

Gene Transfer Technologies to Produce Cyanobacteria

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ABSTRACT

To decipher genetic information, molecular biology has equipped new tools used in trials to improve and reconstruct the evolution and taxonomy of organisms. The avail of molecular methods, in Cyanobacteria, is to study the genotypic relations is a foot. The practicality of macromolecular and chemotaxonomic techniques is scrutinized and their usefulness is guesstimated. The controversial taxonomic problems were solved by using cyanobacterial phylogenetic scheme is based on 16Sr RNA sequence analysis. The morphological features and simple testing methods which are congruent with the genotypic groupings for taxonomic purposes are essential. Genetic engineering has proved to be an important tool in improving various metabolites in Cyanobacteria. It has also contributed in the genetic breeding of Cyanobacteria to obtain highly productive strains. The molecular biology aspects can also be applied to engineer the Cyanobacteria for enhancement in the area of biofuel production. For desirable biotechnological applications of Cyanobacteria, the Genetic tools are crucial.

INTRODUCTION

Cyanobacteria are the quintessential organisms annexed their energy from oxygenic photosynthesis for growth and development. They have uniquely accommodated both a photosynthetic electron transport chain and a respiratory electron transport chain within a single prokaryotic cell [1]. Cyanobacteria in addition are also capable of nitrogen fixation [2-4] these oxygenic phototrophic organisms are often called the bioenergetic 'nonplus –ultra' among living beings. For the growth and proliferation, they utilize, water, sunlight, atmospheric air and exiguous minerals for their needs. The stable accommodation of a cyanobacterium into a non-photosynthetic eukaryote was a milestone in the evolution of all other oxygenic photosynthetic organisms. During evolution, endosymbiosis, occurred once and complement the chloroplast origin [5,6]. This is the basis dogma reason for the voluminous use of Cyanobacteria to cogitate the photosynthesis fundamental processes and other photosynthetic complexes structures [7-11]. Cyanobacteria are chop-chop growing prokaryotic organisms, which can facilely be genetically modified. Cyanobacterium *Synechocystis sp.* PCC 6803 genome sequencing [12], a model organism for the research into photosynthesis and related processes was pondering work. This would not have been possible without the entry of techniques and genetic tools to aid mutational experiments. For the analysis of various aspects of cyanobacterial physiology and development, colossal studies have been made in developing genetic systems. Transformation, electroporation and conjugation systems sustain trenchant gene transfer in disparate cyanobacterial strains. For the precocious studies of cyanobacterial photosynthesis, nitrogen fixation, heterocyst development and metabolism, gene transfer, mingled with the bequest to clone and inactivate genes in these unique and important prokaryotic microorganisms. Almost ten years ago the first conjugation system for Cyanobacteria was described. There are numerous sophisticated gene transfer techniques were developed, cyanobacterial plasmids were isolated, vectors were constructed and the first cyanobacterial genes were cloned. For the genetic analysis of Cyanobacteria, the constituents for assembly into powerful and sophisticated systems were ready by

the late 1980's. Many excellent reviews over the last few years have depicted the progression of many facet of genetic systems in Cyanobacteria [13-18] and several articles have reviewed and described methodologies [19-25]. Extensive tables compiled by [14,26,27] provide valuable information on strain designations, restriction endonucleases, and cyanobacterial plasmids and cloning vectors. In addition, several laboratories are quest of biotechnological pertinence of Cyanobacteria in terms of hydrogen or biofuel production [28-30] or exploitation of interesting pigments or bioactive compounds from Cyanobacteria [31,32]. Transposons having reporter gene can concurrently subdue a gene and report expression of the interrupted gene afford a powerful tool for the identification of environmentally regulated genes. The technique of pulsed-field gel electrophoresis, combined with Southern hybridization analysis using cloned cyanobacterial genes and transposon-tagged as probes, has allowed the construction of the first detailed maps of cyanobacterial chromosomes. Since the available data concerning genetic manipulation systems for many other Cyanobacteria – unicellular as well as filamentous strains- are huge, this review concentrates on the current state of art and on potential applications.

