Engineered Bacterial Cells & Gut Microbiota for The Treatment of Type 2 Diabetes

JiaQi Dai, Yijia Zhang, Yi Song

Abstract

Type 2 diabetes (T2D) is one of the fastest-growing metabolic diseases in the world. In 2021, about 482.94 billion individuals were diagnosed with T2D, with a 10.5 % diabetes prevalence. In 2045, it is projected to have 704.9 million individuals with Type 2 Diabetes. Many clinical studies have found metabolic disorders and chronic inflammatory states in patients with type 2 diabetes, accompanied by intestinal microbiome disturbances. Gut microbiota plays an essential role in metabolism and immune regulation. Therefore, gut microbiota can be a new target for creating treatments and therapies for type 2 diabetes and related diseases. In this review, we will highlight the glucagon-like peptide-1 treatment, the Humulin treatment, and how a healthy lifestyle can control T2D.

Keywords: Gut Microbiota, Type 2 Diabetes, Engineered Bacterial Cells, Lifestyle

1. Introduction

Type 2 diabetes (T2D) is a chronic metabolic disorder that involves impaired glucose conditions, in which there is a decrease in insulin sensitivity, a decrease in glucose tolerance, and an increase in glucose level. For some patients, this condition means the body resists the effects of insulin, a hormone produced by the pancreas that encourages sugar to enter cells. Others do not produce enough insulin to maintain normal blood sugar levels. In either case, the sugar in their body builds up in the blood and causes damage to major organs, which can lead to physical and mental impairment, inconveniences in life, and death in cases of improper treatment [18]. Globally, about 536.6 million individuals have been diagnosed with diabetes, while over 95 percent is diagnosed with Type 2 Diabetes, and about 6.7 million deaths were directly caused by it in 2021 [19].

After years of research, it was discovered that T2D might be determined by gut microbiota. The human gut microbiome is characterized by more than 10 trillion microbial cells from about 1,000 different bacterial communities [18]. It functions as the essential part of our body to absorb energy from food, take in nutrients, as well as break down complex molecules in food. They can affect weight and digestion and protect against the risk of infection and autoimmune diseases [18].

The interaction between drugs and intestinal microbiota has received extensive attention as a scientist has gained more knowledge of the relationship between gut microbiota and T2D. Although several drugs are available to treat T2D, questions related to a variety of person's differences in drug efficacy and potential side effects remain unresolved [22]. It is well known that antibiotics, non-antibiotic drugs, and antidiabetic drugs, can regulate the microbiome and improve diabetes [23]. Drugs control the composition and metabolic capacity of gut microbes. On the contrary, the metabolic activities of the microbiome and its metabolites also affect drug metabolism and action. However, few studies have examined how altering the gut microbiome alters the effects of antidiabetic drugs [22]. In this review, we will highlight the different engineered bacterial cells and gut microbiota for the treatment of Type 2 Diabetes.

