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Horizontal gene exchange in environmental microbiota

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Horizontal gene transfer (HGT) plays an important role in the evolution of life on the Earth. This view is supported by numerous occasions of HGT that are recorded in the genomes of all three domains of living organisms. HGT-mediated rapid evolution is especially noticeable among the Bacteria, which demonstrate formidable adaptability in the face of recent environmental changes imposed by human activities, such as the use of antibiotics, industrial contamination, and intensive agriculture. At the heart of the HGT-driven bacterial evolution and adaptation are highly sophisticated natural genetic engineering tools in the form of a variety of mobile genetic elements (MGEs). The main aim of this review is to give a brief account of the occurrence and diversity of MGEs in natural ecosystems and of the environmental factors that may affect MGE-mediated HGT.

Keywords: horizontal gene transfer, soil, aquatic ecosystems, gut, biofilm

HISTORICAL OVERVIEW

Historically, the first portrayal of horizontal gene transfer (HGT) was made by Griffith (1928), who described the transformation of rough, avirulent live pneumococci into smooth, virulent pneumococci by the addition of factors from dead, smooth, and virulent pneumococci. The term "transformation" at the time had no genetic component in the sense it is perceived today; it simply referred to the "transformation" of pneumococcal phenotype. The transforming factor, unknown until 1944, appeared to be a desoxyribonucleic acid (DNA), thus bringing a genetic context to the original description of transformation (Avery et al., 1944). The discovery of the first mobile genetic elements (MGEs) in bacteria, phages (reviewed in Duckworth, 1976), and plasmids (Lederberg, 1952), has laid the foundation for the development of contemporary molecular biology, which has contributed enormously to many fields of biology, medicine, and biotechnology. This era began in the 1930s and 1950s, when the three main mechanisms of HGT – transduction, transformation, and conjugation – were discovered. The understanding of the fundamentals of the structure-and-function of MGEs allowed us to adapt these mechanisms to serve our needs, in the form of genetic engineering technology, in a number of applications in science, biotechnology, and everyday life.

Another factor that attracted increased attention to MGEs and HGT in bacteria is the problem of antibiotic resistance in pathogenic bacteria. Although the use of antibiotics has revolutionized the treatment of infectious diseases in humans and animals, the emergence of antibiotic resistant pathogens such as *Staphylococcus aureus*, *Streptococcus* and *Enterococcus*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Salmonella* and *Escherichia coli*, and *Acinetobacter baumannii* poses a grave concern. The question, therefore, is: why and how does this happen? Why does a recently introduced and efficient antibiotic, after some years of use, become essentially useless for treatment of an infection? The problem was first encountered in Japanese communities and hospitals in the 1950s when they faced *Shigella* dysentery outbreaks that resisted to treatment by usual antibiotics. Intensive clinical and genetic investigations performed by Japanese scientists during that period resulted in the concept of episome-mediated transfer of drug resistance in *Enterobacteriaceae* (Watanabe and Fukasawa, 1961; Watanabe, 1963). This, along with the following period of antibiotic resistance research, was an enclave within the boundaries of clinical microbiology and bacterial genetics, because it was thought that the antibiotic resistance problem was due to, and could be resolved within, the antibiotic treatment practices of human infectious diseases. The little interaction with other fields of microbiology was another contributing factor to the isolation. The realization that antibiotic resistance research should take a broader than the clinical microbiology approach to identify the factors ultimately leading to the acquisition of antibiotic resistance by pathogens entered the mainstream thinking substantially later. In particular, the placement of the problem within the evolutionary and ecological contexts appeared to be especially fruitful (Aminov and Mackie, 2007; Aminov, 2009, 2010). Two main conclusions emerged from this synthesis: firstly, it is the enormous diversity of antibiotic resistance genes existing in the environmental microbiota that has accumulated during billions of years of evolution; and, secondly, the realization that there are no barriers among the ecological compartments in the microbial world, and that the microbiota of different compartments may easily exchange the gene pool through the MGE-mediated HGT.

Clinical studies focused on molecular mechanisms of antibiotic resistance, genetic elements involved and epidemiology have been developing in parallel to, but not interactively with, the environmental studies of HGT. The first attempts to estimate the frequencies of HGT in natural settings were performed in the 1970s with the use of model bacteria, *E. coli* and *Bacillus subtilis* (Weinberg and Stotzky, 1972; Graham and Istock, 1978). Since then, the approach involving microcosm-based and field studies as well as a range of MGEs has resulted in a better understanding

of how the environmental factors contribute to HGT in natural ecosystems (Van Elsas et al., 2000; Timms-Wilson et al., 2001). The analysis of gene exchange processes in natural settings was facilitated by the introduction of a variety of molecular ecology tools into the microcosm and field studies. These were the markers that allowed distinguishing the donor, recipient, and transformant/transconjugant/transductant populations; DNA hybridization and sequencing; PCR typing; and others (Akkermans et al., 1995; Götz et al., 1996; Smalla et al., 2000; Timms-Wilson et al., 2001). The visualization of gene transfer *in situ* was made possible with the use of the *gfp* gene (Christensen et al., 1996; Andersen et al., 1998; Dahlberg et al., 1998a). These technological advances allowed estimating the actual rates of HGT and factors affecting it in natural ecosystems.

Further substantiation for the important role played by HGT in the evolutionary process, especially among the Bacteria, surfaced during the last decade, commonly referred to as the "omics" era. The advent of high-throughput sequencing makes it possible to determine the genomic structure of many living organisms and apply the retrospective approach to HGT studies. Comparative genomic analyses revealed that, besides the core genes encoding essential cellular functions, the substantial part of bacterial genomes consists of auxiliary genes acquired by HGT (Ochman et al., 2000). The latter group of genes may confer adaptive advantages under certain growth or environmental conditions that may contain antimicrobials, xenobiotics, heavy metals, sucrose, and other compounds. These genes also confer the important characteristics allowing the colonization of new ecological niches governed by biotic factors such as the symbiotic and pathogenic relationships (Koonin et al., 2001).

The range of MGEs involved in the evolution and adaptation of bacteria through HGT is continuously updated and re-classified according to a better understanding of HGT mechanisms. It has been proposed, for example, to unify the heterogeneous classes of MGEs, such as conjugative transposons, integrative "plasmids," genomic islands, and numerous unclassified elements into integrative and conjugative elements (ICEs; Burrus et al., 2002). The basis for this re-classification was that these elements share similar characteristics, such as the excision by site-specific recombination, transfer by conjugation, and integration by recombination between a specific site of elements and a site in the host's genome. Thus, ICEs combine the features of other MGEs, such as bacteriophages (integration into and excision from the host chromosome but no transmission by conjugation), insertion sequences (ISs), and simple transposons (integration into and excision from the chromosome but no horizontal transfer), and conjugative and mobilizable plasmids (transferred from cell-to-cell by conjugation but replicated autonomously; Wozniak and Waldor, 2010). Another, even more radical, proposal was to bring a number of MGEs under the umbrella of genomic islands (GEIs; Juhas et al., 2009). According to this view, GEI would encompass other categories of elements, such as ICEs, integrated plasmids, non-replicative but excisable elements (non-replicating *Bacteroides* unit, NBU; Shoemaker et al., 2000), and even cryptic or damaged prophages.

Another interesting class of MGEs is the so-called gene transfer agent (GTA), which was initially described in *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*; Yen et al., 1979) but was later found in many other bacteria (Lang and Beatty, 2007; Stanton, 2007). Although GTAs are similar to bacteriophages, they have two important differences: they function only in genomic DNA transfers between cells, and there are no negative effects associated with the gene transfer to the recipient. The representatives of another class of MGEs, named IS*CR*s, are known to move by a process called "rolling-circle replication." A function of this process is the concomitant movement of adjacent DNA upstream of their transposase genes (Toleman et al., 2006). A recently discovered novel mechanism of HGT in *E. coli*, which was named "cell-to-cell transformation," involves cell-derived DNA and, similar to Gram-positive bacteria, a putative promoting pheromone (Etchuuya et al., 2011). These examples suggest that there is a continuum of MGEs, partially overlapping in their structure-and-function, rather than separate and strictly defined MGE classes.

