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GUT MICROBIOME COMPOSITION METAGENOMIC ANALYSIS AND POTENTIAL APPLICATION OF PROBIOTICS IN TYPE 2 DIABETES TREATMENT

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SUMMARY OF BIOLOGY DOCTORAL THESIS

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INTRODUCTION

1. The necessary of the thesis

One of the progressive metabolic illnesses, type 2 diabetes is characterized by peripheral insulin resistance and pancreatic beta-cell dysfunction, which impairs glucose metabolism and causes chronic inflammation. Type 2 diabetes is mostly influenced by environmental and genetic factors. According to statistics, there are already more than 460 million adult diabetics globally, and by 2045, there will be 700 million. Patients with persistent diabetes will experience organ deterioration and functional loss in their eyes, kidneys, nerves, and heart, among other organs. Therefore, it is crucial to understand the pathophysiology of type 2 diabetes and how to treat it.

The intestinal microbiota, which consists of bacteria, archaea, fungus, viruses, and protozoa, is seen as a complex ecosystem in the gastrointestinal environment. The immune system, metabolism, food digestion, and overall health of people are all significantly influenced by the gut microbiota. Numerous studies have shown that the gut microbiota affects human health in a significant way and that dysbiosis is linked to a number of disorders, including type 2 diabetes. Probiotics can also help with diabetes treatment by safeguarding the pancreatic islets and -cells, delaying the establishment of type 2 diabetes, and avoiding complications linked to type 2 diabetes. The microbiota can be passed down from mother to child directly in many cases. A healthy adult's stomach has roughly 100 billion microorganisms. More Firmicutes and fewer Bacteroidetes are present in overweight people.

We only comprehended 0.1–1% of the microorganisms in an ecosystem in the past since the study of microbial diversity frequently relied on conventional methods of isolation and culture. Multi-genome analysis employing metagenomics tools is currently the method with the most potential for measuring microbial diversity. The power and most recent developments of systems biology, bioinformatics, and genomics are combined in the Metagenomics tool. Understanding the human gut microbiome, particularly non-isolated bacteria, depends on this technology. The findings of the metagenome study of the human intestinal microbiome will help pinpoint the factors that cause type 2 diabetes as well as potential beneficial bacteria, which will help guide the use of microbes in the treatment of diabetes.

Therefore, we have carried out the thesis: ***"Gut microbiome composition metagenomic analysis and potential application of probiotics in type 2 diabetes treatment"***

2. Research objectives

1) Identify changes in the gut microbiota of people with type 2 diabetes.

2) Explain how alterations in the gut microbiota and factors that lead to diabetes are related.

3) Isolating, identifying potential bacteria, and choosing advantageous strains for those with type 2 diabetes.

3. Research contents

1) Analysis of the 16S rRNA metagenome gene sequencing database to determine alterations in the gut microbiome in the group of patients with type 2 diabetes and non-diabetics.

2) Examine how certain indicators used to identify and forecast type 2 diabetes relate to gut microorganisms.

3) Research on various bacteria strains that have been isolated and characterized that may be connected to type 2 diabetes. Analyze the microorganisms taken from lab mice's blood to see if they can lower blood sugar.

4. New scientific contributions of the thesis

1) This research in Vietnam is the first to create a metagenome database of the intestinal microbiomes of persons with type 2 diabetes and non-diabetics.

2) Evaluation of the biological traits of a few *Lactobacillus* strains isolated from Vietnamese diabetics' stools. Laboratory animals were used to test the *Lactobacillus plantarum* 16 strain's capacity to boost GLP-1 secretion and aid in blood sugar regulation.

5. Scientific and practical significance of the thesis

The findings of the thesis add to the body of knowledge regarding the alteration of the intestinal microbiota in those with type 2 diabetes by identifying and include potential bacterial strains in the diabetic treatment.

CHAPTER 1. LITERATURE REVIEW

Diabetes is a group of diverse illnesses characterized by hyperglycemia and reduced glucose tolerance that can be brought on by insufficient insulin, poor insulin

action, or both. Insulin resistance and deficiency, either of which may exist at the time of the clinical manifestation of diabetes, are characteristics of type 2 diabetes. Fasting hyperglycemia and insulin insufficiency are features of type 2 diabetes; a chronic metabolic disorder brought on by reduced insulin release from pancreatic cells. When cells, particularly those in the liver and muscles, fail to recognize normal insulin levels, it results in insulin resistance or insulin unresponsiveness.

Other significant pathways that may contribute to type 2 diabetes include an absence or insensitivity to the incretin hormone, an increase in lipid catabolism, an increase in blood glucagon, a rise in water and salt retention, and other metabolic abnormalities. The human intestinal microbiota is a microbial community that contains more than 1000 species and roughly 1011 organisms per gram of feces, the majority of which are obligate anaerobes. Its total genome is 150 times larger than that of an average human.

The basic functions of the gut microbiota are as follows: (1) the protective function of the intestinal mucosa by preventing mucosal infections through inhibiting pathogens from entering and maintaining an intact intestinal barrier; (2) metabolic functions, including energy homeostasis, nutrient digestion and bioaccumulation, support for fat metabolism, fermentation of undigested carbohydrates, and production of fatty acids short chain; and (3) immunoregulatory functions, including the production of antibodies and other immune-suppressive substances.

Evidence shows that increasing the amount of fat in a person's diet decreases the quantity of *Lactobacillus* bacteria while increasing the number of Gram-negative bacteria. Additionally, research has revealed *that Lactobacillus reuteri* is more prevalent in obese individuals than *Lactobacillus casei*, *Lactobacillus paracasei*, and *Lactobacillus plantarum*.

According to certain research, patients with insulin resistance have lessened blood's capacity to carry branched-chain amino acids (BCAAs) into cells. In addition to assisting the body in producing more insulin, BCAAs aid in maintaining normal blood sugar levels. The body can absorb BCAAs from diet or can synthesis them through microbes. *Bacteroides vulgatus* and *Prevotella copri* are two bacteria species that are regarded as "producers" of BCAAs. This is a crucial source of BCAAs that lowers the body's level of insulin resistance.

Metagenomics is a method that combines molecular biology with bioinformatics to comprehensively examine both the full genetic makeup of the sample as well as the microbial population that lives there. The functional genomic diversity involved in metabolic processes, species-level microbial diversity, and proposed metabolic pathways have all been clarified using specialist bioinformatics tools. Metagenomics techniques have been used to study the species diversity and composition of microorganisms from a variety of environments, including soil, water, digestive systems, and the digestive systems of many different animals and humans, very successfully in recent years as new generations of sequencing machines have been put to use.

CHAPTER 2. RESEARCH MATERIALS AND METHODOLOGY

2.1. Research Material

2.1.1. Chemicals, biological kits

Chemicals for DNA extraction and sequencing: PowerSoil® DNA Isolation Kit (Mo Bio, USA), QIAamp DNA Sool Mini Kit (Qiagen, Germany), RevertAid First Strand cDNA (Thermo Fisher Scientific Inc, Singapore), TruSeq ® DNA PCR-Free Sample Preparation Kit (Illumina, USA), agarose (Sigma-Aldrich, USA), ethidium bromide (Sigma-Aldrich, USA).

Chemicals for microbial cultivation include NaCl 0.9%, gentitans, lugol, fucshin, and glycerol as well as Oxgall bile salts (Sigma-Aldrich, USA), taurocholate, glycocholate, glycodeoxycholate, and taurodeoxycholate.…

Chemicals for identifying bacteria

Discrimination buffer: NaCl 100 mM, Tris-HCl 10 mM (pH 8), EDTA 100 mM (pH 8), SDS 0.5% w/v, PCI mixture components include phenol, chloroform, and isopropanol in the ratio 25:24:1, and TE mixture components include Tris-HCl 10 mM and EDTA 1 mM.

