

Thematic Issue on Horizontal Gene Transfer

Review article

Horizontal gene transfer between bacteria

Holger HEUER and Kornelia SMALLA*

Federal Biological Research Centre for Agriculture and Forestry (BBA), Braunschweig, Germany

Horizontal gene transfer (HGT) refers to the acquisition of foreign genes by organisms. The occurrence of HGT among bacteria in the environment is assumed to have implications in the risk assessment of genetically modified bacteria which are released into the environment. First, introduced genetic sequences from a genetically modified bacterium could be transferred to indigenous micro-organisms and alter their genome and subsequently their ecological niche. Second, the genetically modified bacterium released into the environment might capture mobile genetic elements (MGE) from indigenous micro-organisms which could extend its ecological potential. Thus, for a risk assessment it is important to understand the extent of HGT and genome plasticity of bacteria in the environment. This review summarizes the present state of knowledge on HGT between bacteria as a crucial mechanism contributing to bacterial adaptability and diversity. In view of the use of GM crops and microbes in agricultural settings, in this mini-review we focus particularly on the presence and role of MGE in soil and plant-associated bacteria and the factors affecting gene transfer.

Keywords: horizontal or lateral gene transfer / mobile genetic element / horizontal gene pool / genome flexibility

EXTENSIVE HGT IN THE EVOLUTION OF BACTERIAL GENOMES

The significance of HGT between bacteria was first recognized when ‘infectious heredity’ of multiple antibiotic resistance to pathogens was observed (Watanabe, 1963). Since then, the assumed importance of HGT has changed several times, but recent advances mainly in whole genome sequencing of bacteria suggested that HGT is a major, if not the dominant, force in bacterial evolution (Berg and Kurland, 2002; Doolittle et al., 2003). Evidence for massive gene exchanges in bacterial evolution was discovered in completely sequenced genomes by deviant composition of acquired genetic elements (guanine + cytosine content, codon usage), high similarity of genes to distantly related species, variation of gene content between closely related strains, and incongruent phylogenetic trees (Koonin et al., 2001). Up to 20% of a typical bacterial genome can be acquired from other species (Ochman et al., 2000). Often remnants of plasmid, phage or transposon-related sequences are found adjacent to genes identified as horizontally transferred, suggesting that these MGE served as vectors for HGT (Ochman et al., 2000). The search of 56 sequenced bacterial genomes for prophage sequences performed by Canchaya et al. (2003) revealed that 40 genomes con-

tained prophage sequences exceeding 10 kb in length, which encoded numerous virulence factors and other adaptive traits. The two sequenced genomes of the bacterium *Xylella fastidiosa*, the leafhopper-transmitted causal agent of citrus cancer, were shown to carry five or six prophages, and prophage occurrence seemed to have influenced genome evolution. With respect to observed large genome differences between closely related strains, Lan and Reeves (2000) proposed the concept of a bacterial species genome defined by a core set of genes which is shared by a large majority of isolates of a species, and an auxiliary/foreign set of genes. Correspondingly, the latter was also called the flexible genome (Hacker and Carniel, 2001), as it is determined by its high plasticity, *i.e.* gene acquisition and loss.

HGT can only affect bacteria that readily exchange genes, and genome analysis of eight free-living bacteria indicated that members of such ‘exchange communities’ have a tendency to be similar in factors like genome size, genome G/C composition, carbon utilization, and oxygen tolerance (Jain et al., 2003). Not all genes seem equally likely to be horizontally transferred (Jain et al., 1999). The modularity of genetic units supports their spread by HGT. The pace of genome innovation is accelerated by HGT, which provides functional modules rather than slowly creating new genes by mutations (Jain et al., 2003).

