Review paper
Pregledni znanstveni prispevek

# HORIZONTAL GENE TRANSFER IN BACTERIA: AN ECOLOGICAL AND EVOLUTIONARY PERSPECTIVE

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## ABSTRACT

In recent years, molecular genetics and genome analysis provided extensive evidence that gene loss and acquisition are likely to be the primary mechanisms by which bacteria genetically adapt to novel or changed environments and by which bacterial populations diverge and form separate, evolutionary distinct species. Three mechanisms of gene transfer have been identified in microorganisms: transformation, conjugation and transduction, but our knowledge concerning horizontal gene transfer in the environment was and still is very limited. Animal gut and in particular the rumen belong to the most complex microbial ecosystems. Conditions in the rumen potentially favour horizontal gene transfer with conjugation and transduction, due to large, diverse and dense bacterial and bacteriophage populations, however, there is very little evidence supporting theoretical assumptions. A brief review of current knowledge on horizontal gene transfer is presented with the emphasis on the available data and views concerning the process going on in the rumen.

Key words: microbiology / bacteria / molecular genetics / horizontal gene transfer / evolution / transformation / conjugation / transduction

# HORIZONTALNI PRENOS GENOV MED BAKTERIJAMI: EKOLOŠKI IN EVOLUCIJSKI VIDIKI

## IZVLEČEK

Molekulsko genetske analize in analize genomov so v zadnjh letih priskrbele številne dokaze o horizontalnem prenosu genov kot osnovnem mehanizmu prilagajanja bakterij na nova oziroma spremenjena okolja in mehanizmu ločevanja bakterij na nove filogenetske linije. Pri mikroorganizmih poznamo tri načine genskega prenosa: transformacijo, konjugacijo in transdukcijo, vendar je znanje o horizontalnem prenosu genov v okolju še zmeraj skromno. Prebavila živali in še posebno vamp sodijo med najbolj kompleksne mikrobne ekosisteme. Razmere v vampu so teoretično ugodne za horizontalni prenos genov s konjugacijo in transdukcijo, glede na obsežno, raznoliko in gosto populacijo bakterij in fagov, vendar je dokazov o prenosu genov zelo malo. V pričujočem prispevku predstavljamo kratek pregled tematike s poudarkom na dostopnih podatkih in pogledih na te procese v vampu.

Ključne besede: mikrobiologija / bakterije / molekularna genetika / horizontalni prenos genov / evolucija / transformacija / konjugacija / transdukcija

## INTRODUCTION

Microorganisms display extraordinary variation in their metabolic properties, cellular structure and lifestyles. Several mechanisms were proposed to be responsible for the differences observed between bacterial and archaeal species. Point mutations leading to the modification or inactivation of existing genes have certainly contributed to the diversification of genes and genomes, however it is difficult to believe, that microorganisms could exploit new environment, by the accumulation of point mutations alone. Instead, there is growing evidence that horizontal gene transfer has played an integral role in the evolution of bacterial and archaeal genome (Dykhuizen and Green, 1991; Smith *et al.*, 1992; Doolitle, 1999; Ochman *et al.*, 2000).

Three mechanisms of gene transfer have been identified in microorganisms: transformation, conjugation and transduction. All three methods have served as elegant tools in the development of genetic methods for prokaryotes, but our knowledge concerning horizontal gene transfer in the environment was and still is very limited. Rumen is one of the most complex microbial ecosystems known, with microbial population reaching more than $10^{10}$ cells per millilitre. Conditions in the rumen, where population density is high and bacteriophages are abundant, potentially favour horizontal gene transfer via conjugation or transduction. However, there is very little evidence supporting theoretical assumptions. This paper present a brief overview of the horizontal gene transfer in the environment and impact of gene exchange on the evolution of bacteria. In addition, evidence for gene transfer in the rumen is reviewed.

## MECHANISMS OF HORIZONTAL GENE TRANSFER IN THE ENVIRONMENT

### Conjugation

Bacterial conjugation is a plasmid or transposon encoded gene transfer mechanism, that require physical contact between cells. First described by Lederburg and Tatum (1946) in *Escherichia coli*, conjugation has since been reported for numerous bacterial species (reviewed by Ippen-Ihler, 1989). Conjugal gene transfer has been shown to occur in a variety of environments such as human and animal gut (Salyers *et al.*, 1996; Scott *et al.*, 1995; Armstrong *et al.*, 1990), rhizosphere (Sullivan *et al.*, 1995; Smit *et al.*, 1993), on plant leaves (Normander *et al.*, 1998), in seawater and marine sediments (Dahlberg *et al.*, 1998) and polluted soils, sludges and water (Top *et al.*, 1994; McClure *et al.*, 1990).

Several types of conjugation are known. The classic examples of self-transmissible conjugative plasmids are the F-plasmid and the plasmid RP4 of *E. coli*. Transfer of such plasmids begins when donor cell produce the pilus, which is encoded by the plasmid and contact the potential recipient cell which does not contain the plasmid. Retraction of the pilus brings the cells into close contact, and a pore forms in the adjoining cell membranes. Formation of the mating pair signals the plasmid to begin the transfer from a single stranded nick at *oriT*. The 5′ end of a single strand of the plasmid is transferred to the recipient trough the pore. During transfer, the plasmid is replicated in the donor, its synthesis being primed at the 3′ OH end of the *oriT* nick. Replication of the single strand in the recipient proceeds by another mechanism using RNA primers. At the end, when mating pair separates, both cells contain double-stranded plasmids. Conjugative plasmids encode all functions they need for the transfer between cells and sometimes they can facilitate transfer of mobilizable plasmid, which encode some but not all of the proteins required for the transfer. Occasionally, conjugative plasmids can integrate into chromosomes, and when such plasmids attempt to transfer (during *Hfr* formation by the F plasmid of *E. coli*), they may take part of the chromosome with them (Firth *et al.*, 1996; Snyder

and Champness, 1997). Conjugation may also be affected by conjugative transposons (Clewel *et al.*, 1995), which may also facilitate plasmid and chromosomal DNA mobilization.

