

NEWCASTLE UNIVERSITY



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Security in the New Era of Synthetic Biology

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ABSTRACT

This report reviews and analyses the legal, security and social implications that accompany synthetic biology research and its applications. Synthetic biology is an emerging field that combines a multitude of scientific disciplines in the design of genetic circuits and synthetic genomes. The science has made significant progress since its initial use in the design of genetic toggle switches. Today its applications are much more diverse in the re-engineering of entire regulatory circuits of microorganisms. Paradoxically, the pace of evolution of this branch of biological science has a self constraining impact on expertise and economics, which in turn, serve to nullify the potential for malicious use. There are no new specific regulatory or control frameworks currently in place for synthetic biology applications *per se*, rather, such prescriptions fall under the umbrella of contemporary genetic engineering regulations. There is pressure for greater transparency and openness of synthetic biology research. This may be achieved by increased publication on open source frameworks. However, transparency of synthetic biology research and cellular mechanisms coupled with the relative ease of accessibility to implement the science raises security concerns

This nascent branch of biology has provided several proof of concept demonstrations that clearly illustrate the important role it will play in strengthening future global securities in the manufacture of foods, medicines and natural materials. There is significant research aimed at reducing reliance on botanical derivatives for medicinal use. It is still too early to assess the full socioeconomic impact that the science may have on smallholding farmers that harvest these natural materials since price stability and less deforestation could provide counter benefits. Synthetic biologists are engineering bacteriophages that systematically invade and disrupt the gene networks of antibiotic resistant bacteria in an effort to overcome the worrying rise of antibiotic resistance. In the search for alternative sources of renewable energy synthetic biologists have engineered bacteria, *Saccharomyces cerevisiae* that are able to produce the next generation biofuel, butanol, from cellulosic biomasses. Synthetic biologists are designing light inducible nitrogen fixation systems aimed at improving crop yields in an effort to address global hunger of the present the future.

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1.0 INTRODUCTION

‘Synthetic biology’ has become a buzzword within the life sciences fraternity since the turn of the millennium, although a precise definition is still to be unanimously agreed. Fundamentally synthetic biology can be divided into two distinct strands: the engineering of novel biological parts and systems, or the re-engineering of natural biological systems. A uniting theme among all synthetic biologists is the adoption of engineering principles through initiatives such as the Registry of Standard Biological Parts, also known as BioBricks (Registry of Standard Biological Parts, 2015).

The advent of BioBricks coupled with enabling technologies such as affordable solid-phase DNA synthesis and essential gene databases has simplified the process of engineering microorganisms. The technology is readily accessible to the extent that thousands of university students are able to engineer personalised microorganisms for the annual international genetically engineered microorganisms (iGEM) competition. Creating a standardised and simplified open source platform has undoubtedly been beneficial for research purposes, however the same ease of access raises security concerns pertaining to dual use research, bio-warfare and bioterrorism.

Synthetic biology is a timely technology with many of the initial applications targeted at the unprecedented grand challenges facing the Human race. Of particular note are: climate change with 2014 being the warmest year ever recorded (Japan Meteorological Agency, 2015), resistance of antibiotics which rapidly increasing, and undernourishment afflicting an estimated 870 million people around the world (Food and Agricultural Organisation of the United Nations, 2015). To compound these problems further, the global population is expected to exceed 10 billion later this century (United Nations, 2013). It is of paramount importance that emerging advanced technologies, such as synthetic biology, be developed and fully utilised to strengthen each of the three major global securities; food, energy and health.

This report aims to review and analyses the legal, security and social implications facing synthetic biology. The security implications cover three aspects:

- Minimising dual use
- Protection of intellectual property
- Strengthening the global securities of energy, food and health

2.0 LEGAL, SECURITY & SOCIAL IMPLICATIONS

The commercial exploitation of synthetic biology will ultimately be constrained by the stringency of the legal and regulatory framework that underpins the science. This section will review the frameworks in place governing applications of synthetic biology. Regulations distinguish which synthetic biology applications will be allowed and those that are outlawed. There are important security issues to be considered, even for those synthetic biology applications that meet regulatory approval. These security issues will be addressed and potential solutions analysed. This section will also give an overview of the likely socioeconomic impact synthetic biology applications will have on some of the world's poorest.

2.1 Legal

Synthetic biology needs a fair and balanced legal framework to operate. If the legal framework is too stringent, as was the case for genetic engineering in Europe, synthetic biology research will be suppressed and the science will not realise its full potential. However if the legal framework is lax or ambiguous, dual use, bioterrorism and bio-warfare concerns will be raised and the public's perception of synthetic biology could be irreparably damaged.

2.1.1 Regulation

To formulate regulations for the emerging field of synthetic biology, one must clearly distinguish this branch of science from its predecessor, genetic engineering. Both fields create genetically modified organisms (GMOs). However a crucial distinction is noticeable during the conceptualisation stages. Synthetic biology has the capacity to build new organisms from the bottom up, and arrange genetic units, rather than modify existing organisms. In this respect synthetic biology more akin to electronics engineering than genetic engineering. A direct consequence of this crucial distinction is that synthetic biology has the potential to rationally engineer microorganisms radically different from or absent in nature. To clarify

this uniqueness from genetically modified organisms from here on organisms derived via synthetic biological methods will be referred to as synthetically modified organisms (SMOs). The complexity of potential SMO's achievable is, in part, due to the diversity of scientific disciplines involved in the emerging field. There are presently 3000 biologists, chemists, engineers, physicists and computer scientists spanning 40 countries contributing to the emerging field (Oldham et al. 2012).

To minimise some of the difficulty regulating the field, synthetic biology has adopted a self-styled open source system via an initiative known as the BioBricks, in which academic and DIY synthetic biologists alike are able to submit biological 'parts' through a publically accessible online catalogue. Each biological part is a DNA sequences that can function as anything from a promoter through to a plasmid backbone; however each part must conform to guidelines that ensure BioBricks are readily assembled in a process analogous to constructing circuit boards in electronics. Proponents of open source claim that it fast tracks research due to its ease of assembly and transparency while also allowing a degree of self-regulation. An exemplar of this open source in action is the annual iGEM competition. In which international universities utilise, and contribute to, the BioBricks system to engineer microorganisms that achieve a set goal such as the detection of pollutants (iGEM University of Cambridge, 2009), production of biofuel (iGEM Rutgers University, 2012), colour photography (iGEM University of Exeter, 2013) or detection of rancid food (iGEM University of Groningen, 2012).

In order to predict likely regulations and controls of synthetic biology applications one must make parallels with the regulation and control surrounding previous genetically modification (GM) endeavours such as GM crops. Until January 2015, the European Union legal arm based in the European Commission (EC) in Brussels dictated the UKs' regulation of GM commerce. Hence, the UK government has had minimal control over commercialization of GM products within the UK for the vast majority of the 21st Century. The EC's stance on GM has been steadfast in its regulation against the commercial use of GM technology. European directives prohibit the commercial growth of GM crops within every EU country including the UK (Gov.UK, 2015). Such restrictive regulations recently lead to a statement in a report by the House of Commons Science and Technology Select Committee declaring that current EU laws are "not fit for purpose" and that "regulatory

reform is no longer merely an option, it is a necessity” (Science and Technology Committee, 2015).