Photosynthetic Microorganisms

Photosynthetic microorganisms are able to produce a multifarious pattern of renewable biochemical products. Cyanobacteria and some microalgae are considered as photosynthetic, auspicious pothunters for many implied applications includes biomass (food supplements and aquaculture feed), environmental applications (biofuels, waste water treatment, CO₂ mollification), high value products production (pigments, polyunsaturated fatty acids, vitamins) [33-37]. These photosynthetic microorganisms boost the need for steadfast, efficacious and economical processes with constant quality products. Cyanobacteria, being photosynthetic organisms, use the sun's energy, H₂O and CO₂ to synthesize their energy storage compounds i.e. carbohydrates, lipids and proteins. These energy storage components from a potential feedstock which can be converted into bioenergy. The main precursors of Cyanobacteria are only sunlight, carbon dioxide, water and minimal nutrients for growth, eliminating the cost of carbon sources and complex growth media. Sunlight is the most without demur available and dirt-cheap resource on earth and the use of cyanobacteria for the production of profitable products and biofuels from solar energy offers a verduous path for the synthesis process. Cyanobacteria decked with photosynthetic capabilities, have higher photosynthesis and biomass production rates compared to plants, convert upto 3-9% of the solar energy into biomass to collate ≤ 0.25 -3% achieved by crops for instance corn, sugarcane [38]. As the need of cyanobacteria increases, continous production systems are attracting interest, according to many authors these systems are the most achievable and successful for the large scale production of the photosynthetic microbes mainly due to decrease of labour costs, lower investments, operational costs and decrease of unprofitable periods [39-45]. Cyanobacteria are ancient autotrophs, responsible for increasing the levels of oxygen and CO₂ mitigation in Earth's atmosphere as they share many similarities with the higher plants and green algae chloroplasts but differs in biosynthetically [46-50]. Cyanobacteria store glycogen instead of starch [51-53] under stress conditions they accumulate polyhydroxybutyrate from acetyl COA [54]. The interest of bioenergy from Cyanobacteria is gaining popularity in research communities focused on photosynthesis, genetic engineering of growth systems and metabolic pathways [55-62]. An avant-garde cyanobacterial cultivation systems has been developed for scaling up practices as well as strain characterization [54]. Cyanobacteria include unicellular and filamentous forms and vary from spherical, oval, fusiform, rod –like to irregular in shape [63]. Cell size ranges from 0.5 μ m to 60 μ m. They are Gram negative has a peptidoglycan cell wall and sandwiched between cytoplasmic and outer membranes [64]. Cyanobacteria, a prokaryote do not have nuclear envelope or a true nucleus, but rather have a nucleoid. The cytoplasm contains the photosynthetic apparatus, called thylakoids, which contains the phycobilisomes [65]. Cyanobacteria are the only members of the domain Bacteria with the ability of oxygenic phototsynthesis. They possess photosynthetic apparatus with two photosystems (PSI and PSII) each with a unique reaction centre (RC) and chlorophyll a (Chl a) and phycobilisomes, which consists of phycobilins covalently bound to phycobilli proteins as peculiar light harvesting systems. In contrast to Cyanobacteria many prochlorophytes contain Chl a and Chl b and lack phycobilins [66,67] and the Cyanobacterium *Acayochloris marina* harvests far-red light with Chl d for photosynthesis underneath minute coral reef invertebrates [68]. Nitrogen fixation was carried out by most of Cyanobacteria [69]. Many filamentous but some unicellular Cyanobacteria move by gliding motility directed by light i.e. phototaxis [70-73]. Cyanobacteria are monophyletic but morphologically and physiologically diverse. They were one of the earliest organisms on this planet and they played a key role in the formation of atmospheric oxygen [74,75]. The early occurrence of Cyanobacteria on the Earth has been concluded from molecular-phylogenetic analysis [67,76-78] studies on the rise of atmospheric oxygen [79] and micro-paleontological investigations [80-82]. Recent research fields indicate that the oxygenic photosynthesis by Cyanobacteria originated about 2,340 million years before present (Ma). Assumably, anoxygenic photosynthesis with one photosystem was a precursror of oxygenic photosynthesis with two photosystems. The three major lineages of bacteria (actinobacteria, Deinococcus and Cyanobacteria) contributed to an early colonization of land [83]. The geological and geochemical data analysis showed that the Cyanobacteria or their ancestors originated in the Archean eon, approximately 2,700 Ma. The increase in the oxygen level after the beginning of oxidative photosynthesis remained very low, about 10⁻⁵ of the present atmospheric level for about 400 million years [84]. (Figure 1).

