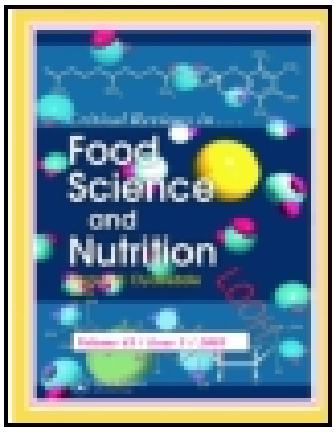

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Synthetic Biology: Applications in Food Sector

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Synthetic Biology: Applications in Food Sector

Running Title: Synthetic Biology

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Abstract

Synthetic biology also termed as ‘genomic alchemy’ represents a powerful area of science that is based on the convergence of biological sciences with systems engineering. It has been fittingly described as “moving from reading the genetic code to writing it” as it focuses on building, modelling, designing and fabricating novel biological systems using customized gene components that result in artificially created genetic circuitry. The scientifically compelling idea of the technological manipulation of life has been advocated since long time. Realization of this idea has gained momentum with development of high speed automation and the falling cost of gene sequencing and synthesis following the completion of the human genome project. Synthetic biology will certainly be instrumental in shaping the development of varying areas ranging from biomedicine, biopharmaceuticals, chemical production, food and dairy quality monitoring, packaging and storage of food and dairy products, bioremediation and bioenergy production etc. However, potential dangers of using synthetic life forms have to be acknowledged and adoption of policies by the scientific community to ensure safe practice while making important advancements in the ever expanding field of synthetic biology is to be fully supported and implemented.

Keywords: Synthetic biology, System biology, Applications, Food and Dairy

1. Introduction

Emerging trends and issues in today's modern world require biology to enter into a new and exciting era of developing effective and customized solutions. From hypothesizing the fundamentals of life, the scope of biological sciences has evolved to applying established principles in association with new technologies in order to facilitate an interdisciplinary interaction between biological, chemical, physical, engineering and computational sciences. Synthetic biology embodies this attempt towards developing a new age technology, where multiple areas merge, complement and facilitate each other. Synthetic biology can thus be defined as the designing and construction of new biological components, devices and systems that do not exist in the natural world and also the redesigning of existing biological systems to perform specific tasks. What differentiates synthetic biology from the traditional methods of genetic manipulation is that it does not involve mere alteration of existing genomes, but provides high throughput construction and engineering of organisms having genotypic characteristics that control synthesis of complex, biologically inspired systems displaying functions that do not exist in nature (Benner and Sismour, 2005; Endy, 2005; Andrianantoandro et al., 2006; Heinemann and Panke, 2006, Tucker and Zilinskas, 2006).

It all begun by the chemical synthesis of a gene by Har Gobind Khorana in 1970s (Khorana et al., 1972) which laid the foundation of developing and synthesizing single DNA strands by exploiting their natural chemistry. However, the term synthetic biology was coined by Hobom (1980) to describe bacteria that had been genetically engineered using recombinant DNA technology. These bacteria are living systems (therefore biological) that have been altered by

human intervention (that is, synthetically). In this respect, synthetic biology was largely synonymous with 'bioengineering'. However, technology especially with regard to synthetic biology has evolved at such a fast pace in recent times (Fig 1) that the term genetic engineering can be termed as '*passé'*. Today, scientists aren't just mapping genomes and manipulating genes, they're building life from scratch. As a result of the race to read and map genomes, it is now possible to sequence tens of thousands of base pairs per minute, and do it relatively cheaply (Erica, 2005). As attention switches from reading to writing, genetic information (and indeed whole organisms), synthetic biologists can now snub their noses at nature's designs in favor of made-to order life-forms. Using engineering concepts borrowed from electronics and computing, synthetic biologists are building simplified versions of bacteria, re-programming DNA as a computing medium and assembling new genetic systems that are human directed. As they do so, a real world technology with enormous applications and implications is fast emerging.

Stemmer et al. (1995) had modulated the polymerase chain reaction and were able to synthesize a large gene and vector system of approximately 2700 bp, leading to the evolution of high throughput designing of mechanisms of DNA manipulation with multifarious applications. By the year 2002, scientists were able to successfully develop functional artificial biological circuitry. The BioBricks foundation marked another milestone by providing open tools and standardized parts for biological engineering. Researchers today are working towards improving the power of DNA synthesis technology, to counter bigger challenges such as inventing new languages and grammar that can enable the evolution of many new genetic 'programmes'. These programmes will eventually be helpful in simulating useful behaviours, such as the production of fuel, food or medicines (Billings and Endy, 2008). The possibility to construct and fabricate new

biological parts and systems with success indicates the possible novel applications of amalgamation of the disciplines of systems biology, modular engineering and fundamental biology to resolve problems arising from traditional biotechnological methods in human diagnostics, therapeutics, food and dairy applications such as food quality monitoring, packaging and finally downstream processing of waste.

Keeping these concepts in perspective, the earlier part of the paper provides an insight into the ever-advancing field of synthetic biology and its dynamism with a specific emphasis on its implications in food and dairy sector. It also outlines the new landscape of synthetic biology by describing its approaches and tools. This is followed by the discussion on the implications of synthetic biology under the broad ambit of the possible social and ethical concerns. The article concludes with discussion on social safety and security concerns associated with the use of synthetic biology and suggests multilevel framework and regulations to monitor righteous use of this ever expanding science.

2. Scope of Synthetic Biology

Before entering into the subject in depth, it is important to differentiate '*Synthetic Biology*' from '*Biotechnology*'. Improving the production of a certain metabolite by tinkering with some of the components of a metabolic network will fall within the realm of biotechnology; whereas, introduction of several exogenous enzymes in an organism to produce a new compound will fall within the scope of synthetic Biology. Similarly, '*Systems Biology'* and '*Synthetic Biology'* can be differentiated. While both disciplines consider modelling and simulation as important tools, they are interrelated and have grown simultaneously (Fig 1). Systems biology aims at the quantitative understanding of natural biological systems, and not at the engineering of new

functions, or properties. Of course, synthetic biology benefits enormously from systems biology studies, since engineering of a biological system requires at least some understanding of it. Systems biology, on the other hand, benefits enormously from engineering concepts applied to network components (e.g., switches, amplifiers and control elements) and network properties (e.g., robustness and modularity), and such studies have provided invaluable insight into module behavior, while abstracting the details of molecular interactions (Ventura et al., 2006).