Many commentators on advanced technologies believe synthetic biology applications will be more far reaching than genetic engineering applications in fields ranging from antibiotic production through to bioremediation. The EU set up an expert ‘Working Group’ in late 2008 to determine if EC laws adequately covered synthetic biology research and its subsequent biotechnological applications. The report, published in 2012, concluded that current GMO directives, 2001/18/EC and 2009/41/EC, were pertinent to synthetic biology “for the foreseeable future” (EU Scientific Committee, 2014; Figure 4). By contrast, a 2014 report by the German Ethics Council, which analysed synthetic biology dual use research concerns (DURC), concluded that new regulations surrounding synthetic biology research in Germany were required. The new German legislation proposes that all synthetic biology research grants pass before a centralized German DURC committee (Edwards, 2014). Germany expects other EU countries will follow suit and similar regulations eventually to be in place globally.

Miller and Kershaw (2015) argue that member EU states must create regulations that embrace synthetic biology, unlike genetic engineering, in order to keep pace with Asia and North America in the biotechnological ‘arms race’. The EU member states seem to be taking heed of such warnings; as of 2013 there were 148 synthetic biology companies based in Europe competing across a plethora of markets (Figure 2). These cumulatively contribute the highest percentage to the global synthetic biology revenue, which stood at £1.8 billion in 2013 (Allied Market Research, 2014) and is expected to grow to £7.2 billion by 2016 (Gov.UK, 2013).

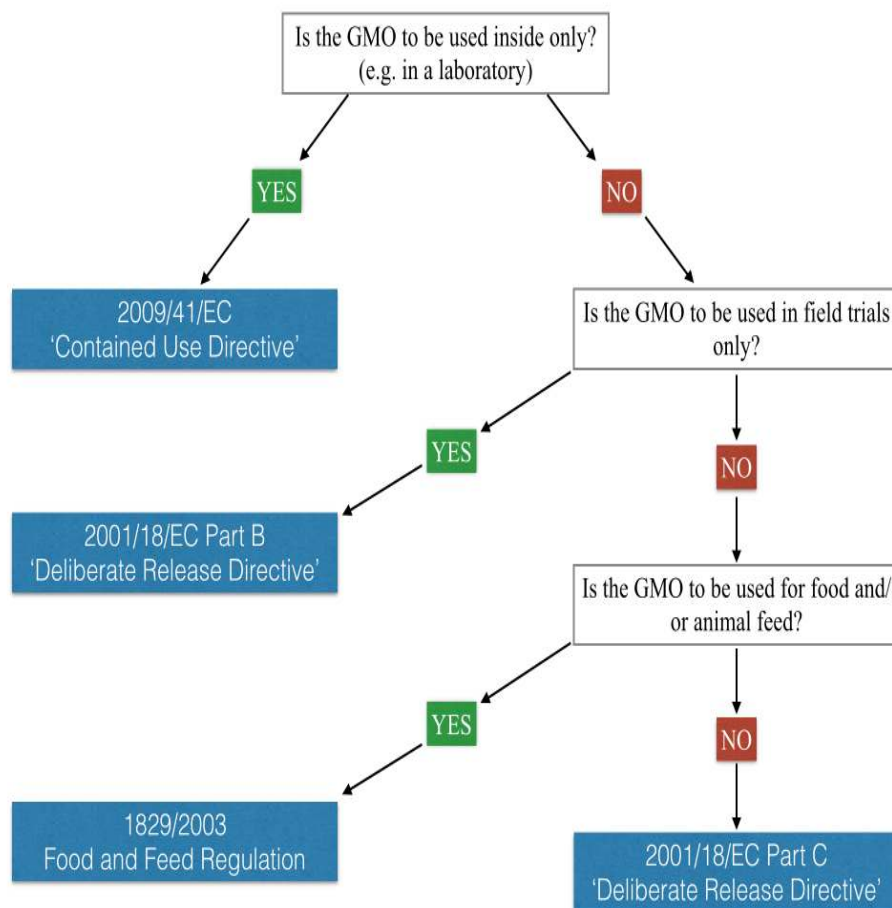


Figure 1 – Flow chart demonstrating how current European Union laws sufficiently govern the use of genetically modification micro-organisms (GMM).

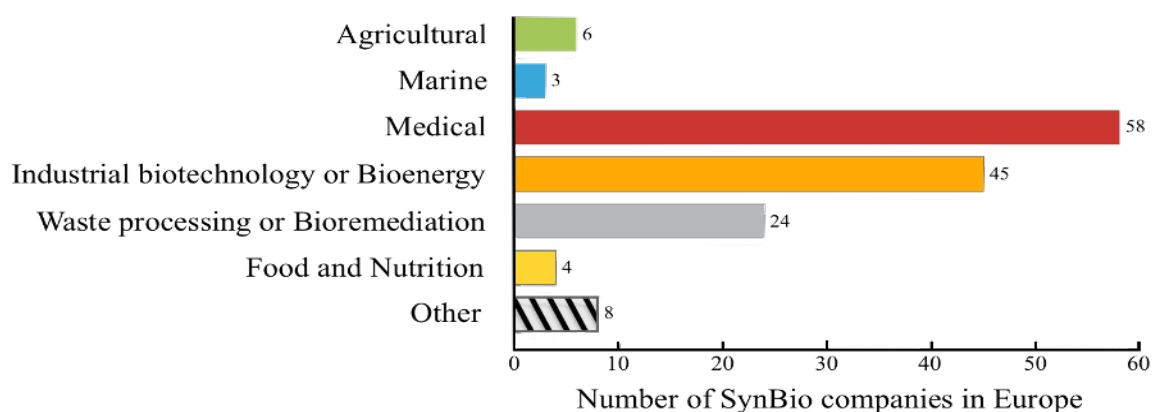


Figure 2 - Number of synthetic biology companies located within Europe during 2013 categorized by sector.
Adapted from ERASynBio Strategic Plan 2014.

2.1.2 Intellectual property

The validity of patents relating to genes, complimentary DNA and genetically modified organisms (GMOs) has been extensively debated at various levels in judicial systems around the world. Four prominent rulings that will form the foundations for synthetic biology patents are listed in Table 2. Using these select previous rulings as a guide SMOs are patentable but BioBricks are not patentable, at least under US law. This is because BioBricks are not markedly different from nature; they are purely isolated genes and other biological parts. The world's first SMO, 'Synthia', was successfully given life at to the J. Craig Venter Institute (JCVI) in 2010 (Gibson et al. 2010). Since Synthia's inception, the JCVI has filed 13 patent 'families' relating to the production of SMOs, but many commentators believe these patents are frivolously vague and merely a deliberate aim to create a monopoly on the applications of SMOs (Chan, 2010), thus stifling growth in similar applications.

Table 1. A selection of court cases that have dictated the biological intellectual property field.

Year, Country	Case	Biological item	Ruling
1911, US	Parke-Davis v. Mulford (Harkness, 2011)	Adrin – therapeutic version of adrenaline	Isolated and purified DNA is patentable
1980, US	Diamond v. Chakrabarty (Dutfield, 2010)	Genetically modified <i>Pseudomonas</i>	GMOs are patentable
2013, US	Association of Molecular Pathology v. Myriad Genetics Inc. (Moore et al. 2015)	<i>BRCA1</i> & <i>BRCA2</i>	Isolated <i>BRCA1</i> and <i>BRCA2</i> are non-patentable. cDNA versions are patentable
2014, Australia	D'Arcy v. Myriad Genetics Inc. (Federal Court of Australia, 2014)	<i>BRCA1</i>	Isolated <i>BRCA1</i> is patentable

A major concern among the synthetic biology fraternity is how patents will be enforced with the inception of advanced technologies such as the 'Biological Teleportation'

device (Venter, 2013). The ‘Biological Teleportation’ device is currently in its prototype stage of development and works by emulating fax machines for biological organisms. A potential method of intellectual property protection would be to incorporate bio-watermarks into the genomes of SMOs. Bio-watermarks are essential for the identification and policing of SMOs that may have escaped containment and for ensuring intellectual property rights are being adhered too. The most basic bio-watermark involves assigning letters of the alphabet to each ‘write’ messages in the form of genetic code within the genome of an SMO, such as *Mycoplasma mycoides* JCVI-syn1.0 (Gibson et al. 2010). A team from the J. Craig Venter Institute printed and assembled the semi-synthetic genome of *M. mycoides* JCVI-syn1.0 from scratch. They were able to insert 5 bio-watermarks into the genome, with information from the bio-watermarks listing 46 authors’ names, an email address, a website address and 3 historical quotes.

