Chapter 13

Escherichia coli as a Model Organism and Its Application in Biotechnology

Vargas-Maya Naurú Idalia and Franco Bernardo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67306

Abstract

Without a doubt, in the past 20 or so years, we have achieved the power of biology in different ways. In the present, we have many tools for developing novel technologies and applications for organism modifications that ultimately let us know many aspects of organisms' biology and, therefore, apply that knowledge for technological purposes. Of all the model organisms and tools for genetic modification available, *Escherichia coli* stands out as a model organism and what we would like to call "molecular biologist tool box." In the present chapter, we aim to review our current knowledge regarding genetic modifications and tools for modifying *E. coli* to generate plasmid vectors, single and multiple gene knockouts, whole genome editing, biosensor generation and applications and synthetic gene circuits and genomes.

Keywords: molecular biology tools, synthetic biology, biosensors, plasmids, bioengineerging

1. Introduction

Who in biology has not heard of *Escherichia coli*? Known to many as the fundamental model microbe and perhaps model organism, *E. coli* is the cornerstone of many important findings in molecular biology and other areas of cell physiology. Perhaps even the first chemo-organo-heterotroph had a similar mass composition as *E. coli*, providing the hits necessary to understand the evolution of modern bacteria [1]. Also called the "workhorse" of molecular biology for its fast growing rate in chemically defined media and extensive molecular tools available for different purposes, *E. coli* is considered the most important model organism of them all. Important findings and Nobel Prizes in biology have been developed in *E. coli*. For instance,

cracking the genetic code [2], unveiling the nature of DNA replication [3], the groundbreaking advances on gene organization and regulation or as we love to call 'the operon' [4, 5], important evidence for the basis of mutations and ultimately to the evolution of organisms [6, 7], and finally, the achievement of a genetically modified organism [8] that skyrocketed several applications of the enormous capacity for manipulating this organism, rendering *E. coli* as a key player in biotechnology (for an excellent review, see Ref. [9]).

The main reasons why *E. coli* is the organism of choice extends and is not limited to its fast growth in chemically defined media; relative cheap culture media; does not form aggregates; industrial scalability; several molecular tools for manipulation; extensive knowledge of its genetics and genomics; extensive knowledge on its transcriptome, proteome, and metabolome, and several strains are considered biosafety 1, which renders it ideal even for teaching and school demonstrations (for example, Bio-Rad transformation kits, URL: http://www.bio-rad.com/es-mx/category/pglo-plasmid-gfp-kits).

Typical *E. coli*, a Gram-negative bacillus bears a rod-shaped, measures only about 1 μm long by 0.35 μm wide, although this can vary considerably depending on the strain and its conditions, there are studies regarding different mutations that affect its size and length considerably. A recent paper showed that this bacterium can grow up to 750 μm in length. By random Tn10 insertion, they found this particular mutant has an insertion at genes *ybd*N and *ybd*M whose function remains uncertain and another mutation that remains to be identified [10]. Interestingly, this mutant strain gives rise to extremely long cells that are viable (capable of cell division) and retain metabolic activity that can be useful for studies involving intracellular localization or optimize cell-surface contact.

In terms of ecology, *E. coli* is a facultative aerobe (either respiration takes place in the presence of oxygen or fermentation in its absence), which bears a sensor for oxygen presence (redox state in the quinone pool) and can activate or repress the required metabolic enzymes, depending on oxygen levels [11–13]. Also, *E. coli* and other Enterobacteriaceae can be the first organisms to colonize human infant intestine due to this capacity to metabolize oxygen or ferment because this facultative bacteria will consume the remaining oxygen in this environment so that other strict anaerobic bacteria are capable of colonizing the intestine rendering the normal microbiota found in humans and also thrive as part of the normal microbiota [14].

Phylogenetically, *E. coli* is a member of the Enterobacteriaceae and is closely related to pathogens such as *Salmonella*, *Klebsiella*, *Serratia*, and the infamous *Yersinia pestis*, which causes plague. Although *E. coli* is mostly harmless, pathogenicity islands have been identified and associated with pathogenesis in *E. coli* resulting in strains that colonize different tissues [15].

The building blocks of *E. coli* consists of about 55% protein, 25% nucleic acids, 9% lipids, 6% cell wall, 2.5% glycogen, and 3% other metabolites [16–18], which for biotechnological applications is important, since carbon flux is often a problematic issue to address in order to generate a novel metabolic pathway or to enhance a current functioning pathway. Also, carbon flux is tightly regulated by sophisticated regulatory networks that require modeling and a basic understanding of the regulatory mechanisms in order to manipulate them and achieve desired goals [19–21].

http://dx.doi.org/10.5772/67306

E. coli is part of the normal microbiota of mammals, rendering the predominant facultative microbe of the gastrointestinal tract and is currently a hot debate on the impact on normal microflora establishment and their role in disease [22].

