Synthetic Biology for Bio-Control: A Mini-Review

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Abstract

Biomolecular systems and cellular capabilities can be engineered by synthetic biology. One of its wide applications is for bio-control. Several teams competing in the International Genetically Engineered Machine (iGEM) competition have utilized the potency of synthetic biology for pest control. Some of their ideas are highlighted in this review. Taking advantages from Indonesia’s rich biodiversity, those ideas can be expanded. Bio-control using E. coli expressing pheromone biosynthesis activating neuropeptide (PBAN) from rice yellow stem borer (Scirpophaga incertulas) can be used to control that pest. The adapted ideas can serve as starting points for research and implementation of bio-control in Indonesia.

Keywords: Bio-control, biodiversity, iGEM, Indonesia, synthetic biology

1. Introduction

Pests need to be treated correctly in order to remove them from the hosts. The use of conventional pesticides is decreasing lately because of their resistant effects and environmental issues [1]. Nowadays, people around the world are changing the pesticides used into any other pest management agents that are more environmentally friendly. Pest bio-control is a strategy used to reduce or even remove pests from a certain environment by using natural agents, such as predators, competitors, parasites, or diseases, without giving residues or polluting the environment [1,2,3]. The use of pest bio-controlling agents is less harmful for other living organisms as well as for the environment, therefore it is a promising solution for pest control systems.

Some examples of pest bio-control which have been used are grouped into natural enemies, microbial pesticides, pest behaviour-modifying chemicals, plant immunization, and genetic manipulation of pest populations [3]. Natural enemies consist of the use of predators, competitors, parasites, and diseases. Poisonous substances produced by microbes can also be used in controlling pests, and are categorized into microbial pesticides. Recently researchers are developing a strategy in pest controlling by using pheromone-like substances which modify pest behaviour. Plant can also be used to control the growth of pests, i.e. by intensifying the immune response. The release of some kinds of genetically modified pests to produce sterility traits is also a bio-control approach [1]. Safety of bio-control implementation is due to some reasons. It uses natural enemies or substances that work specifically to a certain species of pests so other organisms around will not be killed. The use of natural enemies also indicate that the action in bio-controlling system works in less toxic ways as it does not left any chemical residues. On the other hand, the use of biological controlling agents are still developing, because this system needs a deep understanding on the biological aspects of the ecological system included [1,2].

An emerging discipline, synthetic biology attempts to recreate unnatural system in living organism by designing and building novel biomolecular components, network, and pathway to reprogram an organism [4,5]. Synthetic biology has proved its application in branched DNA (bDNA) assay to detect HIV and hepatitis infection. In recombinant DNA technology, synthetic biology searches for interchangeable parts that function in living organisms [4]. Application of systematic design is the central aspect of synthetic biology. Synthetic biology follows a hierarchy as follows: parts, which encode biological function of a molecule; devices, a collection of parts with defined function; and system, set of devices that can perform a task. A construct involving modulation, characterization, and standardization of parts is designed in order to create a new device [6].

As this promising field arises, international competitions are initiated. One of the biggest contributors to development of synthetic biology is iGEM (International Genetic Engineered Machine). The competition involving graduate, undergraduate, and even high school students to participate in full time lab working to characterize or standardize parts called BioBrick. Through iGEM, synthetic biology has been expanding its application to medicine and manufacturing field [6]. Synthetic biology also have some common applications including biosensing, therapeutics, biofuels, pharmaceuticals, and biomaterial [5].

The role of synthetic biology in the field of bio-control has emerged as a promising technology in pest managements. In this field of study, researchers construct the genetic sequences of a microorganism to produce some kinds of metabolites, by altering the behaviour or straightly kill the pests. Using synthetic biology in pest bio-
control is beneficial because it is more controllable than the conventional bio-control methods due to the former's systematic design. In this review, several ideas of bio-control initiated in the iGEM competition are discussed.

2. Synthetic Biology for Bio-control

Strategies of bio-control using synthetic biology mainly focus on producing poisonous substances or pheromone to kill or trap the pests. One example of synthetic biology idea in producing poisonous substances is by producing pest’s metabolism inhibitor. Tokyo NoKoGen 2014 team used this strategy to genetically engineered E. coli to control cockroaches. The bacteria, termed Exterminator coli, is designed to produced 3,3’-diketotrehalose (3-3’dkT) which can inhibit trehalase enzyme needed for energy storage conversion [7]. Several enzymes are overexpressed, such as OtsA, OtsB, and G3DH. G3DH (glucose-3-dehydrogenase) will convert trehalose produced by E. coli into 3,3’-dkT. When cockroaches ingest the bacteria, 3,3’-dkT will be ingested and inhibit trehalase and eventually kill the insect. While OtsA and OtsB are native to E. coli, G3DH must be introduced from other species. One organism capable of producing G3DH is button mushroom Agaricus bisporus [8]. This edible and cultivated mushroom can be found in Indonesia and serve as genetic source for G3DH. Another source for this enzyme is marine bacterium Halomonas sp. α-15 [9]. As a maritime nation, it is not impossible to find other marine bacteria that have G3DH in Indonesian seas.