An additional layer of security could potentially involve utilising DNA absent words as bio-watermarks. DNA absent words are short DNA sequences, between <20 nucleotides in length, which do not occur in any sequenced genome (primes) or do not occur in a specific species’ genome (nullomers) (Hampikian and Anderson, 2007). Primes naturally possess the greatest potential as a watermark as any organism found to contain a prime sequence is inherently manmade. Hampikian and Anderson (2007) reported 60,000 prime sequences after scanning the National Centre for Biotechnology Information genome database. This leaves the possibility open for biotechnology companies to be assigned a prime sequence that acts as a bio-watermark to be implement into every GMO or SMO they create, thus acting as a ‘DNA fingerprint’ belonging to a specific company or institute.

An inherent problem of both previously stated bio-watermarks is their characteristic of being readily identified, thus subsequently easily excisable from a genome. Clelland et al. (1999) initially identified the possibility of combining the complexity of DNA with Second World War microdot technology. There have been numerous publications covering increasingly complex algorithms capable of concealing DNA messages within living genomes (Arita and Ohashi, 2004; Heider and Barnekow, 2007; Jiao and Goutte, 2009; Wong et al. 2003). These messages can be hidden in the sense that sequencing the genome would yield no results. Hence it would require an original encryption table and knowledge of location of the altered bases to read any message hidden within the genome. This second

layer of security encryption lends itself to patent enforcement and would be well suited for the teleportation of SMOs without fear of removal of bio-watermarks.

2.2 Security

Preventing the nefarious of any emerging technology is paramount. This section assesses how the field of synthetic biology is addressing nefarious acts via one of its enabling technology, DNA synthesis. Furthermore in a time when DNA synthesis costs are plummeting, we analyse some of the most recent biocontainment methods being published, before proposing a novel biocontainment method.

2.2.1. Dual Use

Some of the most lethal viruses ever to grace Earth, which had been eradicated during the 20th Century, have been reconstructed recently. Examples include the Influenza A H1N1 virus ('Spanish flu') (Tumpey et al. 2005) and poliovirus (Cello et al. 2002). Other scientists have generated an Influenza A H5N1 virus ('bird flu') capable of airborne transmission (Herfst et al. 2012; Imai et al. 2012) and a super lethal strain of mousepox (Jackson et al. 2001). One should reflect that scientists did not intentionally design either the bird flu virus or mousepox virus, rather they were merely golden viruses plucked from screening thousands during the course of years of research. It is not beyond the bounds of possibility for an organisation, or rouge group, to make the transition from genetic engineering into synthetic biology and engineer such lethal viruses.

A 2012 report by the United Nations Interregional Crime and Justice Research Institute (UNICRI) concluded that within the short-mid term it is 'plausible' that state funded organisations or states with malign intentions, could utilise synthetic biology derived weapons (UNICRI, 2012). Such threats are currently regulated under the provision of the Biological Weapons Convention treaty. However there is no guarantee these regulations would be enforceable. As an analogy of this issue, synthetic biology derived weapons require comparable resources as their chemical counterparts (e.g. chlorine gas). The latter weapons

were globally outlawed by a Chemical Weapons Convention treaty in 1993, (Britannica, 2013). Despite these global restriction there are unsubstantiated reports that chlorine gas was used in Iraq by Islamic State fighters as recently as January 2015 (Reuters, 2015). Hence, treaties are fine on paper but how does the world police and enforce their restrictions?

Opponents of synthetic biology claim the lethal triad of open source biological parts, cheap DNA synthesis and publically accessible research is a ripe recipe for apocalyptic bio-warfare. In reality, synthetic biology is still an embryonic technology requiring much more development. For example, the creation of the world's first SMO by the world leading synthetic biology institute, J. Craig Venter Institute, took 20 scientists (including a Nobel Prize laureate) 15 years and £25 million (Gibson et al. 2010). Previously when security issues have arisen the synthetic biology community has acted swiftly and efficiently to stop such an event occurring. Aldhous (2005) found that of 12 major international DNA synthesis foundries, 50% 'never or did not regularly' check the customer details or the ordered DNA sequence (Aldhous, 2005; Table 1). Such stark findings have led to the creation of regulatory bodies in both Europe (International Association of Synthetic Biology) and the US (International Gene Synthesis Consortium). Both these bodies employ similar protocols that dictate DNA synthesis foundries must verify customer background (name, email and institution) and check ordered DNA sequences against known pathogenic gene databases supplied by the Australia Group. To test these regulations the University of Lethbridge placed orders for 19 potentially dangerous DNA fragments at several DNA foundries. All foundries flagged all 19 sequences as potentially dangerous and subsequently refused the orders (iGEM University of Lethbridge, 2013).

Table 2 - Replies of DNA synthesis foundries when asked if they screen the background of customers or the DNA sequence against a list of known potentially harmful or banned genes. Companies marked in red subsequently formed the International Gene Synthesis Consortium in 2009, which represents 80% of the global DNA synthesis capacity (International Gene Synthesis Consortium, 2013). Adapted from Aldhous, 2005.

DNA Synthesis Company	Scan customers' background or DNA sequence
Bio Basic, Canada	Never
Bio S&T, Canada	Never
Genemed Synthesis, US	Never

Picoscript, US	Not regularly
Bionexus, US	Not regularly
BaseClear, Netherlands	Not regularly
GenScript, US	Regularly
GENEART, Germany	Always
Entelechon, Germany	Always
Blue Heron Biotechnology, US	Always
DNA 2.0, US	Always
Integrated DNA Technologies, US	Always

So are we on the verge of opening Pandoras' box to the age of the personalized super virus? Although the design and synthesis of a 'super virus' is beyond the capabilities of our current knowledge and technology today, it is not improbable in the future. Human genomic data is being generated at unprecedented rates. The US plan to sequence 1,000,000 genomes by 2020 (Regalado, 2015), the UK plans 100,000 genomes by 2017 (Genomics England, 2015) and a complete genome map for Iceland was already reported earlier this year (Gudbjartsson et al. 2015). It is not beyond our imagination to conceive lethal synthetic 'super viruses' designed to target a specific nationality or race. Data from previous DNA sequence studies, such as the 1000 genome project (The 1000 Genomes Project Consortium, 2012), has shown that humans from one geographical location, especially islands, are much more likely to have a specific trait. Thus it can be extrapolated from this finding that DNA sequences of people living in isolated communities are relatively the same, in comparison to people inhabiting other geographical areas.

