BIOTECH SOLUTIONS:

e. coli-Engineered Cellulase for Waste Transformation

Presented by:
LarVase_iGEM

BIOTECHNOLOGY

What is Bio-Technology ?

Biotechnology is a field that utilizes living organisms, cells, and biological processes to create products and technologies that improve life and make the planet better. Biotechnology, with the combination of biology and technology aids medicine, agriculture, and environmental science. Biotechnology helps with solving the world's biggest challenges, from genetically engineering food to dealing with life-saving vaccines.

Bio-Technology in Agriculture

One of the most well-known areas of biotech is genetic engineering, which scientists modifying the DNA of organisms to strength certain traits. For example, crops can be made to be more resistant to bugs or drought, reducing the need for pesticides and helping farmers grow more food in harsh climates.

(genetic modified corn)

Bio-Technology in Medical Field

In medicine field, biotechnology has great contributions to disease treatment such as gene therapy, the discovery of insulin through bacteria, and the development of medicines are some examples of how biotechnology is revolutionizing in healthcare and offering new hope to patients around the world.

BLACK SOLDIER FLIES

Purpose

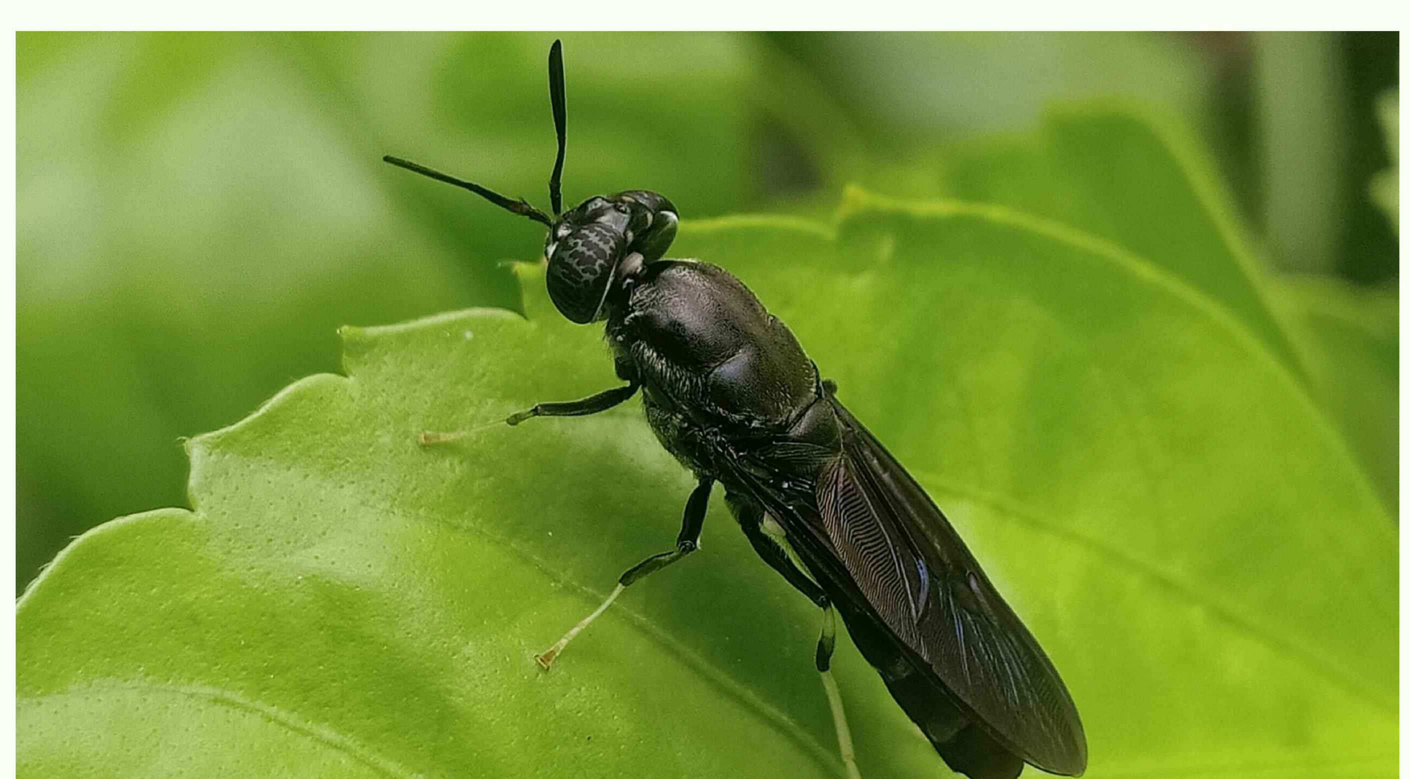
About

Black Soldier Fly (Hermetia illucens) is a bug that benefits the world. It originates from hot and tropical regions and differs from the usual housefly as it is not considered a pest. Rather, it benefits the environment and generates fresh concepts regarding things such as waste management, agriculture, and animal feed production. Its growing value is an example of how our future can become more sustainable with insects playing a key role.

(black soldier fly larvae)

Waste Decomposing

BSF larvae have the **ability to consume twice their weight in organic matter daily and break down food waste**, manure, and agricultural by-products effectively while in the process overwhelming pathogenic bacteria. BSF adults are not fed, hence non-persistent and disease-free. The larvae contain 35-45% protein, 25-35% fat, and trace minerals, making them a better sustainable fishmeal alternative in animal feed. In addition to minimizing landfill waste and greenhouse gases, BSF farming practices also generate useful byproducts such as organic fertilizer and biofuels.



(black soldier fly)