Conjugative plasmids such as RP4, mobilizable plasmids such as IncQ, and conjugative transposons such as Tn916, often display a very wide host range (Davison *et al.*, 1990; Clewel *et al.*, 1995; Salyers and Shoemaker, 1996) and may hence significantly influence the evolution of bacterial genomes and antibiotic resistance gene dissemination.

## Natural transformation

Transformation is the process whereby a cell takes up and expresses extracellular DNA. Natural transformation i.e. the normal, physiological process of certain bacteria should be distinguished from the artificial transformation, which is a laboratory method used with many bacteria for the introduction of cloned genes and plasmids. Our understanding of the mechanisms involved in the uptake and expression of exogenus DNA results almost entirely from studies in three model systems: *Streptococcus* sp., *Bacillus subtilis* and *Haemophilus influenzae* (Stewart, 1989). Despite existing differences in transformation systems among bacteria, four discrete steps are common to all of them: competence development, DNA binding, DNA uptake, and integration into chromosome (Stewart, 1989). Natural transformation is probably a broadly distributed property among bacteria (Lorenz and Wackernagel, 1994). Several species (e. g. *Streptococcus pneumoniae)* were shown to become competent in the natural course of their life cycle (Lunsford, 1998) and some (e. g. *Neisseria gonorrhoeae*) are always in competent state (Lorenz and Wackernagel, 1994). Transformation in a natural environment was first demonstrated by Graham and Istock (1978) and since then many reports have described transformation in water and soil systems (Stewart and Sinigalliano, 1991; Lorenz *et al.*, 1992; Nielsen *et al.*, 1997; Nielsen *et al.*, 2000). In soil DNA can be stabilized by adsorption to sand and clay particles, thereby becoming 100 to 1000 fold more resistant to DNase activity. Adsorbed DNA may retain its transforming ability for weeks or even months (Khana and Stotzky, 1992; Lorenz and Wackernagel, 1994). Other bacteria like *E. coli* are known for some time not to be naturally competent, only being transformable by chemical treatment, osmotic shock or electroporation. Recently, natural competence was shown to develop in *E. coli* at low temperatures in mineral water containing low concentration of $CaCl_2$ (Baur *et al.*, 1996). In human saliva transformation of natural competent *Streptococcus gordonii* was demonstrated (Mercer *et al.*, 1999a), indicating that DNA released from bacteria or food sources within the mouth has the potential to transform naturally competent oral bacteria. Natural transformation of *Stereptococcus bovis*, resident ruminal bacterium, was demonstrated by Mercer *et al.* (1999b), showing that natural transformation is possible in microenvironments established within the rumen.

## Transduction

Transduction is the process of gene transfer whereby a bacteriophage mistakenly packages some of the host DNA in the capsid and transfers it to another bacterium upon subsequent infection (Zinder and Lederburg, 1952). Transduction may be generalized (Masters, 1996), whereby any bacterial gene can be transferred, or specialized (Weisberg, 1987), where only genes located near the site of prophage integration are transferred. Traditionaly, phage-host interaction is believed to be quite specific (Kokjohn, 1989). However, recent findings indicate that gene transfer by transduction can occur across wide taxonomic boundaries, at least in the hot spring environments (Chiura *et al.*, 1998). Bacteriophages are probably very common in most environments (Wichels *et al.*, 1998; Jiang and Paul, 1998), they are relatively stable and protected by the protein coat. Together with the fact that transducting phages have been

discovered for a number of different bacteria (Kokjohn, 1989), one can conclude that transduction is important mechanism of gene transfer in the environment. Transduction of chromosomal and plasmid DNA was seen for example in environmental test chambers in a fresh water reservoir (Morrison *et al.*, 1978; Saye *et al.*, 1987) and, using the same phage, on the leaf surface (Kidambi *et al.*, 1994). A marine phage was shown to facilitate the transduction of a wide host range plasmid to members of a natural marine microbial community (Jiang and Paul, 1998). Bacteriophage mediated transfer has been suggested to explain the distribution of the pyrogenic exotoxin C among different phylogenetic lineages of *Streptococcus pyogenes* (Kapur *et al.*, 1992). Bacteriophages coding for shiga toxin are involved in the pathogeneicity of *E. coli* O157:H7. Recent work shows that such phages are common in sewage (Muniesa and Jofre, 1998), and that they may be the source of genetic diversity among Shiga toxin producing *E. coli* (Muniesa *et al.*, 1999).

The uptake of the DNA by recipient cell does not ensure successful gene transfer unless the transferred sequences are maintained in the recipient's genome. DNA assimilation into the bacterial genome can exploit different processes. DNA can persist within the cell as an episome, which requires selection to avoid loss. Integration of exogeneous DNA can be mediated by bacteriophage integrases or by mobile element transposases. Homologous recombination is unlikely to allow introduction of novel traits, unless highly conserved genetic elements, for instance rRNA genes, serve as transfer vehicles (Strätz *et al.*, 1996).