This organism lacks many interesting features for biotechnology, such as growing at extreme temperatures or pH, the capacity to degrade toxic compounds, pollutants, or difficult to degrade polymers [23]. But as we will see later, this bacterium is capable of doing amazing things and its only limit is our imagination.

E. coli harbors a genome with particular features such as a strikingly organized structure, remnents of many phages, and insertion sequences (IS) and a high transport capacity toward the cytoplasm. In 1997, the complete genome sequence of the K-12 strain was obtained, and a myriad of research was catapulted from that moment on [24]. The complete genome contains a single circular duplex molecule composed of 4,639,221 bp. Regarding its structure, protein-coding regions correspond to 87.8% of the genome, while 0.8% encodes for stable RNAs, and 0.7% consists of noncoding repeats. The remaining 11% encodes for regulatory and other functions. Nevertheless, nearly 34% (1431) proteins are considered orphan or without defined molecular function but in a recent study, it was demonstrated that by homology with distant phylogenetical relationships, they may play a role in defined molecular pathways or processes [25]. From the orphan set in *E. coli*, at least 446 contain some molecular signature that can assess their molecular role. The fact that such a vast portion of the genome remains uncharacterized for defined molecular pathways or roles paves the way for future basic research that may have a strong impact on applied research and development. For biotechnological applications, growth optimization is also a required feature of genetically modified organisms. For *E. coli*, it has been recently shown that growth can be manipulated by removing nonessential genomic sequences that also can lead to the design of important biotechnological strains for industrial purposes [26].

The advancement of genomic, transcriptomic, and proteomic technologies has led to the development of several online resources for the analysis of molecular and physiological aspects of *E. coli*. Some examples are given in **Table 1**.

Escherichia coli **databases**

Name	**URL**	**Description***
EcoCyc: Encyclopedia of *E. coli* Genes and Metabolic Pathways	https://ecocyc.org	A comprehensive database joining together genomic information with biochemical features of *E. coli*
PortEco	http://www.porteco.org	A resource for knowledge and data of the biology of *E. coli* including plasmids, mobile genetic elements, and phages
EcoliWiki	http://ecoliwiki.net/	Community-based pages about everything related to the biology of the nonpathogenic *E. coli*
EcoGen 3.0	http://ecogene.org/	A database dedicated to analyzing and comparing genomic and transcriptomic data

Escherichia coli **databases**		
Name	**URL**	**Description***
Kegg	http://www.genome.jp/kegg-bin/show_organism?org=eco	A powerful resource for understanding molecular datasets in different contexts, also easy access to gene information
RegulonDB	http://regulondb.ccg.unam.mx	Database on transcriptional regulation in *E. coli* K-12 containing knowledge manually curated from original scientific publications, complemented with high throughput datasets and comprehensive computational predictions
E. coli Genetic Stock Center	http://cgsc.biology.yale.edu/	The CGSC Database of *E. coli* genetic information includes genotypes and reference information for the strains in the CGSC collection. An excellent resource for acquiring information and strains for research

*Source: databases website.

Table 1. Relevant *E. coli* resources and databases.

Recently, using the Genome Conformation Capture technique, it was revealed that the linear organization of the genome is also true for the 3D structure, rendering neighboring genes to form small factories that are coregulated or coexpressed and showed a higher probability of forming protein-protein interactions. This organization represents two important aspects of bacterial genomes, first, the compactness in the 3D space, containing pathways in a nonrandom distribution, and second, genes that are closer to each other tend to be coexpressed and form protein-protein interactions favoring the concept of transcription factor even in microbial cells [27].

In the following sections, we will review what we consider the modern tools for genetic engineering *E. coli*, and the future for this microbe that can be considered the toolbox for molecular biology and may be the answer to many problems that humanity may face in the future.

