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Plasmid, its types and uses in Plant Pathology

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Introduction:-

The term plasmid was introduced in 1952 by the American biologist Joshua Lederberg to refer to any extra chromosomal hereditary determinant. Plasmids are transferable extra chromosomal DNA molecules capable of autonomous replication. It usually occurs naturally in bacteria and is sometimes found in eukaryotic organisms e.g. *Saccharomyces cerevisiae*. The size of plasmids varies from 1 to over 400 kilo base pairs (kbp). There may be one copy, for large plasmids, to hundreds of copies of the same plasmid in a single cell, or even thousands of copies, for certain artificial plasmids selected for high copy number such as the pUC series of plasmids. Every plasmid contains at least one DNA sequence that serves as an origin of replication, or ori (a starting point for DNA replication), which enables the plasmid DNA to be duplicated independently from the chromosomal DNA (Yamada *et al.*, 1986*)*. A few types of plasmids can also insert into the host chromosome, and these integrative plasmids are sometimes referred to as episomes in prokaryotes (Brown, 2010).

The plasmids of most bacteria are circular, but linear plasmids are also known, which superficially resemble the chromosomes of most eukaryotes. Frequently plasmids contain some genes advantageous to the bacterial host for example, resistance to antibiotics, production of antibiotics and degradation of complex organic compounds (Minsavage *et al.*, 1990). Bacterial antibiotic-resistance genes encode proteins that inactivate these agents either by preventing their accumulation in the cell or by inactivating them once they have been imported. By utilizing these antibiotic-resistance genes as dominant genetic markers in plasmid cloning vectors, it is possible to select for *E. coli* cells that have maintained high copy replication of plasmid DNA molecules. Plasmids used in genetic engineering are called vectors. Plasmids serve as important tools in genetics and biochemistry labs, where they are commonly used to multiply (make many copies of) or express particular genes. Another

major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacteria produce proteins to confer its antibiotic resistance; it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for, for example, insulin or even antibiotics.

Plasmids as Vectors

Some important features of cloning vectors

A plasmid vector used for cloning is specifically developed by adding certain features:

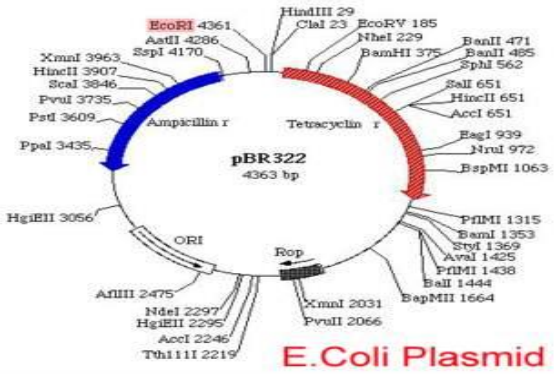
- Reduction in size of vector to a minimum to expand the capacity of vector to clone large fragments. Since the efficiency of transformation of bacterial cells drops drastically when plasmids larger than 15 kb are used, the size of cloning vector should be small, preferably 3-4 kb. In this way, foreign DNA fragments of 10-12 kb can be accommodated.
- It should contain an origin of replication that operates in the organism into which the cloned DNA is to be introduced.
- Introduction of selectable markers.
- Introduction of synthetic cloning sites termed polylinker, restriction site bank, or polycloning sites that are recognized by restriction enzymes. This polycloning site is usually present inside a marker gene so that with the insertion of foreign DNA it will inactivate that marker gene and the recombinants can be selected.
- Incorporation of axillary sequences, such as visual identification of recombinant clones by histochemical tests, generation of single stranded DNA templates for DNA sequencing, transcription of foreign DNA sequences in vitro, direct selection of recombinant clones and expression of large amounts of foreign proteins**.**
- Smaller plasmids can accommodate larger segments of foreign DNA before the efficiency begin to deteriorate
- Artificially constructed plasmids may be used as vector in genetic engeenering. These plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to clone and amplify (make many copies of) or express particular genes.

pBR322

This was one of the first artificial cloning vectors to be constructed, and is undoubtedly the most widely used cloning vector till now. It is a 4.36-kb double stranded cloning vector. This

plasmid vector has been put together from fragments originating from three different naturally occurring plasmids. Figure 1a indicates all the salient features of this vector. It contains CoIE1 ori of replication with relaxed replication control. Generally there are 15-20 molecules present in a transformed *E.coli* cell, but this number can be amplified by incubating a log phase culture of CoIE1, carrying cells in the presence of chloramphenicol. The plasmid contains 20 unique recognition sites for restriction enzymes. Cloning of a DNA fragment into any of these 11 sites results in the insertional inactivation of either one of the antibiotic resistance markers. The gene of interest is spliced into tetR gene cluster, and then the *E. coli* cells are transformed. Thus, three types of cells are obtained:

