



**RETOOLING BACTERIA BY RECOMBINANT DNA TO MILESTONE IN SYNTHETIC
BIOLOGY AND CURE DISEASES**

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ABSTRACT

By manipulating DNA, researchers are trying to create microbes that, once ingested, work to treat a rare genetic condition a milestone in synthetic biology. Microbial biotechnology includes the exploitation, hereditary control and adjustments of miniaturized scale living beings to make business significant items and that likewise includes maturation and different upstream and downstream procedures. Microorganisms produce an astounding exhibit of significant items, for example, macromolecules (for example proteins, nucleic acids, starch polymers, even cells) or littler atoms and are generally separated into metabolites that are basic for vegetative development (essential metabolites) and those which give favourable circumstances over antagonistic condition (auxiliary metabolites). They typically produce these mixes in modest quantities that are required for their own advantage. The appearance of recombinant DNA innovation (likewise alluded to as quality cloning or in vitro hereditary control) has drastically widened the range of microbial hereditary controls. With the progression of recombinant DNA innovation, numerous novel host frameworks have been investigated to create industrially significant items like restorative proteins, anti-toxins, little particles, and biosimilars and so on.

KEYWORDS: Bacteria, rDNA, Re-tool engineer, secondary metabolites, Protein, antibiotic, Enzymes.

INTRODUCTION

Bacteria are a prokaryotic organism which belongs to the monera kingdom of system (R.H. Whittaker, 1969) and to the domain archea (old type bacteria) and bacteria (true bacteria) by the 6 kingdom and 3 domain systems of woes 1990. In ancient time chemoautotroph came to the earth and they have converted reducing environment of the earth to the oxidative environment and these were cyanobacteria. The microbial world has explored with

discovery of microscope. (Galileo Galilei, 1610) prepared microscope with true focusing device and term microscope (Faber, 1625) with the crude type of microscope of their own design there was a scientist known as Antoine van Leuwenhoek who have observed microorganism (bacteria also) and nomenclature them as "Animalcules" or Dierkens, then in 1838 a German scientist C.G.Eherenberg coined the term bacteria.

Table 1: Basic characteristics of some bacteria.

Bacteria	Size	Shape	Stain	Respiration	Nutrition	Motility
<i>E.coli</i>	0.5 micrometer in width and 2 micrometer in length	Rod shape	Gram negative	Facultative anaerobic	Heterotrophic	motile
<i>S. typhi</i>	2-5microns in length and 0.5-1.5 in width	Rod shaped	Gram negative	Facultative anaerobic	Heterotrophic	motile
<i>C. botulinum</i>	3 to 7 microns	Rod shape	Gram positive	Obligate anaerobic	Heterotrophic	motile
<i>C. perfringes</i>	4 to 8 in length and 0.8 to 1.5 in diameter	Rod shape	gram positive	Obligate anaerobic	Heterotrophic	Non motile
<i>M.tuberculosis</i>	Obligate aerobic	rod shape	Acid fast stain	Obligate aerobic	Heterotrophic	Non motile
<i>Nocardia</i>	Obligate aerobic	Rod shape	Gram positive	Obligate aerobic	Heterotrophic	Non motile

*Bergey's Manual of Microbiology

Bacterial characteristic

The characteristic of bacteria they are obligatory aerobic and aerobic; facultative aerobic; and anaerobic due to the nutrition mode of bacteria a number of way which are autotrophic those who obtained energy from the chemical reaction or from environment to be known as chemoautotroph; *Nitrococcus* (Nitrogen bacteria); *Ferro bacillus* (iron bacteria); *Hydroxymonas* (Hydrogen bacteria) respectively. *Lithoautotrophic* bacteria which obtain energy from the sunlight are *Rhodobacillus* and *Thiobacillus*. Those bacteria which obtains nutrition from any other source instead of synthesizing it are classified into three different types namely Saprotrophs (*Pseudomonas denitrificans*), Symbiotic (*Rhizobium japonicum*), Parasitic (*Vibrio cholera*. In the Case of reproduction diversification occurs most of the time bacteria reproduce asexually but under favourable conditions they undergoes sexual reproduction as well as sexual reproduction which is found in the bacteria is not an genuine sexual reproduction but it is just a transformation of the genetic material.

