THE SIGNIFICANCE OF HORIZONTAL GENE TRANSFER IN THE FORMATION OF BACTERIAL BIOFILM

By

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Declaration

It is hereby declared that

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Ethics Statement

This is an entirely new piece of work that has never been published before. It is based on my own honest and thorough study and analysis. All of the sources used in the study are appropriately credited (correct citation).

Dedicated to my beloved

Parents

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Abstract

Different publication and research papers indicate that horizontal gene transfer is interrelated with the process of biofilm formation. Horizontal transfer is also known as HGT. We know that plasmid is a small extrachromosomal DNA molecule which are usually found in bacteria and other microscopic organisms. And we also know that basic criteria of plasmid are replication and replication is maintained in order to guarantee that the number of copies of plasmid of the host remains secure. The rate of horizontal gene transfer during biofilm formation is higher compared with that of during planktonic stage. Under the influence of microbial genetic elements, horizontal gene transfer enforces bacteria for the enhancement of firm association. This happens fundamentally because plasmids are autonomous replicators that boost their own survival by encouraging bacterial interactions. They frequently also carry genes that improve the physical fitness of their hosts. In addition, contemporary study demonstrates that bacteria can be forced to maintain stable social interactions by the so-called mafia qualities encoded on mobile genetic components. Also, it suggests that horizontal gene transfer eventually strengthens the resemblance between bacteria carrying mobile genetic components from a single origin.

Key words

Biofilm, Bacterial biofilm, Horizontal gene transfer, Conjugation, Transformation, Transduction, Plasmid transfer, Conjugative pili, Social mediators, Gene expression, Socio-microbiology.

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List of Acronyms:

Acronyms	Full Form
1. HGT	Horizontal gene transfer
2. ARGs	Antibiotic resistance genes
3. MGEs	Mobile genetic elements
4. ActA	Actin polymerization protein
5. Bap	Biofilm-associated protein
6. bcsA	Bacterial cellulose synthesis
7. CidA	Cell death effector protein
8. Csg	Curly synthesis gene
9. eDNA	Cell death effector protein
10. LAP	Listeria adhesion protein
11. PIA	Polysaccharide intercellular adhesin
12. ARBs	Angiotensin receptor blockers
13. BAFs	Barrier-to-autointegration factor
14. OqxAB	Quinolone and Olaquindox Efflux Pump
15. SoxR	superoxide response protein

1. Goal of the study:

The goal of this paper is to relate the connection of the horizontal gene transfer in the occurrences of bacterial biofilm lifecycle.

1.2 Specific aim:

The goal of this study is to compile all relevant information and offer appropriate guidance based on the knowledge already available from numerous illustrious publications. This study includes information from Jonas, Mette, Lars and many other online articles and well-established publications. The information gathered has been properly cited and offers a comprehensive understanding of interconnection of horizontal gene transfer (HGT) and lifecycle of a bacterial biofilm. It also includes an elaborated study of the factors influencing the transfer of HGT in biofilm, the role of conjugative pili and other accessory plasmid region in biofilm formation. Furthermore, the stages of biofilm formation, emergence of biofilm components and their gene expression are discussed here. In addition, some methodologies for studying HGT and biofilms are briefly presented in this study.

2. Introduction:

In the aquatic environment, Horizontal transfer resistance plays an important role in biofilm. Antibiotic resistance genes or ARG is being spread through horizontal transfer resistance (HGT) for the biofilms of the aquatic environment. Depending on the environment and mobile genetic elements, ARG perform important mechanisms of HGT. HGT mechanism includes conjugation, transformation and transduction.

We know that biofilm is made up of bacterial cells which is encircled in a slimy matrix which is self-produced. This self-produced matrix is made up of extracellular polymeric substances usually exopolysaccharides, nucleic acids and proteins. Bacteria biofilms may adhere to non-living or living surface. We know that in planktonic cells, biofilm exist as free-floating communities in the aquatic environment. Due to growth rate, gene expression and gene transcription, biofilm formed an alternated phenotype which hinders phagocytosis (Important component of body defense system) which shows tolerance to the immune responses. The slimy matrix varies in which biofilms are enclosed vary in size from being micro colony to being large shaped mushroom like structures. This slimy matrix has two functions: 1) It allows the supply of nutrients inside the matrix 2) It engages in removing the waste materials from the deeper biofilm layers.

2.1 Life cycle of a biofilm.

Step 1: Reversible adhesion of microorganisms to a surface through adsorption.

Step 2: Permanent adherence brought on by eps manufacturing.

Step 3: Microcolony formation

Step 4: Biofilm structure maturation.

Step 5: Regaining the planktonic stage or reinfecting other surfaces by dispersing and separating the bacteria from the biofilm.