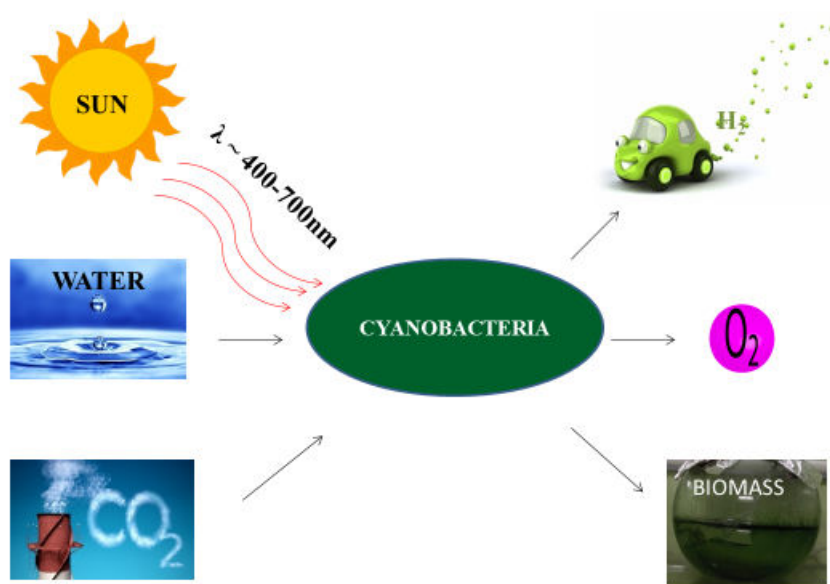


Figure 1: Physiology of Cyanobacteria

Need for Gene Transfer

Cyanobacteria possess genes responsible for the production of bio hydrogen, which is a forethought as an alternate source of energy [85]. Subsistence of their facile, autotrophic growth requirements, Cyanobacteria are promising organisms for bio-fuel production (ethanol) by genetic engineering to scale down the dependency on the depleting fossil fuel reserves [62]. They are also helpful for controlling mosquito borne diseases by expressing insecticidal cry proteins from *Bacillus thuriengensis ssp. israelensis* (Bti) [86]. In order to get the overall triumphant benefits from cyanobacteria in an eco-friendly manner, receptiveness of genetic manipulation to strains is necessary which involves the transfer of genes in a living cell (transformation) both chromosomal and plasmid transformation are possible with Cyanobacteria [87]. Rapidly growing demand and development of bio energy from Cyanobacteria and the profitable production of cosmetics and pharmaceuticals from cyanobacterial natural products, the genetic engineering of Cyanobacteria has been magnetizing to boost up the attention to overcome the biomass problems in industrial applications [88] to modify the metabolic pathway for high value products [89], to architect the bio-bricks for artificial photoautotroph in the promising field of synthetic biology [90,91].

Advantages of Genetically Engineered Microorganisms

During nitrogen deprivation, a glycogen-deficient mutant, *Synechocystis* 6803 (ADP- glucose pyrophosphorylase knock out) secretes pyruvate and α - ketoglutarate. These sugars and organic acids are converted into alcohols for biofuels [92,93]. Sucrose, glucose, fructose, glycosyl- glycerate and lactate export from transgenic Cyanobacteria has also been achieved [94-97]. Controlled parameters ranging from light and nutrient availability to salinity, temperature and pH can be used to find maximal productivity, suspend the cells at a particular doubling rate or determine the limits of cell viability under stress among many other applications [98-100]. The heterologous expression of a plant TPS in a photosynthetic microbe was described in *Synechocystis sp.* PCC 6803 upon transformation with the *Pueraria montana* isoprene synthetase gene [101]. Isoprene (C₅H₈), a volatile hemiterpene product was synthesized at a rate of 4 μg isoprene L⁻¹h⁻¹ [102] similar results were seen in *Synechocystis* successfully transformed with the β - caryophyllene synthase from *Artemisia annua* [103] and the β -phellandrene synthase from *Lavandular angustifolia* [104] permitting accumulation of the sesquiterpene β - caryophyllene and the monoterpene β - phellandrene. All three TPSs were cloned into the *Synechocystis* genome at the psbA2 locus via double homologous recombination, with expression in a light dependent manner [101]. In recent years, the expression of genes in *Synechococcus sp.* strain PCC 7942 was achieved the human carbonic anhydrase gene call used to investigate CO₂ concentrating mechanisms [105] *E.coli* and human superoxide dismutase genes used to investigate oxidative stress [106,107], *E.coli* pet genes used to increase salt stress resistance [108] and *Bacillus thureingensis* larvicidal genes used to develop bioinsecticidal hosts [109,110] are expressed in *Synechococcus sp.* at high levels to produce palpable phenotypes. The genes *pdc* and *adh* from *Zymomonas mobilis* were cloned into

shuttle vector and transformed into *Synechococcus sp.* strain PCC 7942, a significant amount of ethanol was accumulated in the culture medium [111]. Ethanol production from Cyanobacteria have been significantly increased [112,113]. Many enzymes have displayed lowered activity when transferred into Cyanobacteria results in limited production [114,115]. To mitigate the problems of greenhouse gases, renewable strategies for algal recycling of nitrogen have been proposed [116]. The possibilities for engineering the atmospheric nitrogen fixing cyanobacterial strains as production systems to be scrutinized (Table 1) [117,118].

