We attempted to contact politicians, for them to understand the fundamentals of our project and synthetic biology. To do this we wrote an article aimed to be published in POST (Parliamentary Office of Science and Technology).
POST targets parliamentarians to engage in science and be more aware of increasing aspects of research such as medical advances, global communications and also environmental issues. This gives them a better understanding to examine the issues effectively.

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The Biosynthesis of Fragrant Terpenoids in *Escherichia coli*

Overview

- Synthetic biology is an upcoming field of research that is being used to solve many problems.
- The current industrial practices to extract fragrances are dangerous and harmful to the environment.
- Synthetic biology can be used as an alternative to produce fragrances such as lavender, ginger and lemon.
- The positive and negative impacts of using synthetic biology to produce scents in industry.
- This concept can be further exploited by introducing a light-controlled system in bacteria.

Many plants used in the fragrance industry are expensive to grow. Additionally their synthesis involves the exposure to dangerous and harmful compounds. This POST NOTE focuses on using synthetic biology as a strategy to produce fragrant compounds such as lavender, lemon and ginger. This involves manipulation of biobricks and the bacterium *Escherichia coli* to provide an alternative method to current industrial practices.

Background

The problem- Unsustainable practices in the fragrance industry

Many plants used in the scent industry are rare and are expensive to grow. Fragrances such as roses, champaca, ginger and lavender are highly desirable but are expensive to harvest, costing as much as $76 for 1ml of plant oil. [1]. Plant scent extraction is highly inefficient; oils make up around 5% of the total plant mass harvested and average yields of oil after chemical extraction is around 0.5-1%.[2] Acres of land are wasted to generate tiny amounts of useful products, and we believe this land can be put to better use such as planting food crop etc. Some scent plants, for example Indian sandalwood, grow best under specific conditions and temperatures making mass cultivation very expensive. [1,3]

Currently, scent compounds are removed from plants after purification mainly by CO_2 extraction. This involves putting CO_2 under large amounts of pressure to form a liquid. This liquid is then able to diffuse across the membrane, enter the cells and adhere to the aromatic constituents and be removed. This process e.g. in *Zingibirene officinale* affects the bitterness of the plant but not the scent.[3]

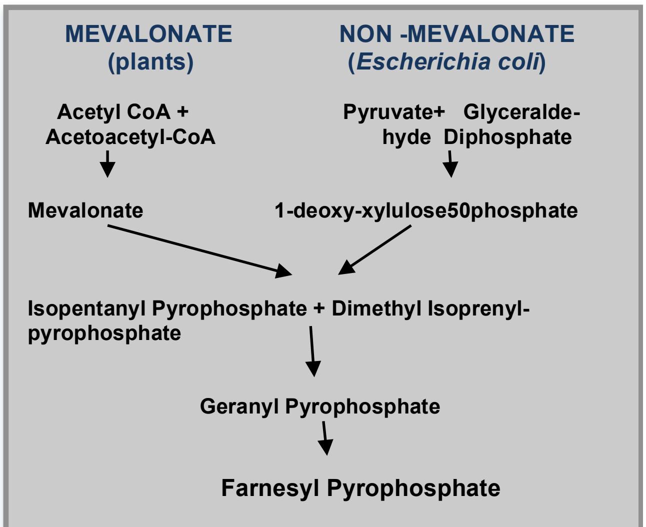
The low output to input ratio, coupled with the high cost of the pressure chamber increase the overall cost of the whole process. In addition, the process is also prone to contamination with pesticides. Since there is an issue of lack of health and safety risks information of this process, pesticides could also be harmful to workers who are not aware of their presence in the processing plant. As with other high pressure procedure, there is risk of explosion. Therefore, it is highly essential that workers are trained to operate the machinery properly and protective clothing must be worn by those who are in contact with the chemicals to reduce the risk of harm which is a cost-adding factor to the process. [4,5]

Synthetic Biology for fragrance production

Synthetic biology is essentially a process where biological parts are standardised: it is the design and engineering of biologically based parts, novel devices and systems as well as the redesign of existing, natural biological systems. It has the potential to deliver important novel applications and improve existing industrial processes – resulting in economic growth and job creation.