Research within synthetic biology can be exploited through one of the two approaches: top-down, or bottom-up (Barmer and Martin, 2008). The top-down approach attempts to eliminate the problem of natural complexity by removing it, e.g. by stripping a genome of all genetic material that is not absolutely essential for replication and functionality (Fig 2). The bottom-up approach uses naturally occurring organisms that appear to have little complexity and adds the required functions by engineering them into the existing genome (Fig 3). From the vast range of research areas in synthetic biology, major emerging areas can be integrated in following headings: making minimal genomes, designing modular components, pathway engineering, expanding the genetic pool, production of artificial cells and creation of synthetic biomolecules described as follows.

2.1. Creating Minimal Genomes

The production of minimal genome microbes follows a top down approach wherein smallest number of genes required for a bacterium to survive is determined. The production of minimal living genomes is undertaken to produce a ‘chassis’ that can have other synthetic pathways added (ETC, 2007); thereby, enabling various products to be made from the same basic organism. *Mycoplasma genitalium* first used by Fraser et al., 1995, was reduced to 386 essential

genes for DNA repair, energy metabolism and other essential processes by 2005. The same team has recently generated semisynthetic self-replicating bacterial cell called *Mycoplasma mycoides* JCVI-syn1.0, successfully transplanted the genome of one bacterium into another bacterium, starting with a digital code in a computer, building the chromosome from four bottles of chemicals, assembling that chromosome in yeast and transplanting it into a recipient bacterial cell (Gibson et al., 2010). Production of efficient fuel alternatives and slowdown of climate change can hopefully be achieved using these basic cells. However, the development of these minimal genome microbes into working systems for the production of fuels or cleanup of environmental contaminants is still at a very early stage.

2.2. Modular Components and the Expanded Gene Pool

Most disciplines in biology struggle with modelling and describing the complexity of systems. However, engineering perspective simplifies the system and limits it to number of 'well characterized, standardized objects' (Pleiss, 2006), which can be modelled using present computing capacity. These standardized objects or interchangeable parts (Benner and Sismour, 2005) can be built into complex systems with known components and their interactions following top down approach of synthetic biology. This means that at every level in the hierarchy in a system, complex machines can be developed from a small number of basic modular elements.

Research presently is intensely focused both on the standardization of these interchangeable parts and also on decoupling of complex systems into more manageable components (Endy, 2005). This allows the researchers dispersed across the world to collaborate independently. A registry of these standardized parts is being developed at MIT, where they are

increasingly known as BioBricks. MIT also runs an annual international competition for undergraduate students to design a system using BioBricks called iGEM (Smolke, 2009). Benner and Sismour, 2005 outlined the two types of interchangeable parts i.e. DNA and proteins being developed by the synthetic biology community. DNA has proven to be an excellent structure for modification since the backbone that supports the base pairs is relatively stable, even when entirely synthetic nucleic acids are added to the sequence. Slight modifications to the DNA polymerase enzyme allow for normal 'reading' of a DNA sequence that contains nucleotides other than AGCT (Benner and Sismour, 2005). The two artificial nucleotides produced by Benner's team are K and X, forming what he calls AEGIS (An Expanded Genetic Information System). However, search for protein-based interchangeable parts has proven more difficult. Engineering proteins by modifying the amino acid sequence in a predictable fashion is a highly complex process due to the secondary and tertiary structures of proteins. As such, attempts to engineer interchangeable parts have so far been successful mostly by DNA modification.

2.3. Artificial Cell Production

The creation of artificial cells operates through a bottom-up process in which life-like cells are built from scratch. A protocell is developed using a system of metabolism, an information-storing molecule and a membrane to hold it together wherein it uses Peptide Nucleic Acid (PNA) in place of DNA (PACE) (Bromley et al., 2008; Ura et al., 2009). This research network aims to produce self-organizing, evolvable, life-like systems to make the next generation of self-repairing computer and robotics technologies to facilitate nanoscale remediation.

2.4. Synthetic Biomolecules

The modification of amino acid sequences after they have been transcribed from genes in the cell can greatly alter the functionality of the sequence. However, the structures of protein have had adverse effect on search for interchangeable parts through protein modification; still, development of synthetic biomolecules have been partly achieved in recent times. A chemical tagging system that utilizes established GM techniques (the LacZ reporter enzyme scaffold) to attach post-translation modifications to amino acid sequences; thereby, producing proteins that have functions such as detecting mammalian brain inflammation and disease based on the expression of reporter gene has been developed (Kasteren et al., 2007). In another study, a synthetic mimic of erythropoietin that has a prolonged circulation time in the body has been constructed (Kochendoerfer et al., 2003).

To date, there are neither fully controllable bioengineered bacteria nor completely synthetic cells or minimal organisms carrying chassis genomes. So far, self-maintaining, self-producing and evolvable protocells have not been realized, but different types of preliminary forms have been constructed or simulated. The synthesis of a small bacterial genome has been achieved and the principle of genome transfer from one bacterium to another has also been established (Glass et al., 2006; Lartigue et al., 2007; Gibson et al., 2008).

This is not to say that synthetic biology has no exciting future as an applied discipline. A successful example has been the production of terpenoid compounds in *E. coli* (Martin et al., 2003) and *Saccharomyces cerevisiae* (Lindahl et al., 2006; Ro et al., 2006) that can be used for the synthesis of the anti-malaria drug. In other cases, important steps have been achieved towards

practical applications. For example, an extensible RNA-based framework has been developed recently for engineering ligand-controlled gene-regulatory systems, called ribozyme switches, that exhibits tunable regulation, design modularity, and target specificity and could be used, to regulate cell growth (Win and Smolke, 2007). Another interesting example is the construction of *E. coli* harboring designed plasmids that invade cancer-derived cells in a density-dependent manner under anaerobic growth conditions (Anderson et al., 2006; 2007).

3. Tools of Synthetic Biology

Synthetic biology is developing the tools and methods that will increase control over regulatory interactions and interactions with the surrounding environment, eventually resulting in an integrative synthetic biology that will allow ground-up cellular optimization. Synthetic biology can be contextualized into three tiers i) Engineering the process of central dogma, ii) Transcription engineering and control, iii) Translational engineering and protein regulation. Efforts at each of these three tiers attempt to control cellular systems and take advantage of emerging tools and approaches. Eventually, it will be possible to integrate these approaches and realize the vision of integrative synthetic biology when cells are completely rewired for biotechnological goals.

