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A Review on Plasmid Curing in Bacterial Systems

***Tricha Kumari*[1], *Rishita Sinha*[1*]**

[1]Department of Biotechnology, GLA College, Nilamber Pitamber University Daltonganj, Palamu 822101 Jharkhand, India.
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ABSTRACT:

Plasmids are extrachromosomal genetic elements that are found in many bacterial species. These circular pieces of DNA can carry a variety of genes, including those that confer antibiotic resistance, virulence factors, and metabolic capabilities. Plasmids are not essential for bacterial survival, but they can provide selective advantages under certain conditions. Plasmid curing is a process by which plasmids are eliminated from bacterial cells, either through chemical, physical, or nutritional treatments. Plasmid curing has been used as a tool to study the roles of plasmids in bacterial physiology and pathogenesis, as well as to understand the mechanisms of plasmid maintenance and replication. This review paper will summarize the current knowledge of plasmid curing in bacterial systems, including the different agents and methods that have been used for plasmid curing, the effects of plasmid loss on bacterial fitness and gene expression, and the potential applications of plasmid curing in biotechnology and medicine. The paper will also highlight the challenges and limitations of plasmid curing, including the potential for unintended effects on bacterial physiology and the difficulty of achieving complete plasmid loss in some bacterial strains. Overall, this review will provide a comprehensive overview of plasmid curing and its importance in the study of bacterial genetics and biology.

Keywords: Plasmid curing; Bacteria; Microorganisms; DNA; Genetic information.

1. Introduction:

Microorganisms, which include bacteria, archaea, fungi, protozoa, and viruses, have different types of genomic material (Parveen et al. 2023). Bacteria and archaea have circular DNA molecules that are found in the cytoplasm. These molecules are known as chromosomes and typically range in size from 0.5 to 10 megabases (Mb), depending on the species. In addition to the chromosome, many bacteria and archaea can also carry one or more plasmids, which are smaller circular DNA molecules that can carry additional genes (Ghoshal et al. 2022). Fungi have linear chromosomes, which are typically found in the nucleus. The size and number of chromosomes can vary depending on the species. Some fungi, such as yeasts, may also have small circular plasmids (Covert 1998). Protozoa have a variety of genomic arrangements, depending on the species. Some protozoa have a single linear chromosome, while others may have multiple chromosomes or a combination of linear and circular chromosomes (Jahn et al. 2002). Viruses can have different types of genomic material, including double-stranded DNA, single-stranded DNA, double-stranded RNA, and single-stranded RNA. The size of viral genomes can range from a few kilobases to hundreds of kilobases, depending on the virus. Some viruses also have segmented genomes, meaning that their genetic material is divided into multiple pieces (Wang et al. 2015). Overall, the genomic material of microorganisms can vary widely in size, structure, and complexity, reflecting the diversity of life in the microbial world.

Bacteria contain several different types of DNA, including (Mazodier et al. 1991):

Chromosomal DNA: This is the main type of DNA found in bacteria, consisting of a single, circular chromosome that carries most of the genes necessary for the cell's survival and reproduction.

Plasmid DNA: Plasmids are small, circular pieces of DNA that can replicate independently of the chromosomal DNA. They often carry genes that provide the bacterium with additional functions, such as antibiotic resistance or the ability to produce toxins.

Transposable elements: These are DNA sequences that can move from one location to another within the bacterial genome. They can cause mutations or alter the expression of genes and are often associated with the development of antibiotic resistance.

Phage DNA: Bacteria can be infected by bacteriophages, which are viruses that can inject their DNA into the bacterial cell. Once inside the cell, the phage DNA can integrate into the bacterial genome, causing changes in gene expression and potentially leading to the production of new virulent strains of bacteria.

Plasmid-like elements: These are DNA elements that are similar in structure and function to plasmids but are integrated into the bacterial chromosome. They can be transferred horizontally between bacteria and can carry genes that provide the cell with new functions or enhance its ability to adapt to changing environmental conditions.

Overall, the diverse types of DNA found in bacteria contribute to their ability to adapt and thrive in a wide range of environments, including those with high levels of antibiotics or other stresses (Dey et al. 2022; Roy and Ray 2019; Roy, Debnath, and Ray 2022).