The electrophoresis solution is: Tris base, 28.6 g of CH3COOH, and 0.5 m of EDTA (pH 8.0) 50 mL, 500 mL H2O, 1 g of agarose, and 100 mL of TAE buffer make up the 1% agarose gel composition.

H2O 8.5 mL, Master Mix 12.5 mL, forward primer 1 mL (10 pmol), reverse primer 1 mL (10 pmol), template DNA 2 mL (200 ng) are the components of the PCR process.

ThermoFisher Scientific's chemicals for bacterial identification.

Chemicals for measuring glucagon, such as sitagliptin and peptide-1 Streptozotocin (Sigma-Aldrich, USA), and an ELISA kit from RayBio® (BioAssay Systems).

2.1.2. Tools and Equipment

The following tools and apparatus were used: Agilent Bioanalyzer 2100 system (Aligent, USA), Sorvall RT 1900W centrifuge (Germany); sterilization pot HA-240MIV (Hirayama, Japan); pH meter (Toledo, Germany); PCR machines Veriti 96-Well Thermal Cycler (Applied Biosystem, USA) and PCR-9700 (Applied Biosystem, USA); Automated sequence reader Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, USA); sequencer Illumina Hiseq 2500 (Illumina, USA); pipetteman (Eppendorf, Germany); 0.8 and 0.22 µm polycarbonate membranes (Milipore, Ireland), WNB14 thermostatic bath (Memmert, Germany) and some common laboratory tools.

High performance compute server with 24 Intel(R) Xeon(R) CPU X5650 @ 2.67GHz, 188GB RAM, 8TB storage and configured on Ubuntu OS version 15.04 Vivid with the latest bioinformatics and statistics software.

Accu check one touch glucometer (Johnson & Johnson, Mumbai, India).

2.1.3. Microorganism strains

Staphylococcus aureus ATCC13709 and *Escherichia coli* ATCC 11105.

2.1.4. Microbial isolation and culture medium

MRS medium for culture and isolation of lactic acid bacteria;

LB medium (g/L) for culturing *E. coli* and *S. aureus.*

2.2. Research Methodology

***2.2.1.* Selection criteria for research participants:**

Type 2 diabetic patients: Patients have a body mass index (BMI) of greater than 30 and have had the disease for at least 5 years. They also have high blood glucose index, as measured by the fasting oral glucose tolerance test (OGTT), and abnormal cholesterol, LDL-C, HDL-C, triglycerides, AST, and ALT levels.

Blood sugar, cholesterol, LDL-C, HDL-C, triglycerides, AST, and ALT were all within normal limits in the control group of non-diabetics, and their body mass index was less than 23.

2.2.2. Methodology of collecting stool samples

Stool samples from type 2 diabetes patients (female, D1-D7; male, D8-D10) and control group (female, C1-C7; male, C8-C10) were provided by the laboratory

department of the Central Hospital of Transport together with information on blood glucose levels, total cholesterol, HDL-C, LDL-C, triglycerides, AST, ALT, and body mass index. Stool samples were taken from residents of Hanoi who had lived there for more than five years and stored in sterile containers. After that, the samples were brought immediately to the lab for analysis while being kept chilled at 4°C.

2.2.3. Methodology for the extraction of microbial DNA from stool samples

Transferring about 200 mg of manure into a little Zirco-prep tube with granules can help smash the sample before the DNA extraction solution is added. Utilize the QIAmp DNA Stool Mini Kit (Qiagen, Germany) to extract DNA as directed by the manufacturer. After separation, the DNA purity and concentration were assessed using electrophoresis on 1% agarose and quantified using a Nanodrop lite (Thermo Fisher Scientific, USA).

2.2.4. Methodology for DNA sequences the V3-V4 region of the 16S rRNA gene

2.2.4.1. 16S rRNA DNA Sequencing from the DNA Metagenome

These DNA samples served as templates for the sequencing of the 16S rRNA gene after the total DNA had been isolated. The V3-V4 region of the 16S rRNA gene is amplified using a set of specialized primers, 341F forward and 806R reverse primers with 6 nucleotides appended at the 5' end to enable simultaneous analysis of samples. The PCR product was then forwarded to First Base Company (Malaysia) for sequencing. Following the directions provided by the manufacturer, sequencing libraries were created using the TruSeq® DNA PCR-Free Sample Preparation kit from Illumina (USA). A system from Agilent Bioanalyzer 2100 (Agilent, USA) was used to evaluate the quality of the library. A 250 bp paired-end read was produced from the library by sequencing it using an Illumina HiSeq 2500 (Illumina, USA).

2.2.4.2. Metagenome DNA sequencing data analysis

Sequencing: The Illumina HiSeq 2500 produces paired-end sequencing reads that are about 250 bp in size.

Determine your taxonomic position: After being processed and examined using Uparse software to establish taxonomic location, the purified reads are classified into Operational Taxonomic Units (OTUs) if there is a similarity of over 97% between them.

Analysis and visualization of diversity The identification of a number of fundamental indices—of which the Shannon, Simpson, ACE, and Chao1 indices are the most significant—was the basis for the study of microbiological diversity (alpha

diversity).

The coordination approach, which is frequently employed in statistical analysis with enormous data sets, is utilized to describe the relationship between the various species compositions and fundamental environmental variability.

Rather of utilizing PCA, genus-level bacterial OTUs were connected to the PCoA matrix when performing principal coordinate analysis (PCoA) to evaluate differences in gut microbiota samples using R software version 2.15.3.

The rarefaction curve was created using a set of taxa (OTUs) that were 97%, 95%, and 90% similar, respectively.

2.2.5. Identification of biological and antibacterial characteristics of isolated Lactobacillus strains.

2.2.5.1. Methodology for isolating and purifying Lactobacillus bacteria

To separate lactic acid bacteria from other types of bacteria, MRS agar medium was treated with 0.5% CaCO3, and at the same time, 0.01% (w/v) sodium azide was used to kill Gram-negative bacteria. The CaCO3 soluble ring that forms around the colony in the presence of lactic acid serves to identify lactic acid bacteria.

Bacteria are cleansed by whisking them on MRS medium until only one colony type is seen there.

2.2.5.2. Methodology for determining the biological characteristic of Lactobacillus

Determination of antibacterial capacity.

Evaluation of acid tolerance.

Assessing bile salt tolerance.

2.2.5.3. Determination of the ability to inhibit growth on pathogenic bacteria

Determine the ability to inhibit *S. aureus* bacteria.

Determine the ability to inhibit *E. coli* bacteria.

2.2.5.4. Species identification based on 16S rRNA gene sequencing

To identify bacteria, proceed as follows:

Separation of total bacterial DNA

PCR reaction

Purification of PCR products

16S rRNA sequencing

Sequencing results were processed with BioEdit software. Phylogenetic tree was formed from Mega X software.

2.2.6. Testing the ability to improve glucagon like peptide-1 in mice of Lactobacillus

2.2.6.1. Treatment of mice

Before beginning the experiment, mice were housed in cages with three to four animals per cage for a week of acclimatization to room settings. The culture room was kept at 25°C with a humidity of 55 ± 5%, with a light cycle of 12 hours and a dark period of 12 hours.

2.2.6.2. Initiation of diabetes in mice

Mice that had been fasting for the previous night were given a single dose of streptozotocin solution (50 mg/kg body weight) in sterile 0.1 M sodium citrate buffer (pH = 4.5). Mice with diabetes had serum glucose levels more than 250 mg/mL.