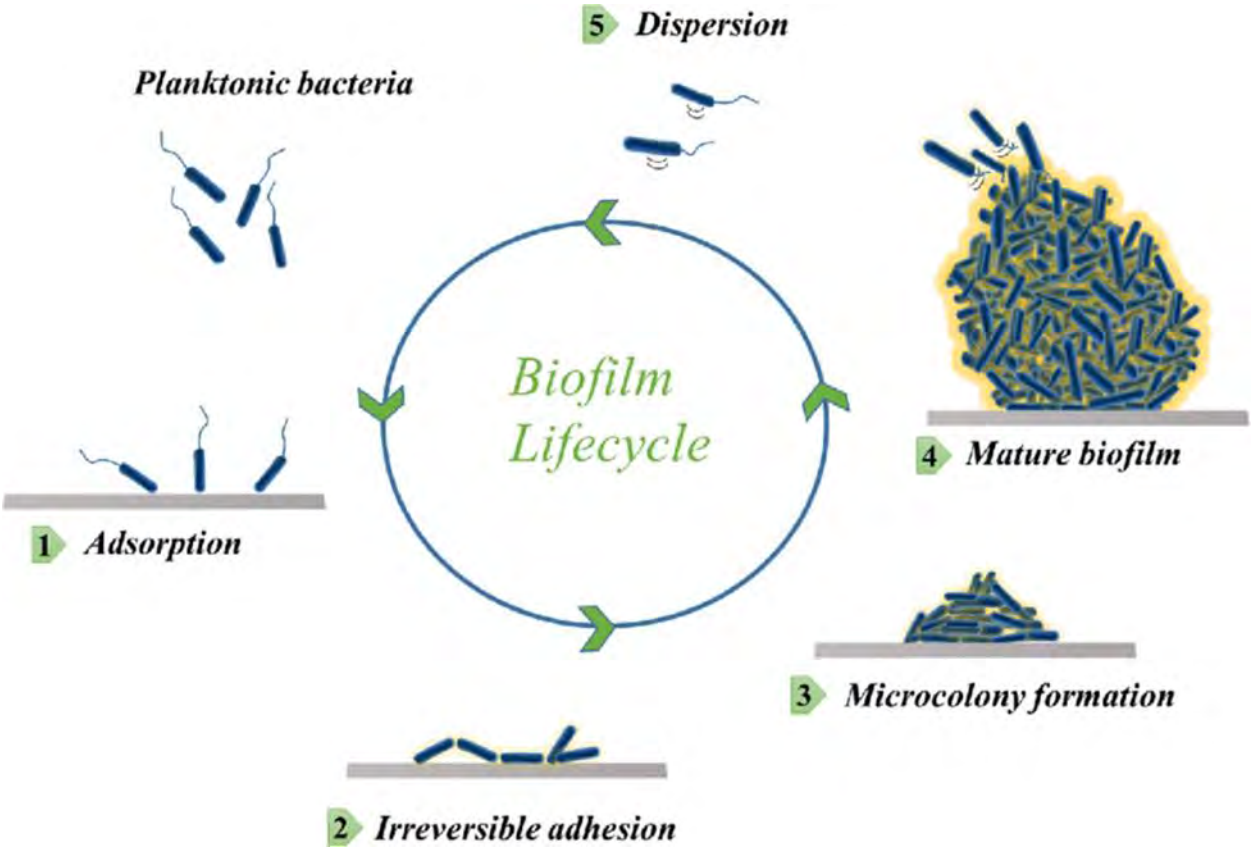

Figure 1: Biofilm lifecycle. [collected from (Flemming *et al*. (2016))]

3. Factors influencing the transfer of HGT in biofilm

Frederick Griffith in 1928 discovered the bacterial gene transfer process i.e., the process of transfer of genetic material from one bacterial cell to another. In the Griffith experiment, it has seen that when an abstract of dead virulent *Streptococcus pneumonia is added to the culture of living non-virulent Streptococcus pneumonia, the cells of this culture became virulent. In the transformation process, the horizontal gene transfer (HGT) of DNA occurs. By taking up the live DNA from the dead virulent bacteria, the living non-virulent bacteria becomes virulent by the method of genetic recombination.*

We know that HGT in bacteria happens in three processes: Conjugation, transduction and transformation. Conjugation happens by direct contact between one cell to another cell. Transduction happens by transfer of bacteriophage mediated DNA and transformation occurs by taking of DNA into the competent cells. Conjugative transfer happens in gram negative bacteria through certain pili and through the mechanism of quorum sensing regulation. On the other hand, conjugative transfer in gram positive bacteria doesn't require certain pili but depends on production and identification of certain pheromones.

3.1 Conjugation

In the planktonic stage of bacterial biofilm, conjugation happens at higher rates within the members of biofilm communities. Horizontal gene transfer or HGT helps this conjugation to occur at higher frequencies (Hausner & Wuertz, 1999; Sørensen *et al*., 2005) When the biofilms are densely populated, it helps the spread of mobile genetic elements (MGEs) at a rapid pace. This quick transfer of genetic materials occurs through conjugative pili. HGT helps the quick transfer of MGEs mainly in the conjugative plasmid. (Andersson & Levin, 1999; Bergstrom *et al*., 2000).

3.2 Transformation

Numerous studies found that horizontal gene transfer of MGEs occur in molecular parasites and vertical genetic transfer of MGEs occur in other pathogenic microorganism (Bahl *et al*., 2007). Biofilm usually consists of plasmids in which high transfer frequencies of MGEs happens host (Andersson & Levin, 1999; Bergstrom *et al*., 2000). Usually in conjugative plasmids, horizontal gene transfer (HGE) of MGEs occurs at successful rate.

Transformation also occurs in biofilm at higher frequencies. The rate of high frequency of transformation of biofilm depends on different elements: Small DNA fragments and Plasmids comprising both encoding and decoding genes for mobilization. (Hendrickx *et al*., 2003; Maeda *et al*., 2006; Etchuuya *et al*., 2011)

3.3 Transduction

Transduction explains the HGT event of bacteriophage from one bacterial host to another. After the formation of new phage particles, DNA that are produced from phage infected bacterial host can transfer into the newly produced phage particles accidently. The DNA which are produced from phage infected bacterial host must enter into the chromosome or plasmid in order to establish stabilization and expression. We know that transformation is the uptake of free DNA from the environment. In most cases the cell reaches capable state that ensures the uptake of DNA. For this, the DNA is inserted into the plasmid or chromosome (circularly) by the genetic recombination process (Webb *et al*, 2004)

The genes in backbone and accessory regions of plasmid encode biofilm-associated factors (BAFs).(Frost *et al*., 2003) In the case of some plasmids, the formation of biofilm plays an important role especially the biofilm-associated factors (BAFs) which are being encoded by backbone genes (such as conjugal pili). As we know that such genes are vital for studying plasmid biology.

4. The role of conjugative pili in biofilm formation

We know that nature of conjugation is that it promotes the cell to cell contact i.e., from donor to recipients. In the backbone of conjugative plasmid, conjugation may result in biofilm formation. It has been found that biofilm formation mainly occurs in the type of E. coli which separates the conjugative plasmid (Reisner *et al*, 2006). Different researchers have found that incF plasmid are mainly responsible for biofilm formation. *E.coli.* harboring derepressed inCF1 and inCF2 conjugative plasmid are differently responsible for biofilm formation through F-pili. In the priming of biofilm IncF plasmid plays an important role. Conjugative Pili is not mainly responsible for cell surface attachment. Cell surface attachment mainly happens due to host biofilm system activation. Several research has indicated that *E. coli* expresses proteinaceous extracellular fibers which is known as curli. And *E. coli* also forms exopolysaccharide colonic acid. incF plasmid generates the appearance of colonic acid and curli in E.coli (May & Okabe, 2008). Therefore, we can say that conjugative pili enables cell

to cell contact, while colonic acid and curli helps in the cell surface attachment and to ensure the firmness of the layout of biofilm.

5. The role of plasmid accessory regions containing biofilm-related factors:

Fimbriae and nonconjugative pili help to enable cell to cell contact as well as cell surface attachment in the accessory region of plasmids. Thus, fimbriae and nonconjugative pili helps to occur biofilm priming. From the research it has been seen that plasmids responsible for biofilm formation encode type 3 fimbriae. Depending on nucleotide sequencing on plasmids, encoded mrkABCDF cassette of the type 3 fimbrae has been found. (Norman *et al*., 2008). Therefore, biofilm primers such as fimbrae in the accessory region of plasmids which have attachment characteristics is advantage to bacterial biofilm.