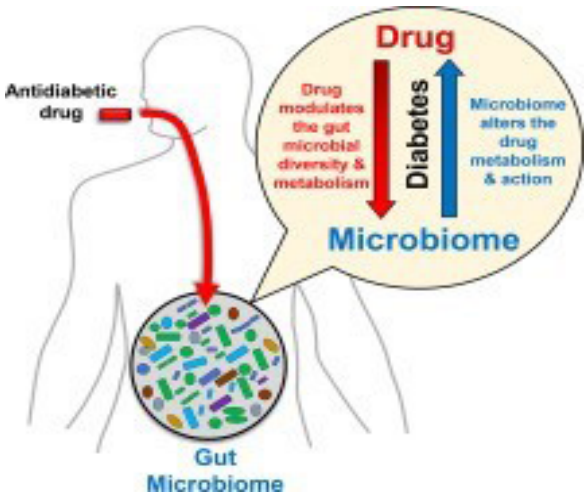

Figure 1. Interaction between antidiabetic drugs and intestinal microbe

2. Glucagon-like Peptide-1

2.1 Introduction

One of the potential treatments for diabetes is engineering

non-insulin generating cells to secrete insulin. Glucagon-like peptide 1 (GLP-1) is an insulinotropic hormone secreted by intestinal epithelial endocrine L-cells into bloodstream after meal intakes [1]. Its main function is to stimulate insulin secretion and inhibit glucagon secretion [2], which it acts additionally on β cells via GLP-1 receptor in glucose-dependent manner through mechanisms involving the regulation of intracellular energy homeostasis and exocytosis, and ion channels [3].
Suzuki et al. discovered that the full-length GLP-1 can stimulate rat epithelial cells to induce insulin production in response to glucose [4]. According to Duan, Liu, and March, human lactobacilli engineered to secrete GLP-1(1–37) were orally administrated to diabetic rats to treat hyperglycemia by converting intestinal cells into glucose-responsive insulin-secreting cells [5].

2.2 Methodology

2.2.1 Aim and Setting

In Duan FF, Liu JH, and March JC's paper, the aim of the experiment mentioned was to use daily oral delivery of GLP-1(1–37) secreting bacteria to convert rat intestinal cells into glucose responsive cells that excrete insulin [5].
L stands for L. gasseri, and LG stands for L. gasseri-secreting GLP-1(1–37) [5]

2.2.2 Main Design and Processes

SEC (USP45-LEISS secretion tag), and SlpA promoter were added to engineer Lactobacillus Gasseri ATCC 33323 to secretes GLP-1(1–37). HIS, a polyhistidine tag was added to the N-terminus, and an enterokinase site (EK) is in between for the protein to separate from the tag after being secreted into the intestine.
First, rats were provided ampicillin-treated (1 g/L) water for 18 hours. Grown Lactobacillus strains LG or L on MRS media were redissolved with 1% sucrose in sterile MRS. The rats were fed with L, LG, or sterile media for 90 days.

Figure 2. Experimental Strain [5]

After 51 days of bacterial feeding, 1g glucose/kg BW was orally administered to STZ-treated rats, and samples of at 0.5, 1, 1.5, and 2 h blood were taken.
After bacterial feeding for 90 days, pancratia and intestines of those rats were removed, measured insulin, and frozen at -80°C for further analysis. The GI (gastrointestinal) tracts were removed, homogenized, and measured weight in 2 mL of fresh MRS medium. Feces were also collected from day 88 to day 90 of feeding.
Separated intestines, pancreases, and livers' total RNA of rats were fed with LG or L.

2.3 Other treatment Involving GLP-1

GLP-1 RAs are drugs tested to be very effective at reducing blood sugar level. Short-acting and long-acting are the two classifications of reducing blood sugar level. Short-acting medications should usually be taken once or even twice a day, and they monitor blood sugar level after meal intake. Examples of it (approved) may include, exenatide (Byetta), lixisenatide (Adlyxin), and oral semaglutide (Rybelsus) [25]. Long-acting GLP-1 RAs, on the other hand, can continue to function after day or even full week after intaking them, which they monitor blood sugar level throughout days. They are typically taken once per week. Examples are dulaglutide (Trulicity), exenatide extended release (Bydureon), liraglutide (Victoza), and semaglutide (Ozempic) [25]. Most of RAs are taken injection, except for Oral semaglutide, which can be in form of pills.

2.4 *Results*

Shown in Figure 2, Bacterial counts were performed for comparison with feces counts and measurements of the colonization level in the upper gastrointestinal tract.

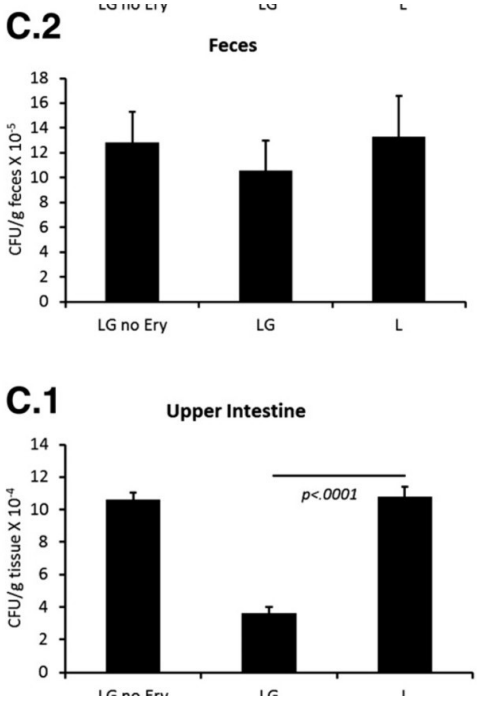

Figure 3. Comparison in Upper GI Tract [5]

Moreover, as shown in Figure 3, the left image of Immunofluorescence was token for GLP-1 expression in the upper gastrointestinal tract of rats fed with LG, while the image on the right from L-fed rats showed no GLP-1 staining.