Terpenoids

Terpenoids are molecules that are derived from the condensation of 5-carbon isoprene units. They are involved in the growth regulation, signalling, and defence mechanisms in plants. Additionally, terpenoids have a characteristic aroma, which explains why they are used in industry for the production of perfumes, cosmetics, household products, etc.[7]
There are two metabolic pathways used to synthesize terpenoids: the mevalonate pathway in plants and the non-mevalonate pathway in *Escherichia coli (fig1)*. Both pathways ultimately lead to the production of the following compounds: Isopentanyl pyrophosphate and dimethyl Isoprenyl pyrophosphate, which are further converted into geranyl pyrophosphate and farnesyl pyrophosphate, which is a precursor of terpenoids. (Box1)[7,8]



Box 1.Mevalonate and Non-mevalonate pathway

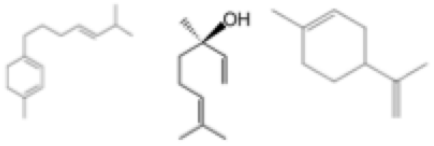
Zingiberene

Zingiberene (Box 2) is the major component (34%) in ginger rhizomes (Zingiber officinale). It is responsible for the pleasant ginger aroma of the plant.

Zingiberene is synthesised by Zingiberene synthase, an enzyme found in ginger rhizomes. Zingiberene synthase converts farnesyl diphosphate into zingiberene and diphosphate in a single enzymatic reaction. Zingiberene ($C_{15}H_{24}$) is classified as a sesquiterpene - a terpenoid that is characterized by the presence of 15 carbon atoms.[9,10,11]

It is extracted via a process known as steam distillation. This involves exposing the plant, in this case ginger, to high levels of steam, whereby the essential oil turns to gas

The organisms used in synthetic biology do not cause disease and have relatively simple genomes that can be easily manipulated
Interestingly, ginger exhibits other properties as well such as anti-ulcer and anti-inflammatory activity which are being further investigated. [12]



Zingiberene R-linalool Limonene

Box 2. The chemical structure of terpenoids.[10,13]

R-Linalool:

R-linalool is classified as a terpenoid (terpenoid) that is found in the essential oil of the *Lavandula* family that contributes to the lavender scent. It is synthesized by the enzyme R-linalool synthase using Geranyl diphosphate and water as substrates, producing R-linalool and Diphosphate as products. Lavender is a scent that is commonly employed by the fragrance industry: e.g. soaps, skin lotions, cleaning products, perfumes and other products. [13,14]

It is noteworthy that R-linalool is not only extracted from lavender plant alone. Other methods to manufacture R-Linalool which are more commonly used in industrial processes is the fractional distillation of the essential oil from plants such as Bois de rose (rosewood), shiu or coriander. Other major sources of R-Linalool include Brazilian rosewood, Chinese and Taiwanese Ho leaf. In some plants, for example *Freesia*, the R-linalool exists as the R-(-) enantiomer only. The chemical synthesis of R-Linalool involves—chemicals such as acetylene and acetone or isoprene hydrochloride. [13,14,15,16]

In the year 2000, approximately 12,000 tonnes of Linalool was produced, out of which more than half was synthesized chemically.[13]

Limonene:
Limonene is a scented cyclic terpene (Box2) from the hydrocarbon family. Limonene is the fragrant compound which is commonly found in lemon and citrus fruits. Usually limonene is harvested from fruits using steam distillation or centrifugal separation. Steam distillation yields around 1%; this is far greater than other distillation techniques which can yield as low as 0.1% [9,10,11]

Limonene is a very common ingredient in cosmetic products, often found in shampoos, lotions, perfumes and other everyday items. The demand for it is huge; as the yield is so small, farming to produce such product is very wasteful.[9,10,11]

Pros and cons of the system

Synthetic biology is being exploited in research as an upcoming solution to many problems and is therefore a relatively new concept to society.

Currently, there are several commercial companies which use synthetic biology to synthesize fragrant terpenoids instead of the more commonly practised methods, which involve the use of harmful chemicals. Additionally, the land that is used to grow lavender can be used to grow other food sources. As with any system, this concept has positive and negative impacts (see Box 3 & 4).

Advantages:

Environment:
Currently essentials oils are extracted from plants using processes such as CO_2 extraction, which requires high pressures, and steam distillation, which requires high temperatures. Both processes are energy-demanding. [4,5] Yields of essential oils obtained through distillation processes are also very low, rarely above 5%, with yields for lavender to be approximately 1% and lemon 2%.[3] This means large amounts of crops are grown to obtain very little of the desired product. The synthesis of the fragrant terpenoids using synthetic biology could allow the production of these fragrant compounds with less energy consumption and less waste products produced.

Creating an environmentally-friendly fragrance industry could increase the awareness of consumers towards the impact of the products they buy on the environment. This may increase the demand for environmentally-friendly products created from other industries and thus promote environmentally sustainable manufacturing in other industries

temperatures. Production of fragrant compounds using synthetic biology could eliminate these extreme working conditions. Furthermore, strains of *E.coli* used is non-pathogenic are often used in current synthetic biology based production.