The type 4 pilli is responsible for the cell surface attachment in bacterial biofilm. From different researches it is seen the type 4 pilli is found in Archaeae (Pohischroder *et al*., 2011). The type 4 pill is found in conjugative plasmids in *E. coli*. The type 4 pili describes the priming module which is situated between an accessory and backbone related gene. From the above discussion we have found that the plasmids related to the biofilm formation are incF(incFl – incFV), incX and incl cluster.

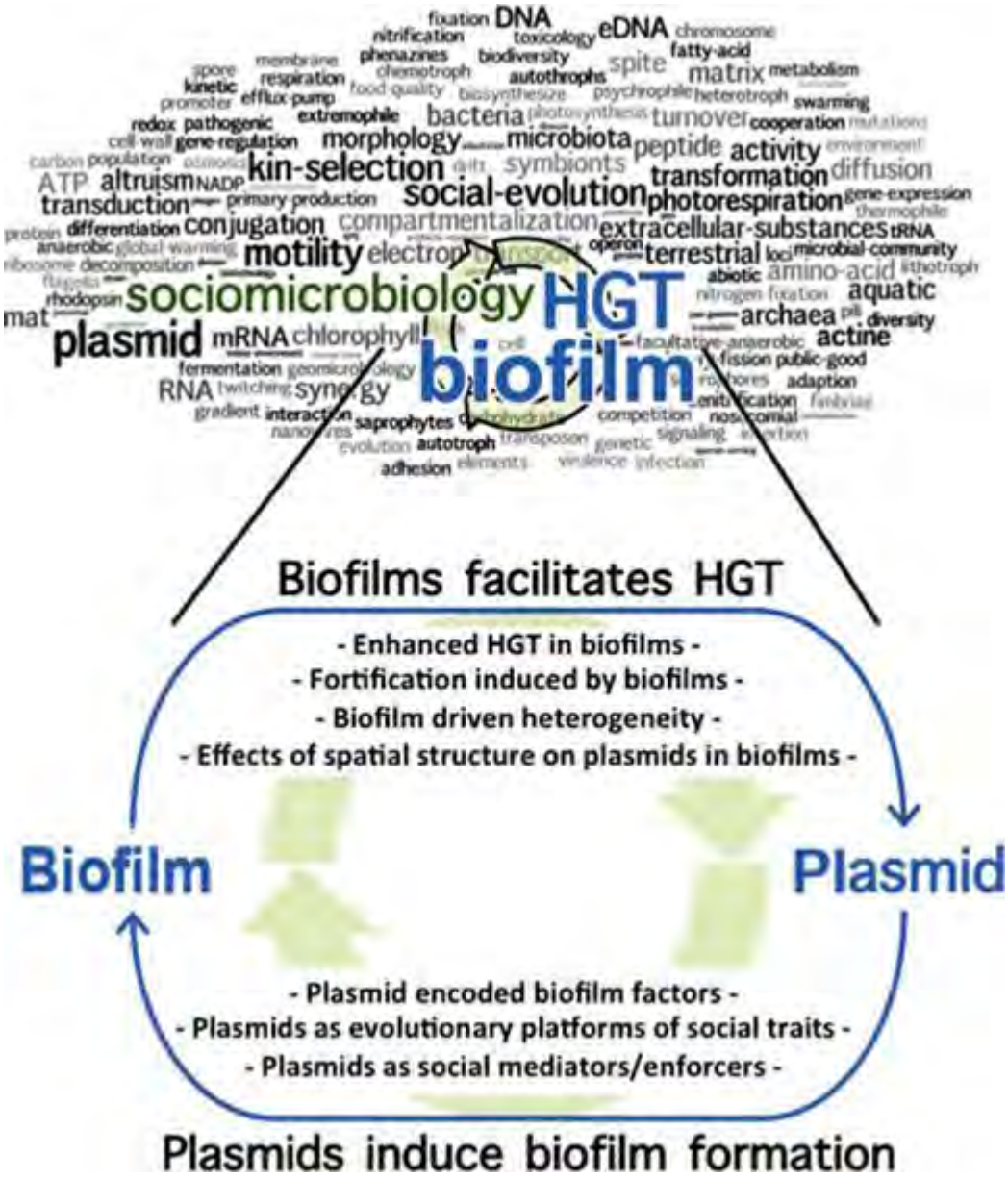

Figure 2: The relationship between plasmid biology and biofilm formation. [collected from (Ghigo *et al*. 2001)]

6. The emergence and reemergence of biofilm components (Plasmid Mechanics):

Plasmids are distinct genetic components that are very common in bacteria. The significance of plasmids in bacteria's evolution as autonomous organisms and potential synergistic symbionts has a significant impact. We now have a better understanding of how MGEs have

horizontally transferred, invented, and reinvented genes and genetic networks to produce new traits shaped by intrinsic selective pressures in various environments over the past two decades. Here are some examples of genes that are thought to have acquired new roles through HGT and course of evolution. These illustrations should emphasize how important MGEs and HGT were in the development of specific original functionalities. For the development of novel characteristics and genetic networks, plasmids serve as evolutionary platforms. This makes the mechanics of plasmids a significant facilitator in the emergence of novel biofilm-promoting features, together with the fact that plasmids depend on the fitness of their host and have the capacity for vertical and horizontal transmission. (Ochman *et al*., 2000; Martínez *et al*, 2008). As a result, the lifestyle strategy of the plasmid will affect how plasmid-encoded BAFs evolve. However, the relationship between plasmids and biofilm development suggests that plasmids play a significant role in influencing bacterial social interactions and, more specifically, in the evolution of microbes. The ability of plasmids to serve as social evolutionary platforms is influenced by a number of factors.

(1) It is significant to note that a plasmid's success depends on the health of its host and the community or population in which it lives.

(2) Because there are multiple copies of a plasmid present inside the cell, genes encoded on plasmids are typically more abundant than those encoded on chromosomes. Consequently, plasmid-encoded genes often exhibit higher levels of expression.

(3) New, stronger promoters might be linked to genes that are transferred from the bacterial chromosome to a plasmid. This typically occurs when insertion sequences, transposons, or integrons mobilize genes or gene cassettes.

(4) In the case of inter-species HGT, gene-regulation is often lost or altered, and genes may, be expressed as a result constitutively or at altered rates.

(5) Plasmid-encoded genes undergo a high rate of turnover.