2.2.6.3. Preparation and quantification of Lactobacillus

The MRS solution was used to cultivate Lactobacillus cultures, which were then incubated for 48 hours at 37°C in an anaerobic Mark II system (Anaero Gas Pack, LE002. HiMedia, India). 1 mL of the culture fluid was diluted to contain 10^7 CFU per mL after incubation. Daily doses of freshly produced Lactobacillus were given to the treated animals for a period of four weeks.

2.2.6.4. Experiment design

Four experimental groups of six mice each were created (n = 6).

Drug and bacterial doses are given orally by syringe once daily for four weeks.

2.2.5.5. Test the ability of sucrose tolerance

Mice were given sucrose (2 g/kg body weight) orally after being fasted for the previous night. The sugar was dissolved in 1 mL of distilled water. After sucrose administration, blood samples were obtained from the tail vein at 0, 30, 60, 90, and 120 minutes. Blood glucose levels were then calculated using a glucometer.

2.2.5.6. Measurement of GLP-1 in serum

On day 28, tail vein samples from the mice given the 2 g/kg body weight-sucrose-sugar were taken at 0, 20, 30, 40, 50, and 60 minutes. A mouse ELISA kit was utilized to quantify GLP-1. The mice were given a little ether anesthesia before blood was taken from the heart after blood was retrieved from the tail vein. Blood samples were centrifuged at 3000 rpm for 5 minutes at 4°C to separate the mouse serum, which was then kept at -80°C for later analysis.

2.2.6. Statistical analysis of data

The statistics are analyzed using IBM SPSS Statistics 22 and Microsoft Excel

software using the t-test method with $p < 0.05$, which is regarded as statistically significant.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Evaluate the intestinal microbiota differences of the study subjects

3.1.1. DNA metagenome isolation results of intestinal bacterial

The QIAamp DNA Kit offers a purification kit (silicon based - in the presence of PCR inhibitors) for up to 30 g of DNA from feces samples collected from research participants. removing the PCR inhibitor by combining the effects of InhibitEX, a single adsorbent resin, and the best buffer.

The results obtained indicate that the samples' DNA electrophoresis images are crystal clear, displaying good quality, and containing all of the DNA that is required. This finding demonstrates the effectiveness of complete DNA extraction.

Using a Nanodrop lite device, the absorbance ratio at 260/280 nm was used to assess the overall DNA's purity. All of the samples had excellent purity, ranging from 1,77 to 2,06. The aforementioned findings demonstrate that the extracted DNA is of high quality and quantity, and that the DNA samples conform to the requirements for next-generation gene sequencing on the Illumina system.

3.1.2. Intestinal microbial diversity index

3.1.2.1. Results of the intestinal microbiota diversity in the female group

Results of biodiversity index calculation: A total of 1,627,646 sequences with high reliability were found, and 99.9378% of them belonged to the bacteria group (Bacteria) and 0.0602 to the archaea group (Archaea). Other microbial group data make up the remaining 0.002% of the data (Others). Stool samples from patients without diabetes (from C1-C7) and samples from people with type 2 diabetes (from D1-D7) made up two groups of sequencing samples. With 97% homology, sequences were grouped into OTUs. The findings revealed that there were 204÷367 OTUs from the control sample, with a mean of 288 (SD 62.796), and 175÷287 OTUs from the type 2 diabetes sample, with a mean of 214 (SD 42.146). This finding indicates that there are more OTUs in the control sample. The OTUs in the samples are all shown to be representative of the species in the samples by the good coverage index in all samples > 0,994. The ACE and Chao1 scores also serve as a proxy for the anticipated number of species. The findings of the alpha diversity analysis demonstrated that the samples' ACE and Chao1 values were inversely correlated with their OTU values (P 0.01). The ACE and Chao1 values are consistently greater than the OTUs across all

samples, indicating that as the number of sequences rises, so will the estimated number of species. The outcomes of the alpha diversity analysis further demonstrate the importance of the variance between Shannon and Simpson values. Shannon and Simpson from the control group's stool had the highest values (6.419 and 0.977, respectively), and the lowest values (4.819 and 0.874, respectively). The type 2 diabetes group's Shannon and Simpson values, however, were the highest from the stool (5.193 and 0.936, respectively), and the lowest (3.495 and 0.758, respectively).

The comparative analysis results of biodiversity indexes:

The rarefaction analysis's findings demonstrated that the microbial OTUs' data coverage was insufficient. In comparison to the control group, the type 2 diabetes group's microbiota tended to be less diverse.

According to a breakdown of the major components using biodiversity indicators (Figure 3.5), the microbial composition of the feces from the type 2 diabetes group (the black square) and the control group (the red circle) is more likely to be separated into distinct groups. This finding demonstrates that type 2 diabetes group and control group's gut microbiota compositions are more similar than each other.

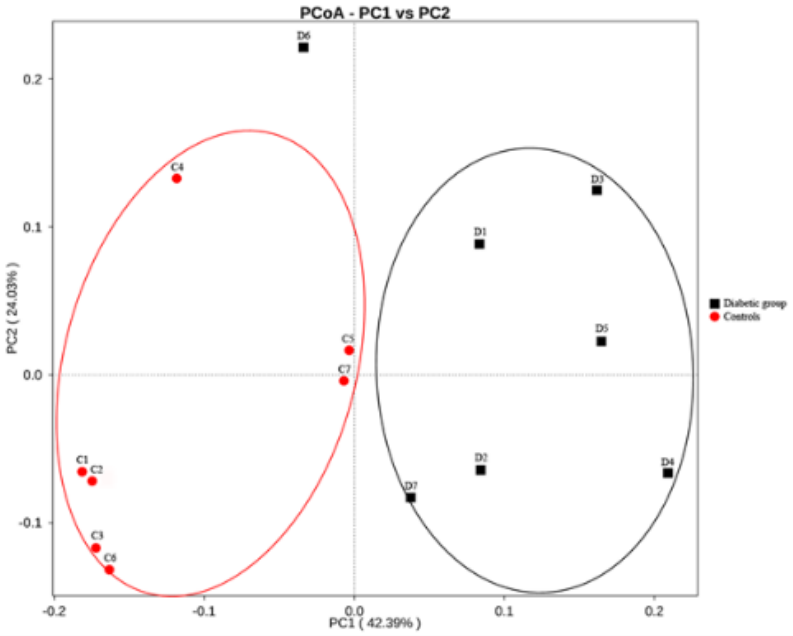

Figure 3.5. Principal coordinate analysis (PCoA) results in stool samples of women with type 2 diabetes and women without diabetes

3.1.2.2. The diversity analysis results of intestinal microbiome in the men group

Sequence analysis revealed that a total of 559,756 sequences had good reliability; 99.9883% of these sequences belonged to bacterial groups (Bacteria), while the remaining 0.01117 percent belonged to the group of archaea (Archaea). Six sets of

sequence reading samples were used, including three sets from men with type 2 diabetes (D8, D9, and D10) that each had 31,369, 12, 5250, and 121,284 sequences and three sets from men without diabetes (C8, C9, and C10) that each contained 23,932, 126,976, and 130,945 sequences. The OTUs in the samples are all indicative of the species in the sample, as seen by the Good coverage index in all samples being > 0.995.

The findings of the comparative examination of biodiversity indicators Because the curves in the analyzed samples have not saturated, rarefaction analysis has not been able to fully quantify the bacterial abundance. In comparison to the control group, the men's type 2 diabetes group's microbiota tended to be more varied.