- Cells that have not been transformed and so contain no plasmid molecules and will be ampS tetS.
- Cells that have been transformed with pBR322 but without the inserted DNA fragment or gene will be ampR tetR. These are transformed cells.
- Cells that contain a recombinant DNA molecule, i.e, the DNA fragment has been inserted into the pBR322 at tetR gene cluster. These cells will lose tetracycline resistance because the fragment has inserted in the middle of tetracycline resistance gene cluster. These are recombinants and will be ampR tetS.



pUC vectors

The name pUC is derived because it was developed in the University of California (UC) by Messings and his colleagues. These plasmids are of 2.7 kb and possess the ColE 1 ori of replication. These vectors contain ampR gene and a new gene called lacZ, which was derived from the lac operon of *E. coli* that codes for β-galactosidase enzyme whuch hydrolysis lactose. The *E. coli* strains, e.g JM103,JM109, usedas hosts for yhe Puc series vectors have the lacZα deleted from their lac operons. When pUC enters such an E.coli cell, the host

genome and the plasmid encode for different parts of the β- galactosidase enzyme, which interact with each other to produce the active enzyme enabling these calls to hydrolyse lactose. B- galactosidase also hydrolyses X gel (5-Bromo-4-chloro-3-indolyl-β-D-galactoside) to yield a blue dye. Therefore appropriate lacZ *E. coli* cells transformed by the pUC vectors behave as lacZ+ and produce blue co;our colonies on a X-gal+ IPTG (isopropylthiogalactoside) containing medium. A polylinker sequence or multiple cloning site (MCS) located within the lacZα provides several unique restriction site for DNA insertion.

Different Cloning Vectors for Different Applications

Sizes of inserted DNA commonly obtained with different cloning vectors:

Cloning Vector	Size of insert (Kb)
❖ Standard high copy number plasmid	< 10
❖ Bacteriophage	9-23
❖ Cosmid	30-44
❖ Bacteriophage P1	70-100
❖ PAC (P1 Artificial Chromosome)	130-150
❖ BAC (Bacterial Artificial Chromosome)	<300
❖ YAC (Yeast Artificial Chromosome)	0.2-2000

Source : Biotechnology expanding horizons by B.D Singh

Gene Transfer in Bacteria

The genetic information encoded in a self-replicating extra chromosomal DNA (plasmid) of bacteria is transferred across a broad range of microorganism through conjugation, transformation and transduction. In case of conjugation the donor cell must have a conjugative plasmid and establish a strong physical contact with the recipient cell. In contrast later two methods do not require any physical contact with in donor and recipient**.**

Conjugation:

Conjugation is a two step process. In first step, the DNA that is covalently linked to recipient is initially transported in a passive manner, trailing on the relaxase where pilus helps in transporting DNA across several membrane barriers in recipient cell and second step is active pumping of the DNA to the recipient, using the already available T4SS transport conduit (Liosa *et al*., 2002). There are reports of inter-kingdom genetic exchange by conjugation. Conjugative plasmid, such as RP4 has extremely broad host range and these plasmids

contribute a lot to genetic diversity. There is much information regarding gene transfer by conjugative mechanism in aquatic ecosystem. Sengelov and Sørensen have selected donor and recipient bacteria in lake water microcosm that received plasmid RP4 by conjugation on filters.

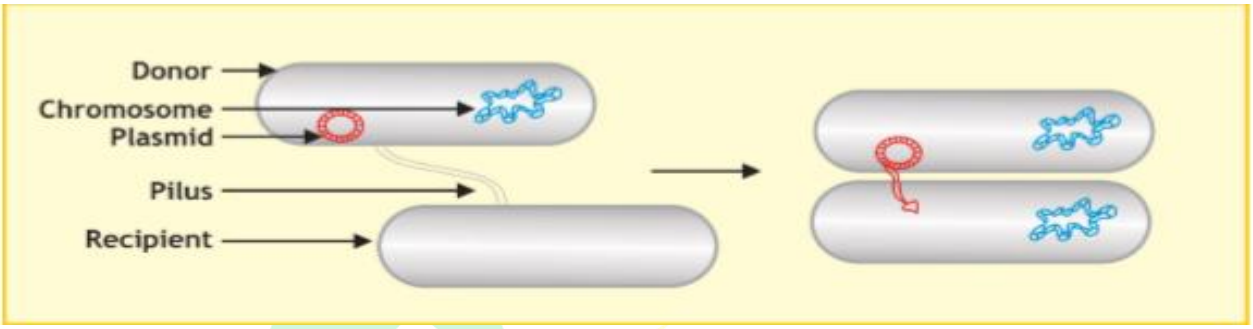


Figure 1: Transfer of gene between bacteria by conjugation

Transduction:

It represents plasmid mediated gene transfer in bacterial community through the bacteriophages which are viruses infecting bacteria. It can be generalized transduction (ability of transducing any gene into bacterial chromosome) or specialized transduction (it can transduce a particular genes). Transduction in freshwater ecosystem is reported in *P. aeruginosa* streptomycin resistance by a generalized transducing phage, F116, in an experiment conducted with a flow-through environmental test chamber for 10 days period. Mean transduction frequencies ranged from 1.4 × 10-5 to 8.3 × 10-2 transductions per recipient (Morrison *et al*., 1978). A pseudo-lysogenic, generalized transducing bacteriophage, UT1, isolated from a natural freshwater habitat, is capable of mediating the transfer of both chromosomal and plasmid DNA between strains of *P. aeruginosa*.