## TRAITS INTRODUCED THROUGH HORIZONTAL GENE TRANSFER

It is becoming increasingly apparent that many genes within prokaryotes have been horizontally acquired, but not all genes are equally likely to be transferred. Genes participating in replication, transcription and translation (informational genes) are less likely to be horizontally transferred than operational genes (Rivera *et al.*, 1998; Jain *et al.*, 1999). Ribosomal RNA for example, which is a part of translation machinery, should be resistant to horizontal gene transfer. Lawrence (1999) concluded, that genes encoding the ribosomal RNA (informational genes) are unlikely to be transferred successfully since the recipient taxa would already bear functional orthologues; moreover, the corresponding product of native genes have experienced long-term coevolution with the rest of the cellular machinery and are unlikely to be displaced. However, few papers were published recently suggesting that even rRNA genes are not immune to horizontal gene transfer. Asai and co-workers (1999) have demonstrated, that 16S rRNA of *E. coli* can be completely replaced by that of *Proteus vulgaris* and that the ribosomal protein L11 binding domain of *E. coli* 23S rRNA can be replaced by the homologous region of yeast 28S rRNA. *Thermomonospora chromogena* genome contains six rRNA operons and one, *rrn*B, exhibits high levels of sequence variation to the other five throughout the entire length of the operon. Comparison of *rrn*B of *T. chromogena* and *rrn*A of *Termobispora bispora* suggests, that horizontal gene transfer is responsible for existence of two different types of rRNA operons in genome of *T. chromogena* (Yap *et al.*, 1999). Horizontal gene transfer was also proposed as a good explanation for 16S rRNA gene heterogeneity observed within strains of *Streptomyces* (Ueda *et al.*, 1999).

### Antibiotic resistance genes

Antibiotic resistant genes make possible for the bacterium to expand its ecological niche to environments where the noxious compound is present. Because the benefit to the microorganism derived from antibiotic resistance is transient it is not surprising that antibiotic resistance genes are associated with highly mobile genetic elements: plasmids, transposons and integrons (Ochman *et al.*, 2000; de la Cruz and Davies, 2000; Rowe-Magnus and Mazel, 1999).

### Pathogenicity determinants

Unlike the acquisition of antibiotic resistance, adoption of pathogenic determinants usually involves a fundamental change in a recipient's ecology. The virulence plasmids of *Yersinia* and *Shigella* are examples of plasmids that create extreme phenotypic changes when they are acquired (Portnoy *et al.*, 1981; Maurelli *et al.*, 1985). Recent studies have discovered that horizontally acquired "pathogenicity islands" are the major contributors to the virulent nature of many pathogenic bacteria. Pathogenicity islands are chromosomally encoded regions that contain large clusters of virulence genes and can upon incorporation, transform a benign organism into a pathogen (Hacker *et al.*, 1997). Some virulence determinants are encoded by bacteriophages and lysogenization by such phage results in a pathogenic variant of the strain (Jackson *et al.*, 1987).

### Metabolic properties

Horizontal gene transfer has also played a significant role in dissemination of genes involved in physiological processes, which have allowed organisms to explore new environments. Metabolic traits are usually complex, and successful mobilisation of such traits requires the physical clustering of genes, such that all necessary genes will be transferred in a single step. As a result, gene clusters and operons, which can be expressed in the recipient cell by a host promoter at the insertion site, will be selected (Lawrence and Roth, 1996). Genetic mechanisms responsible for metabolic traits dissemination are likely to have been the same as in antibiotic resistance genes or pathogenicity determinants (Romine *et al.*, 1999; Di Gioia *et al.*,1998).

## HORIZONTAL GENE TRANSFER AND IMPACT ON BACTERIAL EVOLUTION

In recent years, molecular genetics and genome analysis have provided extensive evidence that gene loss and acquisition are likely to be the primary mechanisms by which bacteria adapt genetically to novel environments and by which bacterial populations diverge, and form separate, evolutionary distinct species (Arber, 1993; Lawrence and Roth, 1999). The question is, how to establish whether a given trait or a genetic region is present in a certain organism as the result of horizontal gene transfer. Horizontal gene transfer may create high degree of similarity between donor and recipient strain for the analysed character. Transferred DNA is introduced into a single lineage and because of that the acquired trait will be limited to descendents of the recipient strain and absent from closely related taxa. The strongest evidence for horizontal gene transfer derives from genetic analysis of the DNA sequences themselves. DNA segments acquired through gene transfer often display restricted phylogenetic distribution among related strains or species. Additionally, horizontally acquired DNA regions display high levels of sequence similarity between strains which are divergent by other criteria (Doolitle, 1999). Bacterial species display characteristic G+C content in their genomes and genes in a particular genome are similar with respect to base composition, patterns of codon usage and frequences of di- and trinucleotides. Sequences introduced through horizontal gene transfer retain the sequence characteristics of the donor and can be thus distinguished from the recipient DNA (Lawrence and Ochman, 1998; Lawrence and Roth, 1999).