Besides waste reduction, the BSF larvae are **rich in protein and fat**, and therefore, can be used as a green poultry, livestock, and fish feed. In an era where the world requires sustainable solutions for waste management as well as for supporting population growth, the Black Soldier Fly presents itself as a naturally occurring ally. Through **recycling waste into productive resources, it facilitates loop closing in the circular economy** and opens the way for a greener future.

MAIN PROBLEM

Waste Issue in Taiwan

Taiwan is grappling with a major food waste problem, with an astonishing 3.84 million tons of food discarded every year——roughly 170 kilograms per person.

In 2022, the country hit a record-high of 11.58 million tons of total waste, as reported by Taipei Times, and food waste made up a significant chunk of it. This isn't just about throwing away leftovers—it's a huge waste of valuable resources and a serious damage on the environment.

In Taiwan's waste production, typically in food, vegetable and fruit scraps make up around 30% to 40% of total food waste, and these materials are particularly high in cellulose and other fibrous compounds. Cooked food leftovers, including rice, noodles, and processed foods, contribute 40% to 50%, often containing added plant fibers.

Purpose

We aim to enhance the effectiveness of BSF by transferring these enzyme-encoding RNA into e. coli for expression that ensures the successful transcription and functional production of the enzyme. This process typically follows mRNA-based expression using plasmids. This method enables the mass production of Black Soldier Fly enzymes in a fast-growing, genetically tractable host like e coli, making enzyme production more scalable and cost-effective.

Enzyme mechanism in BSF

BSF larvae produce a diverse range of digestive enzymes, including Endo-1,4-β-glucanase. The inclusion of endo is often preferred due to its essential role in the complete breakdown of cellulose into usable glucose. These enzymes allow the larvae to rapidly consume and process various organic materials. This enzymatic efficiency makes BSF larvae an excellent mechanism for bioconversion of cellulose.

Enzyme Mechanism

What is Enzyme and e.coli?

An enzyme is a biological catalyst, typically a protein, that speeds up chemical reactions in living organisms without being consumed in the process. Enzymes work by lowering the activation energy required for a reaction to occur, making biochemical processes more efficient. Each enzyme only acts on a particular substrate, which binds to the enzyme's active site to speed up the chemical reaction.