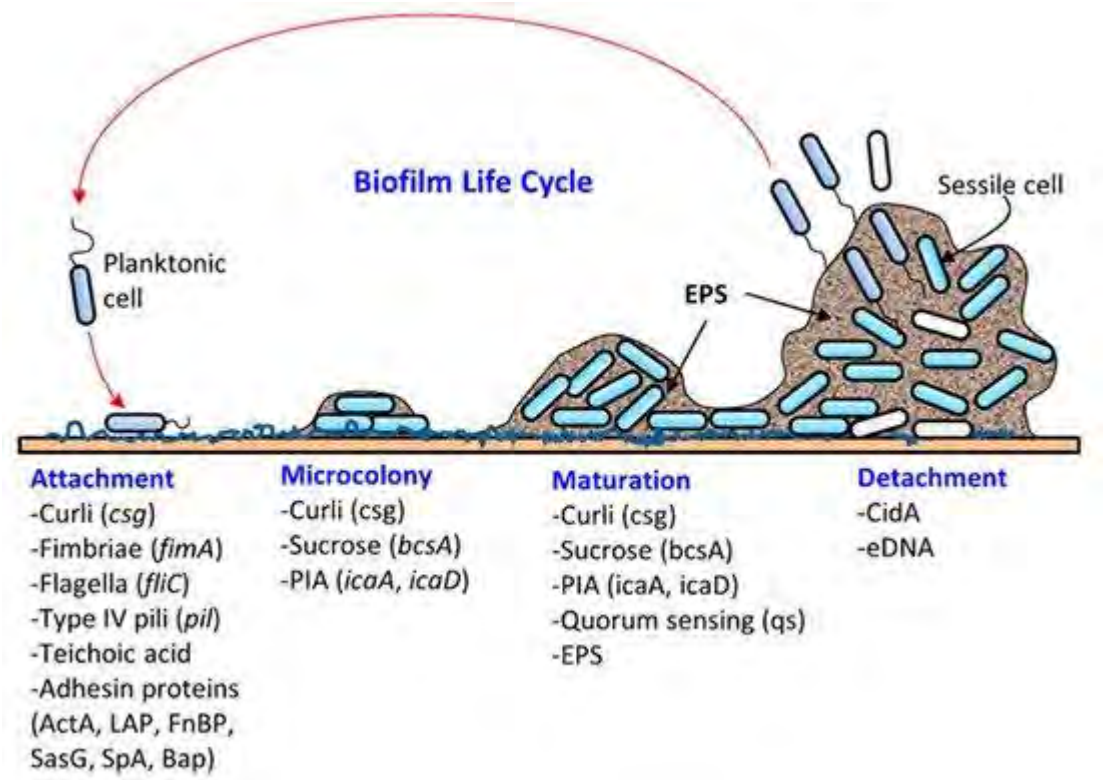

Figure 3: Diagram illustrating the many stages of biofilm formation, including (i) adhesion, (ii) micro colony creation, (iii) maturation with cellular differentiation, and (iv)detachment or dispersion. [collected from (Yaron *et al*., 2014)]

7. Control of gene expression- from genome to plasmids:

Some antibiotic resistance functionalities are one of the most well-researched cases of proteins that are thought to have acquired new functions through HGT via MGEs. The fundamental premise is that housekeeping genes with a variety of functions have evolved into proteins that provide antibiotic resistance, mostly by means of HGT and selection. The OqxAB pump is a good example of this. The OqxAB pump gene is encoded by the chromosome of K. pneumonia where its purpose is unknown but it does not cause antibiotic resistance. (Hansen *et al*., 2007). However, when OqxAB pump is overexpressed on plasmids in other enterobacteria, it gives multidrug resistance. These naturally occurring plasmids have been found in pigs that

were given olaquindox, one of the medications that the OqxAB makes its host resistant to. . (Hansen *et al*., 2007)

Plasmids encoding OqxAB have also been isolated from humans in addition to agricultural animals. Although not specifically linked to biofilms, this example demonstrates how a small number of proteins can alter the phenotypic features of the host cell if they are expressed on a plasmid instead of their regular genetic context. (Kim *et al*., 2009; Zhao *et al*., 2010)

SoxR, a transcriptional factor, is a further example. Although the genes are undoubtedly linked, Dietrich & Kiley (Dietrich & Kiley, 2011) recently claimed that SoxR has distinct roles in enteric and nonentric bacteria. In enteric bacteria (E. coli), SoxR controls just one gene, soxS, which in turn controls several (more than 100) targets. SoxR, a nonenteric bacterium found in actinobacteria, beta-, delta-, and gammaproteobacteria, has been identified direct control over a variety of targets, including the production of biofilms in Pseudomonas aeruginosa and Streptomyces coelicolor. (Dietrich *et al*., 2008). It is thought that the soxR gene was horizontally transferred from a nonenteric to an enteric bacterium. This is an illustration of a single gene that has undergone horizontal gene transfer and has later changed in function in the new host bacteria as a result of recombination processes. (Dietrich *et al*., 2008)

The nucleotide sequence of the chromosomally encoded mrkABCDF in K. pneumoniae and the plasmid-encoded mrkABCDF are nearly identical, indicating a relatively recent mobilization of the mrkABCDF cassette to the incX1 plasmids, which makes it very easy to examine the biofilm priming mrkABCDF system. Recently, significant type 3 fimbriae regulatory elements encoded in the chromosome of K. pneumoniae have been discovered. (Johnson & Clegg, 2010; Johnson *et al*., 2011; Wilksch *et al*., 2011).

The mrkABCDF encoding plasmids lack these regulatory essential components, suggesting that either new regulatory functions have developed or constitutive expression takes place. This illustration shows how plasmids can serve as adaptable and evolutionary templates for systems associated to biofilms. In comparison to those present in chromosomes, the regulatory networks directly linked to the BAFs located on plasmids are typically less complex.

