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Plasmids: Tiny pieces with high efficacy (Review)

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Abstract: Plasmids are of paramount importance in the field of bacterial ecology and evolution due to their facilitation of the horizontal transfer of accessory genes (HGT). It has become evident that, apart from their evolutionary role, plasmids also have the ability to influence the expression of various bacterial traits. Approximately 25% of the plasmids contain the necessary functions to autonomously undergo conjugation, while the remaining plasmids can be mobilized through conjugation at a later time. Given their potential significance, this review centers on the role of these minuscule sequences of nucleotides in various biological activities..

Keywords: Plasmids, (HGT)

Introduction

Plasmids are incredibly important in the world of microbial adaptation. They enable bacteria to develop antibiotic resistance and other metabolic capabilities, as well as diversify their genomes. The process of host cell division allows for the transmission of these plasmids to daughter cells, and in specific instances, they can even be transferred horizontally to other bacteria through horizontal gene transfer (HGT). Nevertheless, there is a lack of comprehensive knowledge regarding the evolution and ecological dynamics of plasmids in various microbial environments and populations. Constructing complete plasmid sequences from short read data continues to pose a significant challenge, despite the sequencing and assembly of thousands of plasmids directly from isolated bacteria (Pellow et al., 2021).

The term 'plasmid' was first introduced by Lederberg in 1952 (Lederberg, 1952) to describe genetic determinants that exist outside of the chromosome. This encompasses the genomes of viruses, chloroplasts, and mitochondria, among others. Currently, the term is primarily employed to denote the simple extra-chromosomal DNAs molecules seen in bacteria, archaea, and certain eukaryotic organisms (figure 1). Bacterial cells normally contain circular, double-stranded DNA units known as plasmids (Dionisio et al., 2019), Plasmids play a crucial role in spreading and acquiring antibiotic resistance and virulence, which can have significant implications for human health (Foley et al., 2021). Typically, they are smaller in size, ranging from 744bp to 2.58Mb, and have the ability to be transferred through HGT (Shintani et al., 2015). Additionally, a bacterium has the

ability to host numerous plasmids, whether they are different or multiple copies of the same plasmid, within a single cell (Beltrán et al., 2021).

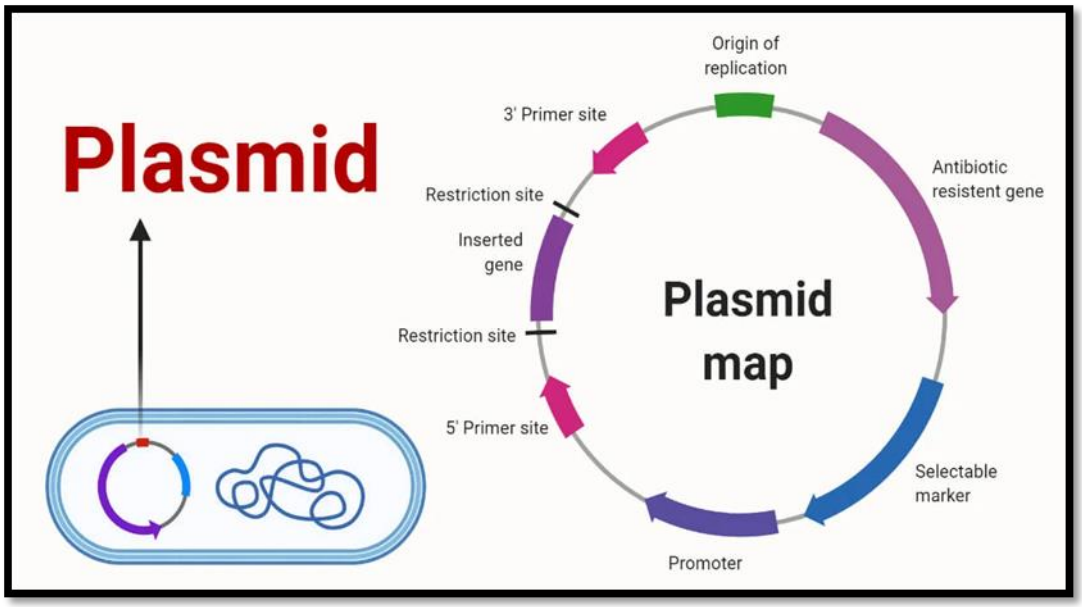

Figure (1): Plasmid main structure designed by Biorender

Numerous plasmids, such as self-transmissible or mobilizable ones, have the ability to be horizontally transferred between mature bacteria through various means, such as conjugation. Plasmids frequently contain genes that provide bacteria with the ability to adapt and thrive in their surroundings. These genes can include antibiotic resistance genes, virulence genes, and other distinctive genetic traits. With the mosaic nature of plasmids, they can also act as carriers for other mobile and mobilizable genetic elements like integrons and transposons, introducing an additional layer of potential recombination and mobility (Kapsak, 2018)