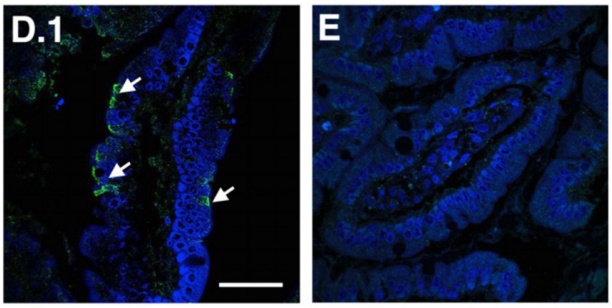

Figure 4. Green staining showing GLP-1 Expressions [5]

Oral glucose tolerance test (OGTT) was also being done. Notably, rats without STZ treatment were fed sterile medium only, and no bacteria were used as controls. Blood glucose is presented on the left, and the graph on the right represents insulin level.

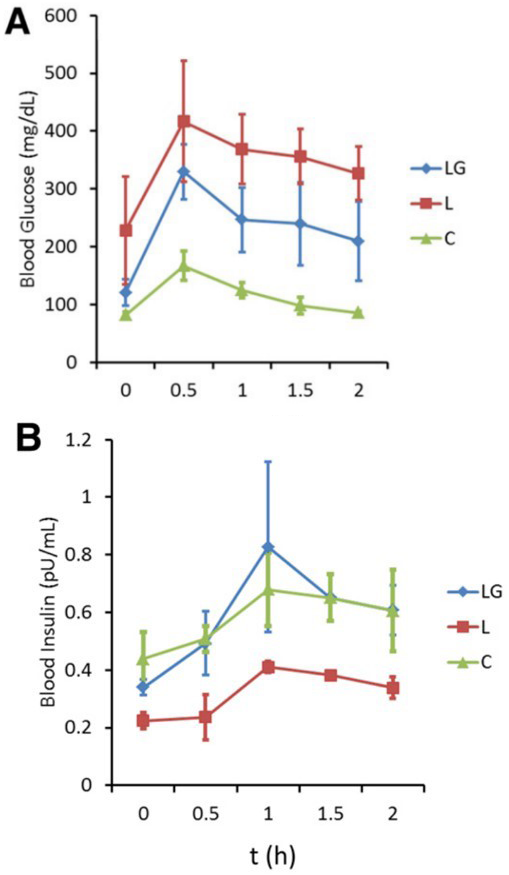

Figure 5. Blood Glucose and Insulin Level Comparisons [5]

The ratio of glucagon-positive cells to total pancreatic cells is presented in the Figure 5.

In summary, rats who received orally administration of GLP-1 secreting Lactobacilli developed insulin producing intestinal cells.

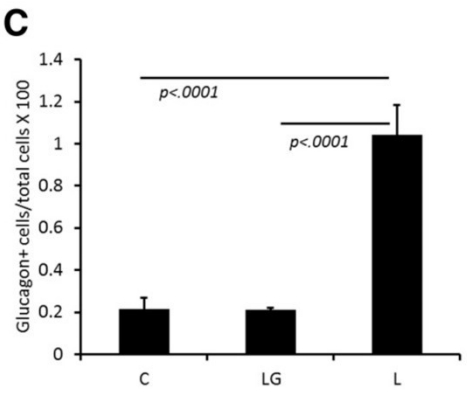

Figure 6. Insulin Producing Cells Level [5]

2.5 *Pros & Cons*

It is not clear so far of how GLP-1 lead to weight loss, but studies have shown that GLP-1 helps suppress appetite and might functions in regulation of meal input [6]. It can also slow down the rate of digestion in stomach, thus decrease the rate of nutrients releasing from food. Moreover, some therapy of GLP-1 can be proceeding once a week. Lastly, it has the potential to reduce Non-alcoholic fatty liver disease, or NAFLD [7]. GLP-1 RAs had shown benefits of cardiovascular health and kidney functions [25].

