BACTERIAL PLASMIDS

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Plasmids, extrachromosomal DNA, were identified in bacteria pertaining to family of *Enterobacteriacae* for the very first time. After that, they were discovered in almost every single observed strain. The structure of plasmids is made of circular double chain DNA molecules which are replicated autonomously in a host cell. Their length may vary from few up to several hundred kilobase (kb). Among the bacteria, plasmids are mostly transferred horizontally by conjugation process. Plasmid replication process can be divided into three stages: initiation, elongation, and termination. The process involves DNA helicase I, DNA gyrase, DNA polymerase III, endonuclease, and ligase. Plasmids contain genes essential for plasmid function and their preservation in a host cell (the beginning and the control of replication). Some of them possess genes which control plasmid stability. There is a common opinion that plasmids are unnecessary for a growth of bacterial population and their vital functions; thus, in many cases they can be taken up or kicked out with no lethal effects to a plasmid host cell. However, there are numerous biological functions of bacteria related to plasmids. Plasmids identification and classification are based upon their genetic features which are presented permanently in all of them, and these are: abilities to preserve themselves in a host cell and to control a replication process. In this way, plasmids classification among incompatibility groups is performed. The method of replicon typing, which is based on genotype and not on phenotype characteristics, has the same results as incompatibility grouping. *Acta Medica Medianae 2007;46(4):61-65.*

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Introduction

The scientist ask themselves are the plasmids live organisms, hence they completely depend upon their hosts, and still they consist of nucleic acid. If we accept the proposed virus definition which says that microorganism is a single unit with continuous parentage and individual evolution history, than plasmids could be considered as live organism in spite of their simple structure (1).

Plasmids, extrachromosomal DNA were at first identified in bacteria from genus Enterobacteriacae; after that they were discovered in almost every single strain observed (2). They were also found in some lower eukaryotes cell (e.g.fungus) (3). There is a common opinion that plasmids are unnecessary for a growth of bacterial population and their vital functions, thus, in many cases can be taken up or kicked out with no lethal effects to a plasmid host cell. In bacterial cell, they usually exist in supercoiled form, but after alkaling lysis and electrophoresis, they could be found in linear, open circle or multiple-supercoiled form (4).

The structure of plasmids is made of circular double chains DNA molecules which are replicated autonomously in a host cell. Their length vary from few kilobases (kb) up to several hundred kb (1kb = 1000 base pairs (bp); 1 bp = 660 dalton (Da); 106Da = 1 megadalton (MDa)). In bacterial cells, some plasmids of small molecules weigths usually may exist in many copies, while in a case of bigger plasmids we have bounded replication and lesser number of their copies consequently (5).

Plasmids can be integrated into the chromosome and then are called the episomes (6). Some big bacterial plasmids, called "co-integrated", may fall into reversible dissociation onto separated units. "Co-integrated" antibiotic resistance plasmids in Proteus mirabilis may dissociated on separated segments with transfer function genes (RTF – replicon) and genes of resistance to antimicrobial drugs (2).

Plasmids transfer

Plasmids are transferred horizontally, among bacteria, with a process of conjugation, that is, by intercellular contact in which donor's

DNA (a cell which gives a plasmid) is transferred to recipient (a cell which receives a plasmid). This process usually is regulated by genes carried by conjugative plasmids, which code creation of pili which are necessary for a contact between cells. It may be possible that there are more genetically different systems of conjugation (7). The conjugation requests the presence of broad genetic region of plasmid DNA (it encompass one third of genom or 33 kb in case of F-plasmid). Plasmids from the same incompatibility group have usually emphasized DNA homology and they code similar F-pili and conjugation systems. During conjugational transfer of DNA occurs the following:

    1.Forming of "a nick" and beginning of DNA transfer at the origin of transfer (oriT),

    2.segregation of two plasmid DNA chains,

    3.transferring one of the chains into recipient cell,

    4.conjugational synthesis of complete plasmid DNA both in a donor and a recipient cells,

    5.creation of circular forms of plasmid replicons.

Thus plasmid replication process can be divided into three stages: initiation, elongation, and termination (8).

F-pilus makes specific contact with potential recipient cell and his retraction leads to close connection between cell membranes of a donor and a recipient. It is supposed that a small canal, formed in this way, allows DNA transfer, or through the pilus itself, which is wide enough to enable passing of single chain DNA, or we have fusion of cell membranes at the contact spot which leads to creation of transmembrane pore as a passage for DNA.