Plasmid DNA is important in bacterial systems for several reasons (Wein et al. 2020):

Antibiotic resistance: Plasmids can carry genes that confer resistance to antibiotics, allowing bacteria to survive in the presence of drugs that would normally kill them.

Virulence factors: Plasmids can carry genes that produce virulence factors, such as toxins or adhesins, which enable bacteria to cause disease.

Metabolic functions: Plasmids can carry genes that produce enzymes involved in the metabolism of certain compounds, such as sugars or hydrocarbons, which can provide the bacterium with a selective advantage in certain environments (Vipparla et al. 2022).

Gene transfer: Plasmids can be transferred between bacteria through a process called conjugation, which can facilitate the spread of antibiotic resistance or virulence genes among bacterial populations.

Genetic engineering: Plasmids can be easily manipulated in the laboratory, making them useful tools for genetic engineering and biotechnology. They can be used to introduce new genes into bacteria, modify existing genes, or produce large quantities of proteins for medical or industrial purposes.

Overall, plasmid DNA plays a critical role in the ability of bacteria to adapt and survive in a wide range of environments, and its study has provided important insights into the mechanisms of bacterial pathogenesis, antibiotic resistance, and gene transfer.

Plasmids are commonly found in bacteria, but can also be found in other types of cells. They are typically much smaller than the chromosomal DNA and can carry a variety of genetic information, including genes that can confer certain advantages to the bacteria, such as antibiotic resistance or the ability to produce certain proteins. Plasmids can be transferred between bacteria, allowing genes to be spread between bacterial populations, and they can also be manipulated in the laboratory for various applications, including genetic engineering and gene therapy (Kado et al. 1981).

Plasmid curing is a process by which plasmids are eliminated from bacterial cells. This can occur naturally or through the use of chemical or physical treatments. Plasmid curing can be useful in research or clinical settings where it is important to distinguish the effects of chromosomal genes from those of plasmid-borne genes (Wang et al. 2021). Natural plasmid curing can occur through several mechanisms, such as replication errors or chromosomal integration of the plasmid DNA. For example, if a plasmid replicates at a slower rate than the bacterial chromosome, it may be lost over time as the cell divides. Similarly, if a plasmid integrates into the bacterial chromosome through a process called homologous recombination, it may become a stable part of the chromosome and no longer behave as an independent element. In the laboratory, plasmid curing can be induced through the use of antibiotics or chemicals that inhibit plasmid replication or partitioning (Green et al. 2021). For example, some antibiotics, such as novobiocin or acridine orange, can interfere with plasmid replication or partitioning, leading to the loss of the plasmid from the bacterial cell. Plasmid curing can also be achieved through physical treatments, such as heat shock or exposure to ultraviolet light, which can damage the plasmid DNA and cause it to be lost during replication. Overall, plasmid curing is a useful tool for studying the roles of plasmid-borne genes in bacterial physiology and pathogenesis, and for understanding the mechanisms of plasmid maintenance and replication in bacterial cells (Zhang et al. 2021).

This review presents valuable insights regarding plasmid curing agents and the associated procedures. In the context of the significance of the plasmid DNAs in enumerating the metal and antibiotic resistance, pathogenicity nodulation, host specificity, metabolic processes, replication maintenance attributes, and conjugal properties, the plasmid-cured derivatives hold immense importance which in turn depicts the novelty of this study.

2. Plasmid curing in bacteria:

Plasmid curing in bacteria is the process of eliminating plasmids from bacterial cells. Plasmids are extrachromosomal circular DNA molecules that can carry additional genes and are not essential for bacterial survival. However, plasmids can provide certain advantages to bacteria, such as antibiotic resistance, virulence factors, and metabolic capabilities. In the laboratory, plasmid curing can be achieved through various methods, including exposure to certain chemicals, physical treatments, or other stressors that interfere with plasmid replication or partitioning (Bahl et al. 2009). Some examples of methods for plasmid curing in bacteria include (Hogan et al. 1982; Watson et al. 1975):

Chemical treatments: Some chemicals, such as ethidium bromide or acridine orange, can interfere with plasmid replication or partitioning, leading to plasmid loss from bacterial cells. Antibiotics, such as novobiocin or nalidixic acid, can also inhibit plasmid replication and induce plasmid curing.