Plasmid genes can be categorized into two groups: those that encode backbone functions and those that encode accessory functions (Norman et al., 2009). The backbone genes are responsible for encoding plasmid functions such as replication and maintenance. On the other hand, the accessory genes encode non-plasmid functions that could be useful to the bacterial host cell (RC & A., 2015). Certain plasmids facilitate the transfer of accessory genes among different bacterial strains and species, even across phylogenetically distant lineages (Hall et al., 2017). HGT plays a crucial role in shaping the evolution of bacteria and has played a significant part in the diversification of bacterial taxa, both genetically and ecologically (Brockhurst et al., 2019; Redondo et al., 2020). Plasmid accessory genes encompass a diverse array of ecological functions, such as toxin resistance, metabolic and catabolic abilities, and the synthesis of virulence factors and anti-competitor toxins (Sen et al., 2011).

Plasmids classification

Since the 1970s, plasmids have been classified based on their incompatibility. The inability to sustainably proliferate two plasmids in the same host cell line, even when they have comparable replication and partition mechanisms, is known as incompatibility (Inc). Just as different species share fundamental genes, incompatible plasmids can be seen as distinct plasmids (species). The phenomenon of plasmid speciation can be attributed to the formation of novel incompatibility groups (Chabbert & Roussel, 1977; Sýkora, 1992). To rebuild evolutionary relationships between plasmids, scientists have utilized diverse methodologies, including the examination of replication

protein sequences (REP classification), conjugative transfer proteins (MOB classification), and even the entirety of the plasmid. (Fernandez-lopez et al., 2017; Francia et al., 2004; Redondo-salvo et al., 2023.; Redondo et al., 2020). The replicant typing process uses the amino acid sequence of the replication initiation (Rep) protein to classify organisms into an Inc category. However, it is important to note that conventional methods may not always confirm whether the plasmid is incompatible with another plasmid of the similar Inc set in the similar host cell line. Grouping plasmids in unidentified Inc groups is made easier by using the replicon typing classification (Shintani et al., 2015). Additionally, the incompatibility groups have been categorized into four main clusters based on genetic relatedness (IncF group (IncF, IncS, IncC, IncD, IncJ); IncP group (IncP, IncU, IncM, IncW); Ti group (IncX, IncH, IncN, IncT); and IncI group (IncI, IncB, and IncK) (Waters & Waters, 1999):

IncP plasmids, group are widely studied and well understood. They are highly prevalent and clinically significant, often carrying multiple antibiotic resistance genes. Additionally, they have a wide range of hosts they can infect. IncP plasmids are known for their remarkable ability to replicate and persist in a wide range of Gram-negative bacteria, making them quite promiscuous in nature. (Adamczyk & Jagura-burdzy, 2003).

IncA/C plasmids, these plasmids are a collection of self-transmissible plasmids with low copy numbers. They vary in size, ranging from 40-230 kb, although there have been reports of smaller conjugative variants measuring 18-25 kb (Rozwandowicz et al., 2018). They are commonly found in various bacteria, including Escherichia coli, Klebsiella pneumoniae, Salmonella enterica, Vibrio cholerae, and Aeromonas hydrophila (Harmer & Hall, 2015).

Each of the three genera has its own system for classifying Inc groups. Staphylococcus has about 18 Inc groups, Pseudomonas has 14 Inc groups, and Enterobacteriaceae has 27 Inc groups (Carattoli, 2009; Sota, M., and Top E., 2008). There are several Inc groups of Pseudomonas that share similarities with Enterobacteria. These include IncP-1 (similar to IncP), IncP-3 (similar to IncA/C), IncP-4 (similar to IncQ), and IncP-6 (similar to IncG/U).

It was ascertained that the subsequent classification could be divided into two discrete clusters: high copy number plasmids (HCPs) and low copy number plasmids (LCPs). Size (tens to hundreds of kilobases), copy number (low), and conjugation frequency are common characteristics of long non-coding RNAs (LCPs). On the other hand, homologous chromosomes (HCPs) don't have a conjugative system, are very small, and have many copies. It should be noted, though, that the conjugative apparatus of other coexisting plasmids can be used to mobilize certain HCPs. (Ramsay et al., 2016).