Even the concepts within the well-established areas of MGE research such as plasmid biology may require further clarifications and adjustments in the light of novel information coming from a broader sampling of microbial diversity and associated genetic mechanisms. For example, the original classification of plasmids into compatibility groups was based on the ability of plasmids to stably coexist in the same bacterium (Datta and Hedges, 1971). Molecular mechanisms underlying the incompatibility were explained basically as a stochastic phenomenon based on the bacterial host's inability to differentiate among similar replicons thus giving rise to homoplasmid segregants as a direct consequence of random assortment during cell division (Novick, 1987). Within the frames of this concept, it is hard to explain the stable inheritance of multiple large plasmids in the Alphaproteobacteria (Pradella et al., 2010). The implementation of a phylogenomic approach to plasmid classification suggests that the Rhodobacterales harbor a set of at least 18 compatible plasmids, which can in principle stably coexist within the same cell (Petersen et al., 2009, 2011). Another concept that is presently undergoing an extensive reassessment is the role of prophages in bacterial genomes. The conventional view of prophages as time bombs that are ready to enter the lytic cycle and destroy the host is gradually shifting toward their perception as a key component in bacterial survival strategies (Paul, 2008). Indeed, the prophages may confer many beneficial properties on the host thus helping to withstand osmotic, oxidative, and acid stresses, as well as contributing to increased growth and biofilm formation (Wang et al., 2010).

While exploring the wealth of data collected by environmental genomics/metagenomics we may get a glimpse of the HGT events of the past, we also understandably wish to know the extent of HGT in the present-day ecosystems, especially in the face of pressing needs such as the acquisition of antibiotic resistance by pathogens or the potential risks associated with genetically modified organisms (GMO). Many microcosm and field experiments aimed at the estimation of HGT rates in natural ecosystems rely on model organisms with well-developed genetics. These organisms, however, have been selected as models due to their characteristics that are important for working under laboratory conditions, such as the simplicity of cultivation, tolerance to high nutrient

concentration, the ease of genetic manipulation, and other characteristics that are not necessarily significant for the survival and multiplication in natural ecosystems. Laboratory strains of *B. subtilis,* for example, have been selected and manipulated to become highly competent, which is important for bacterial genetics studies (Anagnostopoulos and Spizizen, 1961). Many wild type strains of *B. subtilis,* however, are not naturally competent, and the transformation of these "undomesticated" strains is possible only by highly unnatural means such as protoplast electroporation (Romero et al., 2006). Another problem, which may be encountered when accessing HGT in natural ecosystems, is that different MGEs may potentially interact with each other. It is thus important to know the diversity of indigenous MGEs in microcosm and field studies. And finally, due to dispersion and population sizes, microorganisms can be transferred across distant environments (Hooper et al., 2008). This geographical component may substantially contribute to HGT, especially if the transferred genetic material confers the traits important for the survival and reproduction in the "donor" ecological niche. Thus, a certain degree of caution must be exercised when estimating the frequencies of HGT in natural ecosystems.

In this review, HGT is viewed mostly from an ecological and environmental regulation prospective rather than focusing on genetics and regulation at the cellular level. It consists of two parts. In the first part, the soil, aquatic, gut, and biofilm communities are discussed in respect to HGT processes in these ecosystems. In the second part, factors potentially affecting HGT in natural ecosystems are analyzed.

HGT IN NATURAL ECOSYSTEMS

SOIL

Soil ecosystems harbor an extremely broad diversity of microbiota reflecting plant type, soil type, soil management regime, and other factors (Garbeva et al., 2004). To begin with, the physical environment of soil is quite heterogeneous in terms of gaseous, liquid or solid phases (Smiles, 1988). Additional variables include abiotic factors such as temperature, pH, concentration of nutrients and oxygen and moisture content and biotic factors such as antagonistic, commensal, mutualistic, and other relationships among the soil inhabitants. Since the regulation of genetic machinery is highly responsive to the environment, these factors may indeed affect the frequencies of HGT. For example, the rate of conjugal plasmid transfer in soil varies depending on abiotic factors, such as soil moisture and temperature (Richaume et al., 1989), pH (Rochelle et al., 1989), and soil type (Richaume et al., 1992). Water movement in soil may influence the survival and transport of genetically engineered strains (Trevors et al., 1990). As for biotic factors, the presence of earthworms, protozoa, and fungi certainly affects the conjugal plasmid transfer in soil (Daane et al., 1996, 1997; Sørensen et al., 1999; Sengeløv et al., 2000). Bulk soil, however, is generally poor in nutrients, whereas the active metabolic state of bacteria required for HGT is possible in nutritional hot spots such as the rhizosphere, the phyllosphere, decaying plant and animal tissues, and manure-applied soil (Van Elsas and Bailey, 2002).

Indeed, conjugative plasmid transfers among plant phytosphere inhabitants have been detected on multiple occasions (Van Elsas et al., 1988; Lilley et al., 1994; Björklöf et al., 1995; Pukall et al., 1996; Lilley and Bailey, 1997; Kroer et al., 1998). Root growth and exudate production appeared to be the most important factors contributing to the frequency of horizontal plasmid transfer in the rhizosphere (Mølbak et al., 2007). Transfer ratios were about 10 times lower in control soil than in the pea and barley rhizospheres. For some plasmids, an exceptionally broad range of recipients in the rhizosphere was demonstrated (Musovic et al., 2006). It was shown that there was a high frequency conjugal transfer of an IncP-1 plasmid pKJK10 not only to bacteria belonging to the alpha, beta, and gamma subclasses of the Proteobacteria, but also to *Arthrobacter* sp., a Gram-positive member of the Actinobacteria. Compared to filter mating, the plant model (alfalfa sprouts) provided an environment substantially enhancing the transfer of plasmid- and transposon-encoded antibiotic resistance markers between lactic acid bacteria (Toomey et al., 2009).

Application of manure to soil is another hot spot contributing to the increase in local concentrations of MGEs and the frequency of HGT in this ecosystem. This practice, as it has been shown, may enhance plasmid mobilization and survival of the introduced donor strain (Götz and Smalla, 1997). If applied together with an antibiotic, the effect is synergistic and affects the transfer frequencies and composition of MGEs introduced with manure (Heuer and Smalla, 2007). Field application of piggery manure, which harbors a substantial reservoir of broad-host-range plasmids conferring multiple antibiotic resistance genes, results in the dissemination of IncN, IncW, IncP-1, and pHHV216-like plasmids into agricultural soils (Binh et al., 2008). Clinically relevant class 1 integrons are also introduced into soil via similar practices (Binh et al., 2009).

Besides contributing to the accumulation of MGEs and to the HGT increase, the application of manure to soil has other important consequences such as the generation of novel MGE diversity and dissemination of novel phenotypes (for example, resistance to recently introduced antibiotics) among bacterial populations. An example of this kind was described recently (Heuer et al., 2009) and deserves a more detailed discussion. A novel plasmid type with 36% G + C content was captured from manure-treated soil microbiota by conjugation to *E. coli* recipient. The core of plasmids of this type is probably the product of recombination, comprising transfer, and maintenance genes with moderate homology to plasmid pIPO2 and a replication module (rep and oriV) of other descent, correspondingly. These plasmids encode a number of antibiotic resistance genes including *tet*(X) (GenBank accession number FJ012881), which may confer resistance against a recently introduced third-generation tetracycline, tigecycline (Moore et al., 2005). The species of *Acinetobacter* are the putative hosts for these low G + C plasmids in soil ecosystem (Heuer et al., 2009), which is of concern because the closely related multidrug-resistant *A. baumannii* is one of the currently emerging threats in hospitals (Dijkshoorn et al., 2007). Tigecycline is currently proposed as a new treatment choice against *A. baumannii* (Bosó-Ribelles et al., 2008), but this bacterium already poses a significant problem, as the resistance, albeit conferred by a mechanism other than TetX, easily emerges during tigecycline therapy (Peleg et al., 2007; Damier-Piolle et al., 2008). The similarity of structural and replication features among low G + C plasmids from soil and the corresponding plasmids of

clinical *A. baumannii* isolates, together with the stable inheritance of these plasmids in *Acinetobacter* spp. (Heuer et al., 2009), suggests that, once acquired, the plasmids may be easily accommodated by *A. baumannii* in the clinical environment. Given these plasmids carry resistance against the drug of last resort, their possible entry into clinical *A. baumannii* is a highly undesirable scenario. Disappointingly, there is some evidence that at least some clinically relevant resistance genes have an environmental origin (Wright, 2010) suggesting past horizontal gene exchange events between these ecological compartments. Thus, the microbiota of soil, especially of manure-fertilized soil, harbors a wide variety of MGEs enabling extensive HGT within and among microbial ecosystems.