Heat shock: Bacterial cells can be subjected to high temperatures for a short time, which can cause the denaturation and loss of plasmid DNA.

Electroporation: Electroporation involves the use of electrical pulses to introduce foreign DNA into bacterial cells. However, it can also induce plasmid curing, especially if the electrical pulses are too strong or too frequent.

Nutritional stress: Some bacteria require specific nutrients or growth factors for plasmid replication and maintenance. If these nutrients are absent, the plasmid may be lost from the bacterial cell.

Plasmid curing is an important process in studying the biology of bacteria for several reasons:

Identification of plasmid-encoded traits: Plasmids can carry genes that confer selective advantages to bacteria, such as antibiotic resistance or virulence. Plasmid curing can help identify which traits are encoded on the plasmid versus the bacterial chromosome, allowing for a better understanding of the genetic basis of these traits.

Understanding the mechanisms of plasmid maintenance: Plasmids replicate independently of the bacterial chromosome and rely on partitioning systems to ensure that they are segregated properly during cell division. Plasmid curing can help identify genes involved in plasmid replication and partitioning and provide insight into how plasmids are maintained in bacterial populations.

Development of new therapeutic strategies: Antibiotic resistance is a growing concern, and plasmids are a common mechanism for the spread of resistance genes between bacterial species. Plasmid curing can be used as a strategy to reduce the spread of antibiotic resistance by eliminating the plasmids that carry these genes.

Biotechnological applications: Plasmids are commonly used in biotechnology to express recombinant proteins or engineer bacteria to produce novel compounds. Plasmid curing can be used to remove unwanted plasmids from bacterial strains, allowing for the production of more predictable and controlled bacterial strains.

Overall, plasmid curing is a powerful tool in the study of bacterial genetics and physiology, and it has important applications in the development of new therapeutic and biotechnological strategies. Moreover, plasmid curing in bacteria is a useful tool for studying the roles of plasmid-borne genes in bacterial physiology and pathogenesis, and for understanding the mechanisms of plasmid maintenance and replication in bacterial cells (Bahl et al. 2009).

3. Plasmid Curing agents in Bacterial Systems:

There are several agents that can be used for plasmid curing in bacterial systems, including chemical agents, physical agents, and nutritional agents. Here are some examples (Paul et al. 2020; Zaman et al. 2010):

Chemical agents: Ethidium bromide, acridine orange, and sodium dodecyl sulfate (SDS) are chemicals that can be used to induce plasmid curing in bacteria. These chemicals can interfere with plasmid replication or partitioning, leading to the loss of the plasmid from the bacterial cell.

Physical agents: Physical treatments, such as heat shock, ultraviolet (UV) radiation, and high-pressure treatment, can also induce plasmid curing in bacteria. These treatments can damage the plasmid DNA, leading to its loss during replication.

Antibiotics: Some antibiotics, such as novobiocin, nalidixic acid, and rifampicin, can inhibit plasmid replication and induce plasmid curing in bacteria. These antibiotics can target the plasmid replication machinery or affect plasmid partitioning, leading to the loss of the plasmid from the bacterial cell.

Nutritional agents: Nutritional stress can also induce plasmid curing in bacteria. For example, if a plasmid carries genes involved in the metabolism of a specific nutrient, the plasmid may be lost if the nutrient is absent from the growth medium.

It is important to note that different agents may be more effective for plasmid curing in different bacterial strains or under different conditions. Furthermore, some agents may have other effects on bacterial physiology, such as inducing stress responses or affecting the expression of other genes. Therefore, the choice of plasmid curing agent should be carefully considered based on the specific research goals and the bacterial system being studied (Padhye et al. 2012). A technical summary of different examples of plasmid curing agents has been detailed in Table 1.

Table 1. Summary of different plasmid curing agents for microbial systems.