The availability of complete genome sequences provided an opportunity to measure and compare the cumulative amount of horizontally transferred sequences in bacterial genomes. Horizontally transferred genes are recognised by their atypical nucleotide composition or codon usage bias and after correction for genes whose atypical features are due to aminoacid composition, the remaining genes are likely to have been introduced through horizontal gene transfer (Lawrence and Ochman, 1997; Lawrence and Ochman, 1998). Large variation has been observed in the amount of horizontally acquired DNA in analysed genomes. In some organisms,

such as *Rickettsia prowazekii*, *Borrelia burgdorferi* and *Mycoplasma genitalium* almost no horizontally acquired sequences have been observed (Ochman *et al.*, 2000). In others such as *E. coli*, up to 15% of the genome was made of genes introduced through horizontal gene transfer (Lawrence and Ochman, 1998). Very ancient transfer events were not detected by these methods and actual number of transferred genes was underestimated because sequences acquired from organisms of similar base composition and codon usage patterns will escape detection. The same characteristics of genes that suggests a foreign origin, can also help to assess the time of their introduction. Each of the 750 foreign genes in the *E. coli* genome (15% of 4288 genes) was subjected to analysis in order to estimate the time at which each entered the genome. Introduction time varied from 0 to 100 million years ago and the rate of horizontal transfer of genes was estimated to be around 16 kb per million years (Lawrence and Ochman, 1998). Emergence of new phenotipic properties through horizontal gene transfer furnishes several advantages but it can also present several problems to the organism. Since bacterial genomes are not growing ever larger in size, the acquisition of DNA must be counterbalanced by DNA loss. Acquired genes that provide functions allowing for niche expansion may be maintained, while genes providing smaller overall selective benefits may be lost (Lawrence, 1999; Lawrence and Roth, 1999). In some cases, loss of function may be selectable advantage (Maurelli *et al.*, 1998) and reduced genomes of host dependent bacteria, such as *Chlamidia* and *Rickettsia*, attest to the tendency for bacteria to delete more expendable sequences from their genomes (Andersson and Kurland, 1998).

Recent evidence that horizontal gene transfer is a major driving force of bacterial and archaeal evolution is not only dramatic but can also be a threat to the phylogenetic classification which is based on comparative analyses of the nucleotide sequences of genes encoding ribosomal RNAs and some proteins (Woese, 1987). The primary branching pattern of the universal tree of life, separates Bacteria on one side from Archaea and Eukarya on the other. However, findings suggesting that rRNA genes can be subject of horizontal gene transfer (Asai *et al.*, 1999; Yap *et al.*, 1999; Ueda *et al.*, 1999) and evidence supporting gene exchange between bacterial and archaeal domain (Jain *et al.*, 1999; Aravid *et al.*, 1998) and gene transfer from bacteria and archaea to eukaryotes (Pennisi, 1998; Smith *et al.*, 1992) suggest that reticulated tree or a net might more appropriately describe evolution of life (Doolitle, 1999).

## RUMEN HORIZONTAL GENE TRANSFER

### Is it possible and is it useful?

Bacteria are present in large populations ($10^{10}$ cells $ml^{-1}$) in the rumen fluid and they can be also found attached to the substrate particles and the rumen wall. A fairly large population of bacteriophages was also observed in the rumen fluid and in few studies performed the bacteriophages integrated in ruminal bacteria genomes were also found. The necessary requirements for conjugation and transduction are therefore met. Natural transformation on the other hand seems less likely, given the high nucleolytic activity of the ruminal fluid (Flint and Thomson, 1990). However, these are only the technical requirements for the gene transfer. We should also consider whether gene transfer could have any impact on the rumen function. What, if any, may be its role in establishment and functioning of the ruminal community? Despite the circadian and other changes in rumen population brought about by changes of the diet, this community must always be able to supply the animal with volatile fatty acids and (mainly) bacterial protein. Therefore if the degradation of plant fibre and growth on its products, which is dominated by the bacteria, demands a highly specialised and equilibrated community, then gene transfer would be discouraged between its functionally distinct members. However, there is a

daily influx of soil and plant microorganisms into the rumen which remain in the rumen for a given amount of time. They are ingested with food and even being only transient residents they could serve as donors of novel genes for the rumen environment.

## EVIDENCE FOR GENE TRANSFER IN THE RUMEN

### Laboratory transfer of genetic elements by conjugation and natural transformation

These experiments show that the potential for natural gene transfer exists. Flint *et al.* focused on the transfer of tetracycline resistance genes, and succeded to transfer the tetracycline resistance in anaerobic matings between two species of ruminal *Prevotella*. The gene for tetracycline resistance was cotransferred with a 19,5 kbp plasmid pRRI4 which was concluded to carry the tetQ gene (Flint *et al.*, 1988). Although the transfer was of the conjugative type, the plasmid pRRI4 was up to now not shown to code for genes involved in transfer. Therefore, the transfer could have been mediated by some other chromosomal mobilizing element. This was also suspected by Shoemaker *et al.* (1992) when *Prevotella bryantii* succesfully acted as a donor of an *E. coli-Bacteroides-Prevotella* shuttle vector pRDB5 that needed to be mobilized for the transfer. The transfer of tetracycline resistance was also shown between *Butyrivibrio* strains. It was not plasmid associated and it also resulted in a change of the chromosomal restriction pattern observed with PFGE. This suggested the existence of a tetracycline carrying mobile chromosomal element with the size of 40-60kbp (Scott *et al.*, 1997). Not only chromosomal elements, but presumably also parts of the chromosomes can be transferred in such way. This was shown by Gilmour *et al.* (1996) by moving the ability to utilize lactate from a lactate-utilizing to a lactate-non-utilizing strain of *Selenomonas ruminantium* with simple mating procedure. The significance of transient members of rumen microflora as potential donors of various genes was demonstrated by Scott and Flint (1995) who showed that *E. coli* strains isolated from the rumen can successfully exchange their plasmids by conjugation in anaerobic conditions in a medium that included whole rumen fluid. Presumably they can also conjugate with related bacteria, starting the multi-stage intergenera transfer that can lead to the transfer of genes to phylogenetically distant organisms inhabiting the rumen. Recently *Streptococcus bovis,* an important member of the ruminal microflora, was shown to be capable of natural transformation with plasmids in normal culture medium. However, transformation was prevented by ovine ruminal fluid and saliva, being detected at 100 fold dilution of the rumen fluid in the medium (Mercer *et al.*, 1999b). The above described experiments showed that the predominant form of gene transfer in rumen could function via mobile chromosomal elements similar to those found in the human colonic *Bacteroides* species or even the mobilizing non-mobile chromosomal regions. Notably no self-transferable conjugative plasmids were found in strictly anaerobic rumen species.