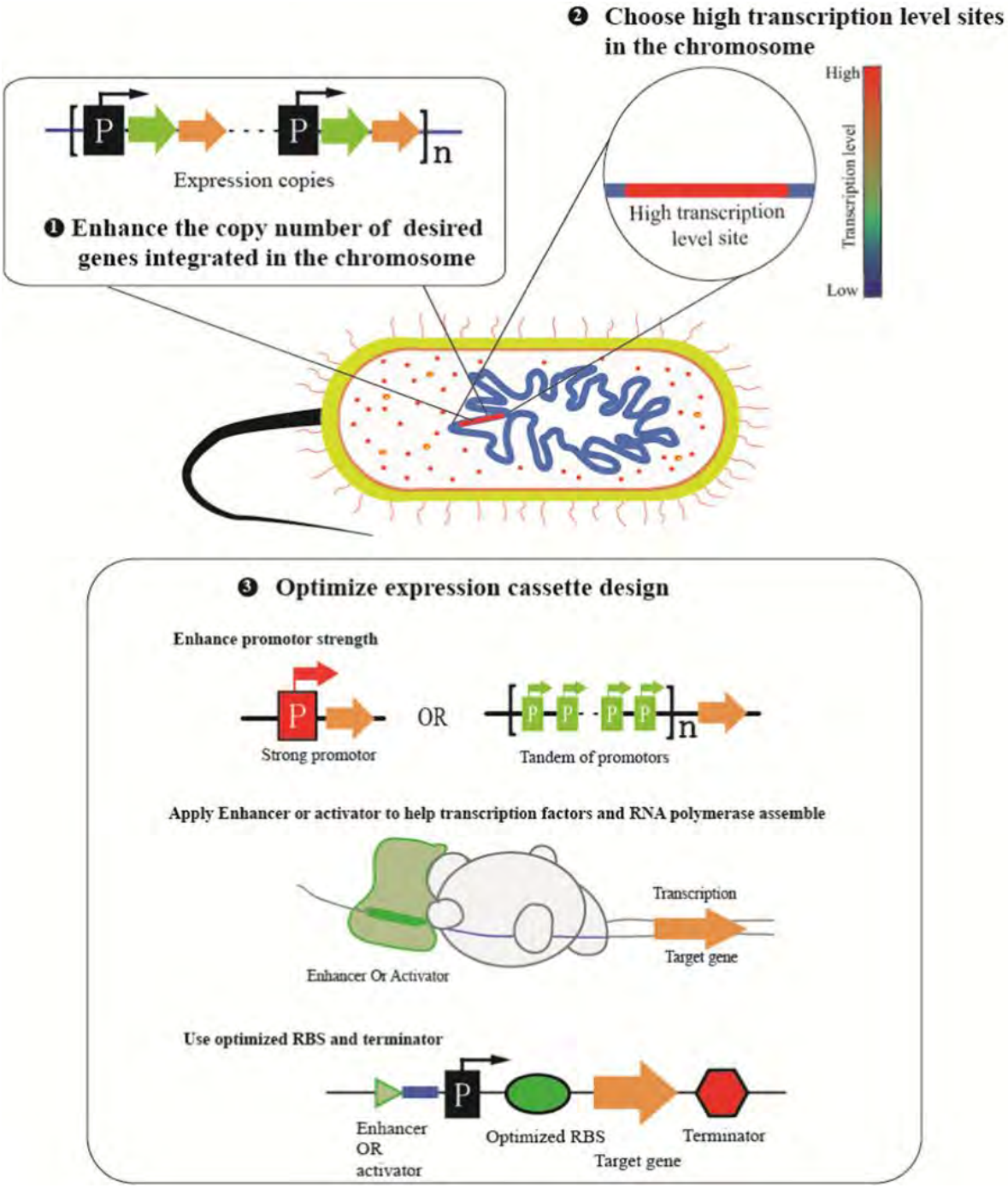

Figure 4: The methods for integrating heterologous genes into the chromosomes of Escherichia coli and techniques to maximize the expression of heterologous proteins. [collected from (Timmermans *et al*, 2010)]

8. Plasmids serving as social mediators:

It has been found that HGT promotes collaboration as MGEs heighten genetic relatedness, enforce cooperation, and, as a result, encourage inter-bacterial cooperation based on computational modeling and sequence analysis of genomic and metagenomic data. The secretome was used in this work as a factor imposing cooperative social relationships. (Nogueria *et al*, 2009)

We quickly discuss the main reasons and link them to the interconnection of biofilm and plasmid biology in this section because many, though not all, BAFs can be regarded a member of the secretome. In 21 genomes—20 E. coli and one Escherichia fergusonii—Nogueria *et al*. (2009) deduced the localization of hypothetical proteins predicted from annotated genes. They demonstrated a trend regarding the localization of core, ancestral, and recent genes (gene classes) by doing this.

Both anticipated secreted proteins and proteins localized at the outer membrane were typically encoded by newly acquired genes, with only a small number of ancestral core genes remaining. This showed that the movable region of the genome is where social traits are generally found. Non-ancestral E. coli genes were projected to belong to the secretory and outer-membrane protein groups much more often than those located elsewhere when human gut metagenomes of unweaned neonates, infants, and adults were analyzed. (Rankin *et al*, 2011). Additionally, they showed how secreted and outer-membrane proteins were more frequently encoded on plasmids than in mobilizable hotspots, and again, more frequently in these hotspots than in non-mobilizable regions of the genomes.

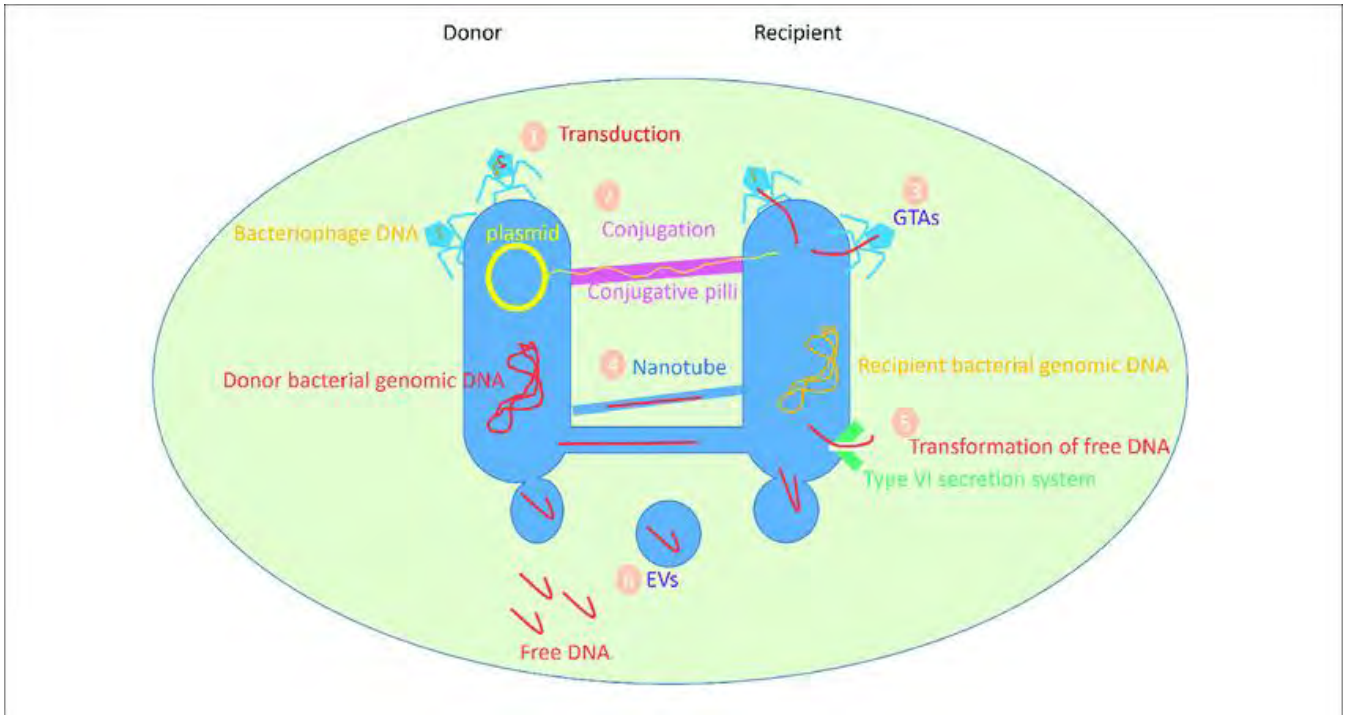

Figure 5: Genetic material is flexiblely exchanged between several creatures of the same or distinct species. It is well established that this phenomena significantly affects the host's genetic, biological, and environmental capabilities. [collected from (Giraud & Shykoff, 2011).)]