Table 1: Bioproducts produced form Cyanobacteria

S.No	Name of the Cyanobacteria	Product	References
1	<i>Synechocystis sp.</i> PCC 6803	Ethanol production by introducing <i>pdh</i> & <i>adh</i> genes from <i>Zymomonas mobilis</i>	[202]
2	<i>Synechococcus elongatus</i> PCC 7942	Isobutyraldehyde & Isobutanol	[203]
3	<i>Anabena, Aphanocapsa, Calothrix, Microcystis, Nostoc</i> and <i>Oscillatoria</i>	Biohydrogen	[204-206]
4	<i>Synechocystis sp.</i> PCC 7002	Hydrogen	[207]
5	<i>S. elongatus</i> PCC 7942	Hydrogen	[205]
6	<i>Synechococcus sp.</i> PCC 7002	Alkane production	[208]
7	<i>Synechocystis sp.</i>	Fatty acids and alkane	[209]
8	<i>Synechococcus sp.</i> NKBG 150 41c	Alkanes and α -olefins	[210]
9	<i>Spirulina</i>	Vitamin B12, β -Carotene, Thiamine and Riboflavin	[211]
10	<i>Synechocystis sp.</i> PCC 6803	Isoprene production	[212]
11	<i>Aphanotheca sp.</i>	Polyhydroxyalkanoate(PHA)	[213]
12	<i>Oscillatoria limosa</i>	PHA	[214]
13	<i>Spirulina sp</i>	PHA	[215]
14	<i>Synechocystis sp.</i> PCC 6803	PHA	[216]
15	<i>Synechocystis sp.</i> PCC 6803	PHA	[217]
16	<i>Synechocystis sp.</i> PCC 6803	(S)-and(R) -3-hydroxy butyrate	[218]
17	<i>S. elongatus sp.</i> PCC 6301	Isobutanol	[219]
18	<i>Synechocystis sp.</i> PCC 6803	PHA	[220]
19	<i>Synechococcus sp.</i>	Glutamate	[221]
20	<i>Nostoc, Arthospira, Aphanizomenon</i>	Food	[222]
21	<i>Synechocystis sp.</i> PCC 6803	Isoprene	[223]
22	<i>Synechococcus elongatus</i>	Glucose, Fructose mixture and lactic acid	[224]
23	<i>Symploca sp.</i>	Bioactive compounds Dolastatin 10	[225]
24	<i>Synechococcus elongatus</i>	Production of 2,3-butanediol	[226]
25	<i>Synechococcus sp.</i> PCC 6308	Extracellular slime by transformation	[227]

Vectors

Many common cloning vectors based on pBR322 i.e., the pUC series of vectors beyond pUC7 and p-Bluescript) have lost the *oriT* (bom) site and cannot be used for conjugation; however, plasmids with a variety of useful features for conjugation to *Anabena sp.* and other cyanobacteria have been created [87,119,120,121]. There is no evidence that most *E.coli* replicons function in cyanobacteria . It appears, however, that IncQ plasmids (pKT210 or pKT230) [122] can transfer

to and replicate in some cyanobacteria after mobilization by the broad-host-range conjugal plasmid, RP4 [123-125]. A mobilizable plasmid based on pBR325 that contains the oriT from an IncP plasmid has been divulged to transfer to and replicate in *Plectonema boryanum* strain UTEX 594 (Table 2-3) [126].

Expression Vectors and Shuttle Vectors

The first cyanobacterial expression vector, pFC1, based on λ regulatory signals, which provide temperature-controlled gene expression in S.7942 [127] or S.6803 [128] and on the conjugative plasmid RSF1010 [129], which facilitates autonomous replication in *Synechocystis* sp. strains PCC6803 and PCC6714 or *Synechococcus* sp. strains PCC7942 and PCC6301 [130]. A powerful substitute vector pMB13 for pFC1, produce proteolytically instable proteins. These vectors are used in *in vivo* studies of Cyanobacteria [131]. The replicating shuttle vectors include two replicons: one that allows replication of the plasmid in *E. coli* and one that allows replication in the host strain. These vectors have been constructed by cloning into a mobilizable *E. coli* plasmid i.e., a plasmid with oriT site, a segment of a cyanobacterial plasmid that includes the genes required for replication. A variety of shuttle vectors for transformation have been constructed using plasmids from unicellular Cyanobacteria. These plasmids typically lack oriT and thus cannot be mobilized [16,20] however mobilizable shuttle vectors that replicate in several unicellular or filamentous Cyanobacteria are available [16,20,119,132-134]. Vectors that include the replication origin from pDU1, a plasmid from *Nostoc* sp. strain PCC 7524 [135-137] allow autonomous replication in several strains of Cyanobacteria [87,119,138,139].