Escherichia coli (e. coli) is a bacterium commonly found in the intestines of humans and animals. It is a:

– Facultative anaerobe, meaning it can survive with or without oxygen.
– Fast growth rate and ease of genetic manipulation, where scientists frequently use e coli as a host organism to express recombinant proteins
– Including enzymes, pharmaceuticals, and biofuels, making it one of the most important model organisms in microbiology and genetic engineering.

Wny Endo-1,4-β-glucanase

In fact, fiber waste has become a significant environmental issue due to its abundance, resistance to natural degradation, and limited avenues for sustainable disposal. Generated from industries such as agriculture and paper production, fiber waste—often rich in cellulose—accumulates in large volumes and poses challenges for landfills and waste management systems.

Natural fibers still take considerable time to break down without the treatment persistent in the environment. In total, it requires 3 main enzymes to digest. Until today, the previous two were fully evaluated, as a result, we are aiming to extend studies focused on the third enzyme - Endo-1,4-β-glucanase

Additionally, this concludes that fiber waste must be enzymatically degraded by such typical enzyme Endo-1,4-β-glucanase into simple sugars for further applications such as biofuel production, bioplastics, or other value-added products.

PROTOCOL

1. RNA Extraction & Reverse Transcription
- Extract total mRNA from BSF samples.
- Perform reverse transcription to synthesize cDNA from the mRNA template.

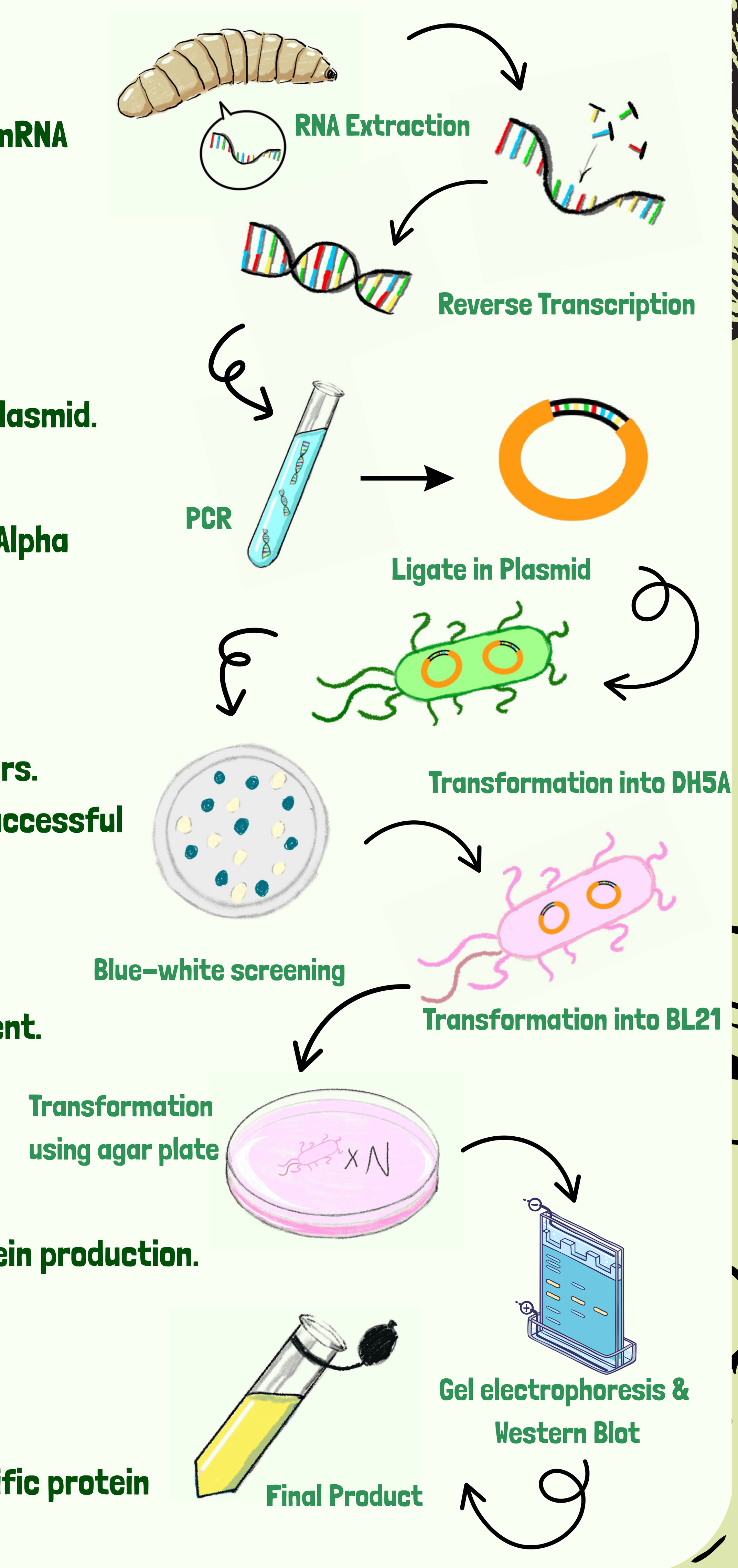
2. PCR Amplification & Cloning
- Amplify the target gene using PCR.
- Insert the amplified gene into a plasmid vector.
- Use restriction enzymes to cut and ligate the gene into the plasmid.