* Corresponding author: k.smalla@bba.de

Real-time adaptation by HGT

While genome sequences retrospectively evidenced horizontally acquired genes over long time periods and their importance in bacterial evolution, there is also much evidence now for HGT being an ongoing process that plays a primary role in the real-time ecological adaptation of prokaryotes. MGE have an essential role in this process by shaping bacterial genomes, promoting intra-species variability and distributing functional genetic modules within exchange communities. Dynamically changing selection forces promote HGT of those genetic modules which allow rapid adaptation to such factors. Consequently, HGT of genetic modules that allowed adaptation to rapidly evolving biotic interactions was frequently observed (recently reviewed by Smets and Barkay, 2005). Such interactions are, *e.g.*, the production of antibiotics by microbes or their use by humans resulting in the spread of antibiotic resistance (McManus et al., 2002; Witte, 1998), the release of xenobiotics or new secondary metabolites and the spread of degradative genes and pathway assembly (Larraín-Linton et al., 2006; Top and Springael, 2003), or pathogenic and symbiotic interactions and the spread of genomic islands (Arnold et al., 2003; Hacker and Kaper, 2000).

Well documented is the example of the dissemination of antibiotic resistance genes by HGT, which allowed bacterial populations to rapidly adapt to a strong selective pressure by medically or agronomically used antibiotics (Heuer and Smalla, 2007; Mazel and Davies, 1999; McManus et al., 2002; Tschäpe, 1994; Witte, 1998). The combinatorial genetic evolution of multi-resistance is facilitated by transposons, IS-elements and integrons. A new class of transposable elements was recently discovered, termed ISCRs, which mobilize DNA adjacent to their insertion site by rolling circle replication (Toleman et al., 2006). The ISCRs studied so far were closely associated with many antibiotic resistance genes, and were often located on conjugative plasmids. Another example of bacterial capabilities that seem to have evolved and spread rather recently by HGT is the capability to degrade man-made xenobiotic compounds. Often the necessary degradative genes are located on IncP-1 plasmids (Top and Springael, 2003), which are the most promiscuous (or broad host range, BHR) self-transmissible plasmids characterized to date (Adamczyk and Jagura-Burdzy, 2003). The DNA sequence of IncP-1 plasmids is typically a mosaic of diverse origin, providing evidence of an active participation in horizontal gene transfer (Heuer et al., 2004; Schlüter et al., 2003; Smalla et al., 2006). Also, MGEs other than plasmids have been shown to carry catabolic genes and to be responsible for their lateral exchange, resulting in the assembly of new pathways. A transposable element that codes for the degradation of biphenyl and 4-chlorobiphenyl was described by Merlin et al. (1999)

and detected in several PCB-degrading bacteria isolated from various environments (Springael et al., 2001). A variety of plasmids involved in chloroaniline degradation was described (Boon et al., 2001; Dejonghe et al., 2002). Recent findings strongly suggest that such MGEs play a very important role in the dissemination of degradative genes among bacteria, and thus in the natural construction of new degradative pathways (Top and Springael, 2003).

Mobile genetic elements also play an important role in the evolution of pathogenic or symbiotic bacteria (Arnold et al., 2003; Hacker and Kaper, 2000; Vivian et al., 2001; Zhao et al., 2005). Comparative genome analysis, *e.g.*, of different *E. coli* strains, revealed that HGT, gene loss and repeated IS element-mediated chromosomal rearrangements played an important role in the evolution of bacterial genomes, and that pathogenicity islands (PAIs) have the potential for ongoing rearrangements, deletions and insertions (Brzuszkiewicz et al., 2006). Genomic differences between the *E. coli* strains compared were not exclusively due to the presence or absence of large PAIs but also due to smaller gene clusters often flanked by MGE (Brzuszkiewicz et al., 2006). The driving force for the acquisition of foreign DNA by HGT is thought to be the need to overcome environmental constraints for survival, and to compete successfully in their ecological niche (Hacker and Kaper, 2000).

