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TYPES OF TRANSPOSONS AND THEIR USAGE IN BIOTECHNOLOGY

Manure-derived antibiotic resistance genes (ARGs) pose a significant environmental concern, and understanding their dynamics in soil is crucial for effective management. This study examines both short- and long-term ARG accumulation in soil with a 40-year history of manure application. While initial manure introduction led to a spike in ARG abundance, resident soil bacteria eventually out-competed manure bacteria, resulting in ARG dissipation within a year. Over four decades of annual manure application, linear or exponential ARG accumulation occurred, with shifts in associated bacteria compared to short-term dynamics. Discontinuing manure application led to a decline in most ARG levels eleven years later, though they remained elevated. This systematic exploration of historical ARG accumulation provides insights into factors influencing their persistence in manure soil. Bacterial transposons, mobile genetic elements, play pivotal roles in biotechnology, facilitating precise gene manipulation for synthetic biology, genetic engineering, and industrial applications. These tools enable the development of microbial organisms with enhanced traits, impacting agriculture, environmental cleanup, and biofuel synthesis. In synthetic biology, bacterial transposons serve as gene delivery vehicles, allowing the creation of artificial circuits and pathways, revolutionizing

pharmaceuticals, bio-derived chemicals, and other biological products. Additionally, they contribute to directed evolution studies, accelerating the discovery of new enzymes and the development of strains with desired traits. In medical biotechnology, bacterial transposons play crucial roles in gene therapy and therapeutic protein production, offering potential solutions for genetic abnormalities and diseases. The ongoing advancements in the biotechnological applications of bacterial transposons underscore their indispensability for diverse research and development efforts.

Keywords: Transposons, Transposase, Insertion sequences, Antibiotic resistance, and Horizontal Gene Transfer.

Introduction

DNA segment known as a bacterial transposon, also referred to as a transposable element, could move around inside the bacterial genome. This mobility is coordinated by enzymes as the transposases, which are the catalysts of transposition. This is where bacterial transposons play an essential role in bacteria evolution driving genetic variability, adaptations, and acquisition of new attributes [1]. At their core, bacterial transposons can be broadly classified into two main types: simple transposons, mostly composed of IS elements, and more complicated transposons that frequently carry a variety of additional genetic material. The development of these elements reflects the variety of their functions and methods of action. The mechanisms governing transposition can be categorized into two main types: cut and paste and copy-paste. In cut-and-paste transposition, the piece of DNA (transposon) is removed from where it was found and inserted into another location in the genome along with target site duplications. In copy-and-paste transposition, a duplicate of the transposon is produced and inserted elsewhere on the genome which leads to increase in transposon copies.

Bacterial transposons do not limit their effects to the reorganization of a genome. They are critical for the evolvement of genetic diversity among bacterial populations and allow them to adapt quickly to different environments. Furthermore, transposons are essential for horizontal gene transfer, which allows genetic material to be transferred from one organism or species to another [2]. This introduction to bacterial transposons covers their structural diversity, transposition methods, and uses in bacterial evolution and adaptation as a preamble to a thorough study of these organisms. Indeed, understanding the functioning of bacterial transposons is essential for deciphering the intricacies of bacterial genetics, with implications spanning from fundamental microbiology to biotechnology and medical fields [3].

Materials and methods

A cell biologist studying this important and fundamental topic Transposons, or «jumping genes,» are mobile DNA sequences that reposition themselves within a genome; they play a role in genetic evolution and adaptation in organisms such as bacteria. They are categorized into simple and complicated arrangements and can travel through cut-and-paste or copy-and paste modes of motion. The catalyzer of transposons excision and insertion is the enzyme transposase which serves as a target for the manipulation in biotechnological practice. Such tools allow for genetic modification, functional genomics, vector creation, metabolic pathway enhancement and site-specific insertion. The way I performed the research was by referring to different scientific and academic sources, such as academic articles, and other research information, so mostly I used the scholarly essay method.

Results and discussion

Bacterial transposons are genetic elements, which help in maintaining the genetic diversity and adaptation of the bacterial genome. Transposons are categorized according to their structure and function with the principal forms being insertion sequences (IS elements) and complex transposons. These transposons have inverted repeats and target site duplications. Their size range and type of content differ, with their input methods being cut-copy or copy-paste [1].

Simple Transposons

IS Elements, or Insertion Sequences

ARE elements being a necessary part of bacterial transposons, which contributed significantly to mobility and recombination of genetic material in bacterial genomes. They include TIRs and a transposase gene, which are short, inverted DNA sequences located at each end of the IS element. The transposase is one of the essential enzymes encoded by the IS element and functions in catalyzing excision of the IS element from its original genomic site and insertion into a new target location. The TSDs are the result of IS elements by creating targets site duplications within a genome through cut-and-paste and copy-and-paste mechanisms [4, 5].