Principal coordinate analysis (PCoA)

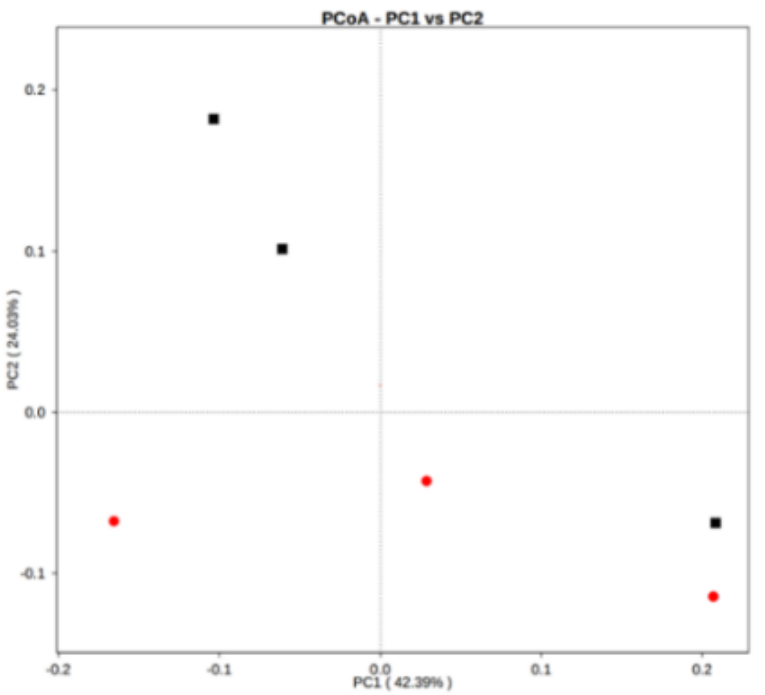

Figure 3.9. Principal coordinate analysis (PCoA) results in male stool samples

Figure 3.9's Principal Coordinate Analysis (PCoA) based on biodiversity indices revealed that there was a tendency for the male group with diabetes (the black square) and the control group (the red circle) to fall into separate groups based on the microbial composition of their faces. In other words, the microbial communities of the males with type 2 diabetes (the diabetes group) and the control group (the control group) were different. The gut microbiota of the control group was more similar to that of the type 2 diabetes group in composition. Normal microbiome balance exists in people without diabetes but is disturbed in those who have type 2 diabetes.

3.1.3. Bacterial species composition

Although studies on the gut microbiota have shown mixed results, gender may play a role in variations in the species makeup of the gut microbiota. As a result, we compare the outcomes between the two sexes.

3.1.3.1. The analysis results of species composition in the female group

The findings of the study revealed that there were 13 primary phyla in total among the read sequences of the two groups. Bacterial OTUs accounted for > 98.56% of all detected OTUs and were concentrated in four primary phyla: Bacteroidetes (30.42%÷73.58%), Firmicutes (11.25%÷49.58%), Proteobacteria (2.97%÷27.05%), and Fusobacteria (0%÷38.49%).

Only the variation in the phylum Fimicutes among the four major phyla is statistically significant. Additionally, the difference between the final two phyla, Synergistetes and Others, is statistically significant, with p values of 0.042 and 0.047, respectively.

The results shown above highlight the traits of the bacterial phyla found in feces. At the phylum level, the findings of this study indicating type 2 diabetes is linked to alterations in the makeup of the gut microbiota are primarily discernible. While Bacteroidetes, Proteobacteria, and Fusobacteria proportions in diabetic patients tended to be higher than in those without diabetes, Firmicutes abundance was markedly reduced. These outcomes are comparable to those mentioned by Larsen et al. Our results are consistent with the idea that dysbiosis and changed gut flora are linked to type 2 diabetes.

Depending on the ethnic group, the human gut flora may differ. The predominant bacterial phyla in the gastrointestinal system vary depending on the research topic. When the Firmicutes/Bacteroidetes ratio is less than 0.8, it indicates that the body's gut bacterial balance is out of whack. Our study's Firmicutes/Bacteroidetes index for type 2 diabetics was 0.49, demonstrating that the Firmicutes phylum's bacteria declined and the Bacteroides phylum's bacteria increased.

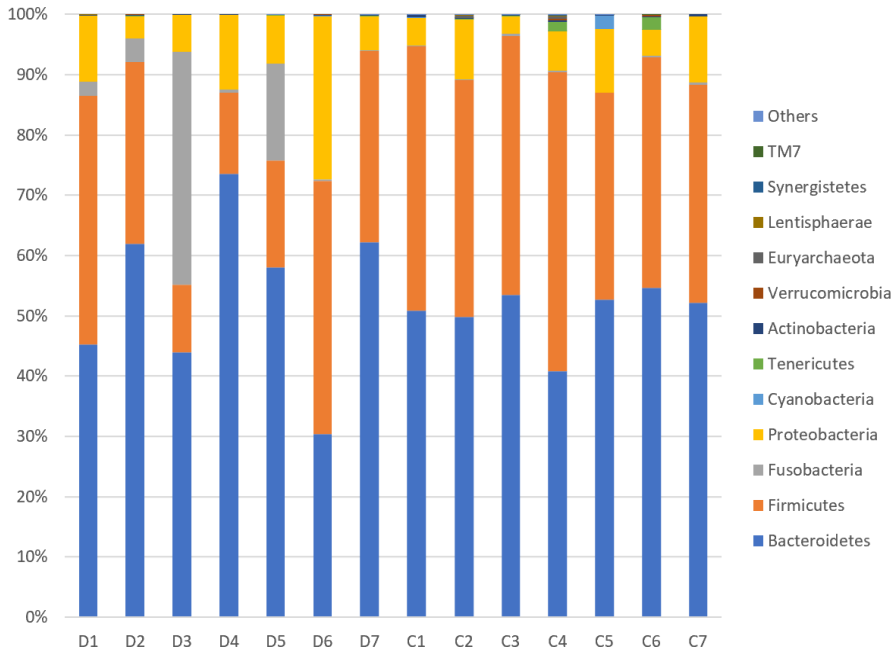

Figure 3.10. Relative phylogenetic richness of feces microbiota in 7 type-2 - diabetic-women (D1-D7) and 7 women without diabetes (C1-C7)

We discovered that only 9 genera out of a total of 94 genera had statistically significant variations in microbial composition between the two groups when comparing the microbial composition at the genus level between the two groups. In contrast to the control group (22.9%), the genus *Bacteroides* significantly increased in the type 2 diabetes group (47.93%) of our study. In 5 of the 8 studies analyzed, it was discovered that type 2 diabetics have higher *Bacteroides* levels. In comparison to the control group, the prevalence of the genus *Prevotella* was lower in patients with type 2 diabetes (0.85% versus 21.48%)..

Because different strains of *Prevotella* in the stomach have different effects on glucose metabolism, some strains are better at improving glucose metabolism than others, which can lead to disorders including metabolic syndrome and obesity.

In the type 2 diabetes group compared to the control group, there were lower levels of the genera *Ruminococcus* and *Desulfovibrio*. This difference was statistically significant ($p = 0.001$ and 0.003, respectively). Similar to the research of Singh et al., our investigation revealed a decline in butyrate-producing bacteria such *Clostridium*, *Faecalibacterium*, and *Roseburia*. The microbiome of type 2 diabetic individuals frequently exhibits this characteristic as well.

The group of people with type 2 diabetes also had a high prevalence of

opportunistic pathogens from the genus *Escherichia*, a lower prevalence of butyrate-producing genera (*Faecalibacterium*, *Roseburia*), and a lower prevalence of mucolytic bacteria (*Akkermansia*). Our findings support several findings from earlier studies, particularly the Murri et al. study.