Approximately 50% of all plasmids have been evaluated as non-transmissible, whereas the remaining 50% is almost evenly distributed between conjugative and mobilizable plasmids (Coluzzi et al., 2022; Smillie et al., 2010); Recent findings indicate that a significant proportion of plasmids, which are typically categorized as nontransmissible, may really be capable of being mobilized. Furthermore, it is possible that mobilizable plasmids constitute the majority of all plasmids (Ares-arroyo et al., 2023).

Broad host-range plasmids are able to establish themselves in a wide variety of hosts, while narrow host-range plasmids are dependent on a certain bacterial group. (IainB, 2009). Topology is another way to classify them, and it has particular bearing on how they affect the biological reproduction process: Circular plasmids predominate, while a small number of linear plasmids have been discovered. (Ravin, 2011; Ventura et al., 2007). In addition to the aforementioned categorization of plasmids according to their general characteristics, it is inherent to establish a methodical biological cataloguing system for plasmid types.

Transferability of DNA

HGT necessitates two fundamental physical processes. Initially, it is important for genetic information to traverse cellular membranes for transmission to the recipient species. Furthermore, in order to guarantee further vertical transmission in the recipient, it is imperative that the genes are associated with a functional origin of replication within a germ line cell. There exists multiple clearly described mechanisms that promote the transfer of genes across bacteria by augmenting one or both of these processes. However, there are still ongoing efforts to identify novel mechanisms and it is highly probable that other mechanisms are yet to be uncovered (Hall et al., 2017). Figure 2 illustrates four distinct routes by which horizontal gene transfer occurs

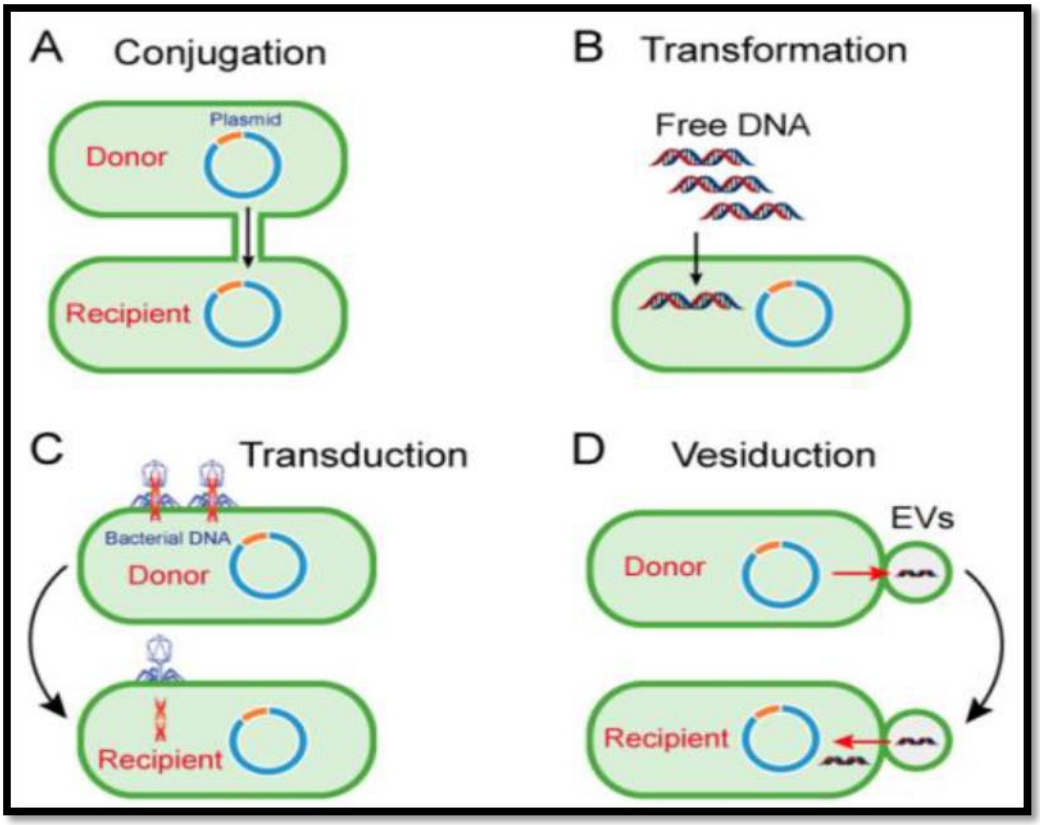

Figure (2): Bacterial ways to transferring DNA ((Liu et al., 2020))