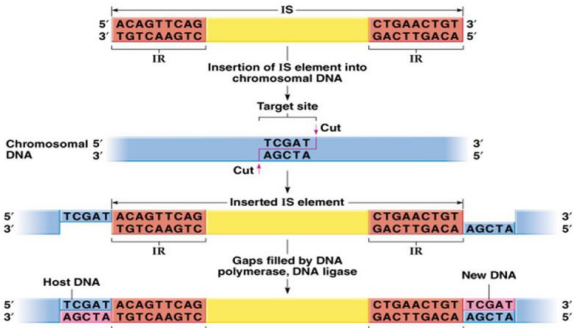

Figure 1 – The IS element carries a single ORF encoding transposase, which cuts and removes it from the chromosome or plasmid. As it moves, it finds target sites for insertion, producing staggered cleavage and sticky ends. This DNA proofreading mechanism results in target site duplication, with one copy of the target DNA on either side

Composite Transposons

Genomic rearrangement occurs due to the transposons, which are mobile genetic elements found in the DNA of some species. Mobile genetic elements or 'jumping genes' are the transposons, which are DNA segments that can hop around in the genome. It is a unique variety of transposon which integrates two IS elements along with the core region. These transposons typically encode many genes for metabolism, pathogenicity, or even antibiotic resistance [7].

Composite Tns reported to include the sequential, composite transposon, Tn5, Tn10, Tn9 and so on; The four major composite Tns are including the Tn903, Tn9, (ampicillin), and also tetracycline in bacterial antibiotic resistance especially of Escherichia coli [6].

Tn5

Tn5 is a gene comprising resistance genes to neomycin/kanamycin, bleomycin, and streptomycin which are present in Gram-negative bacteria like Methyl bacterium, Agrobacterium, Caulobacter, Acinetobacter and Pseudomonas. By inhibiting the IS50 Tase activity, Hfq molecule as an RNA binding protein and gene expression regulator can stop Tn5 transmission in E. coli. It could also block Tn10 transfer and act as an IS10 Tase inhibitor, thus reducing drug resistance in E. coli [6].

Tn10

Tn10 is a tetracycline-resistant gene controlled by IS10R and IS10L, which include tetA, tetR, tetC, and tetD resistance genes. It establishes a Shigella flexneri efflux system and is present in Proteus, Pseudomonas, Salmonella, Klebsiella, and Vibrio. Non-replicative transposition mechanism is found in Tn5 and Tn10, while IS50 and IS6 make up the family of IS4. Additionally, Tn10 transposition regulation is accompanied by H-NS and IHF processes depriving Tns from its abilities to shift and determine the antibiotic resistance of bacteria [8].

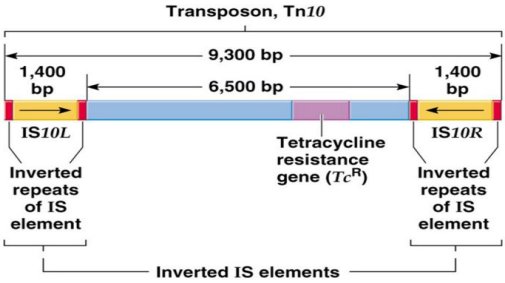

Figure 2 – Tn10 Composite transposition occurs when adjacent IS elements form, enabling transposition when working together, like Tn10, which includes transposase and antibiotic resistance genes

Tn9

Tn9 is a transposon that carries the cat resistance gene - chloramphenicol acetyltransferase, and IS1 flanks this. Besides, IS1 represents one of the first and smallest ISs in bacteria (having less than 770 bp). IS1 contains two ORF named insA and insB and carries resistance to chloramphenicol [8].

Non-composite transposons

Apart from composite Tns, different members of the Tn3 family are "non-composite" and represent carriers of antibiotic resistance genes such as tnpA and tnpR flanked by IRs. Such Tns are extremely important in the genome evolution of bacteria and the spread of antibiotic resistance. The majority of the non-composite Tns are members of the Tn3 family that has subfamilies such Tn21, Tn501, Tn5393, Tn7, Tn5403, and Tn1721. Important non-composite Tns include Tn5053, Tn5041, Tn5652, Tn1013, Tn5036, Tn5541, Tn5090, Tn5060, Tn5051, Tn1331, Tn4430, Tn5044, Tn5563, and Tn402 [6].