Mode of activity	Plasmid Curing Agent (Examples)
Inhibits the replication of the plasmid DNA through intercalation.	Acriflavine, Ethidium bromide, Acridine Orange, Quinacrine.
Restrict the supercoiling of the plasmid DNA by inhibiting the enzyme Gyrase.	Novobiocin, Coumermycin.
Nucleophilic attack on purine bases followed by metabolic activation.	Mitomycin C.
Inhibition of the activity of RNA Polymerase.	Rifampicin.
The plasmid-containing bacteria are sensitive to the SDS since they contain pili on their cellular surface.	Sodium Dodecyl Sulphate.
Sometimes during the process of replication, plasmid-less daughter cells may arise.	Loss of plasmid.
At elevated growth temperatures, the partial or complete deletion of the bacterial plasmid occurs.	Increment in the growth temperature.
These can be only used in the case of thymine requiring auxotrophs.	Starvation of Thymine.
Causing the loss of certain bacterial plasmids.	Formation and regeneration of protoplast.
Incompatibility of the plasmid within the same cell.	Curing incompatibility.

4. Generalized procedure of Plasmid Curing:

Plasmid curing is the process of eliminating plasmids from bacterial cells. A detailed generalized procedure of plasmid curing has been detailed below (Angoli et al. 2012; John et al. 2020)

Select an appropriate method for plasmid curing: There are different methods for plasmid curing such as chemical treatment, heat shock treatment, and selection with antibiotics. The selection of the method may depend on the type of plasmid, bacterial species, and the objective of the study.

Grow bacterial culture: Inoculate a bacterial culture containing the plasmid of interest in a suitable growth medium and grow it overnight or until the culture reaches the mid-log phase.

Apply plasmid curing method: The method used for plasmid curing can vary depending on the type of plasmid and bacterial strain. Here are some examples of plasmid curing methods:

Chemical treatment: Treatment with ethidium bromide, acridine orange, or sodium dodecyl sulfate (SDS) can induce DNA damage leading to the loss of plasmids.

Heat shock treatment: Heat shock can cause damage to plasmids, leading to their loss from bacterial cells.

Antibiotic selection: Some plasmids carry antibiotic resistance that allows bacterial cells to survive in the presence of antibiotics. In this case, it is possible to use antibiotics to selectively kill bacteria carrying the plasmid.

Confirm plasmid curing: After applying the plasmid curing method, it is necessary to confirm that the plasmid has been lost from the bacterial cells. This can be done by performing plasmid extraction, followed by gel electrophoresis, or PCR.

Overall, the plasmid curing procedure can be optimized by testing different conditions and methods to achieve the best results for a particular study or application (Roy and Ray 2020; Roy and Ray 2022; Roy et al. 2022).

5. Effects of plasmid loss on bacterial fitness and gene expression:

Plasmids are small, circular, double-stranded DNA molecules that can be found in many bacteria. They often carry genes that confer benefits to the bacteria, such as antibiotic resistance or the ability to utilize certain nutrients. However, plasmids are not essential for bacterial survival, and bacteria can lose them under certain conditions. The effects of plasmid loss on bacterial fitness and gene expression depend on the specific plasmid and the host bacterium (Song et al. 2023).

Loss of a plasmid can have a range of effects on bacterial fitness, depending on the genes carried by the plasmid and the environment in which the bacterium lives. If the plasmid carries genes that are essential for bacterial survival, such as those that code for antibiotic resistance, the bacterium may become more susceptible to antibiotics and less fit in environments where these antibiotics are present. However, if the plasmid carries genes that are not essential in a particular environment, plasmid loss may not have a significant impact on bacterial fitness (Jian et al 2021).

The loss of a plasmid can also have an effect on gene expression in the bacterium. If the plasmid carries genes that are actively transcribed and translated, the loss of the plasmid can result in a decrease in the expression of those genes. Conversely, if the plasmid carries genes that are normally repressed, their loss can result in an increase in the expression of those genes (Hall et al. 2021). Additionally, plasmid loss can lead to changes in the expression of other genes in the bacterium, as the loss of the plasmid can alter the overall regulatory network of the cell. In summary, the effects of plasmid loss on bacterial fitness and gene expression depend on the specific plasmid and the host bacterium, as well as the environment in which the bacterium lives. Plasmid loss can lead to changes in gene expression and bacterial fitness (Roy et al. 2022).