### Evidence for gene transfer derived from the sequence analysis

Perhaps surprisingly there are more reports on inter-genera and even inter-kingdom transfer than on exchange between closely related species. The latter is probably more common but hard to follow, since it does not result in a gene whose structure (introns, codon usage…) is foreign to the recipient. It was reported by several authors that the genes coding for glycosyl hydrolases (GH) from the rumen fungi of the genera *Neocallimastix, Piromyces* and *Orpinomyces* contained no introns and showed surprising homology with genes that code for rumen bacterial glycosyl hydrolases. This suggested that a gene transfer between bacteria and fungi occurred. Recently Garcia-Vallve *et al.* (2000) conducted a survey of rumen fungal GH sequences. They compared

the codon usage of GH genes to other fungal enzymes and also *F. succinogenes* glycosyl hydrolase genes. The fungal GH genes formed a cluster separate from both other groups, however one rumen fungal sequence was clustered together with *Fibrobacter* genes. The biased codon usage is a general feature of highly expressed genes. But since other non-GH genes from fungi which were included in the analysis are also highly expressed, this could not be the reason for separate clustering of fungal GH genes. Furthermore, the authors constructed a phylogenetic tree based on protein sequences of endoglucanases from GH family 5 including non-rumen fungal GH. The ruminal fungal protein sequences grouped with ruminal bacterial sequences and not with non-ruminal fungal sequences. The same was observed for xylanases. Since ruminal fungi are monophyletic, only a small number of gene transfer events could have sufficed for the present dispersion of the genes of bacterial origin in rumen fungi. The other well documented case of gene transfer to or from rumen bacteria involves the transfer of the tetracycline resistance gene tetQ (Nikolich *et al.*, 1994). This gene was found in human colonic *Bacteroides* species as well as in ruminal *Prevotella* strains. On a phylogenetic tree, that was constructed using tetQ aleles from ruminal *Prevotella* and human *Bacteroides* species, and that was generally consistent with actual phylogenetic distances, one *Bacteroides* alele grouped together with *Prevotella* sequences, indicating horizontal gene transfer. Interestingly, this tetQ alele was identical to the one isolated from oral *Prevotella intermedia*, revealing the movement of this alele from rumen to oral and then colonic microflora of humans.

## POVZETEK

V zadnjih nekaj letih je na podlagi genetskih raziskav in sekvenciranja nekaterih bakterijskih in arhejskih genomov postalo očitno, da je horizontalni prenos genov eden osnovnih mehanizmov prilagajanja bakterij na nova okolja in je najverjetneje odločilno vplival na evolucijo mikroorganizmov. Pri mikroorganizmih poznamo tri načine genskega prenosa: transformacijo, konjugacijo in transdukcijo. Vsi trije načini prenosa genov so bili dokazani v različnih naravnih sistemih, vendar je znanje o horizontalnem prenosu genov in predvsem o obsegu tega prenosa v okolju še zmeraj skromno. Verjetnost prenosa se med geni razlikuje. Geni, katerih produkti sodelujejo pri osnovnih bioloških procesih, t. j. podvojevanju, prepisovanju in prevajanju, se verjetno horizontalno ne prenašajo, oz. se prenašajo v veliko manjšem obsegu kot npr. geni za antibiotsko rezistenco, geni za virulenčne dejavnike in geni, ki sodelujejo v presnovnih procesih. Analizo vloge horizontalnega prenosa genov za evolucijo mikroorganizmov je omogočilo sekvenciranje genomov in obdelava teh sekvenc. Pri nekaterih vrstah, kot so *Rickettsia prowazekii*, *Borrelia burgdorferi* in *Mycoplasma genitalium*, prenosa genov skoraj niso zasledili, medtem ko so pri *E. coli* ugotovili, da je kar 15% genov v genomu tujega izvora. Ugotovitve o pomembnosti in razširjenosti horizontalnega prenosa genov so zamajale tudi obstoječi filogenetski sistem, še posebej, ker so se pojavili nekateri dokazi o horizontalnem prenosu genov za 16S rRNA, ki sodijo med najpomembnejše in najbolj ohranjene dele genoma. Vamp prežvekovalcev je eden najbolj kompleksnih mikrobnih ekosistemov. Razmere v vampu so teoretično ugodne za horizontalni prenos genov s konjugacijo in transdukcijo. Večina preskusov, opravljenih *in vitro*, je potrdila možnost prenosa genov v vampu s konjugacijo in naravno transformacijo. Analiza sekvence gena za glikozil hidrolazo pri vampnih glivah pa je nakazala verjeten bakterijski izvor tega gena, prav tako pa so dokazali tudi prenos gena za odpornost na tetraciklin med različnimi vampnimi in črevesnimi vrstami bakterij.