Tn3

Tn3 is an important bacterial Tn that has two genes, tnpA and tnpR, and a length of 5000 bp. It has the ampicillin-resistant gene and is found in both Gram-negative and -positive bacteria. Tn3 also has blaTEM-1 β-lactamase genes in Gramnegative bacteria. It has a recombination site and is bordered with IRs [6].

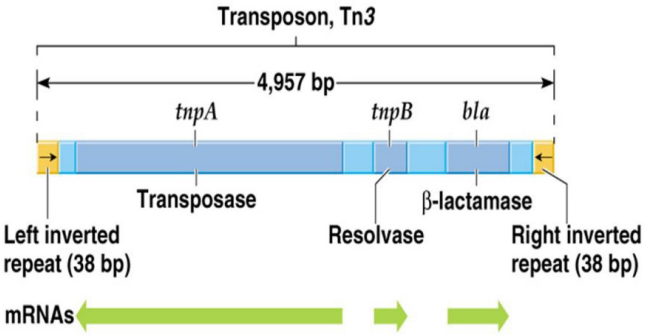

Figure 3 – Tn3 is a non-composite transposon of 5kbp which contains three gene for beta-lactamase (bla), transposase (tnpA) and resolvase (tnpB)

Tn7

Tn7, identified in E. coli 97 is a protein that is 14,000 basepairs long with sequences of Tn7R and Tn7L. It produces five transposition proteins: TnsA, B, C, D and E. Tase contains two subunits, which are TnsA and TnsB, indicating the terminal sequences in Tn7. ATP hydrolysis, TnsC regulates transposition of Tn7. TnsD and TnsE proteins mediate the recognition of the target site for Tn7 transposition, encouraging Tn7 recombination by promoting its transposition. Most antibiotic resistance genes are transported by Tn7 that leads to trimethoprim, streptomycin and spectinomycin resistances [6].

Complex Transposons

Complex transposons are a class of mobile genetic elements and as complex transposons, they take part in the transmission of gene material across the genome Transposons that are complicated are larger and more complex in their structure than simple transposons. They frequently harbor other genes, including those involved in enzyme-related processes [9].

Multiple genes: Unlike simple transposons, which generally comprise a transposable gene and repetitive sequences, complex transposons include

additional genes within the structure of the transposon. These supplementary genes are highly contrasting and could encode processes that include antibiotic resistance, virulence aspects or metabolic enzymes [9].

Reversed Repeats: Just like other transposons, complex transposons often have inverted repeat sequences at their termini the excision and integration of the transposon are facilitated through recognition by the transposase enzyme [10].

Transposase: Complex transposons as simple transposons that rely on the activity of enzyme transposase to catalyze their movement in the genome [10].

Direct Repeats: Besides the inverted repeats at transposon ends, some complex transposons form direct repeats at the target site after insertion. This is brought about by staggered cuts that are made in the integration process [9].

Integration and Excision: Like all transposons, complex ones undergo a process of excision from one position within the genome and insertion into another. This mediation is done by the transposase enzyme [11].

Large Size: Complex transposons are significantly bigger than simple transposons and they sometimes reach over 10 kilobases in length. The incorporation of more genetic information is possible thanks to their larger size [9].

Complex transposons are widespread among different organisms, such as bacterial kingdom where they have been widely investigated for their contribution in transmission of antibiotic resistance genes. Complex transposons harbor several more genes which help in making the host organism adapt well in different environments by giving them a selective edge [12].

Mechanisms of Transposition

Cut-and-Paste Transposition

Cut-and paste move is a conservative means where transposon moves in genome without copying. This process is mediated by the transposase enzyme and incorporates recognition and excision, transposon-excision complex formation and integration. The original DNA sequence retains its integrity and hence, no duplication occurs. This mechanism is prevalent in bacteria where transposons carry the antibiotic resistance genes and adaptive traits. It differs from replicative transposition, which constructs a duplicate of the transposon. The understanding of cut-and-paste transposition is essential in studying genome dynamics and evolution [13].

Copy-and-Paste Transposition

Transposition by copy-and-paste is a replicative mechanism, in which transposons migrate within the genome, leaving behind a double-stranded DNA copy of the original transposon. The transposase enzyme facilitates this reaction, which leads to amplification of the transposon. This mechanism is widespread in eukaryotic organisms such as plants fungi and animals and represents critical

data for evolution genome diversity and trait spread. It also has consequences for the field of genetic engineering and biotechnology that makes use of transposons to insert foreign genes [13].