2. Plasmid and the *E. coli* revolution

Without a doubt, plasmids are the most important tools not only for the manipulation of *E. coli* but also the foundation for the genetic engineering of many organisms, cloning and sequencing, generation of mutants, and many applications in molecular biology. In this section, a brief summary of the myriad of plasmids available will be addressed, providing some of the most important features of plasmids and the up-to-date technologies available for plasmid manipulation and application.

http://dx.doi.org/10.5772/67306

Why plasmids are the basis for genetic engineering? By surveying the literature and commercial sources catalogs, there is a myriad of applications for plasmids: cloning, mutagenesis, protein fusion and overexpression, shuttle vectors from bacteria to a diverse range of hosts, among others. Plasmids must be first presented and then we provide some features that are relevant regarding the importance of plasmids in molecular biology and biotechnology.

Plasmids are extrachromosomal molecules that are self-replicative and sometimes provide interesting features to its host. The term was first coined by Joshua Lederberg in 1952 referring to genetic elements in bacteria that remained as an independent molecule from the chromosome at any stage of their replication cycle [28]. The definition was further refined to all the autonomously replicating DNA molecules to avoid including viruses. These molecules are present not only in eubacteria but also are found in Archea and some lower eukaryotic organisms [29]. In nature, many bacteria contain self-replicating DNA molecules that can be harnessed for molecular biology applications. *E. coli* plasmids were the first ones to be extensively modified for such purposes [30].

In the 1970s, the first generation of cloning plasmids was created, and from that moment on, research in the biological area was enriched with a powerful tool. Plasmids must contain several important features to be used in research: proper size for ease to transform or transfect, selection markers, a replication origin, regulatory elements to control expression, and transcription termination. All features are important when designing a plasmid vector for the desired application, the reader can imagine the goal, and there will always be a way to create the molecular tool for achieving such a goal, and that is possible due to the basic structure of most plasmids used in molecular biology and their modularity [31]. In **Table 2**, we summarize some of the most important features (modules) that plasmids must have in order to serve for different applications. We point out that sequence composition and structure, copy number, selection marker, and special features such as reporter proteins or regulatory elements are the most important features in a plasmid and can influence the outcome of the desired application.

***Escherichia coli* plasmids**

Name	Type of element	Characteristics
ColE1	Replication origin	Generates 15–20 copies of each plasmid molecule. Colicin production. Related to plasmids that confer immunity to phage infections [32]. Found in low copy plasmids such as pBR322 [33]. There are mutations in this replication origin that leads to high copy number plasmids, such as pUC series that can render up to 700 copies per cell [34, 35]
p15A	Replication origin	Low copy number replication origin, estimated in 18–22 copies per cell [36]. This type of replication origin is often found in pACYC and its derivative vectors

Escherichia coli **plasmids**		
Name	**Type of element**	**Characteristics**
pMB1	Replication origin	Versatile replication origin. The original sequence generates 15–20 copies per cell, but a mutant version can lead up to 700 copies per cell [37]. This plasmid contains the *Eco*RI restriction-modification system
pSC101	Replication origin	Five copies per cell [38]
R6K	Replication origin	15–20 copies per cell. Requires the π protein from the gene *pir* for replication [39]. This origin of replication is functional in diverse bacterial species
Amp^r, Kan^r, Cm^r, Tet^r among other	Selection markers	Elements required for the selection and maintenance of plasmids in bacterial hosts. Here are listed the resistance cassettes for Ampicillin, Kanamycin, Chloramphenicol, and Tetracycline, which are the most common selection markers. For additional markers, RAC database contains the information regarding antibiotic resistance traits and their sequence [40] or iGEM website for sequence modules bearing the proper syntax for synthetic constructs
LacZ, CcdB, Green Fluorescent protein (GFP), etc	Additional elements required for positive clone selection, reporter protein fusions among others	Plasmids have been modified so that they contain multiple cloning sites with diverse unique restriction sites, counter selection for positive clone selection. Additional elements such as filamentous origins for single-stranded DNA generation for sequencing of high G+C templates or site-directed mutagenesis

Additional sources: [31], http://blog.addgene.org/plasmid-101-origin-of-replication. https://www.neb.com/, https://www.thermofisher.com/mx/es/home/brands/invitrogen.html, and http://parts.igem.org/Help:Synthetic_Biology, which contains a repository of parts that can be needed for plasmid construction using synthetic methods.

Table 2. Common elements in plasmids used in molecular biology applications.

Some exceptional features of plasmids are they can be used in systems where replication origins (check compatibility first) and selection markers can coexist in the same cell, which can be extremely useful for the coexpression of four different proteins in the same cell (e.g., the four plasmid system developed by Dykxhoorn et al., which are compatible between them [41]); the broad diversity of selection markers and partitioning control elements [30]; cloning capacity [31], which is an important feature for cloning large fragments required for synthetic biology applications or metabolic engineering; reporter proteins useful for selecting positive clones; recombination or assembly technology for easier cloning methods [42, 43], and the

ability to be transferred from one host to another like the case of the OriV from RK2 plasmid [44]. We recommend for further information about replication control of plasmids to refer to del Solar et al. [45].