Initiation is catalyzed most frequently by one or a few plasmid-encoded initiation proteins that recognize plasmid-specific DNA sequences and determine the point from which replication starts (the origin of replication) (8). Transfer of plasmid-specific DNA starts at the specific spot on plasmid molecule recognized as origin of transfer, or oriT. This spot is asymmetric and it is oriented in such a way so genetic region, which controls the transfer, transfers at the end. OriT sequences differ among plasmids and they are very specific. For mobilization of non-conjugative plasmids, an interaction between theirs oriT sequences and products of mob genes is required as well as identification of mobilization system by conjugational system of plasmids.

The enzyme placed in cytoplasm, DNA helicase I, which uncoils DNA chain, is a product of tral genes. In vitro studies have shown that process of uncoiling of DNA may require binding of 70 – 80 molecules protein's enzymes to single chain DNA region of two hundred nucleotides. Multimer created in this way, migrates as a stabile complex along the DNA chain using the energy from adenosine triphosphate (ATP) for uncoiling. The migration develops to 5'-3' direction, and for the rate of DNA helicase I uncoiling it is estimated to be around 1200 base pairs per second. Enzyme which transcripts

unwinding, closed, circled DNA duplex into negative, winding form is named as DNA gyrase. It can be involved in DNA transfer, too. Transfer of a single chain plasmid-specific DNA is always associated with the synthesis and the missing chain replacement along with a DNA polymerase III enzymes activities. The creation of the transferred DNA circled form is based probably on endonuclease enzymes activities – lygase as it is the case with viruses' multiplication. Integration of plasmid-specific DNA occurs by binding of the 5' end of the one molecule to the 3' end of another and vice versa (7). In the process of chromosome integrated plasmids transfer, the integration may play the role in transmission of plasmid-specific DNA (6).

Autonomous plasmid replication is the mechanism for the plasmid replication control. In a given host and a given environment, plasmid exist with determined average number of its copies per cell. All plasmids examined so far control the replication with a negative feedback. Genes and the spots for autonomous replication and its control are basis for plasmid's replicon. They usually consist of initiation of replication, "cop" and "inc" genes which start replication mostly and "rep" genes which code proteins required for replication and its control (1).

Identification and classification of bacterial plasmids

Identification and classification of plasmids are based on genetic characteristics which constantly exist in all of them, as is the case with their ability to preserve themselves in a host cell, and especially regarding control of replication.

However, as there are differences in the control of replication in various plasmids, plasmids in which the control is carried out in the same manner are recognized as incompatible, while the plasmids with different replication control as compatible, usually (1).

Plasmids incompatibility, that is inability of two plasmids to carry themselves in stabile manner in the same cell line (1), was firstly described for F-plasmid. Incompatible plasmids are marked as the members of the same incompatible group. Among the plasmids discovered in enterobacteria, 30 groups like this are described. Plasmids incompatibility testing involves introducing of plasmids (by conjugation, transduction, or transformation) into the strain which carry another plasmid. In addition, two plasmids have to possess different genetic markers in order to follow their segregation. The selection is made on introduced plasmid usually, and daughter cells examined to continuous presence of resident plasmid. If we eliminate the resident plasmid, two plasmids are considered as incompatible, that is as the same group members.

As there are numerous difficulties following this method (numerous control systems of replication, surface exclusivity inhibits plasmid introduction, lack of appropriate genes – examined plasmid markers for selection etc.), a

new method for identification is introduced based on hybridization with specific DNA probes containing genes for replication control.

Technique of "plasmid replicons typing" identification of plasmids is based upon recognition of genotype of different replicons rather than their incompatible phenotypes. This technique is based on estimation of sequences rate between basic resident replicons in the tested plasmid and group of probes DNA replicons isolated from seventeen different incompatible groups. Plasmids from examined strains are immobilized on solid matrix (hybridization of colonies on filter) or they are extracted by agarose gel electrophoresis and hybridized with radioactive rep-probes. Similarity of the sequences is estimated by autoradiography.

It has shown that many of incompatible groups, incompatible grouping and replicons typing have the same results. For plasmids which carry more than one replicon (multireplicon plasmids) or similar, but compatible replicons (family of replicons) the technique of plasmid replicon typing gives most accurate results. Replicon typing is technically easier than testing of groups' incompatibility and it is less time consuming and it is applicable to great number of bacteria especially in epidemiological studies (10).