P. copri, *P. distasonis*, *R. callidus*, and *C. citroniae* were the four species with the most significant statistical differences out of all the species that were evaluated and compared. *P. copri* strains have the ability to metabolize a wide variety of complex carbohydrates and produce a wide variety of SCFAs that are beneficial to health. The ability of *P. copri* to increase hepatic glycogen storage and to create succinate, the chemical that maintains glucose homeostasis by controlling intestine gluconeogenesis, was discovered by study of the 16S rRNA gene sequence. This ability also contributes to an improvement in glucose tolerance. To assist in reducing inflammation, *P. copri* increases the production of cytokines like IL-6 and IL-17. The impact of these factors on human health is the subject of conflicting research findings. The impact of this bacterium on human health, particularly type 2 diabetes, will thus require more investigation.

In this study, *P. distasonis* levels increased in the diabetic group. One of the primary members with significant physiological functions for humans is *P. distasonis*. The published study discovered, in contrast to our findings, that *P. disasonis* abundance was decreased when consuming a high-fat diet. As a result, *P. disasonis* is rarely found in persons with obesity or fatty liver. Additionally, using *P. distasonis* supplements boosts the production of succinate, supports homeostasis, and helps treat metabolic problems.

Ruminococcus callidus species decreased in type 2 diabetes, in contrast to *P. distasonis*. According to earlier research, this bacterium is highly expressed in the intestinal tract of slim persons and is significantly less prevalent in the gut of obese people. Similar to the type 2 diabetes group in our study, there were less of these bacteria there than there were in the control group.

In type 2 diabetics, *C. citroniae* is more prevalent. Trimethylamine N-oxide (TMAO), a chemical linked to adipose tissue inflammation and interference with insulin signal transduction in the liver, is produced in the digestive tract by the bacteria *C. citroniae*, increasing insulin resistance, raising blood sugar levels, and

affecting type 2 diabetes.

3.1.3.2. The species composition analysis results in the male group

Bacteroidetes dominated all samples among the 13 major phyla; the diabetic group's average was 46.60%, compared to 61.25% for the control group (p = 0.21); Bacteroidetes was the most prevalent phylum in all samples. Firmicutes, which had an average rate of 32.47% in the control group and 36.52% in the diabetes group, was the second most prevalent phylum (p = 0.79). Proteobacteria, specifically: in the diabetic group it was 12.58% (p = 0.02), compared to 3.22% in the control samples, was the next major phylum. As evidenced by the three main phyla—Bacteroidetes, Firmicutes, and Proteobacteria—the findings of this study are consistent with those of other investigations.

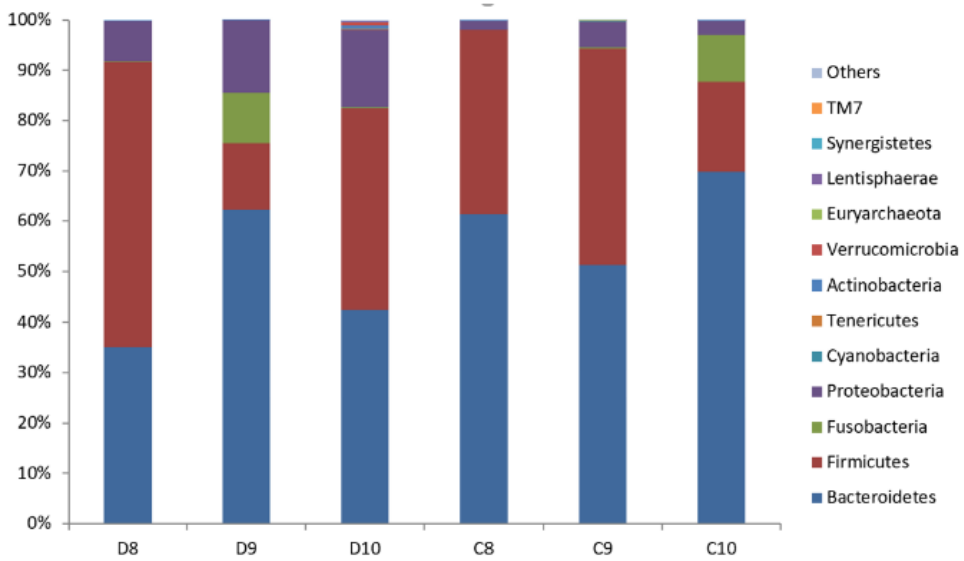

Figure 3.13. The relative phylogenetic richness of feces microbiota in the 3 men with type 2 diabetes (D8-D10) and 3 men without diabetes (C8-C10).

When specifically examining the diversity of genera, we discovered that the type 2 diabetes group contained numerous opportunistic pathogens from the *Desulfovibrio* and *Escherichia* genera, as well as a small number of butyrate-producing bacteria from the *Faecalibacterium* and *Roseburia* genera and mucolytic bacteria from the *Akkermansia* and *Prevotella* genera. The number of microorganisms belonging to the genus *Veillonella* was found to be 0.39 percent in the type 2 diabetes group, compared to 0.08% in the control group when we specifically looked at the richness of the genera (p = 0.025).

There are two distinct and statistically significant species, *R. gnavus* and *V. dispar*, among all the examined and compared species. However, comprehensive research on

the impact of these species on type 2 diabetes is currently lacking.

3.2. Correlation of gut bacteria with glucose and BMI

3.2.1. Correlation between gut microbiota diversity and glucose index and BMI in women

Ten out of the thirteen phyla had a negative correlation with BMI (4 of the phyla, Firmicutes, Lentisphaerae, Synergistetes, and Others, were found to be statistically significant), and eleven out of the thirteen phyla had a negative correlation with the glucose index (only the phylum Actinobacteria was found to be statistically significant). The 10 phyla with the lowest link to BMI also exhibited the lowest association to the glucose index. Although it was adversely connected with glucose, the Proteobacteria phylum was positively correlated with BMI. There is no statistically significant link between glucose and BMI.

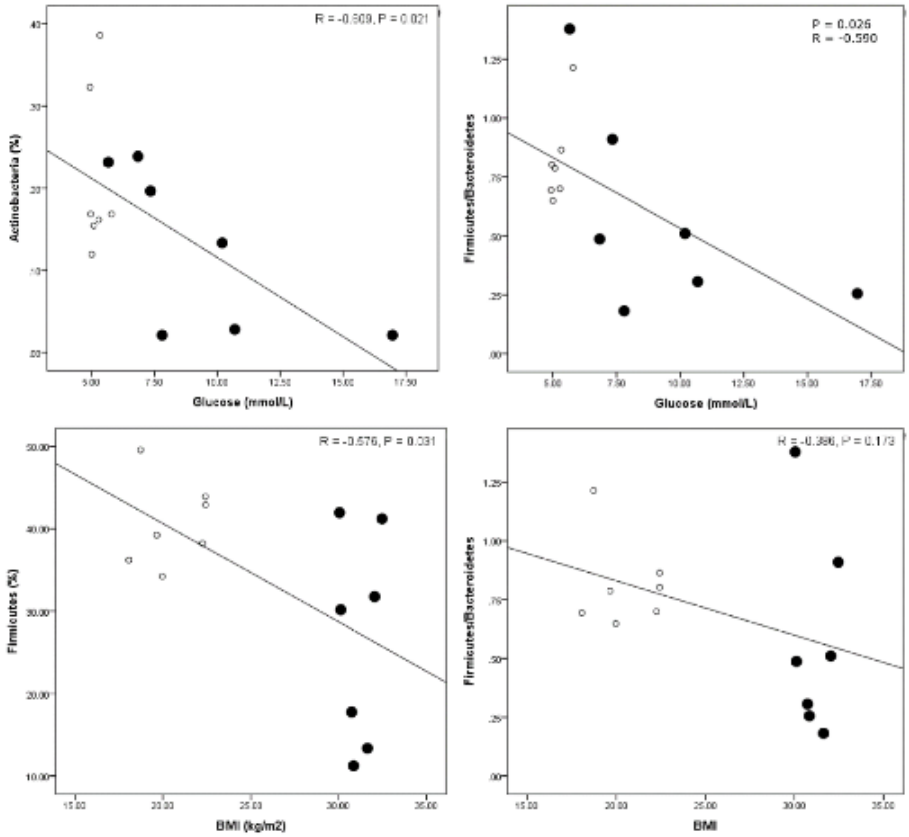

Figure 3.16. Correlation between Actinobacteria, Firmicutes/ Bacteroidetes with glucose index; Firmicutes, Firmicutes/ Bacteroidetes with BMI

Additionally, it was discovered that the ratio of Firmicutes to Bacteroidetes to the glucose index was negatively correlated and statistically significant (R = -0.590, P = 0.026), but the ratio of Firmicutes to Bacteroidetes to BMI was not (R = -0.386, P = 0.173).