Linear plasmids are common in bacteria (particularly in actinobacteria), but thus far, only N15 plasmid prophage has been isolated from *E. coli* [46] and is an impediment for generating knockouts using linear DNA (see Section 3). Recently, a linear plasmid was created to clone unstable fragments bearing repetitive sequences without showing size bias during cloning, active promoter sequences, or sequences with A + T content [47].

One of the biggest impediments for plasmid segregation is the insert size. As discussed in Section 5, we are now facing not only the most exciting age of molecular biology but also the most challenging. In order to develop "bugs to the order," i.e., microbes are "trained" to perform specific tasks [48], from simple pathways to synthesize a specific metabolite to complex genetic circuits that can be controlled for novel environmental responses. In all these cases, plasmids play an important role, from generating site-specific integration of chromosomal fusions to large plasmids that can hold complex constructs for further modification. Another challenging area is the generation of strains capable of producing metabolic products required for the pharmaceutical industry, where metabolic pathways and cell metabolism can be a strong impediment for proper synthesis and purification of relevant precursors [49]. *E. coli* renders an important platform for tackling down impediments for the synthesis of novel compounds.

As part of the information needed for plasmid manipulation, databases and repositories are also relevant for the manipulation and selection of the right plasmid for the applications you want to further exploit. Such examples are given: a powerful plasmid repository is Addgene (https://www.addgene.org/vector-database/) where you can get any plasmid in the repository with minimum fees for shipping and handling. This source is important in many aspects; you can gain knowledge about plasmid sequence, special features, creators, and availability to use since you can acquire them with small fees. Also, Harvard University is currently generating a plasmid repository but still under development (https://plasmid.med.harvard.edu/PLASMID/Home.xhtml). These repositories are an excellent option for accelerating research due to finding already generated constructs useful for ongoing projects. Also, *E. coli* Genetic Stock Center (**Table 1**) is a good source of strains and plasmids for different applications. For a small fee, the strains and plasmids can be shipped worldwide and characteristics can be consulted. Also, the American Type Culture Collection (www.atcc.org) is a source for strains with desired characteristics, as well as knowledge on their features. In plasmid biology, strain selection is fundamental for plasmid stability and proper propagation, as well as for the correct function of special features, such as protein expression [50]. For example, DNA methylation is an important impediment in certain applications such as eukaryotic transfection, proper DAM^- and DCM^- strains for plasmid isolation is required. In protein expression experiments, the repertoire for host selection is big. Most applications require lysogen strains harboring the required RNA polymerase. As stated in **Table 2**, some plasmids require the product from the *pir* gene, so careful strain selection must be taken into account so that plasmids replicate efficiently.

Novel methods such as Gibson assembly, Golden Gate assembly, and AQUA (advanced quick assembly) methods [43, 51, 52] have skyrocketed the possibility to assemble any plasmid with the desired characteristics. These methods are based on designed modules that can either be Polymerase Chain Reaction (PCR) amplified or generated as a complete synthetic construct and then assembled in the desired combination either by an enzymatic process (Gibson and Golden gate) or even enzyme-free methods such as AQUA.

Finally, plasmid biology is still under scrutiny, for their involvement in the mobility of traits that are important for human health such as antibiotic resistance, the distribution of pathogenicity islands, and genome evolution. Recently, novel tools for plasmid mining have been developed and uncover from Next Generation Sequencing data that plasmids can be uncovered and analyzed for further characterization [53, 54].

We are still in the process of truly knowing the potential of *E. coli*, novel tools generated through plasmids in combination with other molecular strategies will lead to new discoveries that will render this organism the basis for important discoveries. In the next section, we will discuss some aspects of gene knockouts and the knowledge we have gained from this versatile organism.

3. Genome modifications to understand *E. coli*

One of the basic questions in biology is: what is life? Defining life imposes a challenging burden both intellectual and experimental. Many attempts have been done to answer this. *E. coli* is a model organism that with all the molecular tools available we can get a step closer to provide sufficient information that will lead us to answer the relevance of genomic information and ultimately what is needed to achieve life [55]. In 1997, its genome was fully sequenced, and with all that information (from *E. coli* and all the organisms that have been sequenced thus far), scientists have aimed high to achieve the knowledge of how many genes are needed for life to be sustained. Molecular genetics have provided many tools for understanding gene structure and function, the most fundamentals are gene knockouts and genome deletions. In this section, we provide aspects that are fundamental for understanding genome structure and function taking our knowledge closer to knowing the minimal core genome of bacterial organisms and the optimization of *E. coli* for biotechnology.