3.1. Engineering DNA

Recombinant DNA methods, DNA sequencing technology, Directed Mutagenesis techniques and molecular biology methods have expanded the domain of DNA tools. Efforts are being made to introduce this synthetic DNA into a generic host in an effort to completely reprogram a cell. In essence, this technology serves as the basis for other synthetic biology tools, since DNA is the vehicle of almost every biological perturbation, regardless of the tier of interest. Adding

synthetic base pairs to expand the basic genetic code as previously discussed coupled with DNA synthesis technology could create a powerful tool for designing synthetic circuits. Regardless of the application, the capacity to engineer DNA using synthetic biology tools provides new access points to the cell unachievable by previous technology.

3.2. Engineering Transcription

Engineering DNA can cause significant alterations in downstream process units. Synthetic optimization of all process units is an important aspect. The first process unit in the central dogma is transcription. Gene expression level can be controlled by promoter engineering with synthetic biology tools. This resulting range of expression affords a more detailed investigation of expression levels beyond traditional wild type, knockout and strong over expression studies. Furthermore, well-characterized promoters enable more precise gene delivery. Proteins involved in transcription can also be engineered to synthetically control a cell. In this regard, another synthetic biology tool termed global transcription machinery engineering (gTME) is aimed to alter the proteins responsible for the processing step of transcription by creating a mutant library of proteins responsible for transcription (such as sigma factors and TATA binding proteins) and subjecting the library to a high-throughput phenotype screen (Tyo et al., 2007). This approach of synthetically rewiring cells at the transcriptional level provides a means of creating large changes within the transcriptome and provides a novel approach to modulating the processing step of transcription. As components of transcription represent a target and provide an access point for synthetic biologists to effect change in biological systems, the interaction networks of transcription factors are also being reconstructed using high throughput data obtained from two-hybrid, co-immunoprecipitation, or bioinformatic mining protocols.

3.3. Engineering Translation and protein regulation

The second major process unit in the central dogma i.e. translation, has also been the subject of recent synthetic biology research. Incorporation of unnatural amino acids into proteins and gene codon optimization are successful examples of synthetically engineering translation machinery. Also modifying mRNA structure can modulate protein levels. Finding of studies on understanding native RNA response to small molecules (Beaker, 2009; 2010) have linked translational control with signaling networks or environmental signals. Also, modifying events such as glycosylation, phosphorylation, and acetylation allow protein function to be modulated and localized within the cell. These events regulate cellular activity through activation, inhibition, and signaling. Protein regulation has been studied in depth since the very beginning of the biotechnological revolution, and is presently becoming influenced by the synthetic biology paradigm. The work previously discussed exemplifies this and opens the door to ground-up cellular optimization.

These tools using the aforesaid approaches can be explored to obtain the best results for bioprocessing applications.

4. Application of synthetic biology in food and dairy sector

Food and dairy industries are facing enormous challenges for developing and implementing systems that can produce high quality, safe foods as well as feeds while also being efficient, environmentally acceptable, and sustainable. To answer these complex set of engineering and scientific challenges, innovation is needed for new processes, products and tools in the food industry. Synthetic biology is now gaining momentum and becoming a worldwide important tool for application in the food and dairy industry as mentioned below.

- Designing methods to enable foods such as soft drinks, ice cream, chocolate or chips to be marketed as 'health' foods by reducing fat, carbohydrate or calorie content or by increasing protein, fibre or vitamin content (Heffernan and Misturelli, 2000; Nakaya et al., 1988).
- Production of stronger flavours, colorings, and nutritional additives, and processing aids to increase the pace of manufacturing and to lower costs of ingredients and processing.
- Development of foods capable of changing their color, flavour or nutritional properties according to a person's dietary needs, allergies or taste preferences (high on the research agenda of food giants including Kraft and Nestlé).
- Smart or innovative packaging to increase food shelf life by detecting spoilage, bacteria, or the loss of food nutrient, and to release antimicrobials, flavours, colours or nutritional supplements in response.
- Re-formulation of on-farm input, production of more potent fertilizers, plant growth treatments and pesticides that respond to specific conditions or targets.

Synthetic biology has the potential to revolutionize the global food system. Novel agricultural and food safety systems, disease-treatment delivery methods, tools for molecular and cellular biology, biosensors for pathogen detection, pesticides, packaging materials, environmental protection, and education of the public and future workforce are examples of the important impact that synthetic biology could have on the science and engineering of agriculture and food systems.

Biological systems producing anti-malarial vaccines, fabrics, flavors, and biofuels have received scientific and popular attention. The application of synthetic biology in medicine, food,

agriculture, cosmetics, and other areas is already a reality and is now being extended to impact food-associated industries. Milk is an essential nutritional food for infants and adults alike. India leads the world in total milk production; however, the development pace of dairy industry is bogging down significantly due to several factors like the tropical climate, unorganized milk production, laxity in quality control, a lopsided demand and supply position coupled with evolution of recalcitrant pathogens in the dairy industry. Synthetic proteins have the potential to change food and dairy production with lot of value addition beneficial for the target consumer population in an affordable way.

4.1. Improving food functionality from health perspectives by engineering microbial communities including probiotics

A growing number of health problems ranging from inflammatory bowel disease (IBD) to obesity and even autism have been linked to disruption in human-associated gut microbiota or alterations of the intimate cross-talk between these microbes and human cells (Grover et al., 2012,a; Panwar et al., 2012; Mallappa et al., 2012). Prospects of probiotics as biotherapeutics are currently being explored as an alternative to drug therapy in the management of these lifestyle inflicted inflammatory metabolic disorders (Grover et al., 2012,b). Probiotics are dietary supplements of live microorganisms which when administered in adequate amounts confer a health benefit on the host. Recently, Kumar et al (2011) evaluated the anti-hypercholesterolaemic effects of two putative probiotic bile salt hydrolase (Bsh)-producing *Lactobacillus plantarum* strains, i.e. Lp91 and Lp21, in rats. The authors suggested that the indigenous *L. plantarum* Lp91 strain has the potential to be explored as a probiotic in the management of hypercholesterolaemia. More recently, Achuthan et al. (2012) investigated the antioxidative