Increased land availability:
With the human population rapidly increasing, land availability for food production is becoming a problem. A report by the Food and Agriculture Organisation for the United Nations in 2011 stated that today's population of around 7 billion is expected to increase to about 9 billion by 2050 (United Nations, 2009). By this time, another one billion tonnes of cereals and 200 million tonnes of livestock products will need to be produced every year (Bruinsma, 2009)[18].The reduction in use of land for fragrance production will free up land for food crop production instead.

Creation of jobs:
With an increased population receiving a higher level of education, there is an increasing number of trained scientists looking for jobs in industry. Additionally, in 2012 statement BBSRC 600M pounds were invested in research councils where 10M pounds were invested in synthetic biology centres based in Bristol, Cambridge/Norwich and Nottingham.[19]

***Scientific advancement/medical research*:**
Terpenoids are not just used in the fragrance industry, they are also commonly found in antimalarial and anticancer drugs. The creation of terpenoids for use in medical products is also being researched [20]. Methods developed in our project could possibly aid research in the pharmaceutical area. It could also aid further synthetic biology research not related to the production of terpenoids by creating a better understanding of the applications of synthetic biology.

***Public image of GMO use*:**
The use of GMOs in synthetic biology to create fragrances may create a 'route of acceptance' to the use of GMOs by the public. Discussions with a number of people with no scientific background showed us that the public may be more likely to accept a product that is created using GM bacteria, as long as the bacteria are not present in the. final product that they would directly consume. This may allow the public to begin accepting the use of GMOs and synthetic biology, thus improving the image of these terms.

Emergence of new fragrance markets:
One of the major benefits of this method is the synthesis of multiple fragrances in each bacterium, as this could allow for a new market to emerge in customisable fragrances. The customisation would be cheap and easy to do and allow for personalisation of fragrances.

Box 3. Best case scenario

- Patent – Money
- Jobs
- Better for the environment
- Medical care
- Clean the land
- Actual extraction of terpenes worked
- Land to make more food
- New environmentally friendly manufacturing process
- Buying products that benefit
- Reduce industrial effect due to this method
- Avoid harmful chemicals
- Safe
- Reduce environmental impact

Disadvantages:

Biodiversity
On an industrial scale, many areas of land currently used to grow a variety of flowering plants may be lost. The land may then be used to grow food crops, and could result in large areas of monocultures. This will cause a loss of plant biodiversity, and could have a knock-on negative impact on other species such as bees. An article in Nature in 2011 stated 'What bees need most, the new pollination studies have shown, is a diverse community of flowering plants that bloom throughout the spring and summer.'[21].

Public acceptance
Society often has a bad image of the use of bacteria and of the use of GMOs. If the public rejected the idea of the project, this could have a damaging impact on public opinion of using GMOs for other purposes.

Risks of release of GMOs to the environment:
The concept of synthesizing terpenoids using *Escherichia coli* involves the use of DNA that carries antibiotic resistance. If a spillage were to occur, the antibiotic-resistant plasmid used could potentially be transferred to other organisms in the environment, passing on drug resistance. This imposes problems to humans because stronger antibiotics or new families of antibiotics will need to be administered during infection.

Tourism:
Many people are attracted to areas of natural beauty when on holiday, and this brings in valuable income for many people who rely on the tourism industry. Removing many areas of land that are currently planted with a variety of flowers may have a damaging effect on this industry in some areas.

Economic damage to third world countries
:Loss of crop sales in poorer countries, and the moving of the production of fragrance compounds from poorer countries to first world countries (which have better scientific facilities, technology and trained labours) could have a damaging impact on the economy of some third world countries. This could result in a greater gap between the economies of first and third world countries.

Box 4. Worst case Scenario

- Bacteria have bad first opinion
- Consequences of genetic meltdown
- Toxicity
- Bee population suffering
- Degradation
- Cost effective?
- People's opinions from bacteria
- Biodiversity
- Public Perception
- Safety
- Greater gap between 1st and 3rd world economy's
- We don't know underlying impact or underlying mechanism behind the project
- Loss of crop sales from poorer countries
- Toxicity of products
- Mess up ecosystem
- Cost Price

Future of the project

Other fragrances can also be produced using synthetic biology such as musk, mint, etc. Additionally the other functions of terpenoids may be exploited, for example in the pharmaceutical industry. The use of this model can be further modified such as the incorporation of a light controlled system. In other words, the genome of the bacterium would contain more than one fragrance e.g. R-linalool and ginger. The desired fragrance to be expressed would be determined by the light that was being used: red or blue.

Endnotes

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