Reproduction

Sexual reproduction sexual reproduction undergoes with in bacteria's by Transformation(Griffith 1928 in mice using *Diplococcus pneumoniae*) Griffith concluded that the R-strain bacteria which was non pathogenic when injected to the mice with heat killed pathogenic S-strain bacteria result of this experiment was death of the mice due to the transformation of the R-strain non pathogenic bacteria to the S-strain pathogenic bacteria then (McCarty,1944), Avery, MacLeod proved this was due to DNA, Transduction (Zender and Lederberg 1952 in *salmonella*) is the process in which foreign DNA is introduced in a cell by a virus and vector, Conjugation (Lederberg and Tatum 1946 *E.coli*) is the process which have pilus transfer their DNA to the other cell, asexual reproduction mostly done by Budding by the formation of the buds; Fission bacteria reproduce by the binary fission but in adverse condition provides bacteria to forms endospore cortex layer of the endospore there is a chemical known as calcium dipicolic-aminopimelic acid.

Table 2: Morphological and Chemical structure of bacteria.

STRUCTURE	MADE UP OF	FUNCTION	REFERENCE
Cell wall of peptidoglycan	NAG (n-acetyl glucosamine) and NAM (n-acetyl muramic acid)	Structure to the cell and provide protection	Bergey's Manual of Microbiology
Flagella	Flagellin protein	Helps in motility	Bergey's Manual of Microbiology
Pili	Pilin	Helps in the conjugation	Bergey's Manual of Microbiology
Mesosome	By the infolding of cell wall	This is mitochondria of the bacterial cell	Bergey's Manual of Microbiology

The morphology, biochemical characterization, physiology, Molecular approaches and application currently made bacteria as tool engineer and needed to be re-engineering in their tool make it more compatible for the biotechnological field and many products useful for human mankind.

Molecular Approaches

The processes for recognition and broken down deoxynucleotide sequences into small parts with restriction endonucleases enzyme like *EcoRI* obtained from *E.coli*. and then it is linked with the plasmid (Extra chromosomal DNA) utilizing with ligase enzymes injected into an animal cell or bacteria cell, via cell division injected along with plasmid they divides and increases in number then, it produces novel characteristics in the cell of bacteria. Molecular biology is a technique to provided mechanistic approaches towards the bacteria as tool engineer.

Plasmids take part in a major role in microbial ecology and evolution as carrier of lateral gene transfer and storages of accessory gene functions in microbial populations. Examples are the involvement of plasmids to speedy adaptation in growth preventive conditions e.g., in the presence of antibiotics or pesticides (San Millan et, al 2018; Gullberg, E et, al 2014 and

Rozwandowicz, M. et al 2018) or to long-term transitions in bacterial lifestyle e.g., pathogenicity (Bruto, M. et al, 2017) or an oxygenic photosynthesis (Brinkmann, H, 2018). Plasmid functions can benefit their bacterial hosts; on the other hand, plasmid replication and gene expression utilizing the host machinery are considered a metabolic burden. *pBR324*, *pBR194*, *pBR32*; BT cotton– *Bacillus thuringensis*, BT Brinjal. Enzymes from bacteria 1963;enzyme were discovered in *E.coli*,the first enzyme was known to link DNA with ligase and other one was known to cut the strand of DNA(restriction endonucleases). *Hind-II* and *Hind-III* are restriction endonucleases enzymes are used in recombinant DNA technology; Bacterial antibiotics Bacterial antibiotics are the chemical substance secreted by a bacteria and poisonous for other microorganisms. Bacterial Antibiotics are the substances or chemical substances secreted by the bacteria and toxic for the other microorganism as well as to other bacteria.

Table 3: Bacterial Antibiotics and their uses.

Bacteria	Antibiotic	Uses
<i>Streptomyces erythraeus</i>	Erythromycin	Used to treat infections caused by bacteria.
<i>Streptomyces venezuelae</i>	Chloramphenicol	Used to treat meningitis, plague, and cholera.
<i>Streptomyces rimosus</i>	Oxytetracycline	Used to treat infection caused he Chlamydia and the mycoplasma organisms.
<i>Streptomyces fradiae</i>	Neomycin	Used to reduce the risk of infection during surgery.
<i>Streptomyces kanamycus</i>	Streptomycin	Used to kill the organism (mycobacterium) that causes infections.
<i>Bacillus subtilis</i>	Bacitracin	Used to prevent minor skin infection caused by small cuts, scrapes, or burns.
<i>Amycolatopsis orientalis</i>	Vancomycin	Used to treat an infection of the small intestine caused clostridium difficile.
<i>Streptomyces niveus</i>	Novobiocin	Used to treat infections by gram-positive bacteria.
<i>Streptomyces noursei</i>	Nystatin	Antifungal antibiotic.
<i>Paenibacillus polymyxa</i>	Polymyxins	To treat infections.