## REFERENCES

Andersson, S.G.E./ Kurland, C.G. Reductive evolution of resident genomes. Trends Microbiol., 6(1998), 263-268.

Aravid, L./ Tatusov, R.L./ Wolf, Y.I./ Walker, R.D./ Koonin, E.V. Evidence for massive gene exchange between archaeal and bacterial hyperthermophiles. Trends Genet., 14(1998), 442-444.

Arber, W. Evolution of prokaryotic genome. Gene, 135(1993), 49-56.

Armstrong, J.L./ Wood, N.D./ Porteous, L.A. Transconjugation between bacteria in the digestive tract of cutworm *Peridroma saucia*. Appl. Environ. Microbiol., 56(1990), 1492-1493.

Asai, T./ Zaporojets, D./ Squires, C./ Squires, C.L. An *Echerichia coli* with all chromosomal rRNA operons inactivated: Complete exchange of rRNA genes between bacteria. Proc. Natl. Acad. Sci. USA, 96(1999), 1971-1976.

Baur, B./ Hanselmann, K./ Schlimme, W./ Jenni, B. Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence. Appl. Environ. Microbiol., 62(1996), 3673-3678.

Chiura, H.X./ Kato, K./ Hiraishi, A./ Maki, Y. Gene transfer mediated by virus of novel thermophilic bacteria in hot spring sulfur-turf microbial mats. Eighth International Symposium on Microbial Ecology, Program and abstract, (1998), 124.

Clewel, D.B./ Flannagan, S.E./ Jaworski, D.D. Unconstrained bacterial promiscuity: Tn916-Tn1545 family of conjugative transposons. Trends Microbiol., 3(1995), 229-236.

de la Cruz, F./ Davies, J. Horizontal gene transfer and the origin of species: lessons from bacteria. Trends. Microbiol., 8(2000), 128-133.

Dahlberg, C./ Bergstrom, M./ Hermansson, M. *In situ* detection of high level of horizontal plasmid transfer in marine bacterial communities. Appl. Environ. Microbiol., 64(1998), 2670-2675.

Davison, J./ Brunel, F./ Kaniga, K./ Chevalier, N. Recombinant DNA vectors for *Pseudomonas*. In: *Pseudomonas*: Biotransformations, Phatogenesis and Evolving Biotechnology (Eds.: Silver, S. A./ Chakrabarty, A./ Iglewski, B.). Washington, DC, Am. Soc. Microbiol., 1990, 242-251.

Di Gioia, D./ Peel, M./ Fava, F./ Wyndham, R.C./ Structures of homologous composite transposons carrying *cba*ABC genes from Europe and North America. Appl. Environ. Microbiol., 64(1998), 1940-1946.

Doolittle, F.W. Phylogenetic classification and the universal tree. Science, 284(1999), 2124-2128.

Dykhuizen, D.E./ Green, L. Recombination in Escherichia coli and the definition of biological species. J. Bacteriol., 173(1991), 7257-7268.

Firth, N.K./ Ippen-Ihler, K./ Skurray, R.A. Structure and function of the factor and mechanism of conjugation. In: *Escherichia coli* and *Salmonella*: Cellular and molecular biology (Ed.: Neidhart, F. C.). Washington, ASM Press, 1996, 2377-2401.

Flint, H.J./ Thomson, A.M. Deoxyribonuclease activity in rumen bacteria. Lett. Appl. Microbiol., 11(1990), 18-21.

Flint, H.J./ Thomson, A.M./ Bisset, J. Plasmid-associated transfer of tetracycline resistance in *Bacteroides ruminicola*. App. Environ. Microbiol., 54(1988), 855-860.

Garcia-Vallve, S./ Romeu, A./ Palau, J. Horizontal gene transfer of glycosyl hydrolases of the rumen fungi. Mol. Biol. Evol., 17(2000), 352-361.

Gilmour, M.M./ Mitchell, W.J./ Flint, H.J. Genetic transfer of lactate-utilizing ability in the rumen bacterium *Selenomonas ruminantium*. Lett. Appl. Microbiol., 22(1996), 52-56.

Graham, J.B./ Istock, C.A. Genetic exchange in *Bacillus subtilis* in soil. Mol. Gen. Genet., 166(1978), 287-290.

Hacker, J./ Blum-Oehler, G./ Muhldorfer, I./ Tschape, H. Pathogenicity islands of virulent bacteria: structure, function and imact on microbial evolution. Mol. Microbiol., 23(1997), 1089-1097.

Ippen-Ihler, K. Bacterial conjugation. In: Gene transfer in the enviroment (Eds.: Levy, S. B./ Miller, R. V.). New York, McGraw-Hill Publishing Company, 1989, 33-72.

Jackson, M.P./ Neill, R.J./ O'Brien, A.D./ Holmes, R.K./ Newland, J.W. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli*. FEMS Microbiol. Lett., 44(1987), 109-114.

Jain, R./ Rivera, M.C./ Lake, J.A. Horizontal gene transfer among genomes: the complexity hypothesis. Proc. Natl. Acad. Sci. USA., 96(1999), 3801-3806.

Jiang, S.C./ Paul, J.H. Gene transfer by transduction in the marine environment. Appl. Environ. Microbiol., 64(1998), 2780-2787.

Kapur, V./ Nelson, K./ Schlievert, P.M./ Selander, R.K./ Musser, J.M. Molecular population genetic evidence of horizontal spread of two alleles of the pyrogenic exotoxin C gene (*speC*) among pathogenic clones of *Streptococcus pyogenes*. Infect. Immun., 60(1992), 3513-3517.