At the same time, nutritionally poor soil ecosystems may also be involved in the HGT process. For example, a deep terrestrial subsurface, which is highly oligotrophic and extreme in terms of physical and chemical conditions, still harbors bacterial populations that carry MGEs and the genetic signatures of past gene transfer events, some of them apparently recent (Coombs and Barkay, 2004; Coombs, 2009). It is still more likely that the HGT rates under nutritionally favorable conditions are higher due to the substantial biosynthetic and energetic requirements of conjugation, DNA uptake, and lytic cycle.

Recent assessments of several types of soil revealed that lysogeny is relatively common in soil microbiota (Williamson et al., 2007, 2008; Ghosh et al., 2008). Similar to the plasmid-mediated HGT, the diversity, and dynamics of bacteriophages is also mostly confined to the nutritionally rich hot spots such as the rhizosphere. The decline in the introduced fluorescent *Pseudomonas* sp. population revealed the presence of large numbers of bacteriophages in the sugarbeet rhizosphere (Stephens et al., 1987). On the other hand, the growth and interaction dynamics of streptomycetes and a bacteriophage investigated under a less nutritionally rich condition of soil microcosm did not reveal such an effect of phages (Burroughs et al., 2000). No measurable impact on the host in terms of reduced growth by the phage was found under these conditions. Interestingly, the burst size of the phage was larger in soil relative to that observed in liquid culture, suggesting that *in vitro* transduction experiments may underestimate the impact of this particular HGT mechanism in natural ecosystems.

In the soil ecosystem, bacteriophages display local adaptation to their bacterial hosts (Vos et al., 2009). Sympatric phages are more infective than are phages from samples some distance away, suggesting that the phage-mediated HGT is highly localized in soil compartments. In model microcosm experiments, the presence of phages greatly reduced the sympatric diversity of the host bacterium but favored the allopatric host diversification (Buckling and Rainey, 2002).

The possibility of natural genetic transformation of *B. subtilis* and *Pseudomonas stutzeri* has been demonstrated in a soil/sediment model system (Lorenz et al., 1988; Lorenz and Wackernagel, 1990). These studies found significantly higher rates of transformation if transforming DNA was associated with mineral/particulate material and was thus protected against the nuclease degradation. The protective effect of DNA absorption on minerals comprising soil and sediments against DNases has been noticed on many occasions (Aardema et al., 1983; Romanowski et al., 1991; Demanèche et al., 2001; Cai et al., 2006). While some naturally competent bacteria, as in the examples above, indeed demonstrate a certain level of transformability, the occurrence of naturally transformable bacteria amongst bulk and rhizosphere soil microbiota is very low (Richter and Smalla, 2007). Various transformation assays were performed, but only transformants with a positive control, *Acinetobacter baylyi* BD413, a highly transformable strain that can be transformed by DNA from virtually any source, were obtained. Thus, the authors concluded that the proportion of native rhizosphere and bulk soil bacteria, which are naturally transformable, is negligibly low.

There have been (and indeed there still are) intensive debates surrounding the issue of possible risks associated with the release of GMO into the environment and, in particular, the impact of transgenic plants. The plant DNA may persist in soil for substantially long periods. In microcosm experiments modeling *in situ* transport of recombinant plant DNA (rDNA) from roundup ready (RR) corn and soybean by leachate water, half-lives of rDNA in leachate water ranged from 1.2 to 26.7 h, depending on the temperature (Gulden et al., 2005). The presence of transgenic DNA in soil where RR corn and soybean were cultivated can be detected by real-time PCR for up to 1 year after seeding (Lerat et al., 2007). In soil microcosm experiments, where the entry of rDNA from decomposing RR leaf biomass into soil was investigated, the corresponding DNA was detectable in soil after 30 days (Levy-Booth et al., 2008). At the present stage of our knowledge, HGT from transgenic plants to terrestrial bacteria is considered to be a rare event (Nielsen et al., 1998). A recent study with the use of an *in situ* visualization technology, nevertheless, demonstrated this process could be observed in real time (Pontiroli et al., 2009). The study, however, employed a highly transformable *A. baylyi* strain BD413, which may lead to the overestimation of natural transformation rates. The current view is that even if HGT from transgenic plants to soil microbiota takes place, it is not expected to influence prokaryotic evolution or have negative effects on human or animal health and the environment (Brigulla and Wackernagel, 2010).

AQUATIC ECOSYSTEMS

More than 70% of the earth surface is covered with water, and the World Ocean is one of the principal components forming the climate and biosphere of the Earth. As the largest habit on the Earth, the World Ocean hosts a large diversity of life and is responsible for nearly the half of oxygen production. Besides, there are many freshwater ecosystems, such as lakes, rivers, and smaller water bodies.

One of the earliest accounts on the occurrence of antibiotic resistance in marine bacteria has found that this phenotype is common within this microbiota (Sizemore and Colwell, 1977). Interestingly, the resistance phenotype was more frequently encountered in bacteria from seawater samples collected offshore than for those collected near shore. Antibiotic resistance was even present in bacteria collected about 522 km offshore and at depths of 8,200 m. In 6 out of 10 bacterial isolates the presence of plasmid DNA was confirmed by ethidium bromide–cesium chloride density gradient centrifugation. In general, however, antibiotic resistance phenotype and the presence of plasmids in marine

bacteria tend to correlate with the degree of pollution, especially by toxic chemical waste (Baya et al., 1986; Young, 1993). A recently applied cultivation-free approach for characterization of coastal *Synechococcus* metagenome relied on flow cytometry-based sorting of cells with a subsequent 454 shotgun pyrosequencing (Palenik et al., 2009). This approach allowed identifying novel plasmids that were not found in model strain genomes of this clade. Plasmids with an enormous size range are also widespread in the *Roseobacter* clade, the representatives of which constitute up to 25% of the total marine bacterial community and thus play a global role in this ecosystem (Brinkhoff et al., 2008). Some representatives may carry up to 12 extrachromosomal replicons suggesting a very sophisticated partition mechanism ensuring their stable inheritance (Pradella et al., 2010). In marine *Vibrio* the majority of plasmids are associated with pathogenic or symbiotic relationship with the host organism (Hazen et al., 2010).

With the advent of high-throughput sequencing technologies, it becomes possible to characterize the marine phage diversity without the isolation and cultivation steps, which are extremely time- and resource-consuming while the outcome is limited to a few phages that we are able to multiply and characterize under laboratory conditions. In fact, the metagenomic approach is the only way to characterize the viral diversity because there is no single gene common to all phages to serve as a phylogenetic marker (Edwards and Rohwer, 2005). Comparative marine virome analyses involving four oceanic regions demonstrated that the global diversity is very high – presumably several hundred thousands of species (Angly et al., 2006). The oceanic regions had different assemblages of marine phages suggesting strong local selective pressures enriching for certain viral types. Metagenomic characterization of viruses within aquatic microbial samples revealed a prevalence of genes encoding microbial physiological functions among viral sequences (Williamson et al., 2008). Screening of 113 marine bacterial genomes for prophages yielded 64 prophage-like elements, 21 of which strongly resembled GTAs (Paul, 2008). Hence, the viral- and GTA-mediated HGT is a common mechanism for generating microbial diversity in the marine environment thus contributing to the survival in different parts of this extensive ecological niche. Moreover, marine phages may directly contribute to the fitness of their hosts. It has been suggested, for example, that the auxiliary genes in marine phages may confer selective advantage to the host through the viral gene cassettes encoding core photosystems, I and II (Sharon et al., 2009).

Historically, the *in situ* HGT rates in aquatic ecosystems have been typically studied with the use of microcosms, and it is understandable that in a number of cases these models have certain limitations in reproducing the vast range of ecological niches present, for example, in the World Ocean. In most cases, the microcosms are limited to modeling the shallow costal or estuarine ecosystems, frequently without the presence of ambient macro- and micro-biota. Laboratory experiments also lack the scale of natural ecosystems and, as a consequence, may discount the role of important variables potentially affecting HGT. Another aspect, which needs to be taken into consideration, is the use of model donors and recipients that have been "preselected" under laboratory conditions to monitor HGT. The HGT results obtained with the use of model organisms, genetic constructs, and microcosms should be interpreted with a reasonable degree of precaution when extrapolating them in an attempt to describe a broader environment.