2.2.2 Biocontainment

As we progress the 21st Century and biotechnological advances continued to outstrip many commentators' expectations, it is imperative we possess full control over SMOs. Irreversible ecological damage caused by the unintended proliferation of SMOs with subsequent vertical and horizontal gene transfer is one of the main fears of the public, pressure groups and scientists alike. The probability of this threat is growing at an ever-

increasing rate as commercial applications, such as *in-situ* bioremediation, involving the intended release of SMOs into the open environment start to be put forward. The first purposeful release of an SMO is already on the horizon. The Glowing Plant Inc., intends to post 100 seeds, capable of producing bioluminescent *Arabidopsis thaliana*, to 8,433 US based customers later this year (Glowing Plant, 2015). However the actual ecological risk may be limited, as Muir and Howard (1999) demonstrated that the offspring of GMOs have decreased fitness, thus they are outcompeted by their wild type counterparts.

Conventional biocontainment methods focus on creating uniplex biocontainment systems such as auxotrophy or toxin kill switches. Auxotrophic GMOs are an excellent method of biocontainment on agar plates, however the mechanism has two fatal flaws for commercial use: scale-up has proved uneconomical to produce sufficient auxotrophic molecules and, natural compounds can mimic auxotrophic molecules, thus circumventing the biocontainment system. Toxin on the other hand, kill switches and decrease the fitness of an organism, creating a negative selection pressure and limiting their commercial uses. A recent joint study by John Hopkins University and the University of Edinburgh focused on ousting traditional uniplex biocontainment systems for a more robust and secure duplex system (Cai et al. 2015). Their proposed duplex system placed the essential histone genes, H3 and H4, under the control of estradiol and galactose inducible promoters respectively. These genes were only activated when a minimum concentration of 30 nM estradiol and galactose was added to induce histone expression. As two essential genes are inducible by two different molecules, no natural molecules would be able to circumvent the system if unintended proliferation in the environment occurred.

Mandell et al. (2015) and Rovner et al. (2015) propose similar mechanisms that focus on creating a dependency upon a synthetic essential amino acid. As essential amino acids can not be synthesized *de novo*, the SMO would be completely reliant upon a continuous exogenous supply. As the amino acid is synthetic no molecule found in nature would be able to circumvent the biocontainment system. In order to create this dependency, both teams engineered an enzyme capable of incorporating the respective synthetic amino acid into the protein during translation upon recognition of a predetermined triplet codon.

Adapting the proposed methods of Mandell et al. (2015) and Rovner et al. (2015), there is scope for creating a reliance upon exogenous synthetic DNA in the guise of unnatural base pairs. Although research is still in its infancy, it may be possible to contain SMOs via artificially expanded genetic information systems (AEGIS). The simplest AEGIS is a six-base system arbitrarily referred to as A-T, C-G, X-Y, with X-Y representing the unnatural base pairs. The most promising unnatural base pairing, dTPT3-dNaM, has a fidelity that is almost comparable to the natural base pairings of dA-dT and dC-dG during PCR (Dhami et al. 2014; Li et al. 2014). Experiments focused on how the cellular machinery of cells react to unnatural base pairs has yield evidence that AEGIS may have a prominent future in synthetic biology. Yang et al. 2011 demonstrate that unnatural base pairs are both excised from and inserted into the genome of *E. coli*, allowing SMOs containing an AEGIS to evolve just as a normal genome would. Malyshev et al. (2014) engineered *E. coli* to express an algal nucleotide transporter and found that uptake of unnatural base pairs via the algal nucleotide transporter was sufficient for the *E. coli* to successfully replicate DNA containing unnatural base pairs. Seo et al. (2009) demonstrated unnatural base pairs are efficiently transcribed by RNA polymerase, while Betz et al. (2012) demonstrated that KlenTaq DNA polymerase can efficiently synthesize DNA strands containing unnatural base pairs.

2.3 Social

Social acceptance of synthetic biology as a technology is pivotal for the successful growth of the science. Public acceptance relies on a correct balance of factual and accessible information pertaining to SMOs and synthetic biology. On the flip side, if synthetic biology applications are accepted, SMOs may end up directly competing against the very same people who accepted it as a technology, in a situation analogous to the introduction of robotics. This section will analyse how the public may be swayed on the issue of synthetic biology, before analysing the impact of some of the initial SMO applications on the developing world.

2.3.1 Perception

The public perception of GM as a technology is largely negative in the UK. This is demonstrated by a limited survey of 180 people carried out by the University of Nottingham in 2006. The survey found that 73% of respondents gained their information of GM purely from the media (Saponja, 2006). Another interesting, and slightly alarming finding, was that

the general public and pupils studying GCSE Biology had similar levels of misconceptions about GM. Much of the negativity and hysteria originates from very effective media campaigns centred on GM crops by pressure groups such as Greenpeace, GeneWatch UK and Friends of the Earth. A particularly successful smear campaign by Friends of the Earth labelled GM food products as “Frankenstein foods” (Friends of the Earth, 1998); a phrase that was subsequently used by major UK news outlets such as the BBC (2008), Independent (2013), Metro (2013) and Channel 4 (2013). Such widespread negative media attention sowed the seeds of doubt about GM in the mind of the UKs’ public. Supporting the view that prejudices were irrational Andrew Miller MP, chairman of the House of Commons Science and Technology Select Committee, earlier this year declared that opposition to GM has been based on “politics, not science” (Science and Technology Committee, 2015).

More recent dissemination of GM science appears to be providing a more balanced and informed view in the public. A 2013 poll of public perception of GM crops by the UK based Independent newspaper found 47% respondents supported GM crops, 42% did not and 11% were undecided (Independent, 2013). The public’s slow, but affirmative change towards acceptance of GM technology, provides a positive outlook for the emerging synthetic biology industry. This apparent swaying of public acceptance is based on a recent public survey conducted by The Biotechnology and Biological Sciences Research Council (BBSRC). The BBSRC found public support for government help creating synthetic biology industries allied to; medicine was 80%, energy 70%, bioremediation 62% and food 55% (BBSRC, 2010; Figure 3). Clearly the data indicates there is significant public demand in the UK to utilize synthetic biology as a future technology but, moreover, it also outlines the public’s reservations about potential environmental damage by synthetic biology which reinforces the need for a robust biocontainment method as outlined in Section 2.2.2.

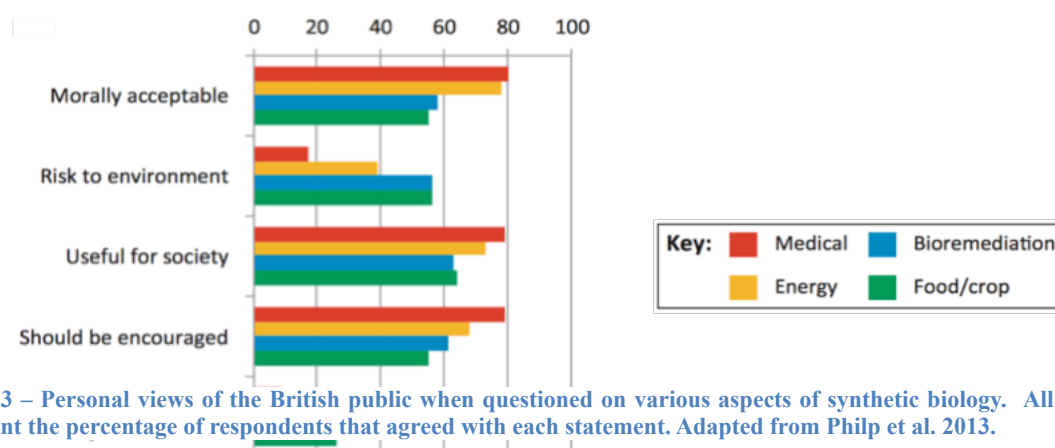


Figure 3 – Personal views of the British public when questioned on various aspects of synthetic biology. All values represent the percentage of respondents that agreed with each statement. Adapted from Philp et al. 2013.