MECHANISMS OF HGT

Horizontal gene transfer (HGT) between bacteria is driven by three major processes: transformation (the uptake of free DNA), transduction (gene transfer mediated by bacteriophages) and conjugation (gene transfer by means of plasmids or integrative conjugative elements). Mobile genetic elements (MGEs) such as plasmids, bacteriophages, integrative conjugative elements, transposons, IS elements, integrons, gene cassettes and genomic islands are the important vehicles in the latter two processes. A brief summary of characteristic properties of MGEs is given in Table 1 (modified according to Dobrindt et al., 2004). In many species, a high proportion of horizontally transferred genes can be attributed to plasmid, phage or transposon-related sequences, since remnants of MGEs are often found adjacent to genes identified as horizontally transferred (Brüssow et al., 2004; Ochman et al., 2000).

Natural transformation

Natural transformation is generally understood as the uptake of free DNA by competent bacteria (Chen and Dubnau, 2004; Dubnau, 1999; Lorenz and Wackernagel,

Table 1. Characteristics of mobile genetic elements (MGEs).

MGE	Properties	Review
Plasmids	Circular or linear extrachromosomal replicons; self-transferable or mobilizable plasmids are vehicles for the transmission of genetic information between a broad or narrow range of species	Thomas, 2000
Bacteriophages	Viruses that infect prokaryotes; can integrate into the host genome and then be vehicles for horizontal gene transfer	Canchaya et al., 2003
Integrative conjugative elements (ICEs)	Self-transferable conjugative elements that integrate into the genome of new hosts like temperate bacteriophages; may promote the mobilization of genomic islands by utilizing conserved integration sites	Burrus and Waldor, 2004
Genomic islands	Large chromosomal regions acquired by horizontal transfer that are flanked by repeat structures and contain genes for chromosomal integration and excision	Dobrindt et al., 2004
Transposable elements	Genetic elements that can move within or between replicons by action of their transposase; flanked by inverted repeats, transposons typically carry genes for antibiotic resistance or other phenotypes, while IS-elements code only for the transposase; multiple copies of the same IS-element promote genome plasticity by homologous recombination	Mahillon and Chandler, 1998
Integrans	Genetic elements that capture promoterless gene cassettes at an attachment site downstream of a promoter, by action of the integrase encoded on the integron; frequently associated with transposons and conjugative plasmids	Hall and Collis, 1995

1994). The uptake of DNA can serve as nutrient source, for DNA repair or as source of genetic innovation (Dubnau, 1999). The uptake of DNA can be followed by an integration into the bacterial genome by homologous recombination, homology-facilitated illegitimate recombination (de Vries and Wackernagel, 2002), or by forming an autonomously replicating element. Natural transformation provides a mechanism of gene transfer that enables competent bacteria to generate genetic variability by making use of DNA present in their surroundings (Dubnau, 1999; Nielsen et al., 2000). Prerequisites for natural transformation are the availability of free DNA, the development of competence, the uptake and stable integration of the captured DNA. While the molecular mechanisms required for natural transformation are being intensively studied for some species, there is very limited knowledge of how important natural transformation is in different environmental settings for the adaptability of bacteria. Mainly two aspects of natural transformation in the environment have been studied: the persistence of free DNA, and the ability of different bacterial species

to become competent and take up free DNA under environmental conditions. Different studies have shown that in spite of the ubiquitous occurrence of DNases, high-molecular free DNA could be detected and its persistence in different environments has been demonstrated (Gebhard and Smalla, 1999; Nielsen et al., 1997; Paget and Simonet, 1997; Widmer et al., 1996; 1997). Natural transformation is considered to also be the mechanism by which competent bacteria could capture DNA from transgenic plants (de Vries and Wackernagel, 1998; 2004; Gebhard and Smalla, 1998). The fate of transgenic constructs in natural settings could be followed by PCR or by selecting specific and directed gene restoration after uptake of transgenic plant DNA (de Vries and Wackernagel, 1998; de Vries et al., 2003; Gebhard and Smalla, 1999).