It has been shown that the presence of marine sediments in microcosms facilitates the uptake and expression of exogenous DNA by transformable marine *Vibrio* sp. (Stewart and Sinigalliano, 1990). It was concluded from this study that sediments were more likely niches for natural transformation than the water column in the marine environment. However, another study of natural plasmid transformation of a *Vibrio* strain in marine water column and sediment microcosms, in the presence of the ambient microbial community, arrived at the opposite conclusion (Paul et al., 1991). The authors explained this discrepancy by a differential experimental setup involving the presence of ambient microbiota in their experiments, while the previous work used sterile sediments. Estimation of transformation rates in estuarine environments based upon the distribution of competency and transformation frequencies in isolates and mixed populations ranged from 5×10^{-4} to 1.5 transformants per day (Frischer et al., 1994). Another aspect of natural transformation is that the structure-and-function of transforming DNA may be affected by the *in situ* microbiota. For instance, a broad-host-range plasmid pQSR50 that was introduced into the indigenous marine bacteria by natural transformation was subjected to the alteration of restriction profiles (Williams et al., 1997). This involved changes in methylation patterns as well as structural rearrangements of the plasmid following gene transfer, thus contributing to the generation of plasmid diversity among the *in situ* bacterial populations.

Conjugative plasmid transfers under simulated marine environment conditions have been demonstrated in many experiments. Transconjugants can be detected even under oligotrophic conditions and at very low population densities of donors and recipients (Goodman et al., 1993). The heterogeneity of the marine environment affects the HGT rates; the plasmid transfer frequency is higher among cells attached to the bead surfaces in the biofilm than among cells in the aqueous phase (Angles et al., 1993). The use of the *in situ* technology with the *gfp*-tagged conjugative plasmid pBF1 from *Pseudomonas putida* suggests that the directly determined rates of horizontal plasmid transfer in marine bacterial communities may be high, ranging from 2.3×10^{-6} to 2.2×10^{-4} transconjugants per recipient (Dahlberg et al., 1998b).

In one of the initial studies, transduction of *P. aeruginosa* streptomycin resistance by a generalized transducing phage, F116, was monitored in a flow-through chamber suspended in a freshwater reservoir (Morrison et al., 1978). The frequency of F116-mediated transduction ranged from 1.4×10^{-5} to 8.3×10^{-2} transductants per recipient during the 10-day incubation period. A recent investigation of phage-mediated gene transfer in freshwater environments used a more advanced technological tool to monitor these events at the single-cell level; the so-called cycling primed *in situ* amplification–fluorescent *in situ* hybridization (CPRINS–FISH; Kenzaka et al., 2010). The P1, T4, and EC10 phages mediated gene transfer from *E. coli* to both plaque-forming and non-plaque-forming Enterobacteriaceae strains at frequencies of $0.3–8 \times 10^{-3}$ per plaque-forming unit (PFU). The rate of transfer of EC10

ranged from undetectable to 2×10^{-3} per total direct cell count when natural bacterial communities were the recipients. This suggests that transduction may involve a wide range of bacteria, not necessarily limited by close relatives, and that the phage-mediated HGT is a frequent event in freshwater environments.

Compared to the freshwater microcosm studies, the corresponding experiments imitating the marine environment yielded lower frequencies of plasmid transduction in the mixed bacterial communities; the values were in the range from 1.58×10^{-8} to 3.7×10^{-8} transductants/PFU (Jiang and Paul, 1998). This is not to say that the overall HGT rates in marine ecosystems are low. Other MGEs residing in marine microbiota may generate an extraordinarily extensive gene flow – one of the highest ever detected. A recent study demonstrated extremely high rates of HGT in marine ecosystems that are mediated by GTAs (McDaniel et al., 2010). The frequencies detected are by many orders of magnitude higher than those of transformation or transduction, with as high as 47% of the culturable marine microbiota being confirmed as gene recipients. Given the wide presence of GTAs in phylogenetically and ecologically diverse bacteria (Stanton, 2007), GTA-mediated HGT may be more common in other natural ecosystems and may generate more extensive gene exchange than previously anticipated.

Comparative genomics and transcriptomics of marine bacteria are consistent with the high rates of HGT in these ecosystems. The co-occurring *Shewanella baltica* isolates from similar depths exchanged a larger fraction of their core and auxiliary genome compared with the isolates from more different depths (Caro-Quintero et al., 2011). These HGT events took place in the very recent past reflecting the rapid adaptation to environmental settings through gene acquisition. Genomic evolution for a cold marine lifestyle and *in situ* explosive biodegradation in *Shewanella* spp. also involved an extensive gene acquisition from deep-sea bacteria (Zhao et al., 2010).

GUT

The frequencies of HGT by conjugation are usually estimated using standard mating techniques, in liquid culture or on the surface of solidified media or filters. The microcosm and field experiments that are better estimates of HGT in the environment usually deal with lower population densities, lower nutrient availability, and generally lower temperatures. The *in vivo* conditions, however, are substantially different, especially in the intestine, where the enormously dense and diverse microbiota performs a number of functions important for the host organism. These functions include the prevention of colonization by pathogens, degradation of dietary (polysaccharides) and *in situ*-produced (mucin) compounds, production of nutrients (short chain fatty acids and vitamins), shaping and maintenance of normal mucosal immunity, and contribution to intestinal epithelial homeostasis. The commensal microbiota is under constant surveillance by the innate (antimicrobial peptides) and adaptive (immunoglobulins) immunity. The effect of innate immunity breaches on gut microbiota can be seen in genetically susceptible hosts, the commensal microbiota of which is formidably restructured compared to normal subjects (Khachatryan et al., 2008).

The discovery of ICEs among the representatives of Bacteroidetes, the major bacterial phylum in the mammalian gut, brought the notion that gene transfer in this ecosystem may be intense (Salyers, 1993), and gut microbiota, therefore, may represent one of the major reservoirs for antibiotic resistance genes (Salyers et al., 2004). Indeed, the taxonomically different representatives of gut microbiota may share the pool of closely related antimicrobial resistance genes (Frye et al., 2011). The role of bacteriophages in acquisition of genes by pathogenic gut microbiota and subsequent evolution toward pathogenicity also pointed to HGT as a crucial event in the development of virulence traits and antibiotic resistance (Calderwood et al., 1987; Barondess and Beckwith, 1990; Brabban et al., 2005). More recent metagenomic studies of the human gut microbiota allowed estimating the role of MGEs without the cultivation bias. The uncultured viral community from human feces contained an estimated 1,200 viral genotypes (Breitbart et al., 2003). Interestingly, unlike the similarity of gut bacterial communities among genetically related individuals, the viral communities are unique to individuals regardless of their degree of genetic relatedness (Reyes et al., 2010). Besides viromes, a recent metagenomic inventory identified a conjugative transposon family explosively amplified in human gut microbiomes (Kurokawa et al., 2007). The culture independent TRACA system was used to sample plasmid diversity in the human gut microbiota (Jones et al., 2010). This study suggested a broad global distribution of some plasmids and plasmid families that are potentially unique to the human gut microbiome. Thus these findings further supported the earlier cultivation-based views that the intestinal microbial ecosystem is extremely enriched by MGEs thus making it the arena of a potentially extensive gene exchange. Indeed, the range of genes that have been exchanged in the past is not limited to the virulence and antibiotic resistance genes but also includes the genes of the core gut microbiome such as encoding bile salt hydrolases (Elkins et al., 2001) or butyrate metabolism enzymes (Louis et al., 2007).

Humans and agricultural animals are the main consumers of antibiotics, for therapeutic, prophylactic, and growth-promoting purposes and, as discussed before, antibiotics may substantially increase the rates of HGT. But what could be other host-mediated factors contributing to the maintenance and transfer of MGEs in gut microbiota? It seems that the *in vivo* environment itself may enhance the transfer frequency and contribute to the stable inheritance of MGEs even in the absence of selection by antibiotics (Johnsen et al., 2002; Dahl et al., 2007). Thus *in vitro* models may substantially underestimate the transfer potential of MGEs. In nutritionally poor environments, such as bulk soil, the presence of earthworms greatly enhances the transfer of plasmid pJP4 from an inoculated donor bacterium, *P. fluorescens*, to the indigenous soil microbiota (Daane et al., 1996). Other soil microcosm experiments, modeled with *E. coli* as a donor of a genetically marked large conjugative plasmid RP4luc, in the presence or absence of earthworms, provided evidence that the gut passage was a precondition for a plasmid transfer to soil microbiota (Thimm et al., 2001). Interestingly, the plasmid was transferred at higher frequencies than detected in filter mating, suggesting once again that the HGT rates in nature are higher than the laboratory estimates. This observation also confirms the earlier notion that microbial ecosystems are not isolated and there is a potential for lateral gene exchange among different microbial ecosystems. If MGEs from

soil have entered the earthworm gut, then they can also enter the gut of animals that are next in the food chain, for example, moles and birds.