Function of Transposons in the Genomes of Bacteria

Transposons contribute importantly to the evolution of bacterial genomes; they also help in competence in adaptation and genetic transmission. Transposons play the following important roles in the genomes of bacteria [15].

Genome Evolution: transposons promote the genome rearrangements and mutations that can increase the plasticity of a bacterial genome. Transposon mobility may generate the genetic diversity needed for the bacteria to adapt to the changing circumstances [15].

Antibiotic Resistance: The most clinically important transmitters of antibiotic resistance genes to the bacterial populations include the transposons. These transposons that bear antibiotic resistance genes can switch between the chromosomes of bacteria and hence they lead to rapid dissemination of refractoriness [16].

Virulence Factors: Transposons that encode for the virulence genes promote the bacteria pathogenicity. Transferring transposons with the virulence genes can enable bacteria to colonize and infect host livings [17].

Metabolic Adaptation: The transposons can also have many genes linked to the metabolic pathways so that it is possible for the bacteria in changing nutritional environments. This is the foundation of bacterial metabolic versatility in the fluctuating habitats. Thanks to transposons, the genomic islands can sometimes contain gene clusters that are associated with special functions such as symbiosis; pathogenicity or even metabolism [15].

Horizontal Gene Transfer: Horizontal gene transfer is the mechanism of the genetic material in other organisms which is promoted by transposons. This mechanism allows the bacteria to acquire new features, promoting their adaptation and evolution [18].

Regulation of Gene Expression: Due to insertion or excision they could control the expression of genes that neighbor them by laying an influence on adjacent genetic matter [18].

Applications and Implications of bacterial transposons

Bacterial transposons can be successfully used in many areas such as genetic research, medicine and biotechnology, agriculture. They are broadly utilized for insertional mutagenesis, which is possible to study the gene function. They also have a vital function in antibiotic resistance research, which helps to comprehend and combat the spread of antibiotic-resistant genes. Essential genes in bacterial

genomes can be identified through transposon mutagenesis, giving information about the physiological processes of bacteria and thus potential drug targets.

Transposons have applications in biotechnology where they are used for genetic manipulation, injecting foreign genes, or modifying native ones, depending on the purpose. They also create mutant libraries of bacteria that are genetically diverse and may be selected for desired phenotypes or utilized in large scale experiments [20].

Research about transposons in bacteria helps us learn about bacterial evolution and genome dynamics. Horizontal gene transfer studies also benefit from the use of engineered transposons for targeted gene delivery in genetic therapy experiments. Transposon-transferred modification through engineered bacteria can help in bioremediation processes for improving the degradation of pollutants or contaminants.

Scientists have developed transposon-based genetic tools, enabling greater precision and control over manipulating bacterial genomes. It is important to understand these applications as they are a source of scientific [19].

Conclusion

Applications of bacterial transposons range widely across many scientific, medical, and biotechnological practices. In genetic studies, antibiotic resistance mechanisms, biotechnology, and genetic engineering they have been used. They have significantly contributed to defining bacterial physiology and genes essential to their functionality by insertional mutagenesis. They are also applied in biotechnology for genetic engineering, allowing the addition of alien genes or the change of native ones. They have also been applied in horizontal gene transfer, shedding a light on the genome evolution of bacterial cells and the acquisition of new traits through genetic transfer. They have also been used to generate mutant libraries, which are important tools for selecting necessary phenotypes and elucidating gene function as well as aiding in bacterial composition analysis. In addition, transposons have been essential in the understanding of evolutionary events in bacterial genomes owing to their function of relocation and altering genomic architecture that drives adaptation by bacteria to changing surroundings. They are also studied for bioremediation and environment, as well as gene therapy that serves to deliver therapeutic genes into target cells. But transposons use presents ethical concerns about the long-term effects, unintended consequences and genetically modified organism release biosecurity risk.