Khanna, M./ Stotzky, G. Transformation of Bacillus subtilis by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA. Appl. Environ. Microbiol., 58(1992), 1930-1939.

Kidambi, S.P./ Ripp, S./ Miller, R.V. Evidence for phage-mediated gene transfer among *Pseudomonas aeruginosa* strains on the phylloplane. Appl. Environ. Microbiol., 60(1994), 496-500.

Kokjohn, T.A. Transduction: Mechanism and potential for gene transfer in the environment. In: Gene transfer in the enviroment (Eds.: Levy, S. B./ Miller, R. V.). New York, McGraw-Hill Publishing Company, 1989, 33-72.

Lawrence, J.G. Gene transfer, speciation, and the evolution of bacterial genomes. Curr. Opin. Microbiol., 2(1999), 519-523.

Lawrence, J.G./ Ochman, H. Amelioration of bacterial genomes: rates of change and exchange. J. Mol. Evol., 44(1997), 383-397.

Lawrence, J.G./ Ochman, H. Molecular archaeology of the *Escherichia coli* genome. Proc. Natl. Acad. Sci. USA, 95(1998), 9413-9417.

Lawrence, J.G./ Roth, J.R. Selfish operons: Horizontal transfer may drive the evolution of gene clusters. Genetics, 143(1996), 1843-1860.

Lawrence, J.G. and Roth, J.R. Genomic flux: genome evolution by gene loss and acquisition. In: Organisation of the prokaryotic genome (Ed.: Charlebois, R.L.). Washington, ASM Press, 1999, 263-289.

Lederberg, J./ Tatum, E.L. Gene recombination in *E. coli*. Nature, 158(1946), 558.

Lorenz, M.G./ Reipschlager, K./ Wackernagel, W. Plasmid transformation of naturally competent *Acinetobacter calcoaceticus* in non-sterile soil extract and groundwater. Arch. Microbiol., 157(1992), 355-360.

Lorenz, M.G./ Wackernagel, W. Bacterial gene transfer by natural genetic transformation in the environment. Microbiol. Rev., 58(1994), 563-602.

Lunsford, R.D. Streptococcal transformation: Esential features and applications of a natural gene exchange system. Plasmid, 39(1998), 10-20.

Masters, M. Generalized transduction. In: *Escherichia coli* and *Salmonella*: Cellular and molecular biology (Ed.: Neidhart, F.C.). Washington, ASM Press, 1996, 2421-2441.

Maurelli, A.T./ Baudry, B./ d'Hauteville, H./ Hale, T.L./ Sansonetti, P.J. Cloning of plasmid DNA sequences involved in invasion of HeLa cells by *Shigella flexneri*. Infect. Immun., 49(1985), 164-171.

Maurelli, A.T./ Fernandez, R.E./ Bloch, C.A./ Rode, C.K./ Fasano, A. "Black holes" and bacterial pathogenicity: a large genomic deletion that enhances the virulence of *Schigella* spp. and enteroinvasive *Escherichia coli*. Proc. Natl. Acad. Sci. USA, 95(1998), 3943-3948.

McClure, N.C./ Fry, J.C./ Weightman, A.J. Gene transfer in activated sludge. In: Bacterial genetics in natural environments (Eds.: Fry, J.C./ Day, M.J.). London, Chapman & Hall, 1990, 111-129.

Mercer, D.K./ Scott, K.P./ Bruce-Johnson, W.A./ Glover, A.L./ Flint, H.J. Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. Appl. Environ. Microbiol., 65(1999a), 6-10.

Mercer, D.K./ Melville, C.M./ Scott, K.P./ Flint, H.J. Natural genetic transformation in the rumen bacterium *Streptococcus bovis* JB1. FEMS Microbiol. Lett, 179(1999b), 485-490.

Morrison, W.D./ Miller, R.V./ Sayler, G.S. Frequency of F116 mediated transduction of *Pseudomonas aeruginosa* in a freshwater environment. Appl. Environ. Microbiol., 36(1978), 724-730.

Muniesa, M./ Jofre, J. Abundance in sewage of bacteriophage that infect Escherichia coli O157:H7 and that carry the Shiga toxin 2 gene. Appl. Environ. Microbiol., 64(1998), 2443-2448.

Muniesa, M./ Lucena, F./ Jofre, J. Comparative survival of free Shiga toxin 2-encoding phages and *Escherichia coli* strains outside the gut. Appl. Environ. Microbiol., 65(1999), 5615-5618.

Nielsen, K.M./ van Weerelt, M.D./ Berg, T.N./ Bones, A.M./ Hagler, A.N./ van Elsas, J.D. Natural transformation and availability of transforming DNA to *Acinetobacter calcoaceticus* in soil microcosms. Appl. Environ. Microbiol., 63(1997), 1945-1952.

Nielsen, K.M./ Smalla, K./ van Elsas, J.D. Natural transformation of *Acinetobacter* sp., *Pseudomonas fluorescens*, and *Burkholderia cepacia* in soil microcosms. Appl. Environ. Microbiol., 66(2000), 206-212.

Nikolich, M.P./ Hong, G./ Shoemaker, B.N./ Salyers, A.A. Evidence for natural horizontal transfer of tetQ between bacteria that normally colonize humans and bacteria that normally colonize livestock. Appl. Environ. Microbiol., 60(1994), 3255-3260.