Another curious factor that may contribute to the enhanced HGT is the presence of ciliates. Ciliates are common in many aquatic ecosystems as well as in the rumen. Their food vacuoles are formed through phagocytosis and follow a particular path through the cell resembling a primitive gastrointestinal tract. It has been shown that ciliates may enhance the rate of conjugal transfer between *E. coli* strains by two orders of magnitude, and the mechanism involved is the accumulation of bacteria in vesicles (Matsuo et al., 2010). The mechanism described may contribute to the dissemination of antibiotic resistance in bacterial populations (Oguri et al., 2011).

The insect gut can also be considered as a hot spot contributing to the enhanced HGT in this ecosystem. For example, the rates of conjugative plasmid transfer between *S. enterica* Newport and *E. coli* in the gut of the lesser mealworm beetle are by two orders of magnitude higher compared to filter mating (Poole and Crippen, 2009). The occurrence of conjugal plasmid transfer and transduction was also observed in the flea and house fly gut (Hinnebusch et al., 2002; Petridis et al., 2006; Akhtar et al., 2009). An assessment of natural transformability of bacteria in the insect gut, however, failed to detect any transformation event, even with the use of *A. baylyi* strain BD413 as a recipient (Ray et al., 2007).

Conditions in the gut can be considered as very favorable for HGT. Firstly, the host provides a continuous inflow of nutrients that allow maintaining the active metabolism of gut microbiota. Secondly, the population densities are extremely high and thus conducive to the HGT mechanisms requiring intimate cell-to-cell contact such as conjugation. Thirdly, it is constant body temperature of homeothermic animals that allows the bacterial cells to perform at optimal efficiency. And finally, the vast diversity of gut microbiota itself may have an "amplification effect" for HGT (Dionisio et al., 2002). In addition to these well-known factors, recent investigations have uncovered the mechanisms of host–microbe molecular crosstalk that may contribute to the frequencies of HGT in the gut.

One of these mechanisms is based on the ability of bacteria to sense and respond to host signals. In particular, bacterial sensing and responding to the level of host stress hormones is a well-established fact (Sperandio et al., 2003; Clarke et al., 2006; Karavolos et al., 2008; Spencer et al., 2010). Bacterial responses to the host stress may also involve a genetic component, which is expressed through the enhanced conjugative gene transfer between enteric bacteria (Peterson et al., 2011). In these *in vitro* experiments, the physiological concentrations of norepinephrine stimulated the transfer of a conjugative plasmid from a clinical strain of *Salmonella* sp. to an *E. coli* recipient. Interestingly, the adrenergic receptor antagonists negated the stimulatory effect of norepinephrine on conjugation. These mediators of host stress may possibly affect HGT under the *in vivo* environment as well.

The issues of potential risks associated with the consumption of GMOs by humans and animals have been addressed in a number of feeding trials. In general, there is a lack of evidence that DNA of transgenic plants, in particular the markers used for their construction, can be taken up by gut microbiota or enter the organs other than the gastrointestinal tract. Neither small fragments of transgenic DNA nor immunoreactive fragments of transgenic protein were detectable in loin muscle samples from pigs fed a diet containing Roundup Ready soybean meal (Jennings et al., 2003). An assessment of the survival of transgenic plant DNA in the human gastrointestinal tract concluded that gene transfer did not occur during the feeding experiment involving GM soya (Netherwood et al., 2004). No traces of the construct or endogenous soybean DNA could be detected in muscle samples of rats fed soybean meal from roundup ready or conventional soybeans (Zhu et al., 2004). Likewise, no traces of transgenic DNA were detected in the milk of cows fed corn silage from an herbicide-tolerant genetically modified variety (Phipps et al., 2005). Plasmid and genomic DNA from GM plants were used in *in vitro* and *in vivo* (monoassociated rats) transformation studies, but no detectable transfer of DNA was found (Wilcks and Jacobsen, 2010). Attempts to detect DNA transfer from transgenic plants to bacteria in the intestine of the tobacco hornworm (Deni et al., 2005) or bees were also unsuccessful (Mohr and Tebbe, 2007).

BIOFILMS

Biofilms are the matrix-enclosed aggregates of microbiota, attached to each other and to biological or non-biological surfaces (Hall-Stoodley et al., 2004). This ancient form of adaptation appeared very early in the prokaryotic phase of evolution to withstand the forces of hostile environmental factors. Indeed, biofilms can be perceived as a form of primitive multicellular organisms, which use the same strategy in their interaction with the environment as their eukaryotic counterparts do. This successful trait, therefore, was replicated in many bacterial and archaeal lineages; and biofilm communities are widespread in many natural ecosystems. It is now widely acknowledged that the majority of microbiota found in natural, clinical, and industrial settings persist in association with surfaces and not in the planktonic state (Costerton, 1995; Davey and O'Toole, 2000). In natural ecosystems, biofilms are usually found in many aquatic or semi-aquatic ecosystems such as rocks and pebbles of most streams and rivers, on the surface of still water bodies, in wastewater treatment systems, in water and sewage pipes, in hot springs, in the subtidal and intertidal solid surfaces of marine ecosystems, on the teeth of humans and animals, in chronic infections of human body, and in many other ecosystems. For studies of HGT in highly organized structures as biofilms, technologies such as single-cell detection of donor, recipient, and transconjugant bacteria, combined with individual-based mathematical models, have been developed to estimate the HGT rates *in situ* (Sørensen et al., 2005). Raman spectrometry and environmental scanning electron microscopy analyses, combined with molecular ecology tools, allow a better understanding of structure-and-function of biofilms including the chemical composition of the matrix, microbiota embedded in the matrix, and the spatial distribution of biofilms (Schwartz et al., 2009).

Most of our knowledge regarding the biology of biofilms came from clinical microbiology. This increased attention to the clinical aspects of biofilm communities was primarily dictated by their role in human disease (Parsek and Singh, 2003; Fux et al., 2005; Lindsay and von Holy, 2006; Estrela et al., 2009; Kaplan, 2010). Biofilm

formation is linked to the pathology of many infectious diseases, and biofilms are notoriously difficult to eradicate because of the increased level of resistance to antibiotics (Stewart and Costerton, 2001; Anderson and O'Toole, 2008; Høiby et al., 2010a). The ability to form highly resilient biofilms has been demonstrated for many human pathogens such as *P. aeruginosa* (for recent reviews, see Harmsen et al., 2010; Häussler, 2010; Høiby et al., 2010b; and Hassett et al., 2010), *Staphylococcus aureus* (Goerke and Wolz, 2010), *S. epidermidis* (Fey and Olson, 2010; Rohde et al., 2010), *Streptococcus pneumoniae* (Moscoso et al., 2009), *S. mutans* (Senadheera and Cvitkovitch, 2008), *Neisseria gonorrhoeae* (Greiner et al., 2005; Falsetta et al., 2009; Steichen et al., 2011), *Campylobacter jejuni* (Haddock et al., 2010; Naito et al., 2010), *Candida* sp. (Morales and Hogan, 2010; Williams et al., 2011), and others. Besides, microorganisms may form mixed-species communities and, in a number of cases, this may promote the development of biofilms (Bamford et al., 2009; Silverman et al., 2010; Teh et al., 2010).

Given their structure-and-function, biofilms are the hot spots for HGT because they provide high population densities and close proximity of cells, cells in biofilms are metabolically active, and the microbiota is protected against harsh environment, predators, and immune surveillance by the extracellular matrix in which the cells are encapsulated. Indeed, HGT occurs with enhanced efficiency in biofilms, and conjugative plasmids themselves contribute to the development, stabilization, and expansion of biofilms (Hausner and Wuertz, 1999; Ghigo, 2001; Molin and Tolker-Nielsen, 2003; Reisner et al., 2006; Burmølle et al., 2008). Conjugal plasmid transfer that is implemented through the synthesis of pili and type IV secretion system contributes to the intimate cell-to-cell contact, thus facilitating the formation and growth of biofilms. Type IV secretion systems use a pilus-based system to mediate DNA or protein transfer (Hayes et al., 2010). The involvement of type IV secretion system in biofilm formation has been demonstrated for a number of bacteria (Shime-Hattori et al., 2006; Li et al., 2007; Luke et al., 2007; Barken et al., 2008; Varga et al., 2008; Bahar et al., 2009; Gibiansky et al., 2010). Protection under the umbrella of biofilms confers a selective advantage for the bacteria as well as aids further plasmid/ICE/GEI transfers. Thus, this positive feedback sustains the diversity of MGEs in biofilms and offsets the fitness cost associated with the carriage of MGEs.