2.3.2 Socioeconomic factors

Synthetic biology has potential to produce stable sources of raw materials in the production of foods, medicines and materials. Traditionally, these products are all prone to annual or cyclic price volatility due to unforeseen anthropogenic or environmental problems affecting raw material supplies. Synthetic biology should be able to deliver constant, and predetermined amounts of raw materials that will stabilise markets thus preventing price fluctuation. However millions of smallholding farmers from many of countries (Figure 4) are reliant on harvesting these raw materials as their sole source of income. Many commentators state that synthetic biology will follow a similar path to robotics, providing economic stability to the minority but taking away jobs and economic stability from societies most vulnerable. The rest of this section discusses three examples of commercial applications of synthetic biology.

The most prominent case reflecting the socioeconomic impact of synthetic biology surrounds semi-synthetic artemisinin. Artemisinin-based combined therapy (ACT) is listed by the World Health Organisation as the most effective treatment for malaria caused by *Plasmodium falciparum* (World Health Organisation, 2015). Natural artemisinin is a botanically derived drug from *Artemisia annua*; a plant that is harvested by 100,000 farmers across China and Vietnam (Figure 4A). A synthetic biology venture to engineer *S. cerevisiae* capable of synthesizing the artemisinin precursor, artemisinic acid, was started in 2003 by Professor Jay Keasling at Amyris, Inc. (California, US). In 2008, Amyris, Inc. gave multinational pharmaceutical company, Sanofi, the royalty-free rights to use the engineered *S. cerevisiae*. Commercialization was realized, one decade on from the project inception, when Sanofi produced 40 million treatments of semi-synthetic artemisinin (Peplow, 2013). The distribution of artemisinin is based on a “no profit, no loss” principle which is in accordance with the funding wishes stipulated by the Bill and Melinda Gates Foundation. The initial release of semi-synthetic artemisinin was marketed at £250 per kilogram, a price towards the upper range of natural artemisinin, £200-270 per kilogram (Peplow, 2013).

Synthetic biology could have an unlikely future within the food industry. Cocoa butter equivalents (CBE) are a cheap palm oil-based substitute for cocoa butter with the same flavour as cocoa butter when blended to make chocolate based foods. Multinational confectionary companies are assessing CBEs as a viable cocoa substitute as there is a significant price disparity: £2200 ton⁻¹ (CBE) and £5500 ton⁻¹ (Cocoa) (Pardomuan and Nicholson, 2014). Cocoa is primarily sourced from 5-6 million smallholding farmers located across West Africa (Côte d'Ivoire, Ghana and Nigeria) and Indonesia (Figure 4B). The livelihood of these farmers could be in jeopardy as Solazyme, Inc. (California, US) aims to commercialise its engineered microalgae that are capable of synthesising CBEs by the end of the year (Solazyme Inc., 2014).

Synthetic biology has gained significant interest from the rubber industry. The natural rubber industry relies upon the procurement of latex, which is supplied by 20 million farmers from South East Asia (Figure 4D). The majority of this rubber is used by the tyre manufacturing industry with each tyre requiring 30% natural rubber in the formulation (Bridgestone, 2014). To create natural rubber via synthetic biology, numerous companies are engineering microbes to express the enzyme isoprene synthase; the enzyme responsible for latex synthesis in rubber trees. This endeavour has forged multiple partnerships between tyre manufactures and synthetic biology companies: Bridgestone and Ajinomoto (Bridgestone, 2012), Goodyear and DuPont (DuPont, 2012), Michelin, Amyris and Braskem (Michelin, 2014).

Other markets at risk in the near future are:

- Coconut oil, Palm oil and Babassu (Figure 4C)
- Saffron (Figure 4E)
- Vanilla (Figure 4F)

It is worth noting that all of the regions highlighted as supplying the raw requirements in Figure 4 are located within some of the most economically deprived areas of the world.

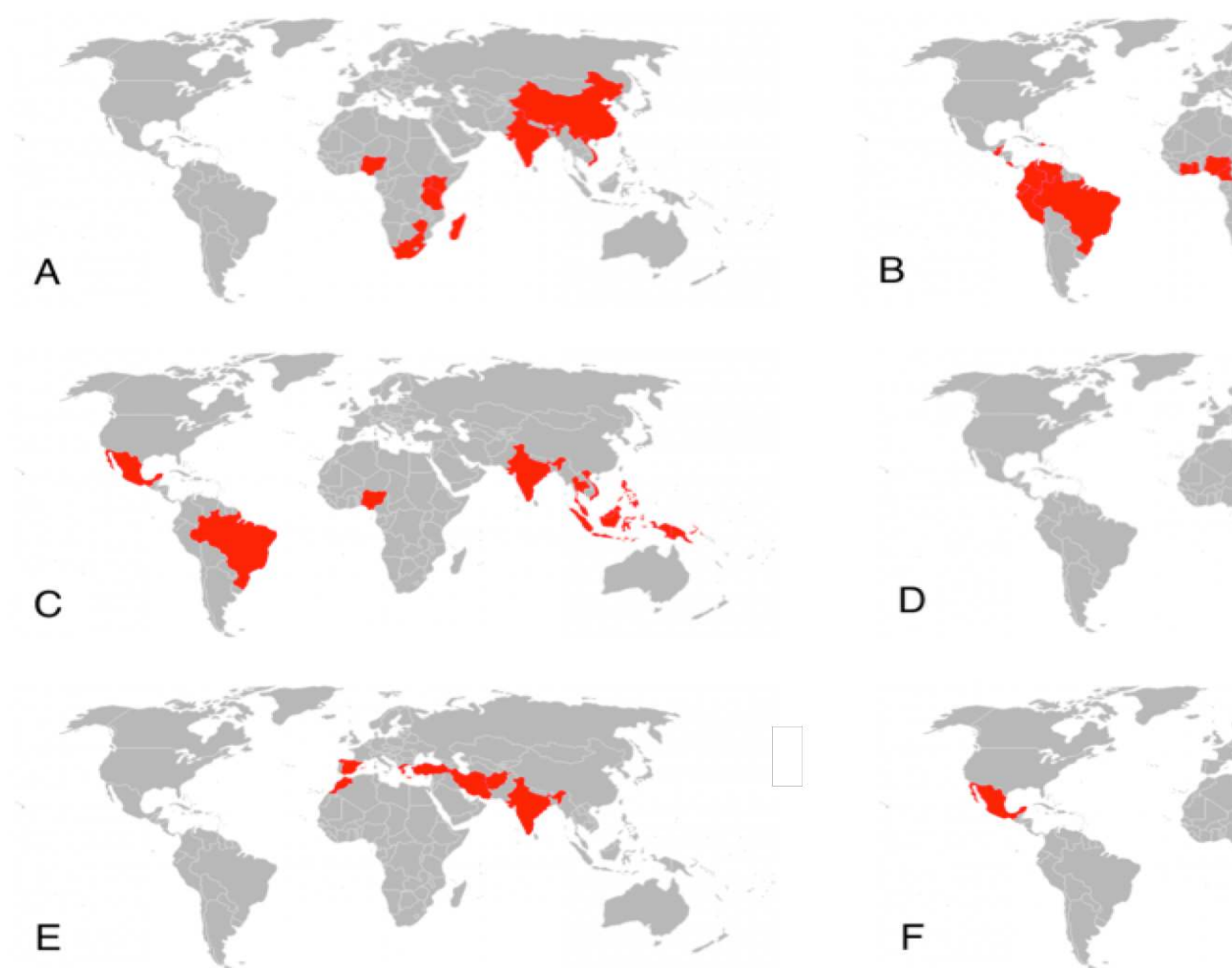


Figure 4. Procurement map showing the countries that provide the raw materials for the production: A) Artemisinin; B) Clove and Babassu; D) Rubber; E) Saffron; F) Vanilla.