Genetic structure and classification of plasmids

Plasmids contain genes essential for their function and existence in a cell (initiation and control of replication). Some of them possess genes which control plasmid stability managing division and transfer functions by conjugation (1). These genes can be found rather in larger plasmids than in smaller ones. However, small plasmids can be mobilized and transferred to another cell by larger, conjugated plasmids (5).

Plasmids are related with numerous biological functions of bacteria: transfer of genetic material by conjugation, bacteriocin and colicin (Col-plasmids) production, generation of antibiotics, heavy-metals resistance, antibiotic resistance (R-plasmids), UV emission resistance (UV), production of enterotoxins *E. coli*, production of chemolysine, camphor metabolism, causes malignancy in plants, restriction, and modification (2).

Beside well described F-, Col-, and R-plasmids there are plasmids that can change some physicological properties of bacteria and make difficulties in identification of microorganisms. For example, lactose fermentation in Salmonella isolates, production of urease in Providencia stuarti and use of citrates and production of H2S in some strains of *E. coli* (11).

Virulence genes could also be found in plasmids. Therefore, in enterotoxic E. coli (ETEC), plasmid of 27 MDa which codes synthesis of fimbria, thermolabile (LT) and thermostable (ST) enterotoxin is described (12). In enteroinvasive *E. coli* (EIEC), invasivity is related to presence of large plasmid around 140 Mda (13). Also, the adherence of enteropathogenic *E. coli* (EPEC) is caused by plasmids (14). It has been noticed that strains of enterohemorrhagic *E. coli* (EHEC) possess plasmid of 60 MDa which codes synthesis of fimbria for adherence bacteria to epithelial cells in tissue culture (12). Similarly, plasmids affect virulence of Shigella (15) and Yerisinia (16) genera and also production of entero- and exfoliative toxins of Staphylococcus aureus (17).

Plasmid – linked resistance is conditioned by smaller genetic sequences – transpozones, which can be introduced in other genetic structures or to fallout from them through the process of transposition ("irregular recombination"). Transposition is the process in which particular sequence from certain replicon, is introduced to another DNA replicon (plasmid or chromosome). Opposite of classic recombination, transposition does not require genetic homology between donor and recipient. Furthermore, neither the product of recA gene, which is obligatory for classic recombination, is required for transposition (6).

Transpozones may contain resistance genes to almost all antibiotics: ampicillin (and the others penicillins), tetracycline, chloramphenicol, trimetoprime, streptomycin, kanamycin, neomycin, gentamycin, and tobramycin. These so-called "jumping genes", which can relocate themselves from one plasmid to another or from plasmid to chromosome, may be the major cause of fast resistance spread among bacteria of different genera (18,19).

While Gram-negative enterobacteriae usually contain one large plasmid for multiple-drug resistance (20), some Gram-positive bacteria, as staphylococcus, often have resistance genes arranged on several smaller plasmids. The instability of these small plasmids obviously leads to different types of antibiograms susceptibility, which is noticed in isolates from one colony, or that isolated at different times from one single patient (5). On the other hand, there are reported evidences that Staphylococcus aureus has took up plasmids' genes for vancomycin (the antibiotic which has long been considered the last line of defence for *staphylococcus*) resistance from *Enterococcus faecalis* by the process of transformation (21).

Resistance plasmids could also be transferred by conjugation among bacteria that belong to different genera. *Salmonella* (S.) typhimurium isolated in Hong Kong and resistant to streptomycin, tetracycline, chloramphenicol, and sulfonamide, possess plasmid of incompatible group H1 discovered in many strains *S. typhi* resistant to chloramphenicol (22).

The same or similar resistance plasmids may have certain geographic distribution. Therefore, plasmids could be isolated from strains from different geographic region as reported for H1 plasmids *S. typhimurium* which coded resistance to ampicillin and tetracycline, ampicillin and kanamycin or, ampicillin, kanamycin and tetracycline. Plasmids of resistant strains from this serotype isolated in Singapore, Malaysia, USA, and Hong Kong have shown mutual similarity. Plasmids isolated from Hong Kong strains were non-autotransferable, unlike to the others, which point out that they are also subjected to evolution (22).