Reporter Genes and Marker Genes

The stability of the transformed foreign gene, the efficiency of expression and frequency of transformation was confined by reporter gene. It is also used to determine the expressed protein and its locality in the transformed cells. GUS and lacZ are the extensively used reporter genes in marine algal transformation. The reporter marker genes subsuming chromogenic, fluorogenic and bioluminescence markers. GUS (β -glucuronidase gene from *E. coli*) system, which produces a fluorogenic product that can be detected at very low level. The reporter gene lacZ and luc expressed in *A. cylindrica* and *Synechocystis* sp. Cyanobacterial colonies carrying lacZ gene produce blue colour i.e, well differentiated from wild type green colour colonies. These *E. coli* promoters are well recognized by *A. cylindrica* and *Synechocystis* sp. [140]. Effective selection marker genes are important to contradistinguish the successful transformants from transformed cells. The selectable markers include two types, the genes conferring antibiotics of higher plants are commonly used in selection of marine algal transformants. The other type is homologous complementation of metabolic mutants, used for chloroplasts transformations. The lists of selectable markers in microalgal were compiled in past reviews [141-143]. The efforts have to be made for alternate markers and standardize marker free selection as there is shooting match in the biosafety [144].

Modes of Gene Transfer Technologies

Conjugation

Conjugation, which is DNA transfer mediated by cell-to-cell contact, is based upon the mobilization of DNA from one bacterium to another bacterium by a broad-host-range conjugative plasmid. Conjugation has been the method of choice for gene transfer in filamentous Cyanobacteria, but is also useful for unicellular Cyanobacteria [119,123,125]. The methodology, first described [119] and has been extensively reviewed [19-20,145]. Three plasmids are consistently concerned in conjugative transfer of DNA to Cyanobacteria. The plasmid to be transferred to the cyanobacterial host (cargoplasmid) must have a site called bom or oriT, that is nicked by an enzyme (the mob product) prior to transfer. The nicking enzyme is usually produced in trans by a second helper plasmid in the same donor cell.

The nicked strand is mobilized from the donor cell to recipient cell via transfer (tra) gene products provided by a third, mobilizing plasmid may be maintained in the same donor cell as the other two plasmids or it can be transferred to from a separate *E. coli* cell to cell containing the cargo and helper plasmids during conjugation. Maintenance of multiple plasmids in one strain requires that the plasmids be compatible i.e., that they have different replicons. In triparental matings, the conjugative plasmid is in one *E. coli* strain, the cargo and the helper plasmids are in a second strain and the third partner is the recipient cyanobacterial cell. The donor DNA is probably transferred as single-stranded DNA; a new strand is synthesized immediately by the host cell, probably during transfer. The transferred plasmid can re-circularize and replicate if the plasmid has a replicon that functions in the recipient cell. The transferred DNA may also recombine with homologous DNA in either the chromosome or in another plasmid in the recipient cell. Although transformation was also utilized for manipulation of filamentous strains, nowadays conjugation is the prevailing method to manipulation of Cyanobacteria genetically [119,146,147]. In addition, conjugation is commonly used for genetic manipulation of unicellular Cyanobacteria (Figure 2) [148, 149].

