which can facilitate lateral gene transfer by transduction. This transduction mechanism is responsible for a process known as lysogenic conversion, where non-pathogenic strains are converted to pathogenic strains by the addition of lysogenic phages containing virulence factors (Krylov, 2003). Some of these phages can induce lysogenic conversion from one *Salmonella* Typhimurium phage type to another (Mmolawa et al., 2002). Figueroa-Bossi et al. (Figueroa-Bossi et al., 2001) discussed the many prophages in *Salmonella* and demonstrated the ability of three *S. Typhimurium* phages Gifsy-1, Gifsy-2 and Gifsy-3 to successfully lysogenize serovars other than *S. Typhimurium*. Virulence determinants which lie on resident prophages can also be transferred between the different families of phages which lie on the genome of one *Salmonella* serovar (Mirold et al., 2001). It has been shown that *Salmonella* Typhimurium can transfer antibiotic resistance determinants within human epithelial cells by conjugation (Ferguson et al., 2002). One of the worrying trends observed in *Salmonella* recently is the formation of resistance and virulence cointegrated plasmids, where the resistance determinants are encoded on the virulence plasmids, and can be disseminated to other serovars (Fluit, 2005).
Conclusion

This review gives a historical account of horizontal gene transfer, illustrating the importance of transfer events in the emergence of new bacterial strains, and also discusses transfer events in certain foodborne bacteria.

The genetic pool consists of a vast array of mobile genetic elements, which in theory can be spread from species to species as every organism has the potential to take up DNA. In essence the likelihood of an organism adopting extraneous DNA is limited by a number of factors: the ability of the organism to take up the DNA, the readiness to deliver DNA and the chance of the organisms being physically close to each other at the same time in the same environment. Every organism that shares a particular ecological niche or organisms passing through can be thought of as potential donors and recipients (Hanssen and Ericson Sollid, 2006).

There are other barriers to horizontal gene transfer which may be significant. Plasmids with the same incompatibility groups cannot survive in the same cell (Novick, 1987); this is a huge consideration to the successful uptake of a conjugative plasmid. A good deal of lateral gene transfer is dependent on bacteriophage and generalized transduction, but the incidence and dissemination of these phages are not known (Lindsay and Holden, 2006). The genetic background of the recipient, and its ability to utilise the incoming genetic sequences have a huge impact on the success of a transfer.

It has been shown in recent literature, that virulence genes have been transferred between the same strains and sometimes between different strains of the foodborne
Running title: Identification of potential recipient organisms

pathogens discussed in this review. *E. coli* has transferred genetic material to a variety of bacteria including: *Klebsiella, Pseudomonas, Enterobacter, Salmonella* and other *Enterobacteriaceae* (Schmidt et al., 1993, Paton and Paton, 1996), as well as transferring genes to other *E. coli* strains, via phages and conjugative avenues (Schwalbe et al. 1990). *L. monocytogenes* has transferred resistance determinants to *Enterococci* and other *Listeria* strains via conjugation (Bertrand et al. 2005, Zhang et al., 2007). Horizontal transfer of resistance determinants in *S. aureus* has not yet been proven (Hanssen and Ericson Sollid, 2006), but the transfer of genes for the preformed toxins which cause foodborne gastroenteritis has been shown to be linked to transducing phages (Novick et al. 2001, Novick, 2003). The *Salmonella* genomic island has been transferred between different *Salmonella enterica* strains and to *E. coli* via conjugation (Doublet et al. 2005), and virulence determinants that lie on resident prophages have been shown to transfer between different *Salmonella* serovars and between the different phage families which resides on the *Salmonella* genome (Mmolawa et al., 2002).

The foodborne bacterial pathogens discussed have transferred different genes to other bacterial strains via conjugation, transformation and transduction. Transfer events recorded have required that the donor and recipient organisms be present in the same general environment, e.g. transfer of non-conjugative plasmids between mixed *E. coli* strains in a biofilm (Maeda et al., 2006). So, recipients of virulence factors and potential emerging pathogens from *E. coli, L. monocytogenes, S. aureus* and *Salmonella* are bacteria such as commensals that are already share an environment like the gastrointestinal tract with these organisms (Farthing, 2004). Future studies are
needed to elucidate the mechanism of pathogen evolution in relation to foodstuffs, and
what part non-pathogenic organisms, like commensals play in this evolution.
Running title: Identification of potential recipient organisms

References


Running title: Identification of potential recipient organisms


Thomas, C. M. (1981) Molecular-genetics of broad host range plasmid RK2. Plasmid 5, 10-19


### Tables

**Table 1. Examples of transfer mechanisms evident in four foodborne pathogens**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Mechanism</th>
<th>Example</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Conjugation</td>
<td>Plasmid transfer between different <em>E. coli</em> strains</td>
<td>Johnson et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasmid transfer between different species</td>
<td>Zhao et al., 2001</td>
</tr>
<tr>
<td></td>
<td>Transduction</td>
<td>Stx-2 conversion of non-Stx-2 <em>E. coli</em></td>
<td>Muniesa and Jofre, 2004</td>
</tr>
<tr>
<td></td>
<td>Transformation</td>
<td>Non-conjugative plasmid transfer in biofilms</td>
<td>Maeda et al., 2006</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>Conjugation</td>
<td>Plasmid transfer between <em>Listeria</em> strains</td>
<td>Charpentier and Courvalin, 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasmid transfer to <em>Enterococcus faecalis</em></td>
<td>Zhang et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Transduction</td>
<td>Generalised transduction</td>
<td>Hodgson et al., 2000</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>Transduction related</td>
<td>Transfer of SapIBov1</td>
<td>Ubeda et al., 2003</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Conjugation</td>
<td>SGI-transfer to other <em>Salmonella</em> and <em>E. coli</em></td>
<td>Doublet et al., 1995</td>
</tr>
<tr>
<td></td>
<td>Transduction</td>
<td>Lysogeny with <em>Gifsy</em>-1, <em>Gifsy</em>-2 and <em>Gifsy</em>-3 in serovars other than <em>S. Typhimurium</em></td>
<td>Figueroa-Bossi et al., 2001</td>
</tr>
</tbody>
</table>