In *E. coli*, several tools for genome modification have been developed. Some of the most important methods involve either the generation of deletion mutants by removing specific genes, one outstanding case is the use of the lambda Red system for inhibiting linear DNA degradation and by homologous recombination, the deletion of specific genes using PCR-derived selection marker cassettes with homologous sequences with target gene [56, 57]. Lambda Red-based method have yielded a total of 4288 genes mutated without lethality (Keio collection), 303 genes were unable to be deleted, from which 37 are of unknown function [58]. This experimental evidence has pointed out one very important aspect of genome structure and function. Genome size increase is the result of horizontal gene transfer or DNA fragment retention that somehow is giving some beneficial features to recipient host, apparently an increase in fitness [26]. The function of genes without evident function is still a relevant area of research since

http://dx.doi.org/10.5772/67306

many of them provide support for fitness and evolution has preserved them, therefore full genome engineering is far more complicated than previously thought.

Larger genomic editions are needed to understand how far we can delete redundant or nonessential sequences. By using Cre/lox recombination, substantial genomic fragments can be deleted or sequentially removed, rendering the nonessential regions (regardless the genes present) from the genome [59–61].

Studies regarding genome size analyzed through deletions of specific genes or complete genomic regions have led on thinking about the minimal genome. In the case of *E. coli*, there are several pieces of evidence (reviewed in Ref. [62]) that points out that at least 23% of the genome can be eliminated gaining genomic stability and normal growth. Also, eliminating insertion sequences can enhance the capacity of *E. coli* to synthesize proteins due to the decrease or insertions on plasmids, and strains exhibit normal growth plus increased genome stability [63].

All these methods rely on basic bacterial genetics founded with *E. coli*, such as transposon-based integration of recombination sequences, λ-recombination of PCR products integrating deletion module cassettes, and the gene-specific knockout methods [62]. Mutations can then be transferred from one strain to the other to generate multiple deletions at once, and other technologies are still limited to either whole genome synthesis with previous knowledge on the structure of the genome.

The most relevant study revealed that genome size has an impact on *E. coli* cell growth, where it is shown that apparently dispensable sequences are needed under restrictive conditions, providing a hint of the still far future of fully functioning cells with all the desired characteristics for biotechnological applications [26]. We envision that genome reduction is a worthy effort, regardless of the method used to generate them. Another important aspect that we have to consider is that all conditions of the mutant strains are exposed to laboratory conditions rendering a behavior close to the ancestor or original strains. Nevertheless, there are also hidden features that must be exploited in order to understand fully the behavior of the genome and the essentiality of genes [62]. Thus far, *E. coli* remains restricted to the use of classic genetic tools and transposon or plasmid-based techniques. We encourage *E. coli* research community to join efforts to enter the synthetic biology era, toward the generation of a fully synthetic *E. coli*. Our excitement is based on the following:

After 6 years, in 2016, the first bacteria operating under a "minimal chemically synthesized genome" was created after the first fully functioning synthetic genome [64, 65].

This research we believe has an impact in the following areas. First, both studies settled the basis for whole genome synthetic biology, which will lead to important findings in many research areas. Second, the extensive transposon-based mutagenesis studies on the genome of *Mycoplasma mycoides* led to the knowledge of the basis of essential genes or quasi-essential genes that have an important impact on cell fitness. Third, all this knowledge led to the design of a complete chemically synthesized genome with all the basic functions, and we now have the basic information for mining existing genomes to look for core modules in the bacterial genomes and design genomes with specific functions. Taking together all the

observations from the synthetic genomes, we envision a bright future for bacterial molecular genetics in many fields of biotechnology, such as the production of molecules for human wellbeing.

E. coli is an extensively studied organism, with all the cumulative data we can ensure that with all this knowledge, we can design tools. In the following section, we comment on the biosensors that are *E. coli*-based.

4. *E. coli*-based biosensors: tools for many applications

In biotechnology, biosensors are broadly defined as any device based on biological part, cell, tissue, or protein complex that are linked to a mechanical sensor or analytical receptor that provides a measurable signal proportional to the analyte in the reaction [66, 67]. *E. coli*-based biosensors using plasmid or chromosomal constructs are useful for the detection of environmental traits or hazards or measuring cellular processes as any standard reporter system [68–70].