3.0 GLOBAL SECURITY

This section assesses the potential of synthetic biology to strength global security by addressing each of the three Grand Challenges of the 21st Century: energy, food and health. The Grand Challenges of this century will span a diverse range of issues ranging from providing sufficient amounts of food for 10 billion people to slowing global warming and preventing antibiotics becoming obsolete.

3.1 Energy

Energy security is fundamental in future policy planning by governments around the world. Energy demand has been rising 7% annually in the newly industrialised countries of Brazil, China, India and Mexico (BP, 2015). Global energy demand in 2013 was 12.7 billion tonnes of oil equivalent (Gtoe) and is predicted to rise to 17.8 Gtoe by 2035 (BP, 2015; Figure 9). Hence reserves of traditional petroleum based fuels stocks are being depleted. There is a strong desire from industrialised nations to have control over their fuel source. Added to this is the continued pressure to reduce global warming. Increasing energy consumption is not detrimental *per se*; rather fossil based energy production is heavily linked to global warming so there is equal ambition to find new sources of non-fossil based fuels. Synthetic biology may offer solutions. In 2013, 62.9% of energy production was supplied by fossil-based sources but only 2.2% was produced via renewable energy. Given such discrepancies, governments around the world have initiated the process of reforming their energy supplies. The EU has mandated that all retail petrol must be compliant with E10 (European Renewable Energy Directive, 2009). E10 is a fuel consisting of 10% ethanol and 90% petrol. In a similar stance to stave of petroleum fuel shortages the US has passed legislation requiring the annual production of 136 billion litres of biofuel by 2022 (Energy Independence and Security Act, 2007).

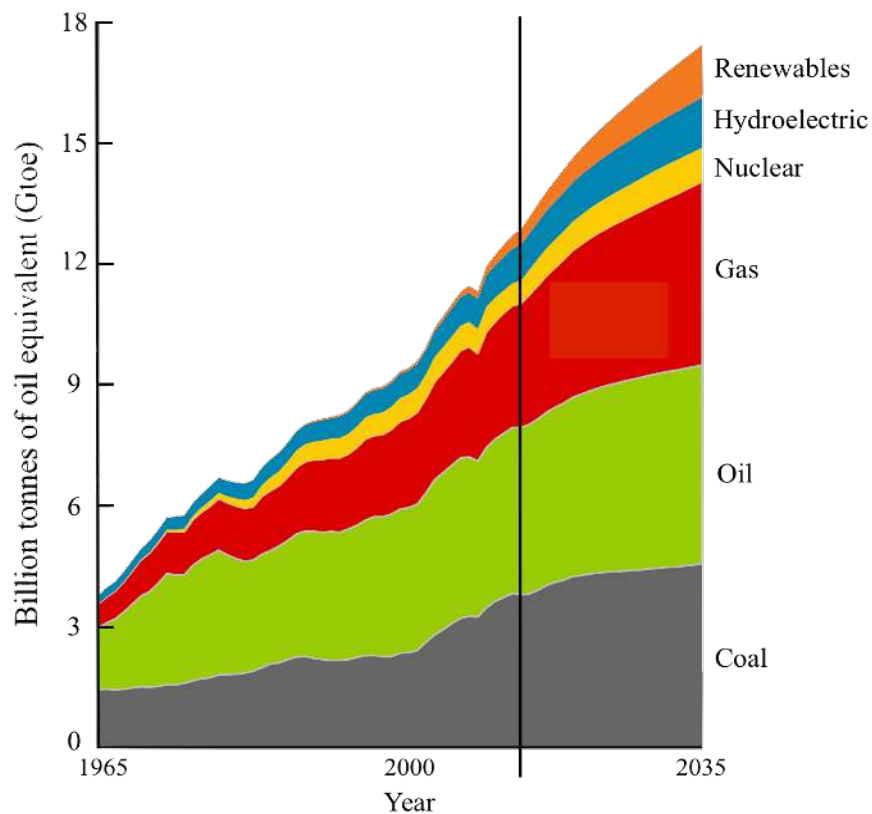


Figure 5. Global energy demand measured in billion tonnes of oil equivalent (Gtoe). Adapted from BP Energy Outlook 2035.

3.1.1. Ethanol

Global biofuel production peaked in 2013 with 115 billion litres (Gl) being produced, of which 90 Gl was ethanol (Figure 10; IEA 2014). Brazil and the US account for 83% (Brazil 27%, US 56% respectively) of the world's ethanol production. Ethanol production requires the use of biomass, typically from geographically dependant crops; corn (US) and sugarcane (Brazil). Synthetic biology can potentially solve these limitations. Using crops to produce biofuel is under criticism for their inefficiency as a sustainable biomass. These crops, especially corn, require large applications of nitrogen fertilizer.

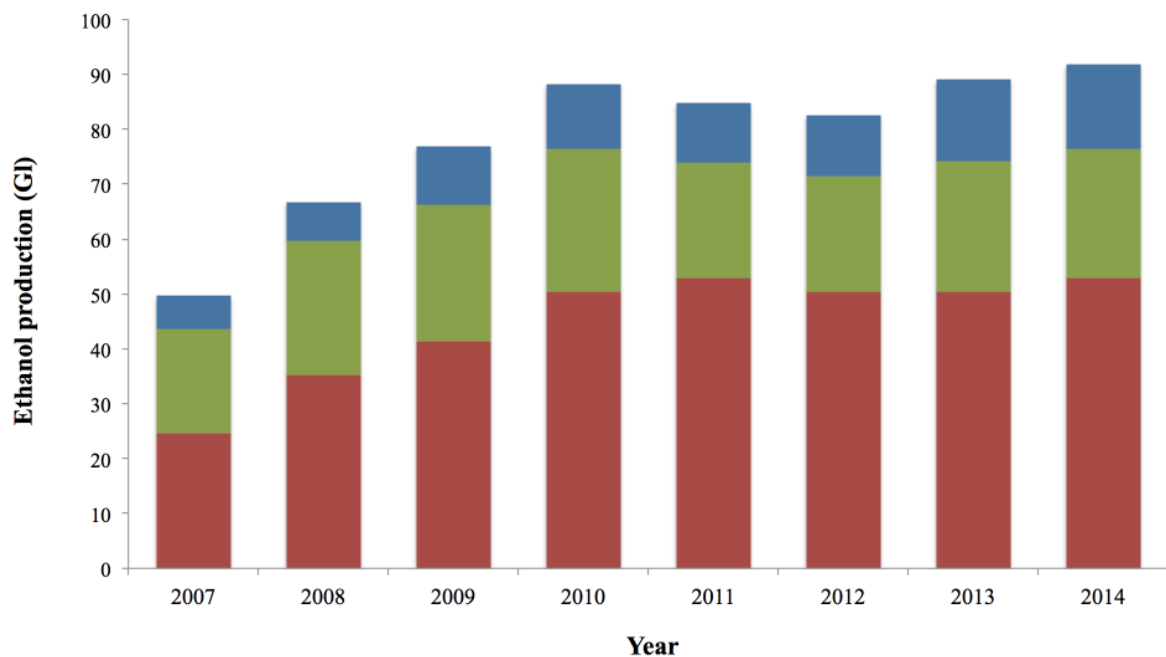


Figure 6 - Global ethanol production measured in billion of litres (Gl) by the United States of America (red), Brazil (green) and Rest of the World (blue) during the period 2007-2013. Data sourced from Renewable Fuels Association, 2015.