potentials of 39 probiotic lactobacilli of Indian gut origin and their ability to augment antioxidant defense enzyme systems in the host cells under oxidative stress conditions. They reported that most of the cultures were moderately to strongly resistant towards 0.4 mM H_2O_2, demonstrated high resistance towards hydroxyl ions and were quite resistant to superoxide anions. Amongst the 39 cultures, *Lactobacillus spp*. S3 showed the highest total antioxidative activity of 77.85 ± 0.13 % followed by Lp55 (56.1 ± 1.2 %) in terms of percent inhibition of linolenic acid oxidation. The expression of catalase gene was also significantly up-regulated by all the cultures at 0.1 mM H_2O_2, conditions. Duary et al., 2012 reported the relative gene expression of pro, anti-inflammatory cytokines and other molecules in 2,4,6-trinitrobenzene sulfonic acid-induced colitis mouse model against *Lactobacillus plantarum* Lp91 by reverse transcription-quantitative PCR. *L. plantarum* Lp91 exhibited strong immunemodulatory properties under in vivo conditions. Synthetic biology is the quest to design and build novel organisms that perform useful functions. Much research in the field is concentrated on using bacteria as a factory. One of its early successes was the development of microbes that produce malaria medicine, wherein Keasling (2008) engineered a yeast strain by inducing multiple pleiotropic drug resistance in it to produce an increased level of anti-malarial drug precursor, artemisinic acid which can be semi-synthetically converted in to Artemisinin, the anti-malarial drug most effective against malaria (Keasling, 2008). In another study, Nisin resistant genes nisFEG and nisI from *L. lactis* were introduced in *Bacillus subtilis* under the control of a synthetic promoter library for higher and cost-effective production of broad host spectrum preservative (Hansen et al., 2009). Synthetic biology has also been explored to engineer micro-organisms appropriately so that they could be used as vehicles for the delivery of target specific drugs/medicines in the diseased part of the

body without affecting the healthy tissues. A Synthetic biology project has led the designers to moot an intriguing application for developing a probiotic that would highlight health problems by changing the colour of faeces. The probiotic would contain bacteria engineered to respond to key chemicals in the body by synthesizing brightly coloured pigments. By drinking it regularly and checking waste every day, it would be possible to monitor the health status. Human gut is the primary organ which responds to nutrients and pathogens alike (Fig. 4). Probiotics colonize in the gut and form a microflora which by competitive exclusion defends against pathogenic infection. Bacteria such as *Akkermansiamuciniphila* (dedicated intestinal mucin degrader) are being used in understanding gut microbiota through metagenomic studies (Van Passel et al., 2011) and synthetic biology in combination with system biology is now being explored to understand and reprogramming of gut micro-flora.

There are new projects coming up which attempt to enhance the health benefits of edible bacteria. The techniques of synthetic biology to create bacteria that target and destroy tumors, fight cavities, produce vitamins and treat lactose intolerance, are in process. Synthetic biology could be used to engineer probiotic lactic acid bacteria used in the production of dairy products like yogurt, buttermilk and curds, to produce Monellin, a heat and pH stable sweetening protein (Chen et al., 2011, Aghera et al., 2011; Templeton et al., 2011). Successful engineering could radically reduce the calorific content of these products and can develop regulatory systems that could be tweaked to suit varied purposes.

4.2. Enhancing the functionality of bioactives/nutraceuticals through synthetic biology

Apart from probiotics, bioactive compounds which are extranutritional constituents that typically occur in small quantities in foods can be used to enhance nutritional value of food and

dairy products. Actinomycete bacteria of the genus *Streptomyces* are major producers of bioactive compounds for the biotechnology industry. They are the source of most clinically used antibiotics, as well as of several widely used drugs against common diseases, including cancer. Genome sequencing has revealed that the potential of *Streptomyces* species for the production of valuable secondary metabolites is even larger than previously realized (de Vos, 2011). Accessing this rich genomic resource to discover new compounds by activating "cryptic" pathways is an interesting challenge for synthetic biology. Detection and production of bioactives through synthetic biology, structure elucidations of novel metabolites, sensors for new metabolites and analysis of samples for different level of bioactives are few concerned areas of research in synthetic biology.

Synthetic biology in agriculture and the food industry involves the incorporation of biocatalysts (living cells or their components) to produce useful and value-added products, also it offers tremendous opportunities to design and produce new or improved agricultural and food products and their manufacturing processes (Tang et al., 2009). This will most likely have a great impact on the food-processing industry. In the increasingly health-conscious society, synthetic microorganisms and specialty enzymes will find enhanced use in improving the nutritional, flavour and storage characteristics and safety of food and dairy products.

4.3. Food Quality Monitoring

There will be a significant challenge for agriculture and the science to provide a healthy, nutritious, wholesome and adequate food supply in coming decades for a rapidly growing population. A problem which ails Indian dairy sector in particular and food processing industry in general is of quality assurance with particular reference to microbial contamination. Quality

assurance in food and dairy industry is of paramount importance because consumers demand safe and wholesome foods and governments impose stringent regulations to ensure food safety and food hygiene. The problem for dairy sector initiates with the milk producing animals, which can deliver heavy load of bacteria to fresh milk due to infections. Even though, there are different reasons for developing and developed economies to combat infection and food contamination, everyone agrees that such undesirable microbes are a threat to human health and must be contained or eliminated. Worldwide there are huge efforts being directed towards the discovery of conventional and non-conventional ways to combat food infections.

The concept of synthetic biology has given rise to identification of novel antibacterial leads which are proving to be effective in combating several food spoilage and pathogenic microorganisms. Sensors or detection systems for rapid detection of spoilage of product components, for quality control, for abuse detection at source and during production chain is possible through a combination of nanotechnology and synthetic biology (Horner et al., 2006; Neethirajan et al., 2009). Synthetic biology can be used to increase the amount of particular nutrients (like vitamins) in food crops. Research into this field is now at an advanced stage. Researchers are especially looking at major health problems like iron deficiency. The removal of the proteins that cause allergies from nuts (such as peanuts and Brazil nuts) is also being researched. Through synthetic biology, scientists aim to produce sturdy plants able to withstand weather extremes, resistant to disease and insects as well also produce better quality food crops that requires less chemical application, such as pesticide and herbicide resistant plants, inexpensive and nutritious food like carrots with more antioxidants, foods with a greater shelf

life, like tomatoes that taste better and last longer, food with medicinal (nutraceutical) benefits, such as edible vaccines; for example, bananas with bacterial or rotavirus antigens.