In **Figure 1**, we depict the basic design for whole-cell biosensors and some applications. Plasmid vectors with all the possible modifications can lead to almost endless combinations. For practical applications, there are commercial vectors that can be used for such purposes or as mentioned in the previous sections, plasmid methods are powerful enough for fast and robust biosensor design.

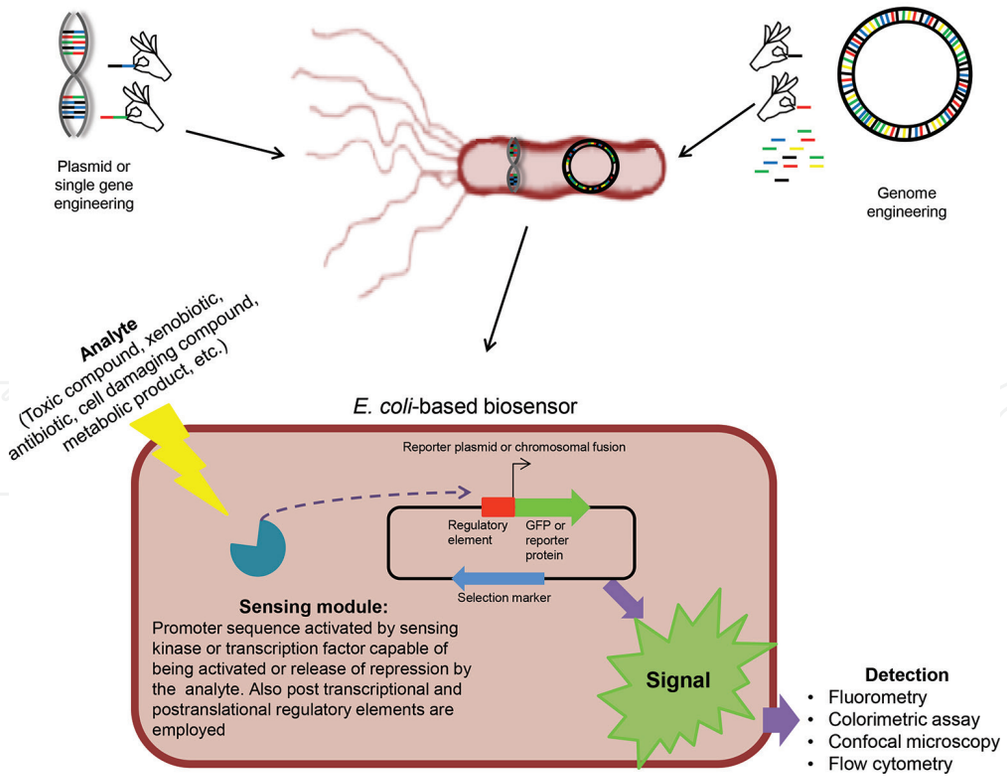

Figure 1. Basic principles for biosensor design. First, the proper design and the experimental creation of reporter strains. With current knowledge, either plasmid creation or whole genome engineering can lead to the creation of a reporter strain. Second, incorporation of such elements into the host cell. Third, sensing module and response measurement. With current reporter proteins and detection technology, it is relatively easy to generate biosensors that can be used in different applications with high sensitivity and selectivity.

http://dx.doi.org/10.5772/67306

The basic design considers the following: copy number, reporter proteins, detection methods, and control elements. The latter is basically the most important feature. As shown in **Table 1**, the available databases provide enough information for promoter selection and design. Bioinformatic tools can make this process easier [71]. Also, generation and detection of this kind of biosensors are cost-effective and easy to generate and reasonably sensitive [71]. In terms of speed, sample analysis with whole-cell biosensors is fast and cheap in comparison with analytical methods. The sensitivity of analytical methods is higher and more accurate, but biosensors are a good alternative for fast detection of hazards. Also, they can be coupled with the controlled production of metabolites of commercial importance.

In the literature, there are several reports where *E. coli*-based biosensors have been successful for detecting different traits: oxidants [72, 73], DNA damaging compounds [74], membrane-damaging compounds [75], protein-damaging compounds [76], aromatic compounds [77–79], xenobiotics [80], antibiotic panels using reporter strains without antibiotic selection [81], etc. The only limitation is the available sensor module and the design. The reporter protein is also important. Stability and reproducibility are two important aspects of biosensor design. In our experience, Green Fluorescent protein (GFP) protein is superior to luciferase, especially that we can detect GFP by various methods (we find flow cytometry, fluorometry, and confocal microscopy our top preferences) without cell lysis or substrate mixtures that are time-consuming [82].