Biotransformation production of hormones steroids

Humulin, (human insulin) 1983, Eli lill an American company manufactured 2 DNA sequence, which was resembling to the alpha and beta chain of insulin after that these two strand or sequence were linked with the plasmid of the bacteria and then it is inserted to the *E.coli* bacteria to produce hormone and it was Humulin.

Microbial assay to determine potency as well pharmacokinetic parameter of drug with use of the simple or complex compound and a microorganism is known as microbial assay.

The utilization of protein biopharmaceuticals in human wellbeing dates from the nineteenth century with the utilization of diphtheria antibody therapy. The antitoxin comprises of immunoglobulins removed from the serum of inoculated creatures that perceive and kill the poison (e.g., pony or sheep). (Park WH et al. 1989; Baxter AG, 2007) actually, a few immunizing agents are accessible to treat evenomation by snakes, scorpions, and wasps, or contaminations. Be that as it may, the utilization of non-human creature antibodies can make touchiness of the patient the creature serum, which is known as serum ailment. (Igea JM., 2013). The twentieth century encountered the utilization of a few molecules coming from creature sources, for example, insulin, development

hormone (GH), glucagon, and asparaginase. (Graf CJ et al, 1999; Lopes AM et al, 2015; Syed YY et al, 2015). However, the discovery of the prion illnesses identified with the organization of hGH revealed another potential hazard related with non-human animal proteins.

This strengthened the requirement for the production of protein pharmaceuticals from different sources. (Powell-Jackson J, 1985)At this time, the biopharmaceutical business took a gander at heterologous expression of protein sedates by methods for recombinant DNA techniques in microorganisms. (Demain AL, 2009). With the advances of atomic science and recombinant DNA, human proteins could be gotten by heterologous expression utilizing *Escherichia coli*, just as other microscopic organisms. The classic model is human insulin, which is utilized to treat diabetes mellitus types I and II (DMI and DMII). At first, insulin was cleaned from the concentrates of ox-like and porcine dish crease. Notwithstanding, the procedure was costly and numerous cases of immune reactions brought about by creature insulin in patients were reported in study of Rosenfeld L, 2002; Leader B et al, 2008. Some example of bacteria(test microorganism) which are used in the assay of corresponding antibiotic along with their identification number in the American type culture collection are described in the table given below.

Table 4: ATCC strain used for Antibiotic formation.

ANTIBIOTIC	BACTERIA	ATCC NO.
Amikacin	<i>Staphylococcus aureus</i>	29737
Bacitracin	<i>Micrococcus luteus</i>	10224
Bleomycin	<i>Mycobacterium smegmatis</i>	607

Sterilization evaluation is an absolute process by which we kill microorganism. In this process we use a number of methods with their corresponding equipment, before

use of such methods their evaluation is important which can be done with use bacteria.

Table 5: Sterilization method for Bio-safety level measurement.

STERILIZATION METHOD	BACTERIA USED FOR VALIDATION
Filtration	<i>Pseudomonas</i>
Dry heat sterilization	<i>Bacillus subtilis</i>
Steam sterilization	<i>Bacillus stercorophilus</i>
Radiation sterilization	<i>Bacillus pumilus</i>
Gaseous sterilization	<i>Salmonella typhi</i>

As per bio safety level I, II, III,

Recombinant DNA and their recent application

Transgenic animals are those which are made by doing some changes in the DNA or genetic material of the animal, this can be done by using bacterial plasmid as a vector in the process of the recombinant DNA technology i.e. Dolly sheep, rosi (a cow), mouse (*Mus musculus*) Genetically modified mice are used extensible in the research as models for human disease.

Two new species of bacteria have been found in the blood of the patients of china, the bacteria were found in the blood of two human patients during blood tests as part of routine medical care. The new bacteria, both of which are in the *enterobacter* genus were found to be resistant to multiple antibiotics. *Enterobacter* are not usually harmful and exist as a part of the healthy gut microflora. However, when these bacteria enter the blood stream, respiratory system or the urinary tract they can cause disease. *Enterobacter* infection in the blood can lead to disease including meningitis and *bacteraemia*, and *enterobacter* in the lungs can lead to *pneumonia* and *Bacteraemia* can lead to serious condition including sepsis septic shock. The researcher named the newly discovered species *Enterobactor huaxiensis* and *Enterobactor chuadaensis* the bacteria were named after the region in the were discovered in Sichuan University, where authors are based. (Wenjing Wu et al, 2019) The discovery of both bacteria was reported in the “International journal of systematic and the evolutionary microbiology” along with the description of the new species and their antimicrobial profiles.