The presence of prophages in the host's genome may have a modulatory effect on biofilm formation and physiology of the host, including central metabolism (Wang et al., 2009). Some prophage-encoded proteins are actually essential for biofilm formation (Toba et al., 2011). The modulatory effect of prophages is expressed in the enhancement of biofilm formation and the resistance of biofilms against adverse environmental conditions (Carrolo et al., 2010; Wang et al., 2010). The biofilm matrix is a conglomerate of polymers usually consisting of extracellular DNA (eDNA), proteins, and polysaccharides. In this respect, a partial phage-mediated lysis of a proportion of microbiota in biofilm, due to spontaneous phage induction, may provide the eDNA supply, thus contributing to biofilm formation and maintenance by the remaining bacterial populations (Carrolo et al., 2010; Gödeke et al., 2011). At the same time, transmission electron microscopy reveals also the presence of intact bacteriophage particles that are enmeshed in the extracellular polymeric matrix of biofilms (Kay et al., 2011). Precise deletion of *E. coli* prophages uncovered the role of e14 and rac proteins in the increased early biofilm formation (Wang et al., 2010). Besides, other prophages contributed to the host resistance against a variety of stresses, including osmotic, oxidative, and acid. These examples reveal the role of the phage-mediated HGT in bacterial evolution and adaptation, especially regarding the acquired ability to form biofilms as well as to withstand environmental stresses.

Natural competence and transformability in bacteria usually correlates with the presence of type IV pili or type IV pilin-like proteins (Averhoff and Friedrich, 2003). While the role of conjugation machinery and pili in biofilm formation and subsequent acceleration of conjugative element transfer is firmly established, it is not clear if the same is true for natural transformation. Given the involvement of type IV pili or type IV pilin-like proteins in natural transformation and in biofilm formation, would this result in a higher rate of transformation within biofilms? Do the large quantities of eDNA in the extracellular matrix participate in natural transformation of bacteria in biofilms? There are very few works that have addressed these questions. Horizontal transfer of non-conjugative plasmids in *E. coli* colony biofilms suggested the possibility of natural transformation within bacterial biofilms (Maeda et al., 2006). Later, however, it became clear that the mechanism of DNA acquisition in the biofilm is more complex and was, therefore, described as a "cell-to-cell transformation," which also involves a peptide pheromone as a regulator (Etchuuya et al., 2011). Other circumstantial evidences of DNA transfer due to close cell-to-cell contact (Van Randen and Venema, 1984; Wang et al., 2007) may be interpreted within the frames of this hypothesis, although the transfer in these cases was between the genera, and the role of pheromones was not shown. The language of intercellular communication used in regulation of various processes within the complex structure of biofilms is indeed the language of pheromones such as *N*-acyl-L-homoserine lactones, furanosyl borate diester, and peptide autoinducers (Dickschat, 2010). Recently, the possibility of transfer of tetracycline resistance by transformation with eDNA within model oral biofilms was described, although without specifying the frequency of such an event (Hannan et al., 2010).

FACTORS AFFECTING HGT

STRESS AND SOS RESPONSE

It has been long recognized that various stress conditions may contribute to increased rates of HGT. For example, UV irradiation or starvation affects the mobility of transposons and ISs (Levy et al., 1993; Ilves et al., 2001). While the lysogenic cycle in the phage development limits its inheritance to the vertical transfer within the chromosome of the host, the stress factors such as induction of the SOS response or amino acid deprivation of the host lead to the lytic cycle and further horizontal dissemination of phage and transduced DNA (Melechen and Go, 1980; Little, 2005). Control of horizontal transfer of ICEs, which are found in many bacterial genomes and which encode a variety of properties besides antibiotic resistance and virulence, may also be regulated through stress, SOS, and other environmental signals (Auchtung et al., 2005; Bose

et al., 2008). In particular, the SOS response, which is induced by DNA damaging agents such as mitomycin C and antibiotics such as fluoroquinolones and dihydrofolate reductase inhibitors, leads to the expression of the SXT activators resulting in more than 300-fold increase of HGT rates (Beaber et al., 2004). Thus the consequences of using SOS response-inducing antibiotics may result in co-selection of other antibiotic resistance genes that are physically linked in a MGE (Hastings et al., 2004). Moreover, the SOS response controls integron recombination thus enhancing the potential for cassette swapping and capture in cells (Guerin et al., 2009). Besides the antibiotic- (see the previous section) and SOS response-regulated excision, transfer, and integration of ICEs, regulatory factors include the stationary phase of growth and the presence of 3-chlorobenzoate (Sentchilo et al., 2003). Interestingly, this chlorinated compound stimulates horizontal transfer of the genes encoding its own metabolism. This may explain the previous results of the authors that suggested the need of specific substrates for genetic transfer to occur in activated-sludge microcosms (Ravatn et al., 1998). The transfer of another class of MGEs, GTA, can also be induced by mitomycin C and oxidative stress (Stanton et al., 2008).

Although it has been initially assumed that the SOS response is triggered exclusively by direct DNA damage, there is evidence that certain stimuli can indirectly generate SOS-inducing signals (Aertsen and Michiels, 2006). Some antibiotics, which do not directly interfere with DNA metabolism, may, nevertheless, induce a genuine SOS response. This was demonstrated, for example, for the β-lactam antibiotics targeting bacterial cell wall (Miller et al., 2004). The resulting effect is the elevated rate of horizontal transfer of virulence genes in staphylococci (Maiques et al., 2006; Ubeda et al., 2006). Furthermore, the presence of a functional SOS response system seems not an absolute prerequisite for the stress-induced increase in HGT frequencies. In naturally competent bacterial species such as *Streptococcus pneumonia* the antibiotic-imposed stress induces genetic transformation in the absence of a SOS-like system (Prudhomme et al., 2006). Another naturally competent bacterium, *Legionella pneumophila*, also lacks a prototypic SOS response system, but UV light, which represents a major source of genotoxic stress in the environment, effectively induces competence development in this bacterium (Charpentier et al., 2011). The authors have hypothesized that competence for natural transformation and, therefore, the ability to acquire and propagate foreign genes may have evolved as a DNA damage response in SOS-deficient bacteria. Thus, Gram-positive bacteria respond to stress conditions by induction of competence for genetic transformation to generate genetic diversity (Claverys et al., 2006). The strategy is combined with SOS induction in bacteria, such as *B. subtilis*, while others, such as *S. pneumonia*, rely solely on competence.

In naturally competent Gram-negative bacteria, the stress conditions may also enhance HGT. *Vibrio cholera,* for example, can acquire new genetic material by natural transformation during growth on chitin, which activates regulatory cascades leading to increased cell density, nutrient limitation, and decline in growth rate and stress (Meibom et al., 2005). Stress in the form of DNA damage induces transcription and translation of competence genes in *Helicobacter pylori*, thus increasing transformation frequency and genetic exchange rates (Dorer et al., 2010).

To what extent is the SOS response induced in natural ecosystems? The impact of solar UV radiation on microbiota may be profound, especially in aquatic ecosystems (Hader, 2000; Sinha and Hader, 2002). One of the major targets is DNA where UV-B irradiation results in the formation of cyclobutane-pyrimidine dimers (CPDs), 6-4 photoproducts (6-4PPs), and DNA strand breaks, thus leading to the induction of a number of repair mechanisms, including the SOS response (Rastogi et al., 2010). In photosynthetic cyanobacteria, UV-B stress is accompanied by additional stresses such as oxidative stress and oxidative damage (He et al., 2002). Despite the fact that future trends for solar UV irradiation of the Earth's surface remain unclear, the increases in UV-B irradiance over the latter part of the twentieth century have been larger than the natural variability (McKenzie et al., 2007). Thus, this factor may play an increasingly important role in accelerating HGT in microbial ecosystems.