With the improvement of DNA synthesis, recoding protein-coding genes for the desired function is expanding the capabilities of transcription factors, and reporter proteins have created novel sensor modules. For example, XilR recoding has led to a sensor that can detect millimolar concentrations of trinitrotoluene and its derivative compounds [83]. By using shuttle vectors, we can generate biosensors that we can transfer from one host to another, which can provide information about differences in physiological responses during the exposure to a given environmental trait.

E. coli plasticity and tools such as BioBrick building (using standardized DNA fragments with compatible ends for fast assembly) can facilitate plasmid and reporter constructs [84, 85]. Correlations of cell growth and physiology with expression patterns from reporter constructs can expand our knowledge of the impact of exposure to the external stimulus on cell physiology. Biosensors based on whole cells are a cheap alternative and can be coupled to portable devices. Using qualitative reporters can be applied in field research [70]. One good example is the detection of parasites without using cold-protected samples or complicated equipment for the detection process [86].

In the following section, we provide our final overview of the impact of *E. coli* in the synthetic biology future.

5. Genetic engineering and synthetic biology of *E. coli*

With the avenue of *in vitro* DNA synthesis to generate larger fragments with increased fidelity along with novel assembly methods, we are now capable of generating large and custom-made DNA molecules with the desired properties or even without the source of a DNA sample

having only the sequence itself. New biological parts (genes, promoter sequence, terminators, etc.), devices (gene networks), and modules (biosynthetic pathways) are only limited by our imagination and the drive to create them. Also, without the advancement of methods for analyzing large amounts of data, bioinformatics, codon optimization software, genome mining, and user-friendly databases, synthetic biology creations are permeating in many laboratories around the world. With this in mind, we will review the current technologies for synthetic genes and genomes, and how this technology can be applied in generating novel regulatory circuits and even whole genomes. In this regard, *E. coli* is the key organism for such endeavors. Why? Well, in this section, we provide some examples that we consider may be helpful in the future of mankind and are in our opinion relevant in the field of genetic engineering and synthetic biology.

Synthetic biology is a relatively new branch of molecular genetics that incorporate engineering principles for modifying several aspects of cell physiology, rewiring genetic circuits, creating novel circuits, and synthesizing custom-made DNA sequences and even genomes [87]. This particular branch of biology needs to be supported by an extensive knowledge of the organism that modifications or even whole genome synthesis is attempted, several novel tools for analyzing big datasets and molecular tools for that particular organism, for the generation of sequences and the computational design of DNA molecules, and a goal that can be achieved with the desired organism. *E. coli* along with *Saccharomyces cerevisiae* are the most studied and well-comprehended organisms in science, and diverse phenotypes have been identified that are helpful for bioengineering [88, 89].

Multiplexing is the novel approach for redesigning organisms to do desired tasks [90]. By cycling through design-build–test framework we can achieve novel features in existing proteins and can further the advancement of genetic engineering. Thus far the most complicated and time-consuming part of this framework is the testing of the novel designs. High-throughput approaches have led to the development of fast and reliable screening methods and advances in this area, such as designed biosensors for the screening of metabolite producing strains or high-throughput methods for product screening, where the use of fluorescent proteins, colorimetric assays, and mass spectrometry are cornerstones for the development of screening methods for assessing success in strain engineering [90, 91]. DNA sequencing and synthesis coupled with good screening methods is the platform for future tools for the development of designed microorganism that can do desired tasks.

With all the technologies available, the advancement of using *E. coli* for biotechnological applications based on synthetic approaches have led to the development of strains capable of synthesizing several novel compounds. In the following lines, we provide some examples that we find important for improving environmental conditions and human well-being.

Synthesis of important metabolites can be difficult, and researchers must face the stubbornness of microorganisms to redirect carbon flux to their own processes, rendering the production of relevant molecules costly and inefficient. But *E. coli* is a flexible platform for the efficient production of molecules for the pharmaceutical industry, metabolites and molecules relevant for food additives, pigments, and more recently complex aliphatic molecules [92].

http://dx.doi.org/10.5772/67306

Several aromatic compounds have been successfully synthesized in *E. coli* due to their biological activities (vitamins and antioxidants, for example), pigmentation (applied in different industrial processes), and fragrance etc. [93]. In the case of the perfume industry, the synthesis of other relevant compounds such as precursors for Ambrox, a highly appreciated odorant for the perfume industry, or the synthesis of Geraniol, a valuable acyclic monoterpene alcohol, is also used in the perfume industry and pharmaceutical applications [94, 95].