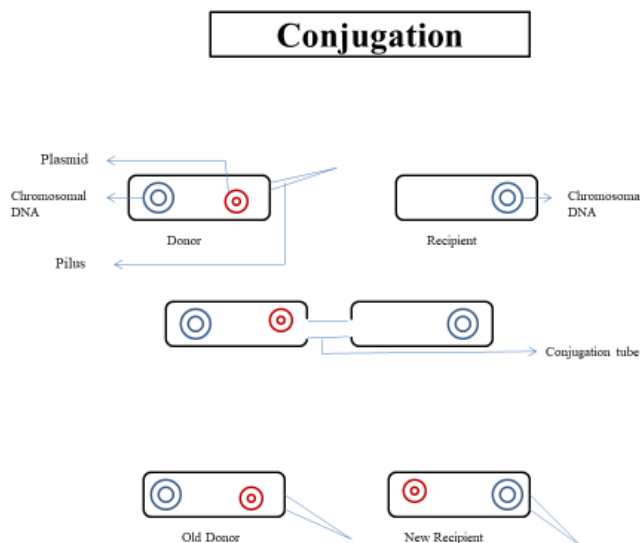


Figure 2: Conjugation in Cyanobacteria

Transformation

Transformation, which is the transfer of free DNA into cells, was first described for *Synechococcus sp.* strain PCC 7943 [150]. Many years ago and remains today the primary means for gene transfer in unicellular Cyanobacteria. Transformation has been thoroughly summarized [15,151] and the methodology has also been described well [152,153]. In addition to the original transformable strain, two close relatives, *Synechococcus sp.* strain PCC6301 [154] and *Synechococcus sp.* strain PCC 7942 [155], are also transformable. These three strains are genetically very kin [156-157]; however transformation has been studied in more detail in *Synechococcus sp.* strain PCC 7942 because it is highly transformable [154]. The bacterium *Synechococcus sp.* PCC 6803 has become a very attractive model organism since the publication of its genome sequence by [158]. This was the first complete genome sequence of photosynthetic organism thus raising not only cyanobacterial genetics and physiology to a higher level but also phototsynthesis research. As *Synechocystis sp.* PCC 6803 is able to grow mixotrophically on glucose with impaired photosystems I and II, several laboratories started to use this organism for the study of phototsynthesis in the eighties of the last century [159-162].

The mechanism of transformation in Cyanobacteria is below par understood; however most of these unicellular strains are naturally competent and the mechanism may claim some peculiarity with other transformable bacteria [151]. One strain in which transformation can be induced is *Synechocystis sp.* strain PCC 6308, which requires CaCl_2 treatment for competency. The ability of heterologous DNA to compete with homologous DNA for uptake in *Synechococcus sp.* strain PCC 7942 [154] and in *Synechococcus sp.* strain PCC 7002 [163] may imply a mechanism similar to that of transformable Gram positive heterotrophic bacteria. Cyanobacteria pop in to be competent during all phases of growth [150,164,165] however, cells are usually transformed during mid-to-late-exponential growth [151]. Transformation is dependent on DNA concentration; it shows single-hit kinetics, with full saturation at concentration as low as $1.0 \mu\text{g}.\text{ml}^{-1}$ in *Synechococcus sp.* strain PCC 7002 and as high as $50 \mu\text{g}.\text{ml}^{-1}$ in *Synechocystis sp.* strain PCC 6803 [151,164,165]. While transformation frequencies are variable between experiments in the same strain, typical values are 10^3 - $10^5 \mu\text{g}^{-1}$ of DNA [151]. Clear indications for a functional link between Tfp and natural competence this term refers to the ability of bacteria to take up extracellular DNA-were first provided by [166] and later by [167]. Many bacteria exhibiting Tfp indeed are naturally transformable [168] and this competence is often dependent on intact piliation, where upon in some cases, Tfp assembly factors rather than the pili structures itself are required for transformation [169]. For naturally transformable bacteria that exhibit Tfp on their surfaces, the presence of these appendages appears to be connected with competence [166,170-172]. Yoshihara et al. [172] suggested a fundamental role of type IV pili in the natural competence of unicellular Cyanobacteria. The gene product of pilB1 exhibits NTPase activity and is regarded as a pilus extension motor that is indispensable for pilus assembly (Figure 3) [173,174].