SUB-INHIBITORY ANTIMICROBIALS

During the recent years the concept of antibiotics as solely killing agents has been substantially revised in the light of new findings suggesting that low concentrations of antibiotics may play a regulatory function in natural ecosystems (Davies et al., 2006; Fajardo and Martínez, 2008; Aminov, 2009). Moreover, the killing/therapeutic and regulatory/sub-inhibitory concentrations of an antibiotic are indeed directed toward different targets in the cell (Hoffman et al., 2005). There is a substantial body of evidence suggesting that the sub-inhibitory concentrations of antibiotics may significantly increase the frequency of horizontal transfer of many types of MGEs. It was noticed a quarter of a century ago that the sub-inhibitory concentrations of β-lactams enhanced the transfer of tetracycline resistance plasmids in *Staphylococcus aureus* by up to 1,000-fold (Barr et al., 1986). Pre-incubation of donor cells of *Bacteroides* sp. in the presence of sub-inhibitory tetracycline accelerates the mobilization of a resident non-conjugative plasmid by chromosomally encoded tetracycline conjugal elements (Valentine et al., 1988). A similar type of exposure of donor *Bacteroides* cells appeared to be a prerequisite for the excision and conjugal transfer of the ICE CTnDOT (Stevens et al., 1993; Whittle et al., 2002). In the absence of tetracycline induction of donor cells, practically no such transfers were detected. Addition of sub-inhibitory tetracycline into the mating medium substantially enhanced the conjugal transfer of another ICE, *Tn*916 (Showsh and Andrews, 1992). A similar stimulatory effect of tetracycline on conjugation transfer was demonstrated for *Tn*925 as well (Torres et al., 1991). A recent study has revealed the induction effect of carbadox and metronidazole on the GTA-mediated HGT in *Brachyspira hyodysenteriae* (Stanton et al., 2008). The induced VSH-1 particles transmitted tylosin and chloramphenicol resistances between *B. hyodysenteriae* strains.

These experiments described above have been performed under laboratory conditions using standard mating techniques, and the question is whether these observations reflect the real *in vivo* situation or these are the effects of specific *in vitro* conditions. The former point of view is supported by a number of experiments using animal models. For instance, the inclusion of sub-inhibitory tetracycline in drinking water resulted in a 10-fold increase of transfer

of an ICE, *Tn*1545, from *Enterococcus faecalis* to *Listeria monocytogenes* in the intestine of gnotobiotic mice (Doucet-Populaire et al., 1991). In gnotobiotic rats, the presence of tetracycline resulted in a higher number of *Tn*916 transconjugants compared to control (Bahl et al., 2004). An astonishing 100% transfer rate of a small plasmid pLFE1 from *Lactobacillus plantarum* to *E. faecalis* was observed in the gut of gnotobiotic mice receiving erythromycin (Feld et al., 2008). These experiments suggest that the stimulatory effect of sub-inhibitory antibiotics on transfer of MGEs is not an artifact of *in vitro* conditions but happens in the real gut of real animals. It needs to be noted, however, that the transfer frequencies were estimated in gnotobiotic animals, which are lacking the highly diverse and dense microbiota in the gut.

The mechanisms contributing to the enhanced movement of MGEs in the presence of antibiotics have been established on several occasions, especially for ICEs. In Tn*916*, transcriptional regulation of the *tra* genes required for the conjugal transfer of this ICE is under the control of *orf7* and *orf8* products (Celli and Trieu-Cuot, 1998; Roberts and Mullany, 2009). Transcripts for these two ORFs are produced from the distant promoter of the *tet*(M) gene as well as from the promoter directly upstream of *orf7*. In the absence of tetracycline, transcription from P_{tetM} is attenuated (Su et al., 1992), and the transcripts are very short, not covering the *orf7* and *orf8* genes. In addition, the product of *orf9* negatively regulates the promoter upstream of *orf7*. In the presence of tetracycline, however, transcription from P_{tetM} extends through *orf7* and *orf8* thus allowing the synthesis of these two proteins that promote transcription from P_{orf7}. The long transcripts from P_{tetM} are also complementary to the *orf9* transcripts thus efficiently reducing the concentration of P_{orf7} repressor. As a result, transcription from P_{orf7} extends downstream of *orf8* through *int* and *xis*, which encode the integrase and excisionase, respectively. Once Tn*917* is circularized, transcription from P_{orf7} also leads to the expression of the *tra* genes thus initiating the conjugation machinery.

Another mechanism, also involving tetracycline as a positive regulator of HGT, is implemented in the *Bacteroides* ICE, CTnDOT (Whittle et al., 2002; Moon et al., 2005). In this regulatory cascade, tetracycline activates the transcription of the *tet*(Q) gene and the downstream genes of the two-component regulatory system, *rteA* and *rteB*. The product of *rteB* activates the transcription of *rteC* system, which, in turn, leads to the elevated transcription of the gene cluster involved in CTnDOT excision. Subsequently, the proteins encoded by the excision genes upregulate the production of *tra* gene mRNA thus activating the conjugation machinery (Jeters et al., 2009). In this complex regulatory system, the stimulatory effect of tetracycline on transcription of its own resistance gene (and concomitantly on the excision and conjugal transfer genes of CTnDOT) is implemented through a translation attenuation mechanism involving the leader region of *tet*(Q) (Wang et al., 2005).

Both regulatory mechanisms of the MGE movement discussed above display a common theme in that the primary switch for this genetic process is based on an antibiotic–antibiotic resistance gene pair. That is, the presence of a sub-inhibitory antibiotic in the environment activates the transcription of a corresponding resistance gene and, concurrently, the genes involved in the mobility of MGEs. In this regard, the interaction of an antibiotic and antibiotic resistance gene resembles a positively regulated switch, with an antibiotic possessing a signaling function, ultimately leading to the activation of horizontal gene exchange in microbiota. The recent works describing concentration-dependent bacterial responses to antibiotics have led to the development of the hormesis concept (Davies et al., 2006; Fajardo and Martínez, 2008). According to this concept, low concentrations of antibiotics may regulate a specific set of genes in target bacteria, while increasingly higher concentrations elicit a stress response, and even higher concentrations are lethal. It has been suggested that antibiotics play a regulatory role in nature at low concentrations unlike the lethal concentrations used in clinical therapy (Aminov, 2009). Given the profound stimulatory effect of low-dose antibiotics on the movement of MGEs, one of the functions of antibiotics in natural ecosystems may be the regulation of HGT between the representatives of environmental microbiota.

Despite the fact that some countries have enacted legislations limiting the non-therapeutic use of antibiotics, in particularly in food animals (http://ec.europa.eu/food/food/animalnutrition/feedadditives/index_en.htm), the use of sub-therapeutic antimicrobials in agriculture and aquaculture of other countries is still widespread. Moreover, the US Food and Drug Administration has recently approved sub-inhibitory concentrations of doxycycline and minocycline for the systemic treatment of skin infections in humans (Del Rosso, 2007). Thus, there is a high probability that the gut ecosystems may continue to be hotspots of horizontal gene exchange involving the resident and transient gut microbiota. The land application of manure with residual antibiotics and antibiotic resistance genes of the gut content may further contribute to the enhanced HGT in the environment (Chee-Sanford et al., 2009).

INDIRECT MECHANISMS OF MAINTENANCE AND DISSEMINATION OF MGEs

The "cooperation" between different mechanisms of HGT can be seen in many examples of mobility among the genetic elements that are normally not mobile on their own. For example, ICEs of *Bacteroides* spp. and large broad-host-range conjugative IncP plasmids R751 and RP4 are able to act *in trans* to excise, circularize, and transfer unlinked integrated elements called NBUs (Li et al., 1993; Shoemaker et al., 1993). Bioinformatics of plasmid mobility suggests that globally about a quarter of all plasmids are potentially mobilizable and, therefore, can be transferred if a compatible conjugation machinery is present (Smillie et al., 2010). In *Sinorhizobium meliloti*, conjugation functions for a 1,683-kb symbiotic megaplasmid pSymB are supplied *in trans* by another megaplasmid, pSymB (Blanca-Ordóñez et al., 2010). The recently described IS*CR* elements are thought to be the key players in IncA/C plasmid evolution serving as antibiotic resistance gene capture and movement systems that are also capable of constructing extended clusters of antibiotic resistance genes (Toleman and Walsh, 2010).

Insertion sequences constitute an important component of most bacterial genomes and are simple transposable elements consisting of inverted repeat (IR) sequences, a transposase gene, and frequently a second recombination regulation enzyme gene (Mahillon and Chandler, 1998). It has been thought that the

transposition of ISs is a rare event within a bacterial genome, but the discovery of a protein called IS-excision enhancer (IEE), which promotes excision events, suggested that the rates could be high (Kusumoto et al., 2011). The IEE activity, therefore, may play an important role in bacterial genome evolution by inducing IS removal and genomic deletion. Another aspect is the transposition of an IS into a conjugative plasmid, an ICE, or a genomic island. This way, the IS elements can be disseminated to many other, not necessarily closely related, bacterial taxa. Together with prophages (Asadulghani et al., 2009), IS elements are the major contributors to the genomic diversification in pathogenic *E. coli* (Ooka et al., 2009). Since the IS elements are ubiquitous in many bacteria, including environmental and commensal species, these mechanisms of diversification are probably not limited exclusively to pathogens.