Side effects that relate to GLP-1 usage are mainly GI side effects, headaches, nausea, diarrhea, vomiting, and nasopharyngitis [25][26].

3. Humulin

3.1 *Introduction*

Humulin is human insulin used for treating diabetes. Before it was developed, diabetics used insulin isolated from the pancreas of pigs and cows. Developed by Genentech, America's first biotech company, it was produced using recombinant DNA technology from a non-pathogenic laboratory strain of E. coli or saccharomyces [20]. Insulin was first discovered in 1921 by Canadians F.G. Banting and C.H. Best. It was first used in clinical practice in 1922 to rescue patients with previously incurable diabetes [24]. The composition of insulin varies from animal to animal. Pig and human insulin have the most similar structure except for one amino acid at the carboxyl end of the B chain. In the early 1980s, genetic engineering technology successfully produced human insulin from microorganisms in large quantities and has been applied in clinical practice [24].

In the 1980s, yeast was used to express insulin through the process of genetic engineering. The product expressed high purity synthetic human insulin, which is similar

in structure to insulin secreted by the body itself. And this product is now called Humulin [24]. Compared with animal insulin, Humulin is less prone to allergic reactions or insulin resistance, so subcutaneous fat atrophy is also reduced.

Today, many people refer Humulin as insulin, however, the insulins that are used to treat human diabetes should be called as Humulin, or human insulin.

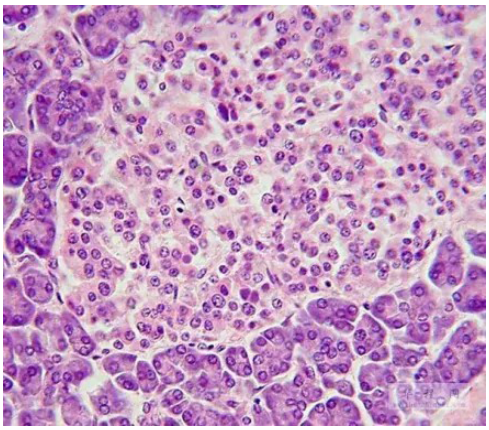

Figure 7. Insulin Beta Cells Under the Microscope

3.2 Methodology

Recombinant drugs are created by inserting genes from one species into a host species (usually yeast or bacteria) that is not naturally present [21]. The gene codes for the desired product, so that the transgenic host organism can grow and be used as a kind of living factory for producing the product. Recombinant human insulin is mainly expressed trough the E. coli express system and the Saccharomyces express system [21].

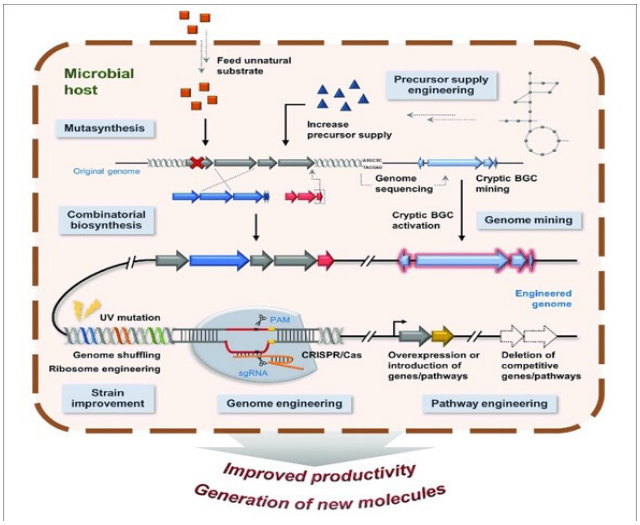

Figure 8. An overview of the Methodology of Humulin production [21].

3.2.1 E. Coli Expression System

By using the E. coli expression system, the insulin precursor (IP) was transformed into an envelope, which was solubilized and refolded with a fully functional polypeptide was obtained [8]. Today, E. coli is the preferred microbe for the mass production of recombinant proteins.