There are incessant reports of synthetic milk being prepared by adulterants and fed to the unsuspecting population thus jeopardizing the health of the people. The synthetic milk generally available is usually composed of chemicals like urea, neutralizers and detergents which can get metabolized into carcinogenic and endocrine disrupting agents. There is a need for development of cost-effective and easy to handle means of detecting such adulterants. Use of biosensors with aid from synthetic biology offers recognizable solution (Fu et al., 2008; Stutzenberger et al., 2007; Cheng et al., 2009). The aptamer based biosensing can provide effective solutions to such problems. Development of biosensors for detecting infectivity in foods and milk at parts-per-billion concentrations is the need of the hour. Synthetic biology could be used to develop Novel Immuno Diagnostics. Such diagnostics eliminate time and effort needed to obtain the natural DNA template of known pathogens and newly described infectious agents. A team at the University of Edinburgh designed and engineered bacteria as biological sensors for detecting arsenic in water. A sequence of genes in the bacteria stimulates them to produce acid if arsenic is present above the safe level for human consumption. The resulting change in acidity can be read cheaply and simply using existing pH test devices. Development of sensing systems for detection of food spoilage, pathogenic microorganisms for frequently used milk adulterants like urea, whey proteins, and detergent is an important step in designing the prototype of the biosensor kit. Sensing system is being incorporated with the suitable signaling system so that the whole biosensor can be developed into “quantitative detection kit” for industries, farmers and unorganized dairy sector level. Screening of small molecule libraries using the chosen gram

negative pathogens and finding their targets and studies for the removal of inactivated bacteria-drug from the milk are very important applications of these biosensors. Artificial gene synthesis can generate multi-epitope and chimeric antigens not possible using natural sequences and hence may show superior diagnostic performance. Artificial gene synthesis provides codon optimization for enhanced recombinant protein production.

Detection and inhibition of adulterants and pathogens in the milk and other agricultural products and by products is need of the hour which will not only address the national need and export potential of global food processing industry but will also lead to development of effective sensing technologies and therapeutics. This comprehensive approach will add lot of value to the product by controlling the adulteration of food and milk and their safety by combating the microbial load.

4.4. Downstream processing of (food) waste

Bioremediation is the use of biological systems to treat environmental contaminants. Synthetic biology approaches are taking advantage of natural biodegradative pathways in certain microorganisms to remediate potent environmental contaminants including heavy metals, actinides and nerve agents. Researchers are now using knowledge of natural processes to develop micro-organisms that can accumulate and degrade substances such as heavy metals and pesticides. For example, a team at Berkley has engineered a strain of *Pseudomonas* to degrade an organophosphate (commonly used as a pesticide) (Mattozzi, 2002). In this study, *Pseudomonas* strain was engineered with the *pnp* operon from *Pseudomonas* spp. strain ENV2030, which encodes enzymes that transform *p*-nitrophenol into ß-ketoadipate, and with a synthetic operon encoding an organophosphate hydrolase (encoded by *opd*) from *Flavobacterium* spp. strain

ATCC 27551, a phosphodiesterase (encoded by *pde*) from *Delftiaacidovorans*, and an alkaline phosphatase (encoded by *phoA*) from *Pseudomonas aeruginosa* HN854 under the control of a constitutive promoter. The engineered strain could efficiently mineralize up to 1 mM (275 mg/liter) paraoxon within 48 h, using paraoxon as the sole carbon and phosphorus source and an inoculum with optical density of 0.03at 600 nm. The organism could utilize paraoxon as a sole carbon, energy, and phosphorus source. Similarly, in an ongoing initiative, the Center for the study of early events in photosynthesis at ASU involves a consortium of approximately 20 researchers that is a key resource for understanding the microbial processes associated in the conversion of CO_2 and nutrient-rich liquids to bioenergy (Zhu et al., 2008; 2010). Researchers have demonstrated expertise in the design, development and optimization of outdoor experimental photo bioreactor systems. Microbial systems can also convert the energy in a range of biomass materials (e.g., animal wastes, human wastes, agricultural product, or photosynthetic microbes) to highly useful energy forms, including methane (natural gas, hydrogen, ethanol, and electricity etc.). While renewable energy production is the principal benefit of these systems, other benefits include waste-stream treatment and the production of high-value compounds (such as fertilizers and nutraceuticals). Efficient use of renewable biomass resources for production of liquid fuel and chemical feedstocks, also efficient treatment and management of agricultural and food processing industries' wastes constitute an important application of synthetic biology in view of environmental concerns and energy conservation.

4.5. Food Packaging/ storage

Food packaging that includes active packaging is a vital component of the food processing to maintain extended shelf life and conservation of the intrinsic nutritional properties

of the product. The use of protective coatings and suitable packaging by the food industry has become a topic of considerable interest because of their potentiality for increasing the shelf life of many food products. Antimicrobial packaging is one of the most promising active packaging systems. The use of such packaging is not meant to be a substitute for good sanitation practices, but it should enhance the safety of food as an additional hurdle for the growth of pathogenic and spoilage microorganisms. Antimicrobial packaging shows promise as an effective method for the inhibition of certain bacteria in foods although barriers to their commercial implementation continue to exist (Neethirajan et al., 2009). Polyvinyl alcohol (PVA), a biodegradable, synthetic polymer is innocuous, non-carcinogenic and has good biocompatibility properties. Apart from this, PVA has excellent film forming properties. Because of its good film forming ability along with high hydrophilicity with outstanding chemical stability, it can be blended with different synthetic and natural polymers and used as a water-soluble film useful for packaging (Tang et al., 2012).

One of the most promising alternatives to plastics made from oil is polylactic acid (PLA). It is biodegradable, safe enough to be used as food packaging, can be processed like existing thermoplastics into coloured or transparent material and can be manufactured from renewable resources such as maize and sugarcane. Although, PLA has been around for decades, it is only in recent years that advances in production techniques, have made it feasible to produce the material commercially. At the moment, PLA is usually made in two stages. First, a source of starch or sugar, which could be an agricultural by-product, is fermented to produce lactic acid; the same substance made by the body during exercise, only in this case it comes from the bacteria exercising themselves in the fermentation process. In the second stage, lactic-acid

molecules are linked into long chains, or polymers, in chemical-reaction vessels, to produce PLA. A recent study by Jung *et al.* (2010) has reported direct production of PLA, in a one-stage process in bacteria. The metabolic pathways of *E. coli* were engineered by knocking out the *ackA*, *ppc*, and *adhE* genes and by replacing the promoters of the *ldhA* and *acs* genes with the *trc* promoter based on *in silico* genome-scale metabolic flux analysis. Using this engineered strain, PLA homopolymer could be produced up to 11% by weight from glucose. Also, P(3HB-*co*-LA) copolymers containing 55-86 mol% lactate could be produced up to 56wt% from glucose and 3HB. P(3HB-*co*-LA) copolymers containing up to 70mol% lactate could be produced to 46wt% from glucose alone by introducing the *Cupriavidusnecator* β-ketothiolase and acetoacetyl-CoA reductase genes. No chemical "post processing" is required. Besides food and drink packaging, PLA is already in use to make some other products, such as medical devices. It also has the potential to be used to make biodegradable clothing, furnishings and hygiene products. Moreover, with further research, synthetically engineered bacteria might be capable of making other sorts of plastics and polyesters from renewable resources.

