HYPOTHESIS

Interference plasmids and their use in combating bacterial resistance

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ABSTRACT

Fighting against pathogenic bacteria that are resistant to antibiotics has become critical for health care worldwide. More than half a million people die every year from infections caused by drug resistant bacteria. Since bacteria acquire resistance to antibiotics very quickly and the development of new antibiotics is a lengthy process, the search for new approaches to stop the spread of bacterial resistance is extremely important. The spread of antibiotic resistance is accomplished mainly by horizontal gene transfer. Scientists are concentrating their efforts on studying the mechanism of this process in order to find a way to stop or reverse it. In this paper, the author gives a brief review of the recent studies on horizontal gene transfer, particularly on incompatibility-based plasmid curing systems. The author examines new possibilities to use the mechanism of horizontal gene transfer for the developing of novel approaches to fight pathogenic bacteria.

INTRODUCTION

The emergence of multidrug resistant bacteria that are not susceptible to known antibiotics is one of the most important and urgent problems of modern health care worldwide. According to Lord Jim O’Neill’s report published in 2016 [1], by the year 2050 the lives of about 10 million people a year will be at risk from infections caused by multidrug resistant (MDR) bacteria if scientists will not come up with a breakthrough in developing new antimicrobials and/or new methods to fight pathogenic bacteria. Even nowadays, 700,000 people worldwide die from infections with MDR bacteria. Not the entire scientific community agrees with this particular prediction [2], but scientists and doctors all over the world consider bacterial resistance as one of the greatest threats to public health.

The first examples of bacterial resistance were observed by Alexander Fleming who discovered the first antibiotic penicillin. Many antibiotics are produced by microorganisms including bacteria – natural antibiotics – and the bacteria-producers are not killed by the antibiotic that they produce. These bacteria use special mechanisms in certain life cycle processes that make them resistant to the action of the antibiotics that they produce. Thus, the bacteria that produce antibiotics were probably the first examples of bacteria with antibiotic resistance. Antibiotic resistance mechanisms can be divided into two categories: general and more specific. One of the examples of general antibiotic resistance mechanisms is the efflux of different antibiotics from the bacterial cell by efflux pumps. This leads to the rapid decrease of the concentration of an antibiotic in the bacterial cell and ensures the survival of the bacteria. Another general mechanism of resistance is the deactivation of an antibiotic in the bacterial cell by enzymatic hydrolysis, acylation, phosphorylation etc., although for every antibiotic the reaction is accomplished with a specific enzyme. Specific mechanisms of resistance include the modification of the structure of the antibiotic target in bacteria, e.g. ribosome and synthesis of short peptides that prevent the efficient interaction of an antibiotic with its target in the bacterial cell. In the course of natural selection, according to the Charles Darwin theory of evolution, bacteria can become resistant to a certain antibiotic as a result of the corresponding gene mutations. Both pathogenic and non-pathogenic bacteria acquire resistance to natural as well as to synthetic antibiotics by means of natural selection and this resistance is passed to the offspring (vertical gene transfer).

Another mechanism of the emergence of bacterial resistance is horizontal gene transfer (HGT) [3-5]. According to the modern concept of HGT, it includes various mechanisms that could be broadly divided into three categories: transduction (transfer of genetic material by means of phages), conjugation (transfer of genetic material on plasmids from the donor bacterium to the recipient bacterium usually through the pili), and transformation (adsorption of genetic material by bacteria from the environment) [6, 7]. Fast spreading of beneficial genetic information by means of HGT between bacteria helps
them to survive in a changing ecological environment in nature. This mechanism is also successfully used by bacteria for defense from antibiotics. By rapid exchange with plasmids that contain antibiotic resistance genes, the pathogenic bacteria survive treatment with antibiotics that leads to the deterioration and even death of infected patients. Some of those plasmids contain genes that make bacteria resistant to several antibiotics – multidrug resistant bacteria – making them the most dangerous type of pathogenic bacteria. Since the development of new antibiotics in the US from the beginning of the discovery process to FDA approval, production launch, and start of marketing takes about 16-17 years and costs around $1.75 billion [8] while bacterial resistance to the new antibiotic can develop in the course of several days [9], it is clear that we are losing the fight against pathogenic bacteria. Therefore, the search for new approaches to stop the spreading of bacterial resistance and to kill the pathogenic bacteria is one of the most important directions in life sciences.

Since HGT is the main mechanism of the spreading of bacterial resistance, lately it has become the subject of intensive research [6, 7, 10-13]. It is believed that the processes of plasmid transfer from the donor bacterium to recipient bacterium (Fig. 1) as well as the maintaining of the received plasmid in bacterium are associated with significant fitness costs [14], although not all scientists agree with that conclusion [15].