Industrial nitrogen fixation via the Haber-Bosch process consumes 1% of the world's energy budget each year (Rowlatt, 2014). A significant proportion of this nitrogen is used as a fertilizer for US corn production. The Haber-Bosch process also produces excessive amounts of the potent greenhouse gas, nitrous oxide. A radical solution to significantly reduce the need for industrially fixed nitrogen combines two synthetic biology research areas: light inducible genetic circuits and re-engineering bacterial nitrogen fixation gene clusters.

Creating light inducible genetic circuits is one of the more progressive research areas of synthetic biology (Shimizu-Sato et al. 2002; Levskaya et al. 2005; Moglich et al. 2009; Tabor et al. 2011). Recently Lee et al. (2013) engineered a light inducible gene expression system within *E. coli* JM1012 via expressing *Hol*, *PcyA* and *Cph8*. In unrelated research, Temme et al. (2012) re-engineered a 20-gene (23,500 base pair) nitrogen fixation gene cluster from *Klebsiella oxytoca* to remove the native regulatory system. Combining these two streams of research it may be possible in the near future to engineer crops capable of fixing nitrogen in specific plant structures such as in the leaves or stems (Figure 11). Furthermore, expression of the nitrogen fixation gene cluster could be regulated to only activate when the intensity of a certain wavelength is exceeded; avoiding energy wasting unnecessary gene

expression or the need for expensive auxotrophic molecules as found in older genetic engineering systems.

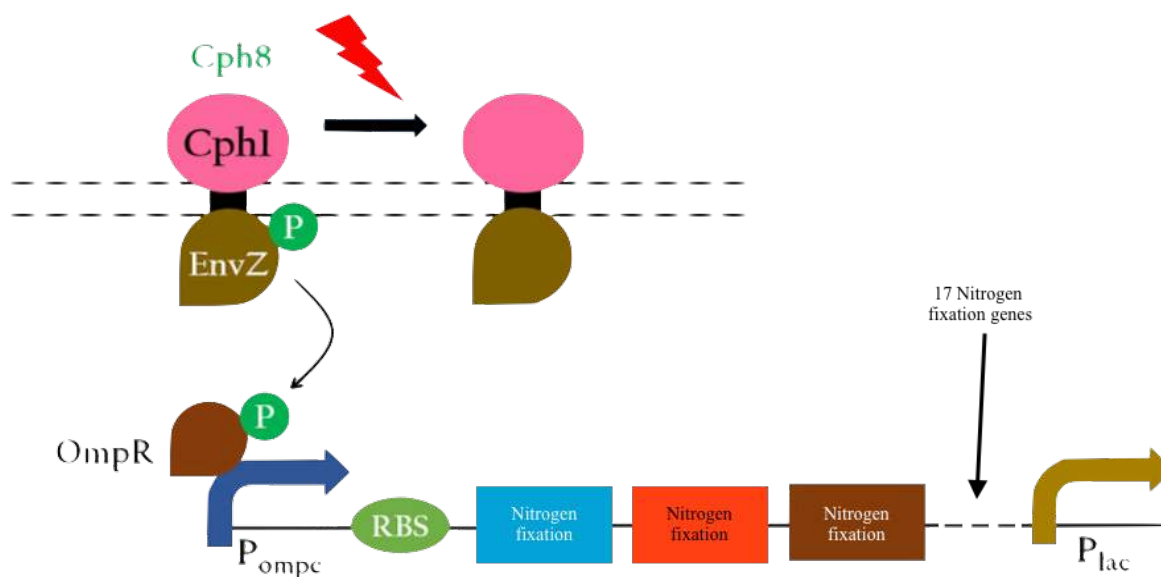


Figure 7 – Schematic diagram outlining how chimeric Cph8 (Cph1-EnvZ) is capable of eliciting photoreceptor regulation of gene expression via red light (620–750 nm). Exposing Cph8 to light wavelengths between 620-750 nm deactivates Cph8, thus repressing the system. However in the absence of light wavelengths 620-750 nm, Cph8 is activated which enables EnvZ-OmpR to autophosphorylate, thus activating P_{ompc} which promotes expression of the nitrogen gene cluster. Adapted from Registry of Standard Biological Parts, 2013.

3.1.2 Butanol

Structurally, butanol has two carbon atoms more than ethanol. However, butanol has two commercial benefits over ethanol. Firstly up to 25% can be blended with petrol without engine modifications (University of Kentucky, 2012). By contrast, ethanol can only be blended up to 15% without specialised engines. Secondly, butanol produces 30% more power per gallon than ethanol (Wass, 2013). Mattam and Yazdani (2013) researched a synthetic biology production method for butanol that is both economic and sustainable. They engineered *E. coli* that is capable of efficiently converting butyric acid into butanol by expressing the following *Clostridium acetobutylicum* genes: *buk* (butyrate kinase), *ptb* (phosphotransbutyrylase) and *adhE2* (aldehyde/alcohol dehydrogenase).

3.2 Food

Food supply is already reaching critical levels and risking future global food security. Reports suggest 870 million people were undernourished in 2013 (Food and Agriculture Organisation of the United Nations, 2013) and nearly half of all deaths in children under 5 were linked to malnourishment (UNICEF, 2015). This bleak situation will intensify as global demand for crops is projected to rise 85-110% by 2050 (Tilman et al. 2011; Ray et al. 2013). This section will review how synthetic biology can improve crop yields thus improving arable-based food security to 2050 and beyond.

3.2.1 Improving crop yield

Yields from traditional crop cultivation techniques are beginning to plateau. GM crops only account for an annual 1.3% increase in crop yield over previous years (Tilman et al. 2011). Tilman et al. (2011) calculated an annual yield increase of 2.4% is required to meet the demands of 2050. As discussed in Section 3.1.1, engineered crops capable of light inducible nitrogen fixation are nearing realisation and may provide massive yield improvements since crops currently rely either upon symbiotic associations with diazotrophs (legumes) or fertilizers to gain sufficient amounts of nitrogen-based compounds.

3.2.2 Limiting crop stress

2014 was the hottest year ever recorded (Japan Meteorological Agency, 2015). Warming reduces the amount of accessible water for plants, which could have dire consequences for future global food security as 70% of current global water usage is devoted to agricultural activities (Food and Agriculture Organisation of the United Nations, 2015). During 2014, California suffered its worst drought in over a decade; with NASA (2014) imaging estimating 42 trillion litres of water is required to replenish supplies. In a similar situation, Brazil is currently in the midst of its' worst drought since 1930 (New Scientist, 2015). If climate change ultimately means that these drought conditions become the norm, then major re-engineering of drought tolerant crops is essential.

Plants minimise water loss by up regulating expression of the hormone, abscisic acid (ABA), which binds to ABA receptors causing stomatal closing. A problem with this system is that it requires an environmental stimulus so is slow to react to drought conditions. The

entire cellular mechanism responsible for up regulating ABA expression and detecting ABA levels was discovered independently by Ma et al. (2009) and Park et al. (2009). Capitalising upon these findings Park et al. (2015) re-engineered an ABA receptor to be activated by a commonly used fungicide, mandipropamid. Engineering crops containing such a system would enable farmers to switch on the drought resistance mechanism in their engineered crops well in advance of any environmental cues, thus saving water and minimising yield losses.