Microbial activity was pinpointed as an important biotic factor affecting the persistence of free DNA in soil (Blum et al., 1997). Cell walls or other debris might play an important role in protecting DNA after cell death (Nielsen et al., 2000; Paget and Simonet, 1997). A more rapid breakdown of DNA was observed at higher soil

humidity and temperature. Both factors are thought to contribute to a higher microbial activity in soil (Blum et al., 1997; Widmer et al., 1996). In the study of Demanèche et al. (2001a) plasmid DNA adsorbed on clay particles was found to be incompletely degradable even at high nuclease concentrations. The adsorption of DNA seems to be a charge-dependent process, and thus the rate and extent of adsorption of dissolved DNA to minerals depends largely on the type of mineral, the pH of the bulk phase, whereas the conformation and the molecular size of the DNA molecules have a minor effect (Lorenz and Wackernagel, 1994; Paget and Simonet, 1994). Since DNA can persist adsorbed on soil particles or protected in plant or bacterial cells, this DNA could be captured by competent bacteria.

Although it is thought that natural competence is widespread among bacterial species (Dubnau, 1999; Lorenz and Wackernagel, 1994), the proportion of bacteria in natural settings that can become competent and the environmental conditions stimulating competence development and are largely unknown. Natural competence is the genetically programmed physiological state permitting the efficient uptake of macro-molecular DNA. Transformation is a tightly regulated process, and requires an elaborate machinery with more than a dozen of proteins involved. For only a rather limited number of bacterial species have the natural transformation systems been studied in great detail (reviewed by Dubnau, 1999). Transformability seems to be a property that is not systematically shared by all isolates belonging to the same species, and transformation frequencies can differ up to four orders of magnitude among transformable isolates of a species (Maamar and Dubnau, 2005; Sikorski et al., 2002). The soil bacterium *Bacillus subtilis* responds to environmental stress with competence development. In *B. subtilis*, competence development is part of a physiological state distinct from vegetative growth or sporulation, which is developed by 10–20% of the cells in the late growth stage under specific nutritional conditions (Berka et al., 2002). In addition to differences in the DNA uptake processes, bacteria do not exhibit the same efficiency to integrate the incoming DNA by heterologous recombination (Sikorski et al., 2002). The majority of studies on transformation in the context of biosafety research have been performed with strain *Acinetobacter* sp. BD413. Recently, the naturally transformable *Acinetobacter* sp. ADP1 strain and its derivative BD413 were shown to belong to the newly described species *Acinetobacter baylyi* (Vanechoutte et al., 2006). This strain can be transformed efficiently with DNA of different sources. A few reports on the development of the competence state under environmental conditions exist. Nielsen et al. (1997) showed that the addition of nutrients can stimulate competence development

of *Acinetobacter* sp. BD413 in bulk soil. Competence development was reported for the plant pathogen *Ralstonia solanacearum* and the co-inoculated *Acinetobacter* sp. BD413 when colonizing tobacco plants. Even more strikingly, for the two soil isolates *Pseudomonas fluorescens* and *Agrobacterium tumefaciens*, natural transformation without any specific physical or chemical treatment was observed in soil (Demanèche et al., 2001b). A peptide-pheromone system, which controls competence in *Streptococcus mutans* functions optimally when cells are living in actively growing biofilms (Li et al., 2001). Biofilms seemed also to facilitate natural transformation of *Acinetobacter* sp. BD413 and did not offer a barrier against effective natural transformation (Hendrickx et al., 2003). Recently, the uptake of DNA by *Pseudomonas* cells during lightning has been described as an alternative mechanism, promoting the uptake of DNA by bacteria which might not possess a sophisticated mechanism for developing natural competence (Cérémonie et al., 2004; 2006). However, the relevance of this mechanism as a gene transfer process in nature is still debated.