Scientists have come up with several packaging alternatives like nano-bricks. In this process, a film combines particles of montmorillonite clay, a soil ingredient used to make bricks, with a variety of polymer materials. The resulting film is about 70 percent clay and contains a small amount of polymer, making it more eco-friendly than current plastics (Choi et al., 2011). Nano titanium dioxide coated with aluminium dioxide has good dispersion property, can implement single granule dispersion, can be used as excellent UV-ray screening agent used in the fields of paint, rubber, fibre, coating material, sun protection products, printing ink and food package etc. (Su et al., 2009).

Polyolefin material integrated with nanophase particles viz. packaging laminate, used in a container for fluid foods e.g. milk or juice, comprises of a layer of polyolefin interspersed with nanometer size clay particles for gas barrier properties, also, "Self-cooling beverage and food container using fullerene nanotubes" have been proposed for better packaging of fluid foods (de Aberu et al., 2007). Ecosynthetix, an environment friendly biopolymer adhesive comprising of starch nanoparticles is being used in adhesive used in packaging of McDonald's burger. When used as an adhesive, it requires less water and thus less time and energy to dry. Other types of polymers explored in food packaging include Durethan® KU 2-2601plastic wrapping and Bayer Nanoparticles of silica in a polymer-based nano composite. Nano particles of silica in the plastic prevent the penetration of oxygen and gas of the wrapping, extending the product's shelf life considerably (Advantage magazine 2004).

Intelligent packaging, on the other hand, provides time-temperature indicators (TTIs) and the emergence of other smart packaging systems offering product differentiation (such as color changing labels), traceability or various interactive features (Brody 2003; Chaudhry et al., 2008). The intelligent package acts as a source of information by sensing the age and quality level of product e.g. ripesense label senses aromatics released from fruits on ripening and signals it through colour change and biosensors for detection of contamination and spoilage. Pathogens indicators e.g. *E. coli* 0157:H7 have also been used for meat, fish and poultry (Horner et al., 2006; Cheng et al., 2009). Barcodes are also provided which give nutritional information, cooking instructions and allergenic information (Oxonica, 2007). ToxinGuard (Toxin Alert, Toronto, ON), an indicator used for pathogen is a patented antimicrobial technology using polyethylene based packaging material and immobilized enzymes. It can detect *Salmonella*,

Listeria, E. coli and *Campylobacter*. It has obtained eighth US patent and is working with US Department of defense testing ToxinGuard for use in bioterrorism prevention. Another system comprising of Radio Frequency Identification (RFID) is a non-contact wireless data communication from where tags of some material are embedded with a chip, programmed and attached for identification, tracking, recall etc (Bell, 2011).

5. Safety and Societal Implications

Current advances in the emerging field of synthetic biology and the improvements in key technologies promise great impacts, not only on future scientific development, but also on the economy. Security threats, in the sense of the intentional release of biological agents, play an important role in the discussion between policy and synthetic biology associated industry. In this context, the DNA synthesis sector is particularly concerned, since ordered DNA fragments could also bear the danger of being misused, which basically requires a careful screening and checking of all the possible outlets including customers. The security concerns are not new to synthetic biology. Biotechnology and even microbiology also had to deal with these concerns of misuse. Although, Martinot and Benner (2004) are confident that artificial genetic systems will not survive outside the lab, research in this field raises profound biosafety questions. Dr. Jonathan King (Pollack,2001), a professor of molecular biology at MIT commented in the *New York Times* and cautioned that "It's a powerful technology, and like all powerful technologies, it needs appropriate oversight and regulation". One possible scenario he suggested is that proteins with artificial amino acids could elicit allergic reactions if used in drugs or in food. Synthetic biology's promoters are hoping that the promise of a very "green" techno-fix, synthetic microbes

that manufacture biofuels cheaply or put a chill on climate change, will prove so seductive that the technology will win public acceptance despite its risks and dangers.

Given that synthetic biology organisms will be artificially created, potential environmental and biosafety risks are impossible to predict. The claim that nano-agrochemicals will reduce the overall use of pesticides should be received critically given similar, unfulfilled, promises made by many of the same companies in relation to crops. Synthetic biology organisms could disrupt, displace or infect other species, alter the environment in which they were introduced to the extent that ecosystem function is likely to get compromised and established within a system such that they become impossible to eliminate (Koide et al., 2009; ETC, 2007). Many synthetic biologists working with fairly simple genetic circuits, report preventing rapid mutation of the circuits as being a key challenge to their work. The potential for synthetic biology organisms, released into the environment, to mutate in unpredictable ways is therefore of great concern. Application in what ultimately may be a mundane fashion and its development will need to be governed at multiple levels and using a range of policies and practices. These may include the establishment of new professional norms in the scientific community (e.g. codes of conduct concerning dual use technology), local and national research oversight, statutory regulation (e.g. new laws and formal regulatory agencies) and international co-operation and treaties. Such a multi-level governance framework will have to provide a robust overarching framework, whilst respecting different national traditions and empowering local enforcement. It will also have to be fully supported by the scientific community and other professional groups involved, through a process of training and awareness-raising. In brief, addressing the important questions raised by synthetic biology should be a policy priority for government, research

funders and the scientific community in order to ensure that it realizes its potential in a way that is ethically acceptable and commands broad public support.