There is a strong correlation between protein cost and location, as shown by the discovery that secreted and outer-membrane proteins have lower biosynthetic energy cost per residue than do proteins in the periplasm, cytoplasm, and inner membrane. (Nogueria *et al*, 2009). Compared to genes that code for proteins, which are normally restricted to the other compartments, MGEs carry social features at a lower cost to the host bacteria. In the study, a large number of secreted proteins were identified as virulence factors, demonstrating how virulence factors might result from bacterial social interactions. (Nogueria *et al*, 2009). Because of a cost that ultimately helps other bacteria that have not invested in the creation of the molecules, the development of outer-envelope compounds that benefit the surrounding bacteria may end up as a fitness loss. Surface-attached outer-membrane molecules, like fimbriae, can be recycled or re-scavenged in contrast to molecules that are secreted into the environment and dissipate away, which lowers the cost of this kind of public benefit. (Nogueria *et al*, 2009). This may also help to explain why the typical BAFs seen on plasmids include things like fimbriae and pili.

9. Methods for analyzing HGT and biofilms:

From the past to the present, genomic study of cultured clonal ARB isolated from the habitats and quantitative PCR have been the main methods used to detect ARGs and ARBs. Assays for filter mating, transformation, and transduction are frequently used in laboratories to confirm the transfer of ARGs. High-throughput next-generation DNA sequencers (NGS) are currently able to directly extract multispecies genomic data from uncultivated bacteria in natural habitats (metagenome) and give enormous amounts of whole-genome data for organisms. The sequencing data are made available for further research, such as categorizing ARGs and locating HGT events, by depositing them on open databases. Many software programs and computational pipelines have been created to date to identify HGT**.** To date, numerous computational methods and pieces of software have been created to find HGT. To predict HGT events in bacterial populations from metagenomic data, Song, Li and Jiang separately developed MetaCHIP and LEMON. (Song *et al*, 2019) (Li and Jiang, 2019). These authors noted the software's accessibility for detecting ARG mobilization. Utilizing the tools could reveal details about how ARGs have previously been distributed within the various microbial communities. The study of antibiotic resistance properties of recently discovered ARBs and mobilization of novel MGEs, whose information is not only obtained from the sequencing data, still require the cultivation-based approach, even though recent environmental ARG research may largely rely on the cultivation-independent metagenomic analysis. (Douglas and Langille, 2019)

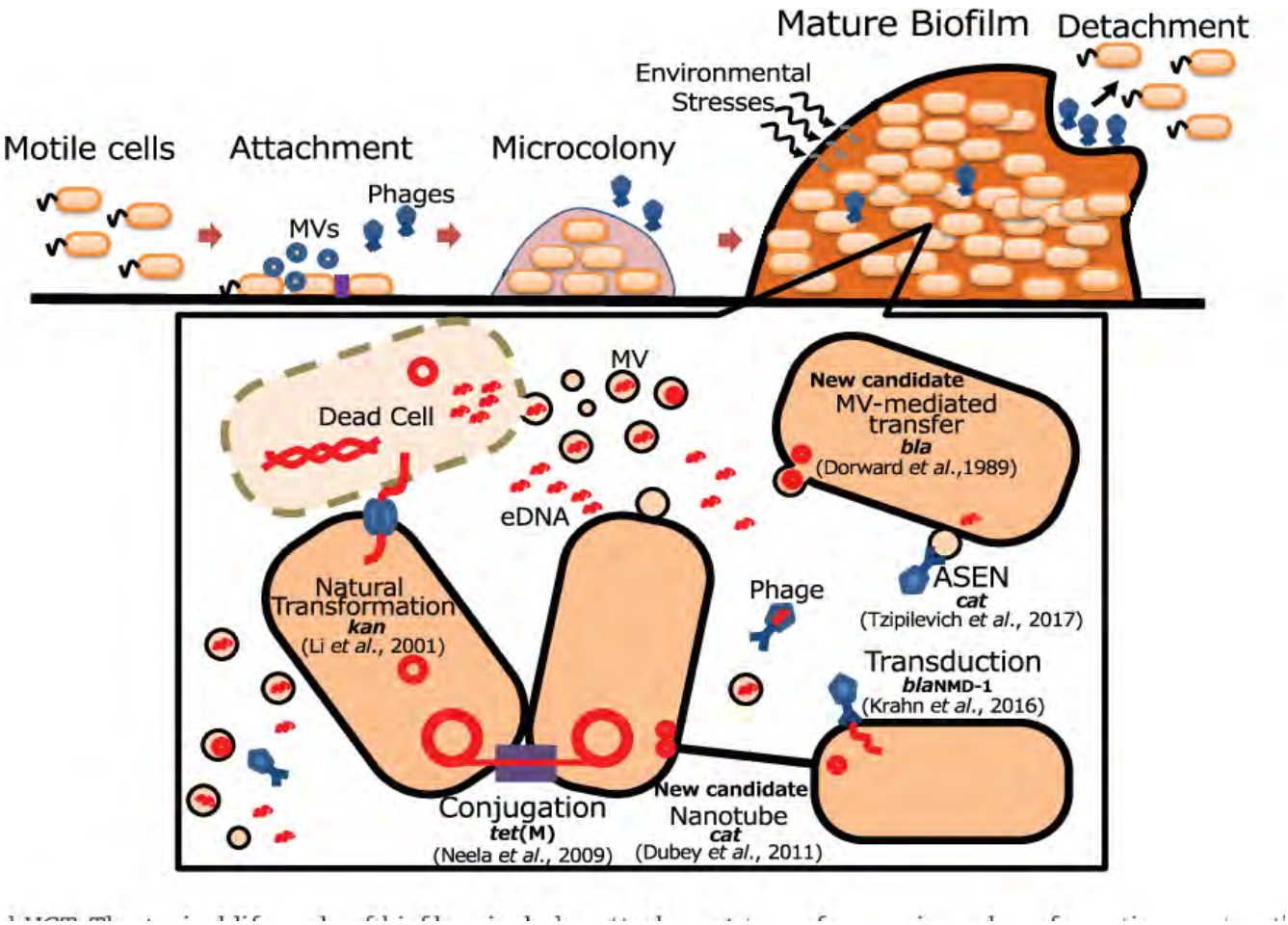

Figure 6: Biofilms typically go through four stages of development: adhesion to surfaces, production of microcolonies, maturation, and detachment. [collected from (Karatan *et al.* 2009)]