Transduction

Transduction is a mechanism of DNA acquisition by which non-viral DNA can be transferred from an infected host bacterium to a new host via infectious or non-infectious virus particles. Host DNA is mistakenly packaged into the empty phage head when the phage particle is produced. Defective phage particles which are released from lysed host cells, can adsorb to new host cells and deliver the DNA carried in the capsid into the new host. The injected bacterial DNA can be integrated into the recipient genome. Although most bacteriophages infect only a narrow range of hosts, this mechanism of gene transfer has the advantage that transducing phages can be rather persistent under environmental conditions, do not require cell-cell contact, and DNA in transducing phage particles is protected (Wommack and Colwell, 2000).

Evidence for the importance of transduction as an HGT process under environmental conditions comes mainly from recent studies on the abundance of bacteriophages in different environmental settings and from bacterial genome sequences. Bacteriophage counts done by means of electron or epifluorescence microscopy revealed that in marine or fresh water samples bacteriophage counts were about 10-fold higher than bacterial counts. The determination of direct bacteriophage counts in soil is obviously more problematic, and only recently studies revealed also a high abundance of viruses in soil (Ashelford et al., 2003). These authors discussed that the average counts of viruses determined in soil (approx. $1.5 \times 10^7 \text{ g}^{-1}$) were most likely an underestimate.

Most of the sequenced bacterial genomes contain prophage sequences (reviewed by Canchaya et al., 2003). Many pathogenicity determinants (toxins) have been acquired via phages, e.g. by *Corynebacterium diphtheria*, *Clostridium botulinum*, *Streptococcus pyogenes*, *Staphylococcus aureus* and Shiga toxin producing *E. coli* (reviewed by Brüßow et al., 2004). Pathogenicity islands (PAI) are large genomic islands that carry one or more virulence gene, which often evolved from lysogenic bacteriophages, and are assumed to be more frequent in pathogenic strains than nonpathogenic strains (Dobrindt et al., 2004; Hacker et al., 2003). However, complete annotation of genome sequences revealed that some nonpathogenic strains can also carry PAIs encoding traits such as adhesins, iron uptake systems or proteases, which contribute to general adaptability, fitness and competitiveness, but lack prominent virulence factors (Grozdanov et al., 2004).

Conjugation

Conjugation is the process whereby a DNA molecule (plasmid or conjugative transposon) is transferred from a donor to a physically attached recipient cell via the so-called conjugation apparatus (Zechner et al., 2000). Although common mechanistic principles are shared by most of the conjugative system, e.g., the synthesis of conjugative pili, there is a remarkable diversity of conjugative systems in Gram-negative and in Gram-positive bacteria. Depending on the shape and characteristics of the plasmid-encoded pili, plasmids transfer better on surfaces, e.g., in biofilms or between planctonic cells (Pukall et al., 1996). Plasmids that do not carry the complete set of genes coding for proteins required for the conjugative transfer apparatus can still be transferred to recipient cells by mobilizing plasmids (Frey and Bagdasarian, 1989), by phages, or by transformation.

A critical property of plasmids is their host range. Host range is not an “all or nothing” property (Heuer et al., 2007). In the environment, certain species are preferred among the potential hosts, and adaptation of host and plasmid to each other can shift the host range (Heuer et al., 2007). Plasmids with a broad host range often appear to have lost restriction sites that are specific for nucleases of the strain to which they are frequently transferring. In addition, they may carry anti-restriction systems that minimize the effect of cleavage by special nucleases that protect many bacteria from invasion by foreign DNA.

Information on the presence of conjugative or self-transferable plasmids in environmental bacteria comes from screening of bacterial isolates, from capturing transferable plasmids into recipients (exogenous isolation) directly from the bacterial fraction of environmental samples, or from sequencing of complete bacterial genomes

(reviewed by Smalla and Heuer, 2006). The proportion of plasmid-carrying bacterial populations is thought to depend on the species, the environmental habitat studied, and the extent of selective pressure. Transfer of conjugative plasmids or transposons has been demonstrated to occur in different environmental settings. Transfer frequencies are mainly affected by the metabolic status of the donor (Johnsen and Kroer, 2006; Pukall et al., 1996). New tools to study HGT by conjugation have improved the knowledge on the horizontal gene pool, and greatly facilitated the identification of factors affecting HGT and the sites where HGT occurs.