The resistance of the newly discovered bacteria to the antibiotics is a concern, as this would make the treatment of the life threatening more difficult. Both new species are resistant to the penicillin and cephalosporin group antibiotic groups are commonly used to treat bacterial infections. Bacterial infection such as *bacteraemia* is usually with the frontline antibiotics and a delay in treatment could cause progression to the sepsis. Sepsis has a high mortality rate and so it is vital this condition is treated quickly.

Recent studies have connected the piece of these microbial networks with changes in the host's wellbeing, conduct, and advancement, yet the causative go between of host-microbiota associations remain ineffectively comprehended. Advances in comprehension and designing these connections require the improvement of hereditary devices to test the atomic cooperation driving the structure and capacity of microbial networks just as

their communications with their host. This survey talks about the present difficulties to rendering culturable, non-model individuals from microbial networks hereditarily tractable- - including defeating hindrances to DNA conveyance, accomplishing unsurprising quality articulation, and applying CRISPR-based instruments - and subtleties late endeavours to make summed up pipelines that streamline and speed up the device improvement process. (Matthew C. Waller et al, 2017) utilize the microscopic organisms present in the human gastrointestinal tract as delegate microbiota to represent a portion of the ongoing accomplishments and future open doors for hereditary apparatus improvement.

The predominant utilization of anti-microbial and the spread of MDR microorganisms raise the possibility of a post-anti-infection time, which underscores the requirement for creating novel therapeutics to target MDR pathogens. The improvement of empowering SB innovations offers promising answers for conveys protected and successful antibacterial treatments. (Krishnamurthy, M., Moore, R.T., Rajamani, S. et al. 2016).

Ongoing advances in manufactured science and natural framework building have permitted the structure and development of built live bio therapeutics focusing on a scope of human clinical applications. Right now, layout how frameworks approaches have been utilized to move from basic constitutive frameworks, where a solitary helpful particle is communicated, to frameworks that consolidate detecting of the in vivo condition, input, calculation, and bio containment. Danino plot models where every one of these capacities are accomplished in various human issue, including malignancy, irritation, and metabolic infection, in various situations, including the gastrointestinal tract, the liver, and the oral depression. All through, we feature the difficulties of creating microbial therapeutics that are both touchy and explicit. At long last, we examine how these frameworks are prompting the acknowledgment of built live bio therapeutics in the facility. (Tal Danino et al. 2018).

By controlling DNA, scientists are attempting to make microorganisms that, once ingested, work to treat an uncommon hereditary condition — an achievement in engineered science. The microscopic organisms are intended to treat an uncommon acquired malady called phenylketonuria, or PKU. Individuals with the condition must maintain a strategic distance from dietary protein in nourishments, for example, meat and cheddar, in light of

the fact that their bodies can't separate a result, an amino corrosive called phenylalanine.

A century prior, Alfred Nissle found that purposeful admission of specific strains of *Escherichia coli* could treat patients experiencing irresistible infections. From that point forward, one of these strains turned into the most regularly utilized probiotic *E. coli* in look into and was applied to an assortment of human conditions. Here, properties of that *E. coli* Nissle 1917 strain are contrasted and other financially accessible *E. coli* probiotic strains, with accentuation on their human applications. A writing search framed the premise of a synopsis of research discoveries revealed for the probiotic Metaphor, Symbioflor, and Colinfant. The nearest family members of the strains in these items are introduced, and their hereditary substance, including the nearness of harmfulness, qualities is talked about. A similitude to pathogenic strains causing urinary tract diseases is perceptible. Memorable patterns in research of probiotic treatment for specific human conditions are distinguished. The eventual fate of probiotic *E. coli* may lay in what Alfred Nissle initially found: to treat gastrointestinal contaminations, which these days are frequently brought about by anti-infection safe pathogens. (Trudy M. Wassenaar 2016)

CONCLUSION

The character and application of bacteria on basis of the fact provided above interpretation of this point is about 100% that bacteria are a tool engineer. Maximum number of assays is performed for the diagnosis of various kind of the disease and also for the research in medical science; pharmaceutical science is about pharmacy where production and research on the formulation (Medicine) takes place .when a new formulation is manufactured, determination of its potency and determination of its pharmacokinetic parameter is important, for this regards microbial assay are performed with use of the microorganism; insertion of the bacterial plasmid to the plant we can make it resistant to the other microorganism and this will lead increase in the growth of the plant and also to its yield even in the complicated conditions thus, bacteria acts a tool engineer in the agriculture field also; rapid ability of adopting an environment and this is the region why day by day bacteria is modifying to itself.

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