10. HGT mechanisms and biofilms are interconnected:

By encoding proteins that are associated with biofilms, several conjugative plasmids promote the growth of biofilms. Three cell-wall anchoring proteins—PrgA, PrgB, and PrgC—are encoded by pCF10, an E. faecalis conjugative plasmid, and they help to increase cell-cell adhesion during the beginning of biofilm formation (Bhatty *et al*. 2015). Genes encoding type III fimbriae, which are crucial in cell adhesion to surfaces, are found on the Klebsiella pneumonia plasmid pOLA52. (Burmolle *et al*, 2008). Numerous conjugative plasmids exist in

Escherichia coli, such as the F plasmid, which encourages biofilm development in a conjugation-pilus-dependent manner. (Ghigo *et al*, 2001).

In streptococci and V. cholerae, natural transformation is known to be intimately related to the development of biofilms; during the establishment of DNA competence, eDNA, a key component of the biofilm matrix, is released. (Ibanez de Aldecoa, Zafra and Gonzalez-Pastor 2017; Veening and Blokesch 2017). Fratricide is a condition that occurs in streptococcal species like *S. pneumoniae* and *S. mutans*. Competent cells boost the production of bacteriocins and enzymes that break down extracellular cell walls, which causes neighboring cells to lyse and release eDNA. (Steinmoen, Knutsen and Havarstein, 2002). In contrast to CSP receptor mutants, Oggioni demonstrated that the addition of synthetic competence-stimulating peptide (CSP) increases the production of S. pneumoniae biofilm. (Oggioni *et al*. 2006). The effectiveness of the transformation correlates with the growth of the biofilm in *S. mutans*. (Li *et al*. 2001). Similar to streptococci, competent *V. cholerae* cells attack nearby cells by injecting effector proteins through a type VI pilus, which causes the recipient cell to die and release eDNA. (Veening and Blokesch, 2017) The *V. cholerae* competence pilus has a function in the generation of eDNA in addition to encouraging cell aggregations by interacting with other pilus during the early stages by destroying the matrix and destroying the embedded cells, phages infiltrate biofilms. (Adams *et al*. 2019).

It appears that the development of biofilms and phages are antagonistic. Recent research has demonstrated the beneficial function that phages can perform in promoting the life cycle of biofilms. Though eDNA is frequently produced as a result of phage-mediated cell lysis, which reinforces the biofilm formations. (Fernandez, Rodriguez and Garcia, 2018). In a different instance, the detachment of the cells from biofilms is caused by the elimination of biofilms by the *E. coli* phage Rac and the *P. aeruginosa* phage Pf4 (Liu *et al*. 2015) (Rice *et al*. 2009). Additionally, the P. aeruginosa prophage Pf4 is implicated in virulence in mice as well as the stabilization of microcolonies, which shapes the mature biofilm structure. Biofilm formation (Rice *et al*. 2009).

11. Conclusion:

In our perspective, understanding the social issues created by microbial interactions is necessary to comprehend how virulence mechanisms, such as biofilm formation, are developed. Understanding the underlying molecular pathways is crucial if our goal is to accomplish the aforementioned objective. This is fundamentally driven by HGT and MGEs like plasmids. In this review, we have hypothesized that there may be a positive feedback loop that encourages both plasmid biology and biofilm growth. The viewpoint encompasses a broad level of connectivity between HGT, MGE, and bacterial social evolution. Therefore, we can prove the significance of HGT is immense in the different stages of formation of bacterial biofilm.

12. References:

1) Hausner M. Wuertz S. (1999) High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. Appl Environ Microbiol65: 3710–3713.
2) Sørensen S.J. Bailey M. Hansen L.H. Kroer N. Wuertz S. (2005) Studying plasmid horizontal transfer in situ: a critical review. Nat Rev Microbiol3: 700–710.
3) Andersson D.I. Levin B.R. (1999) The biological cost of antibiotic resistance. Curr Opin Microbiol2: 489–493.
4) Bergstrom C.T. Lipstick M. Levin B.R. (2000) Natural selection, infectious transfer and the existence conditions for bacterial plasmids. Genetics155: 1505–1519.
5) Bahl M.I. Hansen L.H. Sørensen S.J. (2007) Impact of conjugal transfer on the stability of IncP-1 plasmid pKJK5 in bacterial populations. FEMS Microbiol Lett266: 250–256.
6) Hendrickx L. Hausner M. Wuertz S. (2003) Natural genetic transformation in monoculture Acinetobacter sp. Strain BD413 biofilms. Appl Environ Microbiol69: 1721–1727.
7) Maeda S. Ito M. Ando T. Ishimoto Y. Fujisawa Y. Takahashi H. Matsuda A. Sawamura A. Kato S. (2006) Horizontal transfer of nonconjugative plasmids in a colony biofilm of Escherichia coli. FEMS Microbiol Lett255: 115–120.
8) Etchuuya R. Ito M. Kitano S. Shigi F. Sobue R. Maeda S. (2011) Cell-to-cell transformation in Escherichia coli: a novel type of natural transformation involving cell-derived DNA and a putative promoting pheromone. PLoS ONE6: e16355.
9) Flemming, H. C., et al. (2016). Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563-575.
10) Webb, J. S., Lau, M., & Kjelleberg, S. (2004). Bacteriophage and phenotypic variation in Pseudomonas aeruginosa biofilm development. Journal of Bacteriology, 186(24), 8066-8073.
11) Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: the agents of open source evolution. Nature Reviews Microbiology, 3(9), 722-732.
12) Reisner A. Höller B.M. Molin S. Zechner E.L. (2006) Synergistic effects in mixed Escherichia coli biofilms: conjugative plasmid transfer drives biofilm expansion. J Bacteriol188: 3582–3588.