In general, MGEs are agreed to add some, often small or even not measurable, metabolic burden to their host, although adaptation can occur to minimize this impact (Dahlberg and Chao, 2003; Heuer et al., 2007). Consequently, the prevalence of plasmids is evidence that they can be of benefit to bacteria in the environment, to compensate for any burden they might impose on the cell. The way they do this may not be identical for all plasmids – a small, high copy number plasmid and a large, self-transmissible plasmid may benefit its hosts in different ways. Often traits conferring an improved fitness or ability to colonize environmental niches are located on conjugative MGEs. The broadest view is that MGEs increase the chance of new strains arising with novel or increased selective advantages over their neighbors. Plasmids survive because bacterial communities and their environments are continually changing, so that the variability that an MGE allows increases the speed at which adapted strains arise, and the adapted strains carry the MGE and propagate it faster. Thus MGEs that increase adaptability evolve and will survive at the expense of those that do not.

MGEs IN BACTERIAL COMMUNITIES OF AGRICULTURAL SETTINGS

Prevalence of MGEs in soil or plant-associated bacteria

The use of genomic approaches has revealed a large and untapped diversity of MGEs resident in soil and plant-associated bacteria: plasmids, prophages, pathogenicity islands and integrons. Surveys on the presence of plasmids isolated from plant-associated or soil bacteria have been performed, and revealed that a considerable proportion of bacteria from different environments carried plasmids. Approximately 18% of bacterial isolates from the phytosphere of sugar beets were found to contain plasmids (Powell et al., 1993), and a large proportion of these plasmids were able to mobilize non-self-transferable but mobilizable IncQ plasmids (Kobayashi and Bailey, 1994). The exogenous isolation of MGEs,

which was originally used to retrieve plasmids from river epilithon (Bale et al., 1988), was also successfully used to capture MGEs from soil or phytosphere communities (Smalla and Sobczyk, 2002). Recipients functioning as a genetic sink, and introduced under laboratory or *in situ* conditions, have acquired MGEs conferring selectable traits such as mercury or antibiotic resistance from the bacterial fraction of bulk or rhizosphere soil (Drønen et al., 1998; Heuer and Smalla, 2007; Heuer et al., 2002; van Overbeek et al., 2002). Mercury resistance was used as an effective selective marker to exogenously isolate self-transferable plasmids from the phytosphere of different crops in Gram-negative recipients (Lilley and Bailey, 1997; Lilley et al. 1994; 1996; Schneiker et al., 2001; Smit et al., 1998) or from mercury-polluted soils (Drønen et al., 1998). Capturing degradative genes resident on MGEs has also been successfully demonstrated from soils treated with 2,4-D, but not from untreated controls (Top et al., 1995; 1996). Self-transferable plasmids conferring resistance towards a range of antibiotics were captured from animal manures used for soil fertilization (Heuer and Smalla, 2007; Heuer et al. 2002; Smalla et al., 2000; van Overbeek et al., 2002). Mobilizing plasmids were isolated by Van Elsas et al. (1998), when bacterial communities obtained from the rhizosphere of young wheat plants served as donor in a tri-parental mating. Plasmid pIPO2 was isolated in *R. eutropha* based on its mobilizing capacity. Replicon typing and sequencing of the complete plasmid (Tauch et al., 2002) revealed that this cryptic plasmid of a size of approx. 45 kbp was not related to any of the known BHR plasmids except to plasmid pSB102 (Schneiker et al., 2001). Sequencing of plant-associated bacteria revealed that many phytopathogenic and symbiotic bacteria carry plasmids (Vivian et al., 2001; Zhao et al., 2005), pathogenicity islands (Arnold et al., 2003) or integrons (Gillings et al., 2005).