Normander, B./ Christensen, B.B./ Molin, S./ Kroer, N. Effect of bacterial distribution and activity on conjugal gene transfer on the phylloplane of the bush bean (*Phaseolus vulgaris*). Appl. Environ. Microbiol., 64(1998), 1902-1909.

Ochman, H./ Lawrence, J.G./ Groisman, E.A. Lateral gene transfer and the nature of bacterial inovation. Nature, 405(2000), 299-304.

Pennisi, E. Genome data shake tree of life. Science, 280(1998), 672-674.

Portnoy, D.A./ Moseley, S.L./ Falkow, S. Characterization of plasmids and plasmid-associated determinants of *Yersinia enerocolitica* pathogenesis. Infect. Immun., 31(1981), 775-782.

Rivera, M.C./ Jain, R./ Moore, J.E./ Lake, J.A. Genomic evidence for two functionally distinct gene classes. Proc. Natl. Acad. Sci. USA., 95(1998), 6239-6244.

Romine, M.F./ Stillwell, L.C./ Wong, K.K./ Thurston, S.J./ Sisk, E.C./ Sensen, C./ Gaasterland, T./ Fredrickson, J.K./ Saffer, J.D. Complete sequence of a 184-kilobase catabolic plasmid from *Sphingomonas aromaticivorans* F199. J. Bacteriol., 181(1999), 1585-1602.

Rowe-Magnus, D.A./ Mazel, D. Resistance gene capture.Curr. Opin. Microbiol., 2(1999), 483-488.

Salyers, A.A./ Shoemaker, N.B. Resistancegene transfer in anaerobes: New insights, new problems. Clin. Infect. Dis., 23(1996), S36-S43.

Saye, D.J./ Ogunseitan, O./ Sayler, G.S./ Miller, R.V. Potential for transduction of plasmid in a natural freshwater environment: Effect of plasmid donor concentration and a natural microbial community on transduction in *Psudomonas aeruginosa*. Appl.Environ. Microbiol., 53(1987), 987-995.

Scott, K.P./ Flint, H.J. Transfer of plasmids between strains of *Escherichia coli* under rumen conditions. J. Appl. Bacteriol., 78(1995), 189-193.

Scott, K.P./ Barbosa, T.M./ Forbes, K.J./ Flint, H.J. High-frequency transfer of a naturally occurring chromosomal tetracycline resistance element in the ruminal anaerobe *Butyrivibrio fibrisolvens*. Appl. Environ. Microbiol., 63(1997), 3405-3411.

Shoemaker, N.B./ Wang, G./ Salyers, A.A. Evidence for natural transfer of a tetracycline resistance gene between bacteria from the human colon and bacteria from the bovine rumen. Appl. Environ. Microbiol., 58(1992), 1313-1320.

Smit, E./ Venne, D./ van Elsas, J.D. Mobilization of a IncQ plasmid between bacteria on agar surfaces and in soil via cotransfer or retrotransfer. Appl. Environ. Microbiol., 59(1993), 2257-2263.

Smith, M.W./ Feng, D./ Doolitle, R.F. Evolution by acquisition: the case for horizontal gene transfer. TIBS, 17(1992), 489-493.

Snyder, L./ Champness, W. Molecular genetics of bacteria. Washington, ASM Press, 1997, 129-146.

Sullivan, J.T./ Patrick, H.N./ Lowther, W.L./ Scott, D./ Ronson, C.W. Nodulating strain of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. Proc. Natl. Acad. Sci. USA, 92(1995), 8985-8989.

Stewart, G.J. The mechanism of natural transformation. In: Gene transfer in the enviroment (Eds.: Levy, S.B./ Miller, R.V.). New York, McGraw-Hill Publishing Company, 1989, 139-164.

Stewart, G.J./ Sinigalliano, C.D. Exchange of schromosomal markers by natural transformation between the soil isolate, *Pseudomonas stutzeri* JM300, and the marine isolate, *Psudomonas stutzeri* strain ZoBell. Antonie Van Leeuwenhoek, 59(1991), 19-25.

Strätz, M./ Mau, M./ Timmis, K.N. System to study horizontal gene exchange among microorganisms without cultivation of recipients. Mol. Microbiol., 22(1996), 207-215.

Top, E./ Mergeay, M./ Springael, D./ Verstraete, W. Exogenous isolation of mobilizing plasmids from polluted soils and sludges. Appl. Environ. Microbiol., 60(1994), 831-839.

Ueda, K./ Seki, T./ Kudo, T./ Yoshida, T./ Kataoka, M. Two distinct mechanisms cause heterogeneity of 16S rRNA. J. Bacteriol., 181(1999), 78-82.

Weisberg, R.A. Specialized transduction. In: *Escherichia coli* and *Salmonella*: Cellular and molecular biology (Ed.: Neidhart, F.C.). Washington, ASM Press, 1987, 1169-1176.

Wichels, A./ Biel, S.S./ Gelderblom, H.R./ Brinkhoff, T./ Muyzer, G./ Schutt, C. Bacteriophage diversity in the North sea. Appl. Environ. Microbiol, 64(1998), 4128-4133.

Woese, C.R. Bacterial evolution. Microbiol. Rev., 51(1987), 221-271.

Yap, W.H./ Zhang, Z./ Wang, Y. Distinct types of rRNA operons exist in the genome of the actinomycete *Thermomonospora chromogena* and evidence for horizontal transfer of an entire rRNA operon. J. Bacteriol., 181(1999), 5201-5209.

Zinder, N.D./ Lederburg, J. Genetic exchange in *Salmonella*. J. Bacteriol., 64(1952), 679-699.