The use of exogenous (foreign) protein codons plays an important role in the expression level of recombinant proteins. Foreign protein expression in E. coli can be improved by replacing codons rarely found in highly expressed E. coli genes with more suitable codons [9]. By using protease-deficient strains of E. coli it carries mutations that eliminate the protease production, it can also increase the production of recombinant proteins by reducing protein degradation. Foreign proteins usually accumulate in E. coli in the form of inclusion bodies consisting of insoluble misfolded protein aggregates.

In 1978, Genentech produced recombinant human insulin for the first time in Escherichia coli. The method involves chemically synthesizing cDNA expression encoding the insulin A and B chains in E. coli [10]. After independent expression, the two chains were purified and co-incubated under optimal reaction conditions to promote the generation of intact organisms.

However, some disadvantages limit its use to produce recombinant biopharmaceuticals. Various post-translational modifications (PTMs), such as glycosylation, phosphorylation, proteolytic processes, and disulfide bond formation, are essential for biological activity but do not occur in E. coli [9].

Also, E. coli is a prokaryotic express system which means that it has a lack of post-translation processing. It's express system also contains complicate proteins that are hard to be protect from foreign substance.

3.2.2 Yeast Expression System

Yeast, also known as Saccharomyces, is the preferred host for the expression of various bioactive exogenous proteins requiring post-translational modification with its ability to progress a lot of translation and modification. It is also one of the most frequently used expression systems. These single-celled eukaryotes grow rapidly in specific media, are easier and cheaper than insect or mammalian cells, and are easy to ferment. The yeast expression system is well suited for the mass production of recombinant eukaryotic proteins.

Therapeutic proteins produced in yeast are specifically extracted from Saccharomyces cerevisiae, including hormones and vaccines. Like E. coli, yeast derived recombinant biopharmaceuticals are used to treat infectious diseases or endocrine and metabolic disorders. Saccharomyces cerevisiae has been widely used to

produce recombinant human insulins since the early 1980s, and many commercial recombinant insulins are produced by this yeast expression system [11]. To express and secrete recombinant proinsulin efficiently in yeast, the scientists designed a protein containing natural A chain and B chain lacking C-terminal B30 threonine, which was either fused directly or linked by synthetic short C-peptide [11].

3.2.3 Comparison of the Two System

Although there are many differences is in way each system express human insulin, the product is mainly the same. Which system to use depends entirely on the type of Humulin, the numbers of product needed, and the medicine company's requirement.

Also, notice that these two systems are not the only two system that can express Humulin.

3.3 Pros & Cons of Humulin

3.3.1 *Pros*

This type of control and treatment has been used in the United States for a long time, with enough established cases, safeguards, and insurance. Starting Humulin treatment early in the T2D can keep the human body's Insulin- producing cells working to slow the progression of diabetes. In this case, T2D can be cut from the start. It also works well-lowering the body's sugar, even if the patient has liver or kidney problems, Humulin can still be used, unlike many other hypoglycemic drugs [12].

3.3.2 *Cons*

Humulin is efficient and portable, however, it is expensive, not to mention the automated insulin pumped. Without that, it might be uncomfortable to check blood sugar regularly to take Humulin. Also, it leads to weight gain and fat accumulation, a higher risk of hypoglycemia compared to oral medications, and can cause hypoglycemia (low blood sugar), especially if you do not take it every day or have a random diet and exercise plan [12].

3.3.3 Side Effects

Common side effects of Humulin are low blood sugar, reaction, or redness at the site of injection, allergic reactions, fat deposits under the skin, weight gain, swelling in legs, and headache [12].

3.4 Insulin Options

On the market there are many Humulin choices that are provided to patients today. Humulin not only can be used to treat T2D, but also T1D. Humulin R U-100 is considered as a dietary and exercise adjunct to improve blood glucose control in adults and children with type 1 and type 2 diabetes.

Humulin N Has been found to be safe and effective in controlling blood sugar levels in patients with type 1 or 2 diabetes. The American Diabetes Association recommends human insulins, including Humulin N, as a treatment option in its glucose management guidelines for patients with type 1 or 2 diabetes [17]. In the market, there are many types of human insulin that is widely used to help people manage type 1 and type 2 diabetes. Most of them is used by injecting once or twice a day on your upper outer arms, abdomen, buttocks, or outer thighs.