Many strategies target the pest pheromone. Polytechnic University of Valencia Team [10] constructed a plant that produce moth’s sex pheromones such as the broad spectrum (z)-11-hexadecenal-1-ol (Z11-16:OH) and more specific (z)-11-hexadecenal (Z11-16:Ald) and (z)-11-hexadecenyl acetate (Z11-16:OAc). Z11-16:Ald and Z11-16:Oac are the derivates of Z11-16:OH, so plants producing these pheromones can attract male moths such as Helicoverpa armigera, one of moth species also found in Indonesia as chili pepper pest [11]. The construct to produce sex pheromones consists of four genes: a desaturase (Atr 11), a reductase (HarFAR), an acetyltransferase (EaDACt) and an alcohol oxidase (FAO) that are isolated from different organisms. Three of them (Atr 11, HarFAR, and EaDACt) are isolated from moths. FAO is isolated from a yeast, Candida tropicalis, which can be found in Asia. This method, finally, can be applied in pest bio-control. Another team, Formosa 2014 proposed the idea of pheromone-activating E. coli [12]. As the pheromone biosynthesis is too complex to be carried by E. coli, the bacteria is designed to produce PBAN (Pheromone biosynthesis activating neuropeptide) that can activate pheromones biosynthesis of specific insects. This neuropeptide’s coding sequence is only around 100 base pairs, short enough to avoid metabolic burden. When trapped insects consume this E. coli, PBAN consumed alongside will activate pheromone biosynthesis, thus the insects will produce pheromones to attract more insects into the trap. Therefore more insects are trapped and the population control can be achieved. The PBAN is species-specific and the E. coli can be designed to produce PBAN from several target species. This strategy seems plausible to be implemented in Indonesia. In order to control rice yellow stem borer (Scirrophaga incertulas), we only need to characterize its PBAN and express it in E. coli.

Not only to control pest, bio-control agents can be developed to control or detect pathogens too. We have faced the effects of Panama disease causing banana devastation. It was caused by Fusarium oxysporum f. sp. cubense and cannot be controlled by usual crop protection methods such as fungicides [13]. Now, a novel variant of F. oxysporum was found in Sumatra namely tropical race 4 (TR4), a virulent strain of fungi that attacks Cavendish plantation. It is predicted that this strain is spread largely throughout the world [14]. Inspired by the devastating effects of Panama disease, the iGEM team Wageningen 2014 made a new system called BananaGuard. This project aimed to prevent unproductive banana production due to soil-borne pathogen and to introduce a system into agriculture that will ensure food safety [15]. It consisted of three main combination activities, detection, inhibition, and self-destruction. The BananaGuard was a bacterial platform designed to control the Panama disease using Pseudomonas putida which could detect F. oxysporum presence in the soil by sensing fusaric acid, the phytotoxin produced by the patogen [16], expressing four fungal growth inhibitors, and destroying itself when F. oxysporum cannot be detected anymore.

The iGEM team Wageningen 2014 characterised and validated a putative fusaric acid dependent promoter together with its hypothesized regulator (isolated from P. putida KT2440), cloned the sequence upstream the green fluorescent protein, and transformed in P. putida KT2440 to make the detection system. Three genes (encoding Methionine-γ-lyase, Dimethylsulfide (DMDS) and dimethyltrisulfide (DMTS); Pirl, produce pyoverdine in presence of iron; and Chitinase, overexpresses chitinase activity) and one gene cluster, phiABCDE, able to produce 2,4-Diacetylphloroglucinol (2,4-DAGP) were cloned behind an IPTG inducible promoter to make the inhibition system. Using the Methionine-γ-lyase (MgL) strain, there was an enhanced growth inhibition of F. oxysporum. Indonesia could adapt the concept and develop a new system to prevent repetition of the Panama disease by using different species such as Escherichia coli, a Gram-negative bacteria that has been widely used in biotechnology. This strategy can also be adapted to detect and destroy other pests or pathogens.

3. Conclusion

The development of synthetic biology is advancing greatly aided by iGEM competition. Many interesting novel ideas are addressed in the competition and their potentials in bio-control have been discussed. Provided with mega-
biodiversity, Indonesia can utilize the ideas using their own biodiversity. Therefore, these ideas can serve as the starting point for research and implementation of bio-control in Indonesia.

References