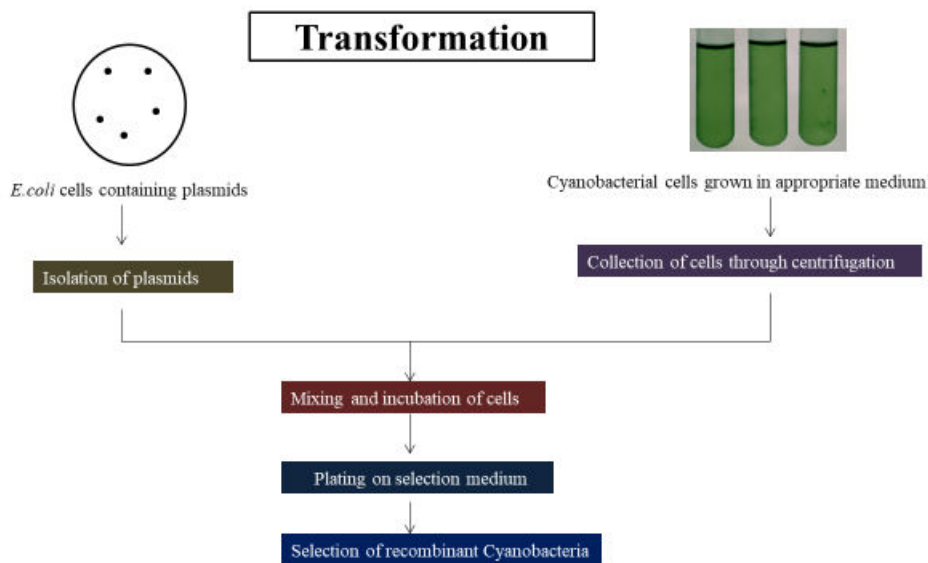


Figure 3: Transformation of Cyanobacteria

Electroporation

Electroporation has also been used to transform several Cyanobacteria, *Microcystis aeruginosa*, *Thermosynechoccus elongatus* BP⁻¹ or filamentous Cyanobacteria [175,176]. Electroporation has been used to introduce DNA into animal cells, plant cells and many bacteria including several Cyanobacteria. Optimum conditions for electroporation of a replicating plasmid in *Anabena nidulans* sp. strain M-131 were field strength of 8 Kv cm⁻¹ and a time constant of 5 ms⁻¹ [175]. As is true for conjugation, restriction of DNA is a significant problem; a single unmodified Avall site reduces transformation efficiency about 100-fold [175].

Electroporation has some advantages over conjugation: *E. coli* cells do not contaminate the transformants; vectors lacking a bom site may serve as donors; and electroporation requires only DNA and washed host cells. The ability to methylate the donor DNA *in vitro*, to produce linear plasmids for transfer (yielding exclusively double recombinants after integration into the chromosome) and to use chromosomal DNA as the donor are potential advantages of electroporation that have not yet been explored (Figure 4).