Numerous studies have demonstrated a correlation between firmicutes and bacteroidetes quantity and ratio and obesity. The study by Schwiertz et al. also shown a significant decline in Firmicutes and a rise in Bacteroidetes in obese individuals, which resulted in a reduction in the Firmicutes/ Bacteroidetes ratio.

Three genera (*Ruminococcus*, *Butyricimonas*, and *Clostridium*) have statistically significant negative correlations with glucose, and six genera (*Oscillospira*, *Ruminococcus*, *Odoribacter*, *Desulfovibrio*, *Butirycimonas*, and *Burkholderia*) have statistically significant negative correlations with body mass index. Only the genus *Bacteroides* demonstrated a statistically significant positive association with BMI (R = 0.577, P = 0.031) among the genera that were positively linked with BMI and glucose.

A negative association exists between BMI and glucose index in 13/19 species and between BMI and BMI in 10/19 species. Nine of these inversely correlated species (*P. copri*, *R. callidus*, *F. prausnitzii*, *L. agilis*, *L. zeae*, *A. muciniphia*, *C. butyricum*, *Ruminococcus biforme*, *Blautia obeum*) out of the 19 species have negative correlations with both glucose and BMI indices. The inverse association between all species and the glucose index was statistically significant for two species (*Ruminococcus callidus* and *R. biforme*), although all species also displayed inverse correlation with the BMI. Only the *P. distasonis* species was positively and statistically substantially connected with both glucose and BMI among the species that were positively correlated with both variables under consideration.

3.2.2. Correlation between gut microbiota diversity and glucose index and BMI in men

Firmicutes and Bacteroidetes were 2 of the 13 phyla with a negative correlation to glucose, and Bacteroidetes, Tenericutes, and Euryarchaeota were 3 of the 13 with a negative correlation to BMI. There was no statistically significant relationship between phyla and glucose and BMI. Proteobacteria showed a statistically significant link with the glucose index and BMI in the phyla where these variables were positively connected.

Six out of the 21 taxa (*Prevotella*, *Ruminococcus*, *Odoribacter*, *Burkholderia*, *Faecalibacterium*, *Roseburia*) had a negative correlation with the glucose and BMI indices, however none of these correlations reached statistical significance.

Veillonella was the only one of the 15 genera that demonstrated a statistically significant positive connection with BMI. Gram-negative anaerobes in this genus can develop into opportunistic pathogens when the body's defenses are compromised.

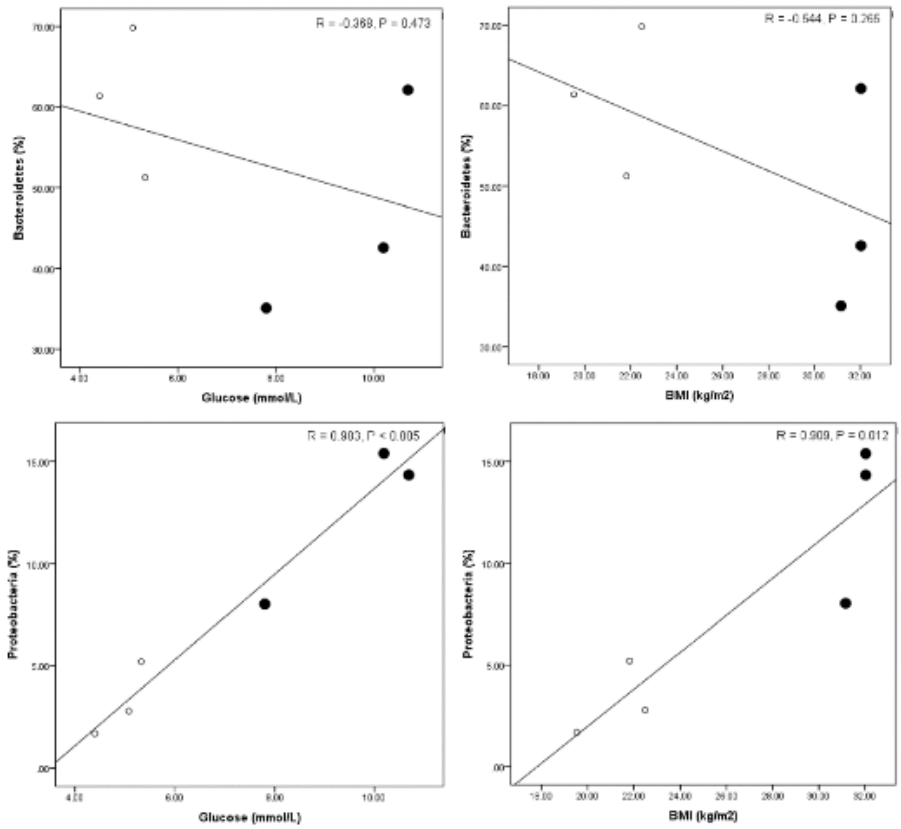

Figure 3.19. Correlation between phyla Bacteroidetes, Proteobacteria with glucose and BMI

There are 6/20 species that are negatively connected with BMI and 8/20 species that are negatively correlated with glucose. None of the species' negative correlations with glucose and BMI were statistically significant. Two species of *Ruminococcus gnavus*, *V. dispar*, are favorably and statistically significant with BMI but not with glucose among the species that have a positive association with the examined glucose index and BMI.

Experimental investigations in mice have demonstrated that the majority of Lactobacillus species, including *L. plantarum*, *L. reuteri*, *L. casei*, *L. curvatus*, *L. gasseri*, *L. paracasei*, *L. rhamnosus*, and *L. sakei*, are advantageous in mouse models of type 2 diabetes. The use of *L. sporogenes*, *L. casei* Shirota, and *L. reuteri* species as monoprobiotics to alleviate type 2 diabetes symptoms has also been demonstrated in a number of human trials. Research on *L. plantarum* species has been extensive, and it lowers mice with type 2 diabetes' blood glucose index.

3.3. Evaluation of the potential application of *Lactobacillus* in the type 2 diabetes treatment

3.3.1. Isolation of Lactobacillus and evaluation of antibacterial effect

3.3.1.1. Isolation of Lactobacillus

By isolating colonies and counting them, we were able to determine that there were (3.9 ± 1.2) x 10^3 CFU/g of *Lactobacillus* strains in the healthy group and (2.7 ± 0.8) x 10^3 CFU/g in the type 2 diabetes group. The research team selected 68 *Lactobacillus* strains with convex, smooth (non-wrinkled), milky, colorless, smooth edges, or lobed colonies from the results of microbial isolation from fecal samples.