Some resistance plasmids may affect Salmonela phagotype. *S. typhimurium* which belongs to the phagotype (phagotype – PT 204), possess plasmid of 63MDa which carry resistance to tetracycline. When this plasmid is introduced in strain PT 36, it disables lysis of the strain which is usually susceptible to all phages. If this plasmid disappears spontaneously from the strain PT 204, than increase the susceptibility to lysis by phage determined with PT 49. PT 204c originated from PT 204 by achieving autotran-sferable resistance plasmid from compatible group H2. If this plasmid is introduced in strain PT 36, its lysis is disabled by two phages, which leads to its conversion in PT 125. It is considered that PT 193 has its origin from PT 204 by achieving autotransferable resistance plasmid from group I2 (23).

Hence, the resistance plasmids can be transferred by conjugation from bacteria genus to another, in some cases they are considered as epidemic rather than bacteria strain. In a hospital environment especially, one plasmid can be found in several different bacteria genera and/or in different serotypes or biotypes of one single strain (24). Outbreaks caused by Klebsiella pneumonia strains, resistant to gentamycin, and Serratia marcescens, were occurred in "Peter Brigham" hospital in Boston (USA) in 1975, and 1976. Examination of strains resistant to genta-

mycin shows that all of them produce the same 2''-aminoglycoside- nucleotide transferase and contain plasmid of 56 MDa which belongs to M incompatible group. This plasmid firstly was found in one biotype of *K. pneumonia* which disappeared from hospital later on. Plasmid was spread out on some strains of *E. coli,* and later it cycled among strains of *S. marcescens*, although it was found in six different bacterial species. Probably, the epidemic plasmid reached the hospital along with one or several strains and after that it was spread out by conjugation onto other strains present in flora of hospitalized patients (25).

Plasmids which have ability of bidirectional transfer between two bacterial strains are called shuttle vectors. They possess two different origins of replication (26).

Numerous plasmids found in clinical bacterial isolates are named "cryptic" as their functions and products of their genes are unknown thus far. The presence of cryptic plasmids can help to identify certain medical important bacterial strains, especially for identification of some strains in genus Enterobacteriacea (5). The differences in plasmids profiles found in many isolates of one epidemic strain show that plasmids can be subjected to significant molecule evolution during an outbreak.

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BAKTERIJSKI PLAZMIDI

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Plazmidi, ekstrahromozomska DNK, bili su najpre identifikovani kod bakterija iz porodice *Enterobacteriacae*, a onda su nađeni kod skoro svakog roda kod koga su bili traženi. Građeni su od kružnih dvostrukih lanaca molekula DNK koji se replikuju autonomno u ćeliji domaćina. Njihova dužina varira od nekoliko kilobaza (kb) do nekoliko stotina kb. Između bakterija, plazmidi se, najčešće, prenose horizontalno, procesom konjugacije. Proces replikacije plazmida može biti podeljen u tri stadijuma: inicijacija, elongacija i terminacija. U replikaciji učestvuju DNK helikaza I, DNK giraza, DNK polimeraza III, endonukleaze i ligaze. Plazmidi sadrže gene koji su neophodni za funkcije plazmida i njihovo održavanje u ćeliji (početak i kontrola replikacije). Neki sadrže i gene koji kontrolišu stabilnost plazmida upravljajući fukcijama deobe i transfera konjugacijom. Smatra se da nisu neophodni za razmnožavanje i vitalne funkcije bakterija, tako da u većini slučajeva mogu biti stečeni ili izgubljeni bez letalnih efekata za ćeliju, domaćina plazmida. Međutim, za plazmide se vezuje veliki broj bioloških funkcija bakterije: konjugacija, produkcija bakteriocina i faktora virulencije, otpornost na antibiotike, teške metale, ultraljubičasto zračenje itd. Identifikacija i klasifikacija plazmida zasnivaju se na genetskim osobinama koje su prisutne i stalne u svim plazmidima: na sposobnosti održavanja u ćeliji domaćinu i na kontroli replikacije. Na taj način, izvršeno je grupisanje na osnovu inkompatibilnosti. Metoda tipiziranja replikona, koja je zasnovana na genotipskim, a ne na fenotipskim svojstvima, daje iste rezultate kao i inkompatibilno grupisanje. *Acta Medica Medianae 2007;46(4):61-65.*

Ključne reči: *bakterija, plazmid*