13) Norman A. Hansen L.H. She Q. Sørensen S.J. (2008) Nucleotide sequence of pOLA52: a conjugative incX1 plasmid from Escherichia coli which enables biofilm formation and multidrug efflux. Plasmid60: 59–74.
14) Pohischroder M. Ghosh A. Tripepi M. Albers S.V. (2011) Archeal type IV pilus-like structures-evolutionarily conserved prokaryotic surface organelles. Curr Opin Microbiol14: 357–363.
15) Pohischroder M. Ghosh A. Tripepi M. Albers S.V. (2011) Archeal type IV pilus-like structures-evolutionarily conserved prokaryotic surface organelles. Curr Opin Microbiol14: 357–363.
16) Ghigo, J. M. (2001). Natural conjugative plasmids induce bacterial biofilm development. Nature, 412(6845), 442-445.
17) Ochman H. Lawrence J.G. Groisman E.A. (2000) Lateral gene transfer and the nature of bacterial innovation. Nature405: 299–304.
18) Martínez J.L. (2008) Antibiotics and antibiotic resistance genes in natural environments. Science321: 365–367.
19) Yaron, S.; Romling, U. Biofilm formation by enteric pathogens and its role in plant colonization and persistence. Microb. Biotechnol. 2014, 7, 496–516.
20) Hansen L.H. Jensen L.B. Sørensen H.I. Sørensen S.J. (2007) Substrate specificity of the OqzAB multidrug resistance pump in Escherichia coli and selected enteric bacteria. J Antimicrob Chemother60: 145–147.
21) Kim H.B. Wang M. Park C.H. Kim E. Jacoby G.A. Hooper D.C. (2009) oqxAB encoding a multidrug efflux pump in human clinical isolates of Enterobacteriaceae. Antimicrob Agents Chemother53: 3582–3584.
22) Zhao J. Chen Z. Chen S. et al. (2010) Prevalence and dissemination of oqxAB in Escherichia coli isolates from animals, farmworkers, and the environment. Antimicrob Agents Chemother54: 4219–4224.
23) Dietrich LEP Kiley P.J. (2011) A shared mechanism of SoxR activation by redox-cycling compounds. Mol Microbiol79: 1119–1122.
24) Dietrich LEP Teal T.K. Price-Whelan A. Newman D.K. (2008) Redox-active antibiotics control gene expression and community behavior in divergent bacteria. Science321: 1203–1206.
25) Johnson J.G. Clegg S. (2010) Role of MrkJ, a phosphodiesterase, in type 3 fimbrial expression and biofilm formation in Klebsiella pneumonia. J Bacteriol192: 3944–3950.

26) Johnson J.G. Murphy C.N. Sippy J. Johnson T.J. Clegg S. (2011) Type 3 fimbriae and biofilm formation are regulated by the transcriptional regulators MrkHI in Klebsiella pneumonia. J Bacteriol193: 3453–3460.
27) Wilksch J.J. Yang J. Clements A. et al. (2011) MrkH, a novel c-di-GMP dependent transcriptional activator, controls Klebsiella pneumonia biofilm formation by regulating type 3 fimbriae expression. PLoS Pathog7: 1–22.
28) Timmermans, J., & Van Melderen, L. (2010). Post-transcriptional global regulation by CsrA in bacteria. Cellular and Molecular Life Sciences, 67(17), 2897-2908.
29) Nogueria T. Rankin D.J. Touchon M. Taddei F. Brown S.P. (2009) Horizontal gene transfer of the secretome drives the evolution of bacterial cooperation and virulence. Curr Biol19: 1683–1691.
30) Rankin D.J. Ginty S.E. Nogueira T. Touchon M. Taddei F. Rocha EPC Brown S.P. (2011a) Bacterial cooperation controlled by mobile elements: kin selection and infectivity are part of the same process. Heredity107: 279–281.
31) Giraud T. Shykoff J.A. (2011) Bacterial cooperation controlled by mobile elements: kin selection versus infectivity. Heredity107: 277–278.
32) Jiang X, Ellabaan MMH, Charusanti P et al. . Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. Nat Commun. 2017;8:15784.
33) Li B, Qiu Y, Jiang X et al. Real-time study of rapid spread of antibiotic resistance plasmid in biofilm using microfluidics. Environ Sci Technol. 2018;52:11132–41.
34) Song W, Wemheuer B, Zhang S et al. . MetaCHIP: community-level horizontal gene transfer identification through the combination of best-match and phylogenetic approaches. Microbiome. 2019;7:36.
35) Douglas GM, Langille MGI. Current and promising approaches to identify horizontal gene transfer events in metagenomes. Genome Biol Evol. 2019;10:2750–66.
36) Karatan, E., & Watnick, P. (2009). Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiology and Molecular Biology Reviews, 73(2), 310-347.
37) Bhatty M, Cruz MR, Frank KL et al. Enterococcus faecalis pCF10-encoded surface proteins PrgA, PrgB (aggregation substance) and PrgC contribute to plasmid transfer, biofilm formation and virulence. Mol Microbiol. 2015;95:660–77.
38) Burmolle M, Bahl MI, Jensen LB et al. Type 3 fimbriae, encoded by the conjugative plasmid pOLA52, enhance biofilm formation and transfer frequencies in Enterobacteriaceae strains. Microbiol. 2008;154:187–95.

39) Ghigo JM. Natural conjugative plasmids induce bacterial biofilm development. Nature. 2001; 412:442–5.
40) Ibanez de Aldecoa AL, Zafra O, Gonzalez-Pastor JE. Mechanisms and regulation of extracellular DNA release and its biological roles in microbial communities. Front Microbiol. 2017;8:1390.
41) Veening JW, Blokesch M. Interbacterial predation as a strategy for DNA acquisition in naturally competent bacteria. Nat Rev Microbiol. 2017;15:621–9.
42) Steinmoen H, Knutsen E, Havarstein LS. Induction of natural competence in Streptococcus pneumoniae triggers lysis and DNA release from a subfraction of the cell population. Proc Natl Acad Sci USA. 2002;99:7681–6.
43) Oggioni MR, Trappetti C, Kadioglu A et al. . Switch from planktonic to sessile life: a major event in pneumococcal pathogenesis. Mol Microbiol. 2006;61:1196–210.
44) Li YH, Lau PC, Lee JH et al. . Natural genetic transformation of Streptococcus mutans growing in biofilms. J Bacteriol. 2001;183:897–908.
45) Veening JW, Blokesch M. Interbacterial predation as a strategy for DNA acquisition in naturally competent bacteria. Nat Rev Microbiol. 2017;15:621–9.
46) Adams DW, Stutzmann S, Stoudmann C et al. . DNA-uptake pili of Vibrio cholerae are required for chitin colonization and capable of kin recognition via sequence-specific self-interaction. Nat Microbiol. 2019;4:1545–57.
47) Fernandez L, Rodriguez A, Garcia P. Phage or foe: an insight into the impact of viral predation on microbial communities. ISME J. 2018;12:1171–9.
48) Liu X, Li Y, Guo Y et al. Physiological function of Rac prophage during biofilm formation and regulation of Rac excision in Escherichia coli K-12. Sci Rep. 2015; 5:16074.
49) Rice SA, Tan CH, Mikkelsen PJ et al. The biofilm life cycle and virulence of Pseudomonas aeruginosa are dependent on a filamentous prophage. ISME J. 2009; 3:271–82.