3.3.1.2. Evaluation of antibacterial effect

Out of 68 isolated *Lactobacillus* strains, 17 strains were chosen that had antibacterial ring diameters ranging from 5.2 to 20.3 mm and were resistant to both tested strains. With a broad resistance ring diameter ranging from 16.3 to 20.3 mm, 5/17 *Lactobacillus* strains, including *Lactobacillus* 02, 13, 15, 16, and 17, have demonstrated significant resistance to both strains of *S. aureus* ATCC13709 and *E. coli* ATCC 11105.

3.3.2. Determination of probiotic characteristics of Lactobacillus

Only *Lactobacillus* strains that can withstand pH and bile salts are suitable for use as probiotics. The viability of 17 strains varied from 21 to 96% at pH 2.0. There were 8 strains total, with *Lactobacillus* 01, 02, 03, 04, 13, 15, 16, and 17 having a high percentage of viable cells (82–96%). 16/17 strains demonstrated viability ranging from 90 to 97.3% in MRS medium supplemented with 0.3% bile salts (w/v); strains with high viability cell counts > 95% are *Lactobacillus* 04, *Lactobacillus* 09, *Lactobacillus* 16, and *Lactobacillus* 17.

3.3.3. Identification of Lactobacillus bacteria

We have found 3 strains of *Lactobacillus* 17, *Lactobacillus* 16, and *Lactobacillus* 13 that include all 3 priceless probiotics features out of the 17 strains investigated for their antibacterial capacity, tolerance pH 2.0, and bile salt content of 0.3%. The findings demonstrated that all three strains of *Lactobacillus* 13, *Lactobacillus* 16, and *Lactobacillus* 17 are members of the *L. plantarum* species.

We discovered that strain *L. plantarum* 16 predominated the other 02 strains in three different *L. plantarum* strains, including 13, 16, and 17. Due to this, we utilized

strain *L. plantarum* 16 in the subsequent experiment.

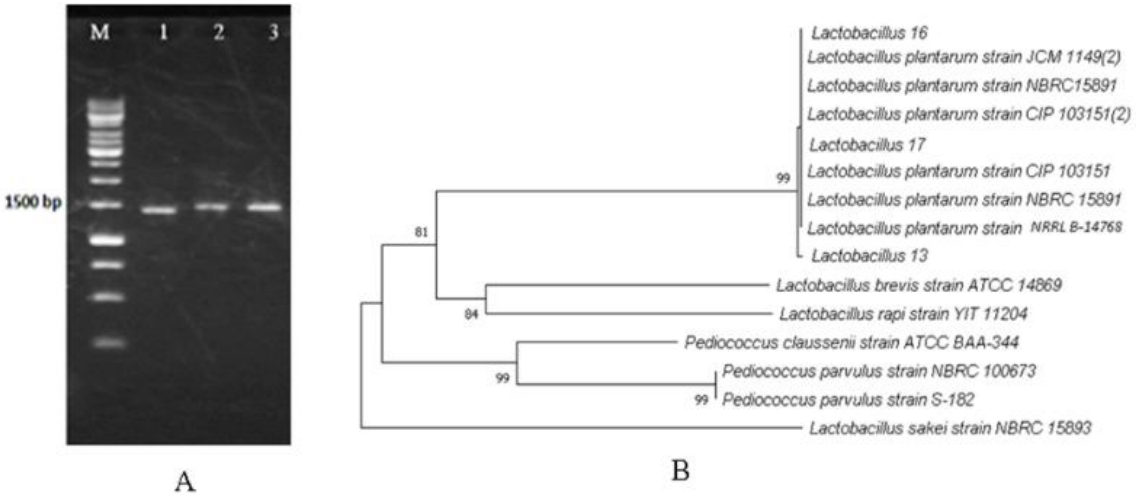

Figure 3.26. A. PCR products of 3 strains: M: Maker; 1: *Lactobacillus 13*; 2: *Lactobacillus16*; 3. *Lactobacillus17.* B. Phylogenetic tree

3.3.4. Evaluating the effect of increasing GLP-1 secretion and sucrose tolerance of L. plantarum 16 in mice

3.3.4.1. The effect of increasing GLP-1 secretion in serum

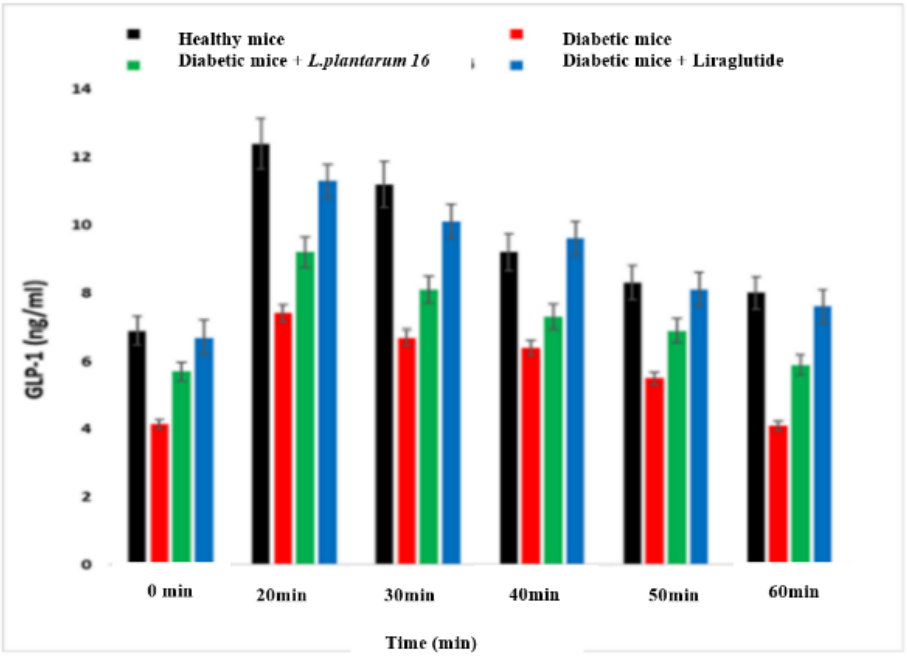

Figure 3.27. GLP-1 concentration in mouse serum at the end of the trial

When compared to healthy mice, untreated diabetic mice showed a statistically significant and significant drop in GLP-1 levels (p 0.01). After 4 weeks of therapy with Liraglutide, diabetic mice had significantly higher GLP-1 levels (p 0.01). In particular, when diabetic mice were given L. plantarum 16, their GLP-1 levels increased similarly to those of the group receiving liraglutide after 4 weeks. (Figure 3.27).