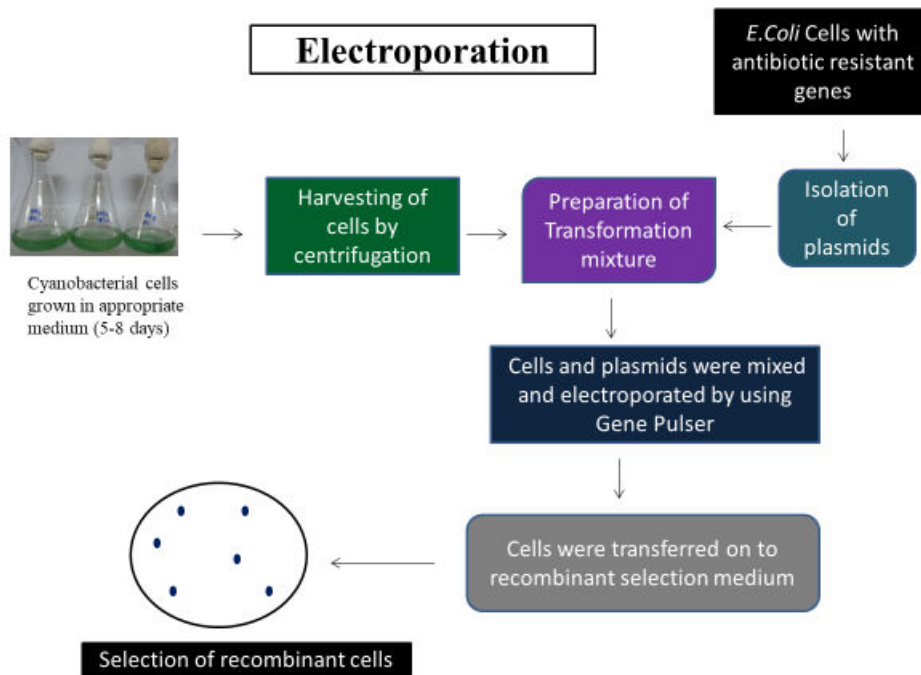


Figure 4: Electroporation of Cyanobacteria

Applications of Genetic Engineering

The genetic engineering offers the forecast asset and permits the introduction of numerous divergent desirable genes at a single event and rebate the time. The first report of transgenic plants [177] has been accelerated and used for practical ends of crop improvement. The plant genetic engineering practices involve mainly two important technologies cellular and molecular biology. *Bacillus thuringensis* engross world’s leading position as biopesticide, accounting for ca. 90% of biopesticide sales [178]. Gene encoding in sweet potato, trypsin inhibitor in transgenic tobacco results in severe growth retardation of *Spodoptera litura* caterpillars fed on it [179]. The osmolytes like mannitol fructans, proline, trehalose, ononitol production by genetic engineering increase resistance to drought but the mechanism of these osmolytes in protection yet to be discovered [180]. In transgenic tobacco plants the tolerance of salt stress and drought, due to over express of inositol methyl transferase gene (IMT1) from ice plant (*Mesembryanthemum crystallinum*) increased by the accumulation of methylated form of inositol, D-ononitol [181]. Mannitol, a photosynthetic product of higher plants and many algae aggrandize the tolerance to water-deficiency mainly through osmotic adjustments [182]. Mannitol dehydrogenase (mtlD) was introduced into wheat results in the increment of water stress tolerance [183]. The genes AtGolS1 and AtGolS2 shows tolerance to drought resistance due to accumulation of galactinol and raffinose, which are osmoprotectants in *Arabidopsis* plants [184]. Bacterial fructan gene was engineered in sugar beet and tobacco plants showed drought stress tolerance [185,186]. Many crops were genetically modified, implicates insect resistance, plant pathogen and herbicide resistance and also for slow ripening, seedless fruits and increased sweetness (Table 3) [187]. Soybean, potato, cotton, corn and canola occupies largest area of engineered crops [188,189]. The genes Adh and Pdc introduced into hairy roots of *Arabidopsis thaliana* to improve the low oxygen conditions [190]. The production of fatty alcohols in genetically engineered *E.coli* by fatty acyl Co-A reductases from Jojoba [191], mouse [192], *Arabidopsis thaliana* [193]. By using metaboloc engineering *E. coli* strains produce 1.6 mg zeaxanthin/g dry weight [194]. Many antibiotic resistant genes are successfully used for microalgae transformant selection, includes chloramphenicol [195], hygromycin [196], spectinomycin [197,198], streptomycin [198], paromomycin [199,200-256]. The biotechnological importance of *Spirogyra*, produces bioactive substances (Table 2).

Table 2: Different activities produced by Cyanobacteria

S.No	Name of the cyanobacteria	Activity	References
1	<i>Synechococcus</i>	Antibiotic resistance Ampicillin	[228]
2	<i>Weistiellopsis prolifca</i> ARM 365, <i>Hapalosiphon-hibernicus</i> ARM 178, <i>Nostoc muscorum</i> ARM 221, <i>Fischerella</i> sp. ARM 354 & <i>Scytonema</i> sp.	Antibacterial activity against <i>P.striata</i> , <i>B.subtilis</i> , <i>E.coli</i> & <i>Bradyrhizobium</i> sp.	[229]

3	<i>Anabena</i> sp.	Antibacterial activity against <i>S.aureus</i> , <i>E.coli</i> , <i>P.aureginosa</i> , <i>S.typhi</i> & <i>K. pneumonia</i>	[230]
4	<i>Nostoc commune</i>	Antibacterial activity (by <i>Noscomin</i> , diterpenoid compound) against <i>B. Cereus</i> , <i>S.epidermidis</i> & <i>E.coli</i>	[231]
5	<i>N. commune</i> <i>N. insulare</i> <i>Nodularia harveyana</i>	Antifungal activity (by nostofungicide) against <i>Aspergillus candidus</i> (norhormane 4,4'- dihydroxy biphenyl) against <i>Candida albicans</i>	[232,233]
6	<i>Nostoc ellipsosporum</i> <i>Scytonema varium</i> <i>Scytonema</i> sp.	Anti-viral activity (Cyanovirin-N, ScytovirinN, Sulfoglycolipid against HIV)	[234-236]
7	<i>A. quadruplicatum</i> PR-6	Insecticidal	[237]
8	<i>Spirulina maxima</i>	Thrives under alkaline (pH~ 11) & high salinity (1.2M Na ₂ CO ₃)	[238]
9	<i>Arthospira</i>	Skin care	[239]
10	<i>S. elongatus</i>	Ability to grow in absence of light	[240]
11	<i>Synechocystis</i> sp. PCC 6803	Tolerance to butanol	[241]
12	<i>Synechocystis</i> sp. PCC 6803	Tolerance to butanol	[242]
13	<i>Synechococcus</i> sp. NKBG 042902	Promote plant germination by transformation	[243]
14	<i>Synechococcus</i> sp. PCC 7002	Natural transformation	[244]
15	<i>Synechococcus</i> sp. NKBG042902 YG 1116	First marine unicellular cyanobacterium transformed by electroporation	[243]

Table 3: Biodegradation, remediation and absorption activity of Cyanobacteria

S.No	Name of the cyanobacteria	Degradation activity	References
1	<i>Synechocystis</i> sp. PUPCCC64, <i>Westiellopsis prolifica</i> , <i>Nostoc hatei</i> , <i>Anabena sphaerica</i>	Degrade organophosphorus, organo chlorine insecticides in aquactic environments	[244-246]
2	<i>Anabena doliolum</i>	Removes copper & iron	[247]
3	<i>Oscillatoria</i> , <i>Synechocystis</i> , <i>Pleurocapsa</i>	Degradation of petroleum compounds	[248]
4	<i>Synechococcus</i> sp.	CO ₂ removal	[249]
5	<i>Oscillatoria</i> sp.	UV absorption	[250]
6	<i>nidulans</i>	Removal of outer membrane by lysozyme treatment	[251]

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