6. Application of plasmid curing in biotechnology:

Plasmid curing is the process of eliminating plasmids from bacterial cells. Plasmids are circular, double-stranded DNA molecules that exist independently of the bacterial chromosome and can confer various beneficial traits to their host bacteria, such as antibiotic resistance or the ability to produce certain metabolites (Norman et al. 2009). However, plasmids can also have negative effects on the bacterial host, such as increasing the metabolic burden on the cell or increasing the risk of horizontal gene transfer to other bacteria (Roy et al. 2022).

Plasmid curing has several applications in biotechnology, including (Banik et al. 2023; Lavanya et al. 2011; Li and Roy 2023; Zou et al. 2022):

Elimination of antibiotic resistance: Plasmid curing can be used to eliminate antibiotic resistance genes carried by plasmids, which can help to reduce the spread of antibiotic resistance in the environment.

Production of plasmid-free cells: Plasmid curing can be used to generate plasmid-free cells for research or industrial applications. For example, in the production of recombinant proteins, plasmid-free cells can be used to avoid the risk of contamination by plasmid DNA.

Study of plasmid biology: Plasmid curing can be used to study the biology of plasmids, such as their replication, maintenance, and transfer mechanisms. By removing plasmids from bacterial cells, researchers can study the effects of plasmids on bacterial physiology and investigate the mechanisms by which plasmids are eliminated or maintained in bacterial populations.

Development of new plasmid vectors: Plasmid curing can be used to generate bacterial strains that lack endogenous plasmids, which can then be used as hosts for the development of new plasmid vectors for gene expression, protein production, or other biotechnological applications.

Overall, plasmid curing is a useful tool in biotechnology that can be used to manipulate bacterial cells and plasmids to achieve specific goals, such as the elimination of antibiotic resistance, the production of plasmid-free cells, or the study of plasmid biology (Roy et al. 2023; Su et al. 2022).

7. Limitations and challenges of plasmid curing:

Plasmid curing is the process of eliminating plasmids from bacterial cells. Plasmids are circular DNA molecules that are separate from the chromosomal DNA and can replicate independently. They often carry genes that confer advantages to the bacteria, such as antibiotic resistance or the ability to metabolize specific compounds. Plasmid curing can be useful in situations where the plasmids are undesirable, such as when they carry antibiotic resistance genes that could transfer to other bacteria and contribute to the spread of antibiotic resistance (Daly et al. 1994).

However, there are several challenges and limitations to plasmid curing, including (Wallis et al. 1995; Matsui et al. 2015):

Selectivity: Plasmid curing methods are often not selective, which means they can also eliminate plasmids that are beneficial to the bacteria. This can lead to a loss of important traits and a decrease in bacterial fitness.

Effectiveness: Some plasmids are more difficult to cure than others, and not all plasmid curing methods are equally effective. The success of plasmid curing can also depend on the bacterial strain, growth conditions, and the specific plasmid being targeted.

Stability: Even if plasmids are successfully cured, they can sometimes be reintroduced through horizontal gene transfer. This can occur through conjugation, transformation, or transduction, and can result in the reacquisition of undesirable plasmids.

Genetic modifications: Plasmid curing methods can sometimes lead to unintended genetic modifications in the bacteria, which can have unpredictable effects on their physiology and behavior.

Safety concerns: Some plasmid curing methods involve the use of toxic compounds or radiation, which can have negative effects on the environment or human health.

Overall, plasmid curing can be a useful tool in certain situations, but it is important to carefully consider the potential challenges and limitations before using this approach.

8. Potential of plasmid curing for unintended effects on bacterial physiology:

Plasmid curing can have unintended effects on bacterial physiology, particularly if plasmids carry genes that are important for the bacteria's growth, metabolism, or virulence (Sengupta et al. 2011). Plasmids can carry a wide range of genes, including antibiotic resistance genes, virulence factors, and genes involved in stress response, quorum sensing, and other cellular processes. Therefore, removing plasmids from bacterial cells can have a significant impact on their phenotype and fitness.

Here are some potential unintended effects of plasmid curing on bacterial physiology (Swift et al. 2001; Volke et al. 2022):

Growth rate: Plasmids can carry genes that are important for bacterial growth, such as genes involved in nutrient uptake or metabolism. Removing these plasmids can slow down the growth rate of the bacteria and make them less competitive in the environment.