3.3.4.2. Sucrose tolerance efficiency

A sucrose tolerance test was conducted after 4 weeks, and the maximal blood glucose level was attained in 60 minutes. The blood sugar level of the group receiving *L. plantarum* 16 treatment was marginally higher than that of the group receiving Liraglutide treatment. Diabetes-related mice that weren't treated with *L. plantarum* 16 had considerably higher glucose levels ($p < 0.01$) than those that were. *(Figure 3.28).*

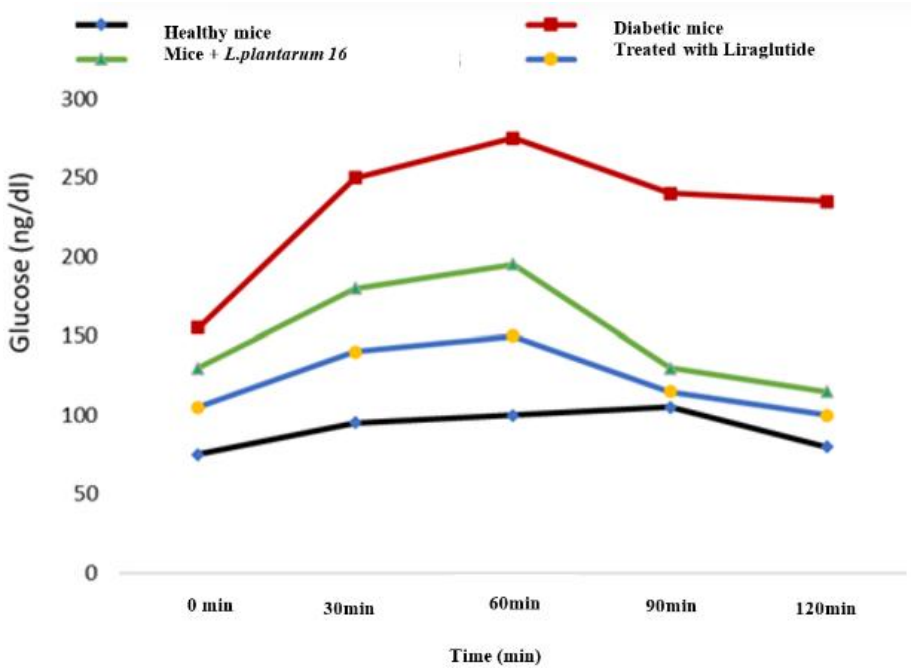

Figure 3.28. Effects of treatment on mice's blood glucose levels after oral sucrose

We predicted that taking *L. plantarum* 16 orally could affect the levels of the GLP-1 hormone. We evaluated the serum concentration of GLP-1. Streptozotocin-induced diabetes mice had considerably lower levels of the GLP-1 hormone than the control group after 4 weeks of testing. These findings show a correlation between elevated GLP-1 levels and the advantageous metabolic effects of *L. plantarum* 16. In this work, we discovered that diabetic mice with *L. plantarum* 16 had elevated GLP-1 levels and hypoglycemia.

The gut microbiota has been linked to the regulation of GLP-1 production in previous research, albeit the exact mechanism of action and the bacterial species involved are yet unknown. In this study, we investigated a microbial strain originating from Vietnam's capacity to stimulate GLP-1 secretion for the first time.

Probiotics are described as live microorganisms that, when taken in sufficient quantities, promote the health of their host. The advantages of *Lactobacillus*

probiotics, including their anti-tumor and antibacterial properties, immune system enhancement, and ability to restore the gut microbiota following antibiotic treatment, have been demonstrated in several research. Through increased GLP-1 secretion and enhanced pancreatic beta-cell function using various *Lactobacillus* strains, Manaer et al. showed a significant hypoglycemic impact in diabetic rats. Previous studies have demonstrated that using *L. plantarum* alone or in combination with other probiotics improves the effects of other probiotics on stress, antioxidants, and anti-hyperglycemia.

In this work, we discovered that administering *L. plantarum* 16 to diabetic mice elevated GLP-1 levels and decreased blood sugar. We predicted that taking *L. plantarum* 16 orally would have a greater impact on GLP-1 hormone alterations. Numerous studies have demonstrated that probiotic therapy boosts incretin hormone production, which is linked to higher insulin hormone levels by repairing pancreatic islets due to antioxidant activity, GLP-1 remodeling, and GLP-1 secretion, which is involved in the regulation of the gut microbiota. Our research revealed that diabetic mice had significantly decreased body weights; this abrupt weight loss may have been caused by lipolysis in muscle tissue since lipids are involved in the production of new glucose.

After 4 weeks of therapy with *L. plantarum* strain 16, mice's body weight, however, dramatically increased. Increased insulin levels may be connected to the process underlying diabetic therapy, which would improve blood sugar regulation and prevent weight loss.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUDE

1. When comparing the gut microbiota of the two study groups, the female control group had more OTUs than the female group with type 2 diabetes (on average, 288 versus 214). Males who had type 2 diabetes had more OTUs than those who did not (medians 252 and 217, respectively) throughout the analysis of the male group.

The Proteobacteria phylum was different between the two groups ($p = 0.021$) in the male group, where the amount of read sequences was concentrated in three phyla (Bacteroidetes, Firmicutes, and Proteobacteria). Only one genus and two species showed statistically significant differences when genus and species differences were

analyzed.

The amount of read sequences was concentrated in four phyla (Bacteroidetes, Firmicutes, Proteobacteria, and Fusobacteria) and accounted for > 98.56% when the species composition of the female group was examined. Only the variation in the phylum Fimicutes among the four major phyla is statistically significant. Analysis of the differences in genera and species between the two groups revealed that there were 9 genera and 4 species that differed and were statistically significant.

2. When we looked at how some markers for type 2 diabetes diagnosis and prediction related to intestinal bacteria, we discovered:

Four phyla (Firmicutes, Lentisphaerae, Synergistetes, and Others) had a negative correlation with BMI and were all statistically significant in females. The Actinobacteria phylum also had a negative correlation with the glucose index. Three genera have a statistically significant negative connection with glucose, while six genera have a negative association with BMI. Of these, two genera (Ruminococcus and Butyricimonas) have a statistically significant negative correlation with both indices.

Only the Proteobacteria phylum showed a statistically significant positive correlation between glucose index and BMI in men. Only the genus Veillonella showed a positive correlation with statistically significant BMI among the genera. In the female group, 2 species (Ruminococcus callidus and Ruminococcus biforme) had a significant negative correlation with BMI, and 1 species (P. distasonis) had a positive and significant correlation with both indices. In the male group, no species had a statistically significant correlation with glucose, but there were two species that had a positive correlation with BMI (R. gnavus and V. dispar).

3. isolation of probiotic-specific bacteria and evaluating it in diabetic mice showed that:

Three strains of the probiotic bacterium Lactobacillus plantarum species—Lactobacillus 17, Lactobacillus 16, and Lactobacillus 13—converge on the desirable traits of probiotic bacteria. We discovered that strain L. plantarum 16 outperformed the other 02 strains in our study on the ability of 03 strains of Lactobacillus to survive in a pH 2.0, 0.3% bile salt, and antibacterial environment. L. plantarum 16 treatment for 4 weeks elevated GLP-1 levels and reduced blood glucose in diabetic mice.

RECOMMENDATIONS

In order to get more trustworthy results, more research on more patient samples is needed.

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2. Xuan Thach Tran, Thi Hien Vu, Thi Thu Ha, Thi Hoa Nguyen, **The Hung Hoang**, Duc Hoang Le, Quyen Van Dong, Nam Trung Nguyen and Thi Tuyet Nhung Nguyen, *Screening bile salt hydrolase activity of Lactobacillus isolated from Vietnamese human origins*, Vietnam Journal of Biotechnology, 2022, 20(3): 527-536.

3. **The Hung Hoang**, Thi Minh Tu Hoa, Thi Thu Uyen Pham, Thi Thanh Huyen Le, Thi Hien Vu, Thi Thu Ha, Hoang Duc Le, Trung Nam Nguyen, Thi Tuyet Nhung Nguyen, *Characteristics of Lactobacillus strains isolated from Vietnamese patients with type 2 diabetes*, Vietnam Journal of Biotechnology, 2022, 20(1): 99-108.

4. **The Hung Hoang,** Duc Hoang Le, Thi Thanh Huyen Le, Thi Tuyet Nhung Nguyen, Hoang Ha Chu, Nam Trung Nguyen, *Metagenomic 16S rDNA amplicon data of microbial diversity of guts in Vietnamese humans with type 2 diabetes and nondiabetic adults*, Data in Brief 34 (2021) 106690.

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