6. Afterword

Realizing the immense potentials of synthetic biology in almost all the possible areas particularly with regard to food and nutrition having direct impact on human life, the food and bioprocessing industry finds it extremely valuable technology and will see great advances with intelligent innovations in the upcoming years that eventually will result into drastic improvement in food quality and safety. Synthetic biology in conjunction with nanotechnology has led to the development of highly advanced and powerful analytical tools such as biosensors and diagnostic devices with improved sensitivity and selectivity to monitor food processes and assure food quality measurements along the production lines. Apart from this, this new technology may provide solutions to some of the problems facing the planet including climate change, food security and infectious diseases. There are several companies viz. Ambrx, Amyris Biotechnologies, Synthetic Genomics, which are developing products ranging from biopharmaceuticals to minimal genomes as chassis for energy applications. Moreover, the timeframe of application of these technologies in actuality has considerably abridged with efficiency improvements in gene synthesis machines, which are now speeding up very fast. According to Carlson (2004), "Within a decade, a single person could sequence or synthesize all the DNA describing all the people on the planet many times over in an eight-hour day or sequence his or her own DNA within seconds". In the race to synthesize life, as discussed in the review, several milestones have been achieved. A major breakthrough has been achieved by J. Craig Venter's team (Smith et al., 2003) wherein they created first synthetic bacteria *M.*

mycoides JCVI-syn1.0. This attempt to build an artificial chromosome has been synbio's most high-profile projects. However, unlike living cells which reproduce themselves and also evolve and mutate, scientists have not yet learnt how to synthetically design evolving microbes that can be easily understood. Synthetic biologists would need to show that they know-how their creations will behave from generation to generation or indeed over hundreds of generations since microbial organisms reproduce quickly and in near future venter's team assures to achieve the same. Designing of Synthetic Immune System which explores systems of designed yeasts that bring healthcare into the home, allowing individuals to measure and control their own health is also an ambitious project. This personalization of synthetic biology and medicine functions through outsourcing the body's own immune system to technology, for example, yeasts that can sense what the body needs and provide it through biosynthesis of drugs and vitamins. These kinds of designer projects explore the future of synthetic biology, bringing the micro-scale details of engineered genes and bacteria to a human scale. Though these significant synthetic biology advancements are set to change the world, making it a better place in future, researchers have to be attentive towards its societal implications and not to divert attention and resources from other approaches that are less front page friendly, but nonetheless sustainable and de-centralized.

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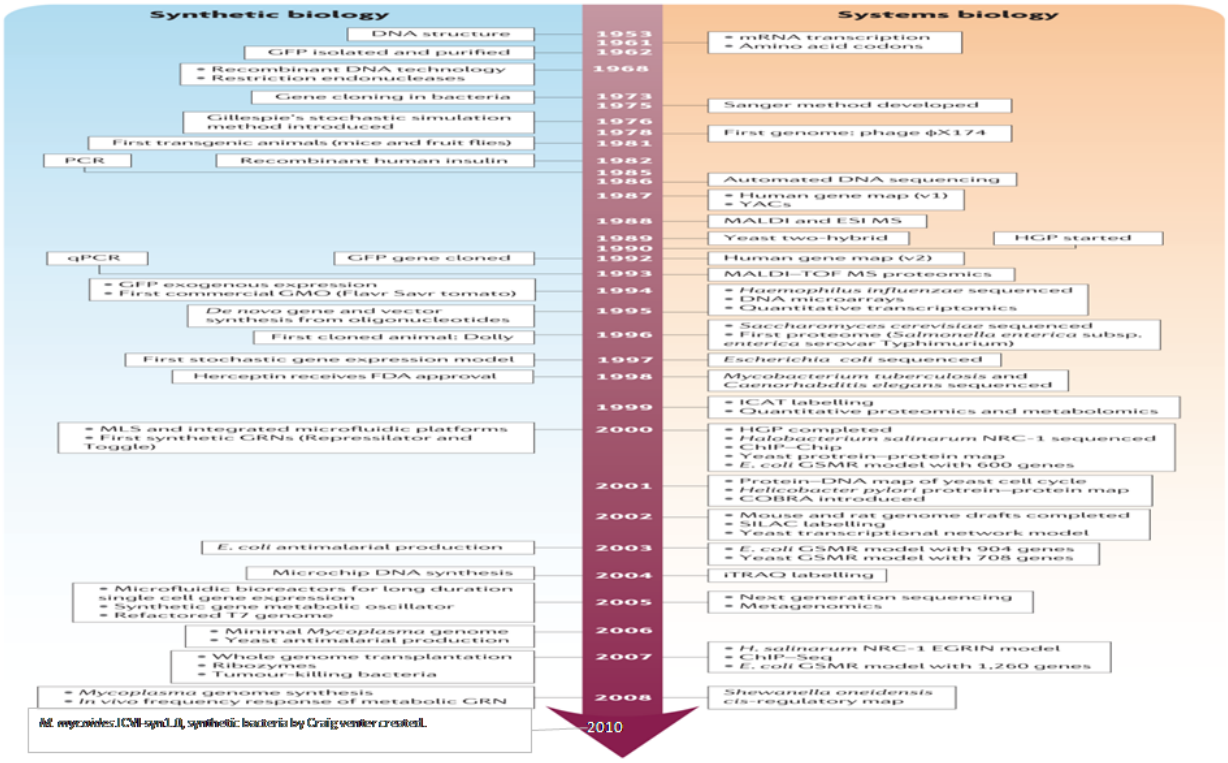

Fig 1: Milestones towards the development of Synthetic Biology vis a vis Sytem Biology [Reprinted by permission from Macmillan Publishers Ltd: [Nat Rev Microbiol], (Koide et al., 2009), copyright (2009)