Stress response: Plasmids can carry genes that are involved in stress response and adaptation, such as genes for heat shock proteins, oxidative stress response, or DNA repair. Removing these plasmids can make the bacteria more vulnerable to environmental stressors, such as temperature shifts or exposure to toxins.

Virulence: Plasmids can carry genes that are important for bacterial virulence, such as genes for toxins, adhesins, or secretion systems. Removing these plasmids can reduce the pathogenicity of the bacteria and make them less able to cause disease.

Antibiotic resistance: Plasmids can carry genes that confer resistance to antibiotics, which can make the bacteria more difficult to treat with antibiotics. Removing these plasmids can restore the susceptibility of the bacteria to antibiotics, but it can also increase the risk of antibiotic treatment failure.

Genetic instability: Removing plasmids from bacterial cells can lead to genetic instability and the emergence of new mutations or rearrangements in the chromosome. This can have unpredictable effects on bacterial physiology and behavior, and it can potentially lead to the emergence of new pathogenic strains.

Overall, plasmid curing can have unintended effects on bacterial physiology, and it is important to carefully consider the potential risks and benefits of this approach before using it in a particular context. The impact of plasmid curing on bacterial fitness and virulence should be thoroughly assessed to avoid unintended consequences.

9. Difficulty in achieving complete plasmid loss in some bacterial strains:

Achieving complete plasmid loss can be difficult in some bacterial strains for several reasons (Lewis et al. 1996; Nathan et al. 1997):

Replication: Plasmids can replicate independently of the chromosomal DNA, which can make them difficult to eliminate. In some cases, plasmids may have a higher copy number than chromosomes, which means they are present in multiple copies in each cell. This can make it more difficult to completely eliminate all plasmids from a bacterial population.

Stability: Plasmids can be stably maintained in bacterial cells for many generations, especially if they carry genes that confer a selective advantage to the bacteria. In some cases, plasmids can integrate into the chromosome, making them even more difficult to eliminate.

Heterogeneity: Bacterial populations can be genetically heterogeneous, with individual cells exhibiting different levels of plasmid copy number or gene expression. This can make it difficult to achieve complete plasmid loss, as some cells may continue to maintain plasmids even after treatment.

Antibiotic resistance: Plasmids that carry antibiotic-resistance genes can provide a selective advantage to bacterial cells in the presence of antibiotics. This can make it difficult to eliminate these plasmids, as the antibiotic treatment may only kill off the susceptible cells while allowing the resistant cells to survive and continue to maintain the plasmids.

Genetic barriers: Some bacterial strains may have genetic barriers that prevent plasmid loss, such as toxin-antitoxin systems that promote plasmid maintenance or restriction-modification systems that prevent foreign DNA from entering the cell.

Overall, achieving complete plasmid loss in bacterial strains can be challenging, especially in the presence of selective pressures such as antibiotic resistance. Various methods have been developed to increase the likelihood of plasmid loss, such as plasmid incompatibility, mutagenesis, or the use of bacteriophages, but it is important to carefully consider the limitations of these approaches and their potential impact on bacterial fitness and genetic stability.

10. Conclusion:

In conclusion, plasmid curing is a powerful tool for studying bacterial genetics, physiology, and pathogenicity. Plasmids can carry a wide range of genes that can have significant impacts on bacterial phenotype and fitness, and removing these plasmids can provide insights into the function of these genes and their role in bacterial physiology and virulence. Plasmid curing can be achieved using various methods, such as chemical treatment, mutagenesis, or the use of bacteriophages, and the choice of method should be carefully considered based on the specific goals of the study and the characteristics of the bacterial strain being studied. However, it is important to recognize the potential limitations and risks of plasmid curing, including the difficulty of achieving complete plasmid loss, the potential for unintended effects on bacterial physiology, and the risk of genetic instability and the emergence of new pathogenic strains. Therefore, researchers should carefully assess the potential risks and benefits of plasmid curing before using it in a particular context, and they should take appropriate measures to minimize the potential risks and to ensure the safety and ethical integrity of their research. Overall, plasmid curing is a valuable tool for studying bacterial genetics and physiology, and it has the potential to provide important insights into the mechanisms of bacterial pathogenesis and the development of new strategies for controlling bacterial infections.

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