3. Transformation into e. coli (1st round)
- Introduce the recombinant plasmid into 1st e. coli strain DH5Alpha (likely for cloning and selection).
- Use heat shock method for transformation.

4. Screening & Verification
- Place e. coli on antibiotic agar plates and incubate for 24 hours.
- Perform blue-white screening (LacZ system) to check for successful cloning:
  - White colonies = Recombinant DNA present.
  - Blue colonies = No insert (LacZ remains functional).
- Perform DNA sequencing to confirm the correct gene is present.

5. Plasmid Extraction & Secondary Transformation
- Extract the recombinant plasmid from e. coli.
- Transfer the plasmid into a secondary vector (e.g., pET22b).
- Transform the plasmid into expression e. coli (BL21) for protein production.

6. Protein Expression & Analysis
- Culture transformed e. coli on LB agar plates.
- Perform gel electrophoresis to verify protein expression.
- Use Western blot to confirm β-glucosidase production (specific protein detection).



Potential results

We Are Aiming For: A recombinant BSF enzyme expressed in e. coli, useful for bioconversion, industrial enzyme production, or synthetic biology. e. coli colonies should grow on antibiotic plates, with white colonies in blue-white screening indicating successful insertion, verified by DNA sequencing.

- e. coli expressing the BSF enzyme (e.g., Endo-1,4-β-glucanase) successfully grows.
- Western blot detects the target protein, confirming its expression.
- Functional enzyme activity (e.g., Endo-1,4-β-glucanasee activity assay shows protein digestion)

If successful, this experiment could enable the production of recombinant BSF enzymes for applications like bioconversion, waste management, biofuels, or cellulose digestion.

Applications

This engineered e. coli can be applied in waste treatment facilities, compost, or even in portable waste treatment systems for disaster relief or remote locations. Because bacteria reproduce very rapidly and are also simple to culture, our system can also reduce food waste, agricultural residues, or even low-grade industrial organic waste more effectively than current methods.

It not only treats the worldwide problem of organic waste generation, but it may also assist in offsetting greenhouse gases that typically result from garbage decomposing in landfills. With optimal and scaled-up production, our results would be a significant part of forthcoming sustainable urban waste management and more sustainable agriculture. It's new to integrate nature's solutions with advanced biotech to address actual issues.

Future Plan

Enzyme Powder

Over time, we aim to turn our modified e. coli into a viable and eco-friendly product. A key goal is to harvest and purify the enzymes, making them into a powder that can be easily stored and shipped. This enzyme powder can simply be sprinkled onto food waste to foster decomposition, offering a straightforward solution to the treatment to food waste.

With powder product, we would also like to investigate mass production like insulin to make it easily accessible to general public. By expanding the generate process and maximizing enzyme recovery, we believe that we can make the product widely available and inexpensive. This would provides a simple and green solution for households, restaurants, wastewater treatment facilities, and even more.