Recently, it was shown that the plasmid transfer between bacteria intensifies in the presence of stress factors like certain metal salts, UV light, small concentrations of antibiotics and/or toxins, etc. [16]. It should be mentioned that many antibiotic resistance plasmids contain a so-called 'addictive' system – genes that encode for long living toxins and short living antitoxins. Bacteria containing this kind of plasmid cannot lose it since it would then be killed by the toxin. On the other hand, this toxin-antitoxin addictive system ensures that the plasmid will be copied in the course of the cell division and that the offspring bacterial cell will contain the copy of this plasmid [17-19].

In general, the more we learn about HGT the more we understand that it is a very complicated process that depends on a number of factors, such as the conditions in bacterial colony or colonies, fitness costs associated with plasmid transfer and maintaining, adaptive benefits of the plasmid (e.g. antibiotic resistance genes), etc. [12, 14, 18, 20]. It should be noted, however, that if in the course of the treatment of an infected patient with a certain antibiotic even a small number of pathogenic bacteria will survive by acquiring the resistance to this antibiotic it can lead to the death of the patient since the resistant bacteria will spread very fast in the body of the patient [20]. That is why even if the HGT in bacteria seems to be inefficient under certain conditions, it poses a serious threat to the health and life of the infected patients.

Fig. 1. Conjugative plasmid transfer. The bacterium bearing a plasmid (donor) is shown in blue, and the bacterium acquiring a plasmid (recipient) is shown in magenta. The plasmid DNA is shown in black. Plasmids are small circular DNA molecules inside the bacterial cell that can replicate independently. In the course of conjugation, plasmid-encoded proteins are produced. These proteins assemble into the type IV secretion system protein complex that performs several functions: spanning the donor cell envelope, the formation of pilus that mediates the contact between the bacterial cells, and the recruitment of the relaxase and a few accessory proteins to the secretion channel. In step 1 (nicking), the plasmid-encoded relaxase nicks one strand of the plasmid double-stranded DNA, forming the transfer DNA (T-DNA). In step 2 (transfer), the conjugation machinery transports the T-DNA into the recipient cell. In step 3 (DNA synthesis) in the recipient cell, the relaxase ligates the 5’- and 3’-ends of the DNA to form a covalently closed single-stranded DNA circle. The complementary DNA strand is synthesized to generate a double-stranded DNA circle in the donor and recipient bacteria.
PLASMID CURING IN BACTERIA

Since it is widely recognized that the spreading of antibiotic resistance in bacteria is accomplished mainly by means of HGT, scientists are focused on studying the mechanism of HGT [21] and on searching for methods that prevent HGT in bacterial colonies [22]. Research is being actively conducted in the following areas: plasmid curing compounds, using phages for plasmid curing, plasmid curing by CRISPR/Cas system, and incompatibility-based plasmid curing systems.

a) Plasmid curing compounds

It has been shown that a number of compounds can be used for plasmid curing in vitro [23]. These compounds have varying structures, belong to different classes of chemical compounds, and consequently have different mechanisms of action. In addition, the mechanisms of action of some of these compounds are still poorly understood. Some examples of plasmid curing agents are: antibiotics (e.g. quinolones [24]), detergents (e.g. sodium dodecyl sulfate [25]), natural compounds derived from plants [26], psychotropic drugs and heterocyclic compounds [27, 28], etc. Since plasmid conjugation is a multistep process (Fig. 1), different compounds disturb different steps of this process: some antibiotics (e.g. nolvobionic) inhibit the GyrB subunit of bacterial DNA gyrase and thereby affect the plasmid DNA supercoiling; unsaturated fatty acids prevent transfer of the genetic material from the donor cell to the recipient cell [29] (step 2, Fig. 1). DNA intercalating agents, such as ethidium bromide [30], acidine orange, and acriflavine [31], are used in laboratory studies as efficient plasmid curing compounds, but have no prospect for practical application because of their high toxicity. Phenothiazines – compounds that are used as psychotropic drugs – have also shown significant plasmid curing activity in vitro and are more likely to be used in medical practice in the future [27].

b) Phages as plasmid curing agents

As was discussed in recent publications (e.g. [32]), phages that target bacterial secretion machinery can effectively cure plasmids from bacterial colonies. A bacterium donor uses pilus for the contact with a bacterium recipient over the course of plasmid transfer by conjugation (Fig. 1). Since the pilus targeting phages kill bacteria with high pilus expression, it leads to the inhibition of conjugation and eventually to plasmid curing. Jalasvuori et al. [33] studied the phage targeting the bacterial mating pair complex encoded by conjugative plasmids and showed that this is another example of bacterial plasmid curing by phages.

c) Plasmid curing by CRISPR/Cas system

A number of studies (e.g. [34, 35]) showed that the CRISPR/Cas system can be used for plasmid curing in bacteria. CRISPR/Cas, which is also known as the bacterial ‘adaptive immune system’, enables bacteria to recognize the invading foreign genetic material – DNA or RNA sequence – and recruit the corresponding Cas proteins for its cleavage. Therefore, the bacterial CRISPR/Cas system that targets the DNA of plasmids containing antibiotic resistance genes will degrade these plasmids, which will eventually prevent the spread of antibiotic resistance by plasmid conjugation in the bacterial colony.