A. Conjugation When one cell transfers its DNA to another, this process is called gene transfer, a donor bacterium expresses a complex macromolecular membrane traversal structure known as a 'conjugative pilus'. This structure serves as a physical connection for the movement of DNA between the donor and recipient cells (see figure 1, A) (Guglielmini et al., 2012). The transfer of DNA involves the presence of a nucleotide sequence known as the "origin of transfer" (oriT), which is identified by the conjugative machinery (Smillie et al., 2010) in addition, the transportation of single-strand DNA occurs through a type-IV secretion system (T4SS). (Shen et al., 2020). The transfer of genetic material in Gram-negative bacteria occurs through the pilus, whereas in Gram-positive bacteria, the transfer takes place via surface adhesion (Kohler et al., 2019).

B. Transformation The process of bacterial genome integration involves the uptake of free extracellular DNA fragments (as shown in figure 1, B) and their integration into the bacterial genome, either through chromosome integration or incorporation as plasmids. Due to

environmental constraints and the host's physiological limitations, this process is anticipated to proceed at modest rates. (Smillie et al., 2010). The Staphylococcus mecA gene exploits a mechanism that provides resistance to β-lactam drugs (Maree et al., 2022). Natural transformation occurs through the process of DNA uptake from the surrounding environment. A conserved opening in the cell membrane allows for the uptake of double-stranded DNA molecules, and this process is made possible by the contraction of pili, which are fibers on the surface of the cell. Bacteria that are dying, including those that are lysed by phage, can yield DNA substrates for transformation, and donors can also actively secrete them (Prince et al., 2017).

C. Transduction The process entails the transmission of bacterial DNA through bacteriophages (as depicted in figure 1, C**)**, is the process by which bacteriophages infect other bacteria by enclosing non-phage DNA within viral particles (Modi et al., 2013). Bacteriophages facilitate the transport of DNA, safeguarding it against degradation caused by environmental DN-ases and physical causes (Liu et al., 2020). Conjugation and transduction mechanisms serve to safeguard DNA from any environmental harm subsequent to its departure from the donor cell. Bacteriophages have the capability to transmit DNA pieces exceeding 100 kilobases (kb) in size. Additionally, instances of transduction between distinct genera have been documented, such as the transfer of a 5620-bp plasmid containing a kanamycins resistance gene between the Serratia and Kluyvera genera through the bacteriophage MAM1 (Matilla & Salmond, 2014).

D. Vesiduction : Alludes to the process by which two bacterial cells exchange DNA by use of extracellular vesicles (EVs) (Fulsundar et al., 2014). The release EVs from bacterial membranes was initially documented in the 1960s (Knox et al., 1966). Through this process, the donor bacteria releases a vesicle from its membrane that contains genetic material (Soler & Forterre, 2020) (figure1, D). likewise, extracellular vesicles (EVs) can serve as DNA carriers that can safeguard DNA from degradation caused by restriction enzymes, DNase, or other physical and chemical factors. As a result, EVs play a vital role in horizontal gene transfer (HGT).. Yaron et al. initially demonstrated in 2000 that extracellular vesicles (EVs) derived from E. coli O157:H7 are accountable for the transmission of virulence genes (Yaron et al., 2000). The exact process behind vesiduction remains elusive, as does the secretion of the vesicle and the recognition and incorporation of the vesicle through fusion by the recipient cell (Liu et al., 2020). Membrane fusion (Naor et al., 2012) and Genetic elements can be transferred across bacteria through intercellular connections facilitated by nanotubes (Dubey & Ben-yehuda, 2011).

It is worth noting that a significant number of these mechanisms are not actually regulated by the bacteria themselves. Instead, they are governed by semi-autonomous segments of DNA that prioritize their own self-interest rather than serving as functional tools for bacterial gene exchange.

Conclusions

New There are several ways in which genomic traits can be passed down through generations, but in Prokaryotes, there are four main pathways that are particularly significant and accurate. There are various mechanisms that facilitate the transfer of plasmids between bacteria, such as conjugation, which appears to enhance efficiency. These methods have had a significant impact on the evolution of bacterial species. Further investigation is required to uncover the underlying genes responsible for the effects of plasmids and gain better control over these mechanisms. Truly, to truly grasp the intricacies of plasmid ecology and evolution, it is essential to move beyond studying plasmids in isolation and instead examine them within a broader context. By considering how plasmids interact with other genetic elements, we can gain a more comprehensive understanding of their life-styles

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