4. The Unattainable of T2D Drugs and Treatment

Notice that both GLP-1 and Humulin cannot cure T2D. The effect of them is just to manage and control T2D. To today, there's no diabetes cure, however, diabetes can be treated, control, and prevented by the correctly use of drug and a healthy lifestyle to reach the goal of manage blood sugar level.

5. Other Treatments Besides Gut Bacteria

As type 2 diabetes is caused by the inability of the pancreas to produce sufficient insulin and the inability of the cells to react to insulin and intake sugar, which is known as insulin resistant, the long-term treatment of diabetes would begin with developing a healthy diet and a routine of monitoring and recording the fluctuation of blood sugar level. In the diet, patients would be asked to eat vegetable which are composed with balanced amount of starchy, like potato, and non-starchy plants, like broccoli; variety kinds of fruits; whole grains which includes bread, pasta, and rice; different kinds of protein, from lean meat, fish to eggs and nuts; and nonfat or low-fat dairy. The diet provides the patients with specific kinds of fats and proteins to prevent and lower the chance of further diseases caused by diabetes. Furthermore, patients would be suggested to exercise more to lower their blood sugar level. The patients would be asked to do minor exercising like walking while talking on the phone and watching television; doing chores such as working in the garden and cleaning in the house; having bike rides and walking in the park; and doing different kinds of light activities and stretches including leg lifts, torso twists and arm stretches. However, over exercising without any regulations or preparations would easily cause hypoglycemia or hyperglycemia if the patients are not carefully testing and recording their blood sugar level and managing their drug intake, and with the development of the disease, diabetes would likely cause other serious health problems, such as heart disease, vision loss and kidney disease, which can interfere with the curing plans of the patients.

Besides exercising and keeping a diet, medicines can also be taken to treat diabetes. For instance, acarbose can prevent α-glucosidase from digesting carbohydrates, and metformin can inhibit the liver from releasing glucose, reduces insulin tolerance and appetite. However, both mentioned medicines have side effects such as diarrhea and low blood sugar. For metformin in specific, it can be inhibited due to imidazole propionate which is a chemical produced naturally by gut bacteria that has shown a great connection with a downgrade of the medicine and the effectiveness of the glucose metabolism [12].

Moreover, according to American CDC, till 2022, there are already 37.3 million people who have been diagnosed with diabetes which includes 28.5 million adults. As more and more people have been diagnosed, more and more treatments and research are developed based on genetic editing and gut bacteria [19]. In general, genetic editing and gut bacteria are still some of the best and most promising ways of treating type 2 diabetes.

6. Discussion

In this paper, we looked over many ways of treating diabetes. From the basic ways of controlling blood sugar level to the chemical compounds produced by genetic engineered gut bacteria, they provide a great conclusion of different methods researchers have tried to solve the great problem of curing type 2 diabetes. From this research, we can glance into the future of engineered gut bacteria and type 2 diabetes. The ways of controlling blood sugar and producing more insulins are some of the problems we have already cracked, but many problems of medicine and various ways of monitoring and controlling blood sugar still need new solutions. The side effects of weight loss, nausea, diarrhea, the suppressing effect of other chemicals still needs more tests and experiments to find more treatments and to solve the side effects from the medicines. All in all, the researchers have already found many keys to this serious health problem, the future in genetic editing and gut bacteria is a great and promising direction in treating diabetes.

7. Conclusion

In summary, this essay highlighted different engineered bacterial cells and gut microbiota for the treatment of Type-2 diabetes. The effectiveness of Glucagon-like Peptide-1, Humulin, and healthy diet and routine for treating Type-2 diabetes has been proven by many experiments. Research investigated the method of orally administrating engineered human L-cell that secrets insulin to diabetic rats to treat hyperglycemia. Humulin was also produced via recombinant DNA technology from a non-pathogenic laboratory strain of E. coli or Saccharomyces. However, to ensure treatments are effective for the long term, healthy diets and regular monitoring of blood sugar level must be applied. As more and more various ways of treating type-2 diabetes are developing, it will be easier to treat or even fully cure it in the future.

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