Since the carriage of MGEs may be associated with a considerable fitness cost, certain mechanisms of stable inheritance have been selected within the MGEs. One of these mechanisms is a toxin–antitoxin (TA) system consisting of two components, a stable toxin and its labile antitoxin. Type I and II TA systems were found on plasmids in the 1980s (Ogura and Hiraga, 1983; Jaffe et al., 1985), and type III is a more recent discovery (Fineran et al., 2009). The general mechanism of action is that if an MGE with TA system is lost during cell division, the concentrations of a labile antitoxin rapidly decreases thus releasing a stable toxin, which kills an MGE-free cell (Van Melderen, 2010). Thus, TA systems contribute to the stable maintenance and dissemination of plasmids and genomic islands in bacterial populations despite the associated fitness cost.

The size of MGEs varies from small IS elements (typically 700 to 2,500 bp; Mahillon and Chandler, 1998) to large symbiotic megaplasmids of *Sinorhizobium meliloti* (1.35 and 1.68 Mb; Barnett et al., 2001; Capela et al., 2001). The majority of large self-transmissible genetic elements have a sufficient capacity to carry multiple genes, including those encoding antibiotic, heavy metal and biocide resistances, metabolism of various substrates and xenobiotics, symbiosis with the host, and other auxiliary functions. The fact that R plasmids mediate resistance to mercury, nickel, and cobalt was first described more than 40 years ago (Smith, 1967). Subsequent research by other groups demonstrated that the genetic linkage between antibiotic resistance and mercury resistance in enterobacteria had occurred prior to the late 1950s in Japan (reviewed in Liebert et al., 1999). At the same time, Enterobacteriaceae strains collected by E. D. G. Murray from 1917 to 1954 contained very few antibiotic and mercury resistant bacteria, despite the finding that 25% of the strains carried conjugative functions (Hughes and Datta, 1983). Thus, the frequency of MGEs even in the "pre-antibiotic era" was sufficiently high but not associated with the adaptation to anthropogenic factors such as antibiotics or heavy metals.

The exceptional natural genetic engineering capabilities of bacteria have been profoundly demonstrated during the "antibiotic era" (Aminov, 2010). To withstand the massive pressure of antimicrobials used by humans, commensal, and pathogenic bacteria were able, within a relatively short period of time on the evolutionary scale, to mobilize a huge reservoir of antibiotic resistance genes, often from the environmental bacteria (Aminov and Mackie, 2007; Cantón, 2009; Wright, 2010). The main genetic engineering tool used by bacteria to collect antibiotic resistance genes is integrons, the genetic platform that is involved in the acquisition and functional expression of exogenous gene cassettes (Mazel, 2006). The discovery of superintegrons that contain hundreds of auxiliary genes and may occupy a significant part of many bacterial genomes has changed our initial interpretation of integrons as merely a mechanism of collecting antibiotic resistance genes. The presence of toxin/antitoxin cassettes in superintegrons further facilitates the stabilization of large cassette arrays consisting of many ancillary genes (Cambray et al., 2010).

The physical linkage of numerous and functionally diverse groups of genes within the high-capacity MGEs has implications for their persistence in the environment. Selection that is imposed toward even a single component/gene of an MGE will automatically select for the whole MGE. Thus, we should see the co-selection effect in phenotypes of bacteria that carry MGEs. Indeed, bacteria in metal-contaminated areas appeared to be more tolerant to metals and antibiotics than in control sites (Stepanauskas et al., 2005; Baker-Austin et al., 2006; Wright et al., 2006). Treatment of agricultural soils with copper may lead to a significantly higher incidence of antibiotic resistance phenotypes in indigenous soil microbiota (Berg et al., 2005). In freshwater microcosms, amendment with metal concentrations representative of industry and mining-impacted environments increased the frequency of antibiotic resistance in the microbial communities (Stepanauskas et al., 2006). Genetic mechanisms responsible for the co-selection phenomena in the contaminated environments are presently poorly understood, but it is clear that the MGE-driven HGT is the main adaptive trait in bacteria inhabiting industrially contaminated aquatic (Wright et al., 2008) and soil (Top et al., 1995; Sobecky and Coombs, 2009) ecosystems.

QUORUM SENSING

The quorum sensing (QS) systems are widespread among a variety of microbiota and initially they were recognized as population-density-sensing mechanisms based on the best-studied prototype, the QS network, which regulates the *lux* operon in *Vibrio fischeri* (Eberhard et al., 1981; Fuqua et al., 1996). Since then it has become clear that the QS is involved in regulation of a much broader range of functions and activities such as pathogenicity, extracellular enzyme production, antibiotic biosynthesis, and others (Bainton et al., 1992; Jones et al., 1993; Passador et al., 1993; Pirhonen et al., 1993; Pierson et al., 1994). The QS is indeed a universal language of communication not only among the bacteria but also in the inter-kingdom interaction (Shiner et al., 2005).

One of the earliest indications of the QS involvement in HGT came from the studies of conjugal transfer of the *Agrobacterium tumefaciens* Ti plasmids (Zhang and Kerr, 1991; Piper et al., 1993; Fuqua and Winans, 1994). The Ti plasmid encodes a regulatory system, consisting of the acyl-homoserine lactone (AHL) synthase TraI and the transcription factor TraR. TraI synthesizes AHLs, mainly *N*-(3-oxo-octanoyl)-L-homoserine lactone (OOHL), while TraR is an OOHL-dependent transcription activator of conjugative transfer genes. Molecular mechanisms of this activation that leads to the enhanced conjugal transfer of the Ti plasmids are well understood (Costa et al., 2009; Qin et al., 2009).

Another well-explored area is the QS regulation of transfer of large symbiotic plasmids in rhizobia (Danino et al., 2003; He et al., 2003; Tun-Garrido et al., 2003). Analysis of plasmid transfer in several rhizobia species has revealed a regulatory relay that is specifically poised to detect AHLs made by different cells and to respond to these signals by up-regulation of conjugal transfer genes. In turn, the production of AHLs, *N*-(3-oxo-octanoyl)-L-homoserine lactone, and *N*-(octanoyl)-L-homoserine lactone, is regulated by a complex interaction of plasmid- and chromosome-encoded genes in donors and recipients in response to environmental cues. Recently it has been shown that the transfer of an ICE of *Mesorhizobium loti*, which carries genes for a nitrogen-fixing symbiosis with *Lotus* species, is also regulated by a QS mechanism (Ramsay et al., 2009). The two conserved hypothetical genes, which are essential for the QS-mediated excision and transfer, can also be found on putative ICEs in several alphaproteobacteria, indicating a broader presence of this HGT regulatory mechanism.

Recent investigations have identified an important role played by QS in regulation of phage-mediated HGT. For example, acyl-homoserine lactones (AHLs), the essential signaling molecules of QS in many Gram-negative bacteria, can trigger phage production in soil and groundwater bacteria (Ghosh et al., 2009). Interestingly, in the *recA* mutant of *E. coli* the induction responses of lambda to AHL remained unaffected, suggesting that this mechanism does not involve an SOS response.

CONCLUDING REMARKS

The high rate of horizontal gene exchange in natural ecosystems is evident from both retrospective and prospective types of studies. The microbial world around us can be seen as a giant microbiome, with the continuous flow of genes between its different compartments. This flow is sustained by a variety of sophisticated natural genetic engineering tools, MGEs, which have been selected during the evolution as providing the means for re-shuffling the available genetic material and picking the best responses possible to cope with the continuously changing environmental challenges. The recent relatively short history of the "antibiotic era" (Aminov, 2010) demonstrates the ultimate success of this strategy and urges us to rethink our own when interacting with the microbial world. Continuous discoveries of novel MGEs and mechanisms of HGT, together with the findings of unexpectedly high HGT rates in natural ecosystems, indicate that we are still far from the understanding of the true extent of HGT in nature. The contribution to the better understanding may be envisaged as the combination of retrospective and prospective approaches. On the dry lab side, the history of past HGT events, which is recorded in the wealth of genomic/genomic information, can be more vigorously interrogated on the basis of our knowledge about MGEs and with the help of bioinformatics tools that are able to detect the events consistent with HGT. On the wet lab side, it is the development of *in situ* technologies that are more sensitive, less intrusive and applicable to the field studies. The microcosm experiments should model real environmental situations, working with native microbiota, with a lesser dependence on model organisms. These developments may help to elaborate better strategies to deal with the pressing needs such as the emergence of novel infections and opportunistic pathogens as well as antibiotic resistance genes.

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