Over the duration of the 20th Century the average life expectancy of a person living in the United States of America increased by ~25 years (Mapping History, date unknown). Scientific, most prominently medical, advances made during the 20th Century such as antibiotics, X-ray imaging and organ transplants contributed massively to this impressive increase. However as the average age of the world population increases ‘new diseases’ such as cancer, cardiovascular disease and dementia replace the ‘old diseases’. A new wave of medical advances is required to prevent a recession in the population average ages of many developed countries.

3.3.1 Antibiotic Resistance

During 2012 there were 450,000 incidents of multidrug resistant tuberculosis spanning 92 countries (World Health Organisation, 2014). Antibiotic resistance has all the hallmarks of becoming the single most important health related problem of the 21st Century. The rate of antibiotic resistance is now proliferating faster than new antibiotics are being identified and commercialised. During the past decade synthetic biologists have been engineering microbial strains capable of producing novel antibiotics or weakening antibiotic resistance mechanisms, thus increasing the bacterium’s susceptibility to conventional antibiotics. A couple of select synthetic biology research projects are discussed here.

Lu and Collins (2007) used synthetic biology techniques to engineer lytic bacteriophage T7 (ϕ T7) to express the biofilm-matrix degrading enzyme, dispersin B; this resulted in a death rate of 99.997% of ϕ T7 treated biofilms. Utilising a different mechanism Lu and Collins (2009) engineered M13mp18 bacteriophage (ϕ_{lexA3}) to overexpress the ‘SOS DNA repair system’ repressor gene, *lexA3*, by placing it under the control of a synthetic inducible promoter. As ϕ_{lexA3} is non-lytic, infection does not alert natural bacterial defence mechanisms, allowing for efficient suppression. Antibiotic susceptibility experiments combining ϕ_{lexA3} and ofloxacin resulted in a 5000-fold increase in cell death of antibiotic resistant *Escherichia coli* K12 compared to using ofloxacin alone. Using the combined treatment on mice resulted in 80% survival rates of mice containing antibiotic resistance bacteria, compared to 20% using ofloxacin alone.

3.3.2 Cancer

The probability of a person living in the UK being diagnosed with cancer during their lifetime has nearly doubled from 25% to 40% over the last 40 years (Cancer Research UK, 2015). Cancer-targeting bacteria have been imagined as a cancer cure for centuries (Pawelek et al. 2003). In a promising synthetic biology twist intelligent anti-cancer bacteria are engineered to communicate with cells and distinguish healthy cells from cancerous cells. To achieve the ‘communication’ stage Anderson et al. (2006) engineered strains of *E. coli* MC1061 to only invade β -integrin mammalian cells via expressing the invasion gene (*inv*) from *Yersinia pseudotuberculosis*, which was induced by the anaerobic *fdhF* promoter. Also engineered into the system was the *lux* operon from *Vibrio fischeri*. This operon enabled cell density detection via quorum sensing. Thus the engineered *E. coli* only invaded mammalian cells during high cell densities and hypoxic conditions, characteristics associated with cancerous cells. Trials of the engineered *E. coli* demonstrated their capability at detecting and invading cervical carcinoma, hepatocarcinoma and osteosarcoma cells. (Anderson et al. 2006)

3.3.4 Gut Microbiota Engineering

An individual’s gut microbiota consists of all the bacterial cells residing within the gut lumen. These bacterial cells roughly outnumber their human cells by a factor of 10 (Rajpal and Brown, 2013). DNA sequencing is gaining traction at an increasing rate. Scientists are now able to map the bacteria that constitute the microbiota and engineer a personalised microbiota consisting of ‘intelligent’ bacteria capable of preventing infections (Duan and March, 2010) or deliver therapeutic molecules (Duan et al. 2008).

Duan and March (2010) engineered *E. coli* Nissle 1917 to express cholera autoinducer 1 (CAI-1) and autoinducer 2 (AI-2) from *V. cholerae*. CAI-1 and AI-2 are used as the molecules by *V. cholerae*, during quorum sensing, as a mechanism of detecting the *V. cholerae* density in a specific area such as the human gut. If *V. cholerae* density is already high within the gut then proliferation is stopped. Thus the engineered *E. coli* are able to artificially halt the pathogenesis of *V. cholerae* by expressing CAI-1 and AI-2. Mice trials found that mice that had the engineered *E. coli* within their microbiota’s had 80% reductions in gut cholerae toxin binding levels (Duan and March, 2010).

Duan et al. (2008) engineered *E. coli* Nissle 1917 to express the pancreatic and duodenal homeobox gene 1 (PDX-1) under the control of a glucose inducible promoter. As glucose passes through the gut, the promoter is activated and PDX-1 is transcribed and translated. *E. coli* then secretes PDX-1, which in turn stimulates β cells and intestine epithelia to produce insulin.

4.0 CONCLUSIONS

The numerous publications in journals and iGEM projects clearly support the notion synthetic biology is a rapidly emerging science. Applications are diverse, with many focused at addressing shortages in essential or strategic resources such as energy, food and health. With few exceptions, many applications are still in an embryonic research stage. Surveys suggest the UK general public are cautious in accepting synthetic biology, an empathy founded following controversies over GMO's during the last couple of decades. There is a view greater public acceptance of this emerging branch of biology will only occur through continued openness and transparency of research activity by those involved. This process has already started through the formation of an open source platform, greatly improved public relations in comparison to genetic engineering and education of next generation scientists via iGEM.

Overarching regulation and control of synthetic biology applications within Europe is presently covered under European Commission Scientific Committee directives pertaining to GMO's. That is, there are no new and specific regulations intended for this emerging branch of science. However member states are starting to introduce tighter regulations pertaining to research activities within the field. Germany, for example, has recently introduced legislation requiring all synthetic biology research grants to go through a centralized German DURC committee for approval. The relative ease by which one can order DNA online for the creation of GMO's has led to the establishment of self-governing synthetic biology bodies in Europe (International Association of Synthetic Biology) and the US (International Gene Synthesis Consortium). Both these bodies employ similar protocols in an attempt to prohibit DNA synthesis foundries intentionally or unintentionally assisting nefarious acts.

The very nature of open access to research information on mechanisms for building synthetic organisms, coupled with an apparent lack of robust regulation, raises serious security concerns. Surprisingly, resources required to design, manipulate and build biological systems with capability to process information, produce energy, synthesize chemicals, provide food and enhance human health are minimal. It is possible to order DNA fragments online from DNA synthesis foundries and circumvent controls designed at preventing the manipulation and creation of potent organic toxins. The ease by which one can access synthetic biology shows the science is highly vulnerable to misuse by organisations or rogue groups with malicious intent.

An aspect of security, the protection and policing of bona fide intellectual property is currently under development. One method utilises bio-watermarking to assign letters of the alphabet written as specific short code sequences to ‘write’ messages within the genome fragments. A further possibility I believe is worth consideration is to use DNA absent words as bio-watermarks. DNA absent words are short DNA sequences, between ≤ 20 nucleotides in length, which do not occur in any sequenced genome (primes). Any organism found to contain a prime sequence is inherently manmade.

Many synthetic biology applications are aimed at replacing natural botanical raw materials, used primarily in medicines, with semi-synthetic versions. This undoubtedly has socioeconomic impact. It will stabilise raw material supply and probably not only stabilise but also reduce costs in the long term to the benefit of the modern world. There are concerns the substitution of natural with synthetic raw materials will have a detrimental impact on the livelihood of smallholding farmers in the poorest countries of Africa, South East Asia and South America where most of these botanical materials are harvested. It’s too early to speculate what the overall impact will be since secondary benefits may be derived that paradoxically support smallholding farmers through improving market confidence during anthropogenic or environmental downturn, thus removing price fluctuations and, supporting local ecology through less deforestation.

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