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ТРАНСПОЗОНДАРДЫҢ ТҮРЛЕРІ ЖӘНЕ ОЛАРДЫҢ БИОТЕХНОЛОГИЯДА ҚОЛДАНЫЛУЫ

Көңнен алынған антибиотиктерге төзімділік гендері (ARGs) айтарлықтай экологиялық алаңдаушылық тудырады және олардың топырақтағы динамикасын түсіну тиімді басқару үшін өте маңызды. Бұл зерттеу көңді қолданудың 40 жылдық тарихы бар топырақта қысқа және ұзақ мерзімді ARG жинақталуын зерттейді. Бастапқы көңді енгізу ARG молшылығының өсуіне әкелді, бірақ резиденттік топырақ бактериялары ақырында көң бактерияларын жеңіп шықты, нәтижесінде бір жыл ішінде ARG диссипациясы болды. Қырық жыл бойы жыл сайынғы көңді қолдану, қысқа мерзімді динамикамен салыстырғанда байланысты бактериялардың ығысуымен сызықтық немесе экспоненциалды ARG жинақталуы орын алды. Көңді қолдануды тоқтату он бір жылдан кейін ARG деңгейлерінің көпшілігінің төмендеуіне әкелді, бірақ олар жоғары болып қала берді. Тарихи ARG жинақтауының бұл жүйелі зерттелуі олардың көңді топырақта сақталуына әсер ететін факторлар туралы түсінік береді. Бактериялық транспозондар, жылжымалы генетикалық элементтер биотехнологияда маңызды рөл атқарады, синтетикалық биология, гендік инженерия және өнеркәсіптік қолданбалар үшін нақты гендік манипуляцияны жеңілдетеді. Бұл құралдар ауыл шаруашылығына, қоршаған ортаны тазартуға және биоотын синтезіне әсер ететін жақсартылған белгілері бар микробтық агзаларды дамытуға мүмкіндік береді. Синтетикалық биологияда бактериялық транспозондар генді жеткізу құралы ретінде қызмет етеді, бұл жасанды тізбектер мен жолдарды жасауға, фармацевтикалық препараттарды, био-туынды

химиялық заттарды және басқа да биологиялық өнімдерді өзгертуге мүмкіндік береді. Сонымен қатар, олар бағытталған эволюциялық зерттеулерге үлес қосады, жаңа ферменттердің ашылуын және қажетті белгілері бар штаммдардың дамуын жеделдетеді. Медициналық биотехнологияда бактериялық транспозондар гендік терапияда және емдік ақуыз өндірісінде маңызды рөл атқарады, генетикалық ауытқулар мен ауруларға ықтимал шешімдер ұсынады. Бактериялық транспозондардың биотехнологиялық қолданыуындағы жалғасып жатқан жетістіктер олардың әртүрлі зерттеулер мен тәжірибелік-конструкторлық жұмыстар үшін қажет екенін көрсетеді.

Кілтті сөздер: Транспозондар, Транспозаза, енгізу реттіліктері, Антибиотиктерге төзімділік, гендердің көлденең трансферті.

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ТИПЫ ТРАНСПОЗОНОВ И ИХ ИСПОЛЬЗОВАНИЕ В БИОТЕХНОЛОГИИ

Гены устойчивости к антибиотикам (ARG), полученные из навоза, представляют собой серьезную экологическую проблему, и понимание их динамики в почве имеет решающее значение для эффективного управления. В этом исследовании изучается как краткосрочное, так и долгосрочное накопление ARG в почве с 40-летней историей внесения навоза. Хотя первоначальное внесение навоза привело к резкому увеличению численности ARG, резидентные почвенные бактерии в конечном итоге вытеснили навозные бактерии, что привело к рассеиванию ARG в течение года. За четыре десятилетия ежегодного внесения навоза происходило линейное или экспоненциальное накопление ARG со сдвигами в ассоциированных

бактериях по сравнению с краткосрочной динамикой. Прекращение внесения навоза привело к снижению большинства уровней ARG одиннадцать лет спустя, хотя они оставались повышенными. Это систематическое исследование исторического накопления ARG дает представление о факторах, влияющих на их стойкость в унавоженной почве. Бактериальные транспозоны, мобильные генетические элементы, играют ключевую роль в биотехнологии, облегчая точные манипуляции с генами для синтетической биологии, генной инженерии и промышленного применения. Эти инструменты позволяют развивать микробные организмы с улучшенными характеристиками, влияя на сельское хозяйство, очистку окружающей среды и синтез биотоплива. В синтетической биологии бактериальные транспозоны служат средствами доставки генов, позволяя создавать искусственные цепи и пути, производя революцию в фармацевтике, химических веществах биологического происхождения и других биологических продуктах. Кроме того, они способствуют направленным исследованиям эволюции, ускоряя открытие новых ферментов и разработку штаммов с желаемыми характеристиками. В медицинской биотехнологии бактериальные транспозоны играют решающую роль в генной терапии и производстве терапевтических белков, предлагая потенциальные решения для генетических аномалий и заболеваний. Продолжающиеся достижения в биотехнологическом применении бактериальных транспозонов подчеркивают их незаменимость для разнообразных исследований и разработок.

Ключевые слова: транспозоны, транспозаза, инсерционные последовательности, устойчивость к антибиотикам, горизонтальный перенос генов.

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