Factors influencing HGT in soil

Soils usually provide only restricted resources supporting microbial growth, resulting in limited population densities and activity. This in turn restricts those microbial processes that are dependent on density and activity, such as all HGT (Timms-Wilson et al., 2001; Van Elsas et al., 2003; 2006). However, particular sites in soil and the phytosphere have been shown to provide good conditions for bacterial colonization and activity, resulting in the occurrence of locally-enhanced densities of active cells. These sites are often conducive to HGT processes, and are regarded as “hot” spots for bacterial gene transfer activity. Soil treatments with manure, decomposing plant material or plant-derived root exudates are considered to stimulate HGT in soil (Götz and Smalla, 1997; Heuer and

Smalla, 2007). The plant species-dependent bacterial diversity in the rhizosphere (Costa et al., 2006a; 2006b; Smalla et al., 2001), which is believed to be caused by different root exudation patterns and differences of root shape, has recently been shown to also influence plasmid transfer frequencies (Mølbak et al., 2007). Key abiotic and biotic factors that affect the extent of HGT in hot spots in the phytosphere or in soil have been reviewed recently (Van Elsas et al., 2003; 2006). The importance of selection on the impact of gene transfer processes in soils was discussed in a review by Top et al. (2002). Recently, Heuer and Smalla (2007) showed that treating soils with manure or with manure supplemented with sulfadiazine resulted in significantly increased abundance of genes conferring resistance to sulfadiazine, of MGEs (integron gene cassettes or plasmids), and increased transfer frequencies of exogenously isolated MGEs conferring antibiotic resistances. The presence of nutrients as well as of surfaces which can be colonized is particularly important, as such sites are known to support large densities of metabolically-active micro-organisms. Using both microcosm and *in situ* experiments, HGT between bacterial hosts has been shown to occur in soil and in the phytosphere (Lilley and Bailey, 1997; Mølbak et al., 2003; Normander et al., 1998; Pukall et al., 1996; Sengeløv et al., 2001). The use of marker genes in combination with microscopic tools or flow cytometry allowed monitoring of HGT processes by conjugation *in situ* at a single cell level (Sørensen et al., 2005).

Contribution of MGEs to the adaptability and diversity of soil or plant-associated bacteria

Nowadays, HGT is viewed as an important and common process by which also soil or plant-associated bacteria adapt to changing environmental conditions, broaden their range of environmental niches by increasing their fitness and competitiveness. Although MGEs have been detected in many soil and plant-associated bacteria, their function often remains unclear. The presence of a mercury resistance operon cannot explain why *Pseudomonas* isolates from the phytosphere host large plasmids of greater than 300 kb which carry plant-inducible genes (Zhang et al., 2004). Sequence analysis of one of these *Pseudomonas* plasmids revealed that the functions of almost all putative genes still need to be investigated to get an idea about their contribution to host fitness in the phytosphere.

Vivian et al. (2001) reviewed the role of plasmids in phytopathogenic bacteria and stressed the paucity of knowledge concerning almost all aspects of plasmid biology among phytopathogenic bacteria. This notion can be extended to the molecular biology and ecology of most MGEs associated with plant-associated or soil bacteria.