d) Incompatibility-based plasmid curing systems

Investigation of the mechanism of conjugal plasmid transfer in bacteria led scientists to the conclusion (e.g. [23, 36]) that different plasmids can coexist in the same bacterium unless these plasmids share a very similar replication system. The early exclusion system will prevent the uptake of a conjugation plasmid of the same ‘exclusion’ type as the plasmid that bacterium already has. On the other hand, under strong selection conditions, the entering plasmid displaces the incompatible plasmids that are already in this bacterial cell. This approach can be used for the eradication of plasmids with antibiotic resistant genes from bacteria by interference plasmids over the course of HGT. Kamruzzaman et al. [37] showed that plasmid interference can be used for the replacement of antibiotic resistance plasmids by the corresponding plasmids with deleted antibiotic resistance genes in pathogenic bacteria in vivo. After that, pathogenic bacteria become susceptible to antibiotics again and infection can be cured by the administration of antibacterial drugs. In order to achieve the efficient uptake of reference plasmids by the bacterial cells that contain the antibiotic resistance plasmids, the authors of this paper used a purifying selection in their experiments (in bacteria and mice). Interference plasmids were constructed so that they did not carry the antibiotic resistance genes and the toxin-encoding gene of the original plasmids, but contain the same replication system that made them ‘incompatible’ with the original antibiotic resistance plasmids. In addition, another gene, which provides resistance from tetracycline, was introduced into the interference plasmids. This ensures the high efficiency of uptake of the interference plasmids by bacteria (and the further replacement of original antibiotic resistance plasmids by these plasmids) in the presence of tetracycline because only bacteria with interference plasmid containing tetracycline resistance gene will survive in these conditions. Since interference plasmids did not contain a toxin-antitoxin ‘addiction’ system, they were cured from bacteria in the course of several days and bacteria became susceptible to antibiotics including tetracycline.

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Developing this approach, we came up with a much more efficient method of fighting the multidrug resistant bacteria. According to our method [38], in addition to the tetracycline-resistant gene, the interference plasmid contains the antibiotic A encoding gene and ligand-dependent transcription factor sequence linked to a promoter that is known in the art [39-42]. Since the transcription factor for the antibiotic A encoding gene is ligand-dependent, the synthesis of antibiotic A is
Interference Plasmids

The synthesis of an antibiotic encoded by plasmid in the bacterial cell has several significant advantages compared with oral or intravenous antibiotic administration:

- The amount of antibiotic necessary to kill bacteria in this case is much smaller because it is not spread in the bloodstream but is synthesized in the bacterial cell;
- It should not be delivered to the bacterial colony by the bloodstream;
- An antibiotic synthesized in the bacterial cell will act right away that significantly reduces the possibility of any side reactions at physiological pH;
- The molecule of this antibiotic should not have the special physicochemical properties necessary for penetration into the bacterial cell through the membrane (or membranes) and bacterial cell wall;
- Because of the above-mentioned advantages, the probability of side effects from this antibiotic is negligible.

It is known that bacteria are adapting very fast to any changes in the environment, including the presence of antibiotics, but this approach will give bacteria practically no time to come up with a new resistance mechanism.

Recently, the lytic bacteriophages cycle and especially the action of phage-derived lytic proteins – holins, endolysins, and spanins – draw the close attention of the scientific community in connection with the development of new approaches to fight the pathogenic bacteria [46]. Holins and endolysins are used in the last stage of the phage lytic cycle to release the progeny from the bacterial cell: holins form the pores in the inner bacterial membrane and release endolysins that destroy the bacterial cell wall. Currently, the discussion is ongoing in the scientific literature about the possibility of using these proteins as antimicrobial agents [47]. On the other hand, these proteins are already used in a number of processes in biotechnology [48], e.g. for breaking-up the bacterial membrane and cell wall in order to release the product of fermentation [49–51]. In fact, it was shown recently that holins and endolysins encoded by the corresponding plasmids could be synthesized in the bacterial cell that inevitably leads to bacterial cell lysis. The synthesis of these proteins and consequently the bacterial cell lysis was induced by xylose, by the reduced concentration of Mg2+ ions, or 3-methylbenzoate [49]. On the other hand, Borrero-de Acuna et al. [52] developed an efficient inducible lytic system for the Gram-negative bacteria using the lysozyme encoding plasmid.

Therefore, the interference plasmids encoding holins and endolysins (instead of antibiotic A) can be used for fighting the pathogenic bacteria using the above-described approach. In this case, after the interference plasmid will be acquired by the pathogenic bacteria, the synthesis of holins and endolysins will be activated by the administration of the corresponding ligand (e.g. xylose) leading to the fast lysis of the bacterial cell. This process also has one important advantage – bacterial resistance to the phage lysins is an extremely rare event [53] – e.g. no resistance to the action of endolysins has been reported to date [54].

CONFLICT OF INTEREST

The author does not pursue commercial or financial interests.

CITATION

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