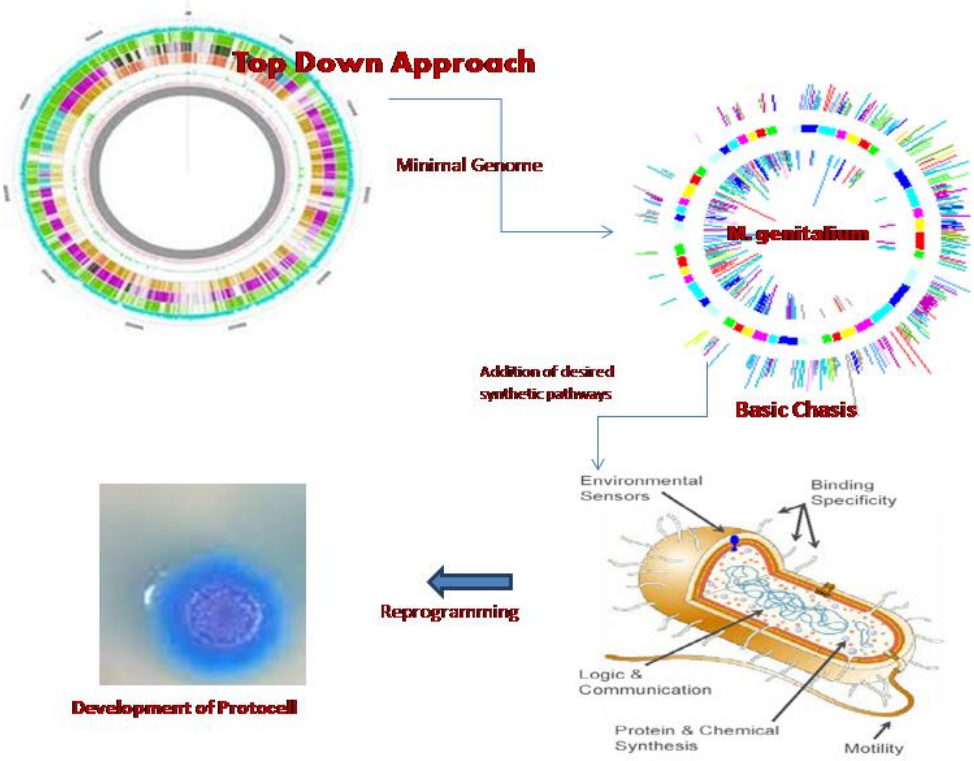

Fig 2: Top Down Approach in Synthetic Biology

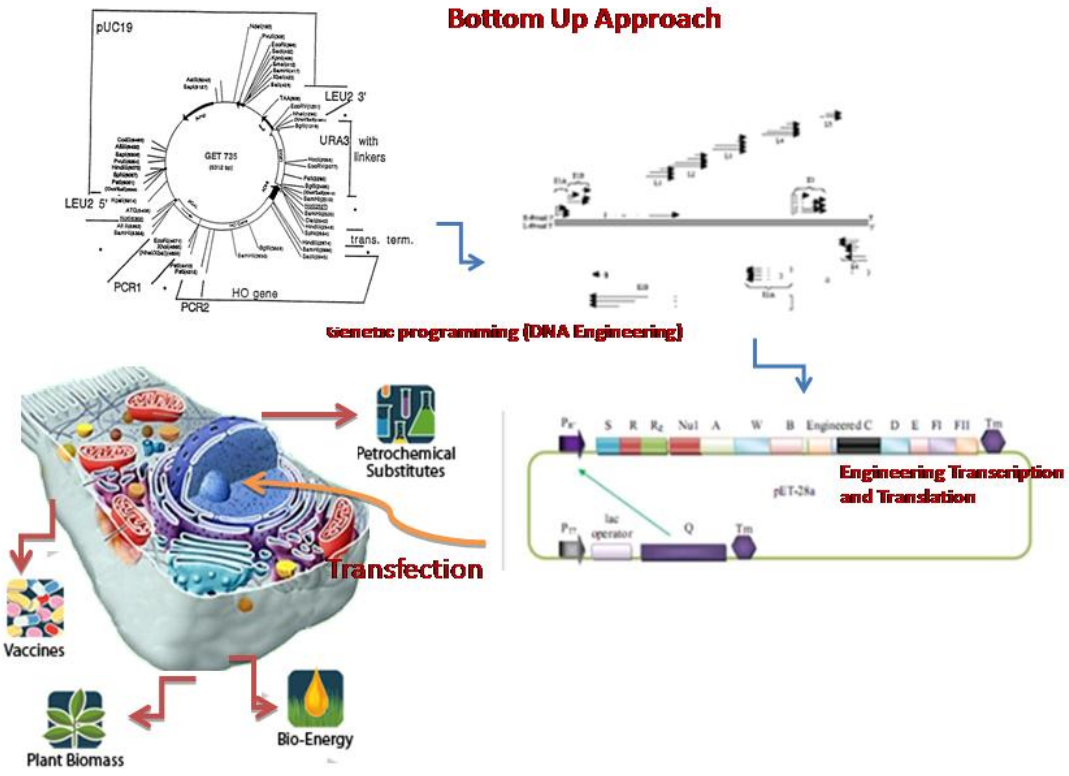

Fig 3: Bottom Up Approach in Synthetic Biology

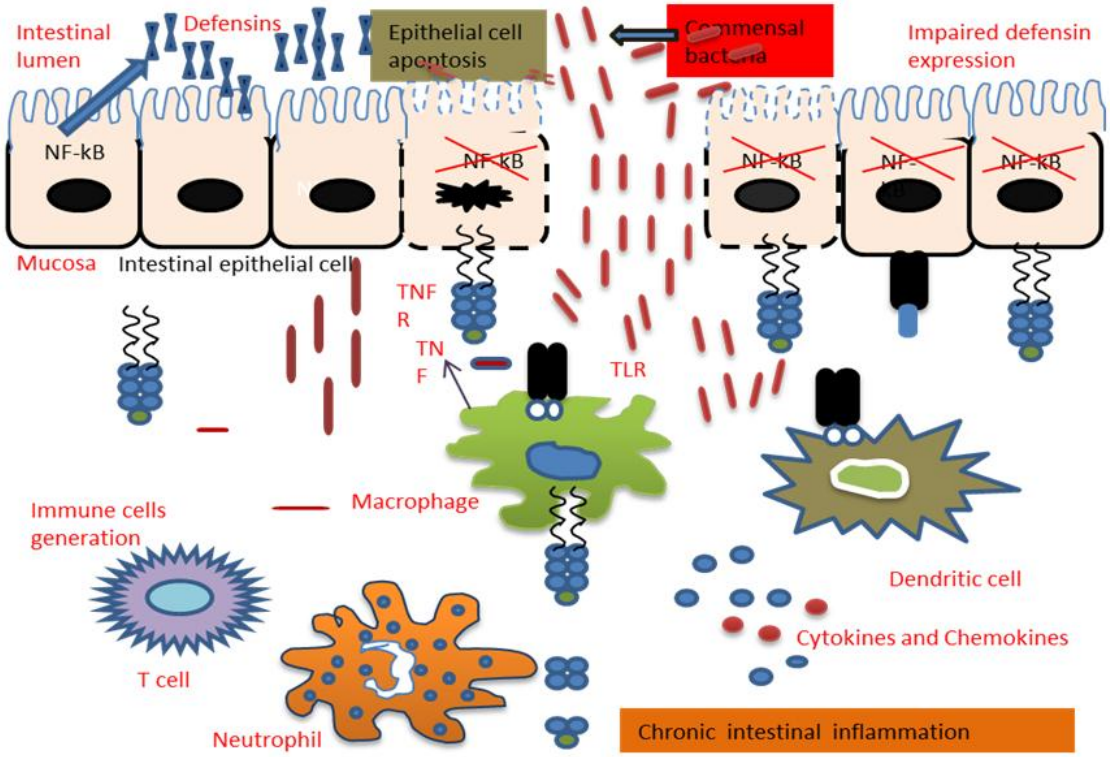

Fig 4: Cross talk between the commensal gut flora, pathogens, dietary ingredients and the host epithelial cells to maintain the gut microbial homeostasis