This is even more surprising, as a substantial number of genes involved in pathogenicity and host specificity of phytopathogens have been assigned to plasmids. Traits encoded on plasmids found in phytopathogenic strains range from cytokinin, ethylene, indolacetic acid, UV resistance, streptomycin or copper resistance, toxins such as coronatine to avirulence genes (Vivian et al., 2001). While avirulence genes or toxin-encoding genes are often found on plasmids, the genes encoding the specialized protein secretion machinery (termed type III secretion system), which is required to deliver proteins to plant cells, are more often located on PAIs in the chromosome (Arnold et al., 2003). Exceptions are *Ralstonia solanacearum* and *Erwinia herbicola*, where the TTSS is located on a 2.1 Mb plasmid and 150 kb plasmid, respectively. Genes involved in pathogenicity are thought to be acquired by HGT mediated by phages, conjugative transposons or plasmids. Many genomic islands in phytopathogenic bacteria probably originated from plasmids encoding beneficial traits that integrated into the chromosome. PAIs are usually flanked by direct repeats, indicating that they were integrated into the host genome via homologous recombination (Arnold et al., 2003). Sequencing complete genomes of phytopathogens revealed that PAIs can occur on plasmids and the chromosome, and have similar features as the ones studied in great detail in human pathogens (Arnold et al., 2003; Hacker and Kaper, 2000). Genomic islands have usually G+C content that is lower than the surrounding genome. A lower G+C content may indicate the presence of acquired DNA, or have a direct functional role by maintaining low expression levels or silencing of laterally acquired genes (Lucchini et al., 2006). Integrons best known for their potential to assemble antibiotic resistance gene cassettes in clinical bacteria were also reported to occur in different soil bacteria. Gillings et al. (2005) explored the importance of integrons as source of *Xanthomonas* genome diversity. In general, strains belonging to the same pathovar had identical sets of gene cassettes, and these had no similarity to the gene cassettes found in different pathovars. So far, the *Xanthomonas* cassettes are phenotypically cryptic, and future research is required to clarify whether their gene products contribute to the adaptation of the phytopathogen to their particular plant host (Gillings et al., 2005). Furthermore, integrons might be an important mechanism for incorporating horizontally acquired genes into the chromosome of its host.

The presence of plasmids and the conjugative transfer process was recently shown to contribute to biofilm formation (Ghigo, 2001; Reisner et al., 2006), and this might also have implications for colonization in the rhizosphere.

MGE-driven bacterial adaptation to man-made selective pressure such as antibiotics, copper or herbicides

used in agriculture is also quite well documented. Increased abundances of bacteria with MGEs carrying respective resistance genes or degradative genes and transfer frequencies were observed in polluted soils compared to the control (Heuer and Smalla, 2007; Top et al., 1995). Identical nucleotide sequences of resistance genes in bacteria from antibiotic-treated farm animals and humans suggested transfer of these genes between these habitats, raising the risk of acquisition of resistances by human pathogens through the use of antibiotics in agricultural animal feed (Tschäpe, 1994). In a recent study, it was shown that bacteriostatic compounds like tetracycline could increase spread and establishment in the intestine of transconjugants that acquired a resistance-conferring plasmid (Licht et al., 2003). Although only a few aspects of the flexible gene pool of soil and plant-associated bacteria could be highlighted in this mini-review, these examples should be sufficient to elucidate the enormous importance of bacterial genome plasticity for survival and successful colonization of new niches and adaptation of soil or phytosphere bacteria to ever changing environmental conditions.

CONCLUSIONS

It is now generally accepted that HGT played an important role in the evolution of bacterial genomes. It is also a major mechanism for real-time adaptation of bacteria. The mosaic structure evident in many bacterial genomes sequenced reflects the selective pressure for genetic plasticity. The rapid accumulation of bacterial genome sequences and the development of powerful tools allowed – and will continue to allow – new insights into the horizontal gene pool, *i.e.* the horizontally transferred genetic modules and their mobile genetic vehicles. Bacterial species are constantly diversifying by sampling the horizontal gene pool, which enables populations to rapidly adapt to ever-changing environments. However, most aspects of their ecological niche remain constant, and select for the less variable part of the genome which code for the main characteristics of the species.

The risk assessment of a genetically modified organism must consider the potential for and, more importantly, the likelihood of any adverse effects of HGT to bacteria in the environment. If there is any phenotype associated with the inserted gene(s) which is potentially unwanted in another host background, such as toxicity, pathogenicity, increased virulence, resistance to antibiotics, competitive advantage, utilization of novel substrates, or greatly expanded host range, then a close examination of the potential for gene transfer is warranted. Otherwise, if there is already a significant existing gene pool in the environment for genes imparting a particular inserted trait, evaluation of the exposure components of

risk seems to be gratuitous, in view of the tremendous genome plasticity of bacteria in nature.

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