Another relevant area was *E. coli* stepping in to biofuel production. The twentieth century is characterized by the human dependence on fossil fuels. They participate in a myriad of processes, and the demand is increasing. In order to alleviate the demand, scientists have turned to the development of novel technologies for biofuel production by the conversion of carbon sources into usable fuel. There are several reports where *E. coli* have been successfully engineered for the synthesis of branched-chain fatty acids or short-chain fatty acids that can ultimately lead to the mass production of fuel precursors or useful materials derived from oil [96–98]. Perhaps, the most promising future is a fully replicated fossil fuel, i.e., a mixture structurally and chemically identical to the fossil fuels that are currently under use, which is a mixture of aliphatic n- and iso-alkanes of various chain lengths [99]. Also, a more complete metabolic atlas of *E. coli* is needed, and recent efforts have mapped the metabolic flux from this bacterium further [100].

Another important field where *E. coli* is making an important contribution powered by synthetic biology is the antibiotic production. From the biomedical standing point of view, increasing antibiotic resistance in pathogens is a burden for humankind, and the discovery of novel compounds is a time-consuming task. Recent efforts with known polyketides have started to give good production rates in *E. coli*, favoring the process of antibiotic production from different sources, eliminating the need for host growth standardization, and inducing conditions [101].

Due to the modularity of polyketide synthases, they are excellent candidates for engineering, for either the production of novel compounds from existing gene clusters (through module shuffling, mutagenesis or deletions), or by the introduction of novel environmentally sequenced gene clusters and heterologous production [102, 103]

Therefore, the production of diverse metabolites or biological or industrially relevant molecules can be successfully achieved in *E. coli*. Success stories have been published more frequently. With the advancement of high-throughput technologies, more sensitive detection, and analytical tools, and better DNA synthesis, the future for *E. coli* is brilliant.

6. Future

With all the research that is currently conducted in *E. coli*, we envision that the future for this versatile microbe is bright. Gained knowledge on *E. coli* is overwhelming, nearly 340,000 research papers with the "*Escherichia coli*" keyword available in Pubmed, versus 115,000 using

"*Saccharomyces cerevisiae*" as a keyword for an example. Several genomes from environmental sources have been sequenced. Thousands of research papers aimed to assess the metabolic and genetic potential of this organism have been published. Now we need to start joining forces for boosting the true potential of this wonder microbe.

With all the data available, we are on the verge of really important findings and novel biotechnological procedures using *E. coli*. Recently, with an extensive analysis of human microbiota, we are approaching exciting times where all the knowledge can be applied to help people during certain diseases related to colon microbiota imbalances [104]. Findings suggest that microbiome can be manipulated to improve certain metabolic pathways [105].

In the rest of the biotechnology fields, we have enough information that suggests *E. coli* will be in the spotlight for quite some time. Even with novel organisms that have been proposed to substitute it, like *Vibrio natriegens*. Recently, Lee and collaborators analyzed the genome sequence and growth properties of the fast-growing bacterium *V. natriegens* [106], where authors created a novel platform for genetic engineering in one of the fastest growing bacteria. In previous reports, it has been shown that *V. natriegens* are capable of being transformed with *E. coli* plasmids [107], rendering that all the molecular tools available thus far may be compatible with this new system. This bacterium as *E. coli* renders only one important drawback: it may be pathogenic to oysters [108]. But as with old *E. coli*, we should be careful when managing genetically modified organisms. With recent efforts to generate lab-contained organisms, *E. coli* mutants that can only survive in the presence of synthetic amino acids have been created [109]. Despite all the molecular tools developed so far for *E. coli*, the future is still open for a novel molecular toolbox. Until a novel toolbox is standardized and incorporated into the everyday life of the laboratories around the world, we will keep exploiting the capabilities of *E. coli*, one wonderful bug.

Acknowledgements

The authors acknowledge the generous support from CONACyT-CIBIOGEM grant CB-4O-040116, CONACyT grant CB-2012-01 182671, and Guanajuato University grant 1081/2016 (DAIP). NVM acknowledge the support from CONACyT for a scholarship grant. We also acknowledge the support from Dr. Felipe Padilla Vaca.

Author details

Naurú Idalia Vargas-Maya* and Bernardo Franco

*Address all correspondence to: naudalia@hotmail.com

Biology Department, Division of Natural and Exact Sciences. University of Guanajuato, México

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