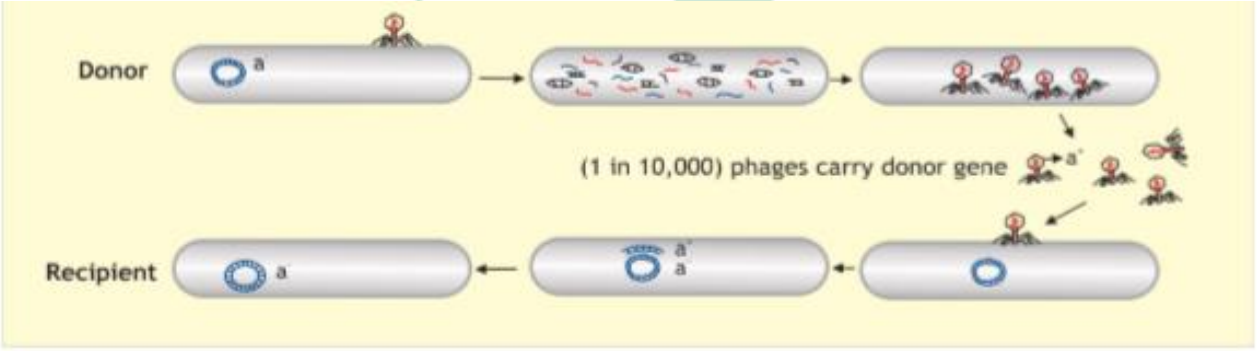


Figure 2: Gene transfer through transduction occur between bacteriophage and bacteria

Transformation:

It is the most common method of bacterial gene transfer in nature. The process requires competent cells which is ready to accept extracellular plasmid and further stable replication inside recipient cells. Artificial transformation by preparing competent *E. coli* bacterial cells in lab is a common and widely used method in gene cloning. But there are many naturally occurring competent bacteria which participate in natural transformation like *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* (Lorenz and Wackernagel, 1994).

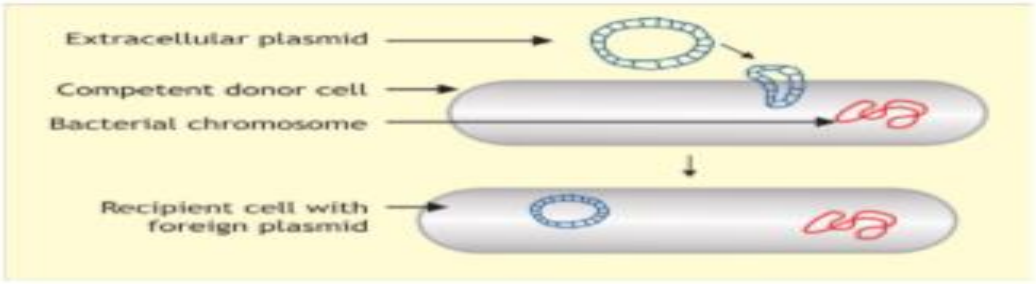


Figure 3: Extracellular plasmids are transferred to competent cells by the process of transformation.

Types of plasmids

Plasmid are classified as F plasmids, colicinogenic (Col) plasmids, Resistance (R) plasmids, Degradative plasmids and Tumour inducing (Ti) plasmid.

Uses of plasmids in plant pathology

(A) Mainly used in production of transgenic plants by introducing beneficial gene into host cell.

(B) Also used in transfer of genes from one bacterium into another.

(C) Development of resistance plant against several diseases by using Ti plasmid in plant against disease such as crown gall tumour. The plasmid is rendered avirulent by curing it, prior to its use as a vector.

(D) Used in gene cloning as vector:-The plasmids most commonly used recombinant in DNA technology replicate in *E. coli*. Generally, these plasmids have been engineered to optimize their use as vectors in DNA cloning

(E) Plasmid in Gene Therapy: Used in the insertion of therapeutic genes in the human body to fight against disease. Easy to manipulate and their replication in the bacterial cell is easy. No harmful effects of plasmids like the viral vectors Plasmid vectors are one of many

approaches that could be used for this purpose.

(F) Plasmids in recombinant DNA technology: - For drug delivery, use plasmids to insert the desired drug into the body. Involved in the antibiotic resistance and used to kill harmful bacteria from the body. Also used for insertion of human insulin in the human body.

Role of Plasmid in Bacteria

- ✓ Bacteriocin production
- ✓ Pathogenicity and host specificity
- ✓ Nutrient capabilities
- ✓ Hormone production
- ✓ Toxin production
- ✓ Copper and antibiotic resistance

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