

CAPITAL UNIVERSITY OF SCIENCE AND  
TECHNOLOGY, ISLAMABAD



# Functional Characterization of Probiotics Strains Against Metabolic Syndrome

by

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A thesis submitted in partial fulfillment for the  
degree of Master of Science

in the

Faculty of Health and Life Sciences

Department of Bioinformatics and Biosciences

2024

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*Every tough work needs our deep efforts as well as guidance of  
elders.*

*I dedicate my thesis to my parents ,teachers and especially my husband whose  
encouragement help to achieve all the goals.*



## CERTIFICATE OF APPROVAL

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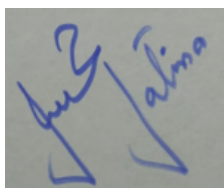
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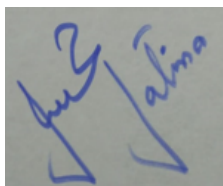
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## *Acknowledgement*

In the name of Allah who is most Gracious and Most Merciful. All praise is for Him and His Holy Prophet Muhammad (P.B.U.H) who has given me strength to complete my thesis.

First of all I would like to express my thanks to Capital University of Science and Technology (CUST) Islamabad for giving me this opportunity to do MS Biosciences. My deepest and special appreciation goes to my sweetest and most intelligent supervisor Dr. Syeda Marriam Bakhtiar, Head of department, CUST for her continuous support, encouragement and guidance throughout this journey. Everytime when I needed her she was available. She helped me in easiest possible way to accomplish this task.

I would like to acknowledge the role of all other teachers of my department Dr. Arshia Amin, Dr. Sania, Dr. Sohail Ahmad Jan who made us able to accomplish this task.

Finally I express my gratitude to my beloved Parents and my Husband. They supported me and helped me financially and morally to get my target and today I am able enough to achieve my goal.

**(Saher Fatima)**

## *Abstract*

In comparison with antibiotics , probiotics are now widely studied and used to treat and prevent many digestive, immunological, behavioral , gastrointestinal , cardiovascular and metabolic disorders. Metabolic syndrome is a disease which is combination of many different factors and diseases like cardiovascular disorders, obesity, cholesterol etc. Various probiotic strains were characterized using software and tools and best of them were collected to check their potential probiotic role. Members of genus *Bifidobacterium* have large genome sizes that protein encoding open reading frames .Pangenome analysis of this genus includes 14% genes that encode for carbohydrate metabolism enzymes. They produce metabolites that are very useful for increasing immune function and maintaining barrier of intestines. Acetic acid is being produced by many *Bifidobacterium* strains that are helpful in being used as probiotics. They use metabolic pathway to synthesis a compound of aromatic lactic acid which help in anti-inflammatory action during their mode of action. Some strains also produce various vitamins which helps them in signaling and effect the host by effecting its physiological state. *Bifidobacterium* genus strains were identified as major contributors to treat metabolic syndrome by controlling obesity, lipid and cholesterol level. Comparative analysis and Bacterial Pangenome Analysis was used to annotate and functionally analyse the genes of 11 probiotic strains of *Bifidobacterium* genus. Core genes were identified and their potential probiotics characteristics were checked. Gene ontology and KEGG pathways used to identify the role of genes and their pathway .By getting all the data about COG analysis and pathways of genes we come to know that J category genes of *Bifidobacterium* genus strains are present mostly and involved in ribosomal and protein synthesis , translation and biogenesis. These genes can help in treating metabolic syndrome if these probiotics are used. No pathogenicity and virulency being detected so *Bifidobacterium* genus strains have potential to be used as probiotics against carbohydrate ,lipids metabolism, cholesterol high level and obesity so that metabolic syndrome can be treated.

**Keywords :** Metabolic syndrome, *Bifidobacterium* genus strains, probiotics

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# Abbreviations

<b>BPGA</b>	Bacterial Pangenome Analysis
<b>COG</b>	Cluster of Orthologous Groups
<b>CDC</b>	Centre for Disease Control and Prevention
<b>GO</b>	Gene Ontology
<b>MetS</b>	Metabolic Syndrome
<b>NCEP</b>	National Cholesterol Education Program
<b>WHO</b>	World Health Organization

# Chapter 1

## Introduction

### 1.1 Background

The diseases which can not be communicated have more powerful impact on society and health of people. The Metabolic syndrome is a disease which is collection of non-communicable disorders including insulin resistance, hypertension, and hyperlipidemia, has been reported in a number of studies undertaken in both the general population and adult populations with the disease.[1][2]

The variables that raise the risk of many diseases like Diabetes type II , heart diseases like myocardial infarction, arteriosclerosis, clotting and all diseases that are causing death of people are when collectively considered refereed to as ‘metabolic syndrome’.[1] [3]

The syndrome is made up of a number of variables, including obesity in visceral area , hypertension, heart problems, dyslipidemia,hypercoagulation, and hereditary diseases that are vulnerable. [4] Body mass index, family history, physical activity, dietary habits, stress levels, sleep smoking, alcohol, lack of knowledge and malnutrition all are the major risk factors that are leading in prevalence of metabolic syndrome among people.[2][5]

## 1.2 Pathophysiology of Metabolic Syndrome

Metabolic syndrome pathophysiology consist of various mechanism which are very complex to understand and still not fully cleared. Still this discussion is under process that several of metabolic syndrome are ether falling under the same pathological category or they have some different unique pathophysiology that have to be considered separately.[3]

Genetic factors are those factors which have the highest contribution in prevalence of this syndrome but along with them there are various other too . Like dietary habits , lifestyles of people have influenced this syndrome in many cases. High intake of proteins, lipids or fats is also a major concern when coming to the diseases like obesity which is effecting metabolic syndrome at top level. Obesity is such a disease that induces many pathways that will lead to severity of metabolic syndrome.

The mechanism that are being involved in the rise of progression of this particular syndrome includes resistance against insulin , nervous and hormonal activation , increase in inflammation . They are playing a very impressive role in increasing cardiovascular diseases and diabetes that will lead to metabolic syndrome[5]

## 1.3 New Approach in Treating Metabolic Syndrome

Obesity is the main cause of metabolic syndrome. Management strategies widely used are dietary habits, pharmacogenomics, polypharmacy, probiotics and prebiotics, putative mechanisms. Many probiotics and postbiotics are being used to treat MetS. A lot of probiotics strains are being identified that reduce the onset of risk factors of metabolic syndrome. Identifying the strain that has the potential to treat metabolic syndrome without causing any effect is difficult. [6]

All probiotics strains have different characteristics and mode of action for treating different variables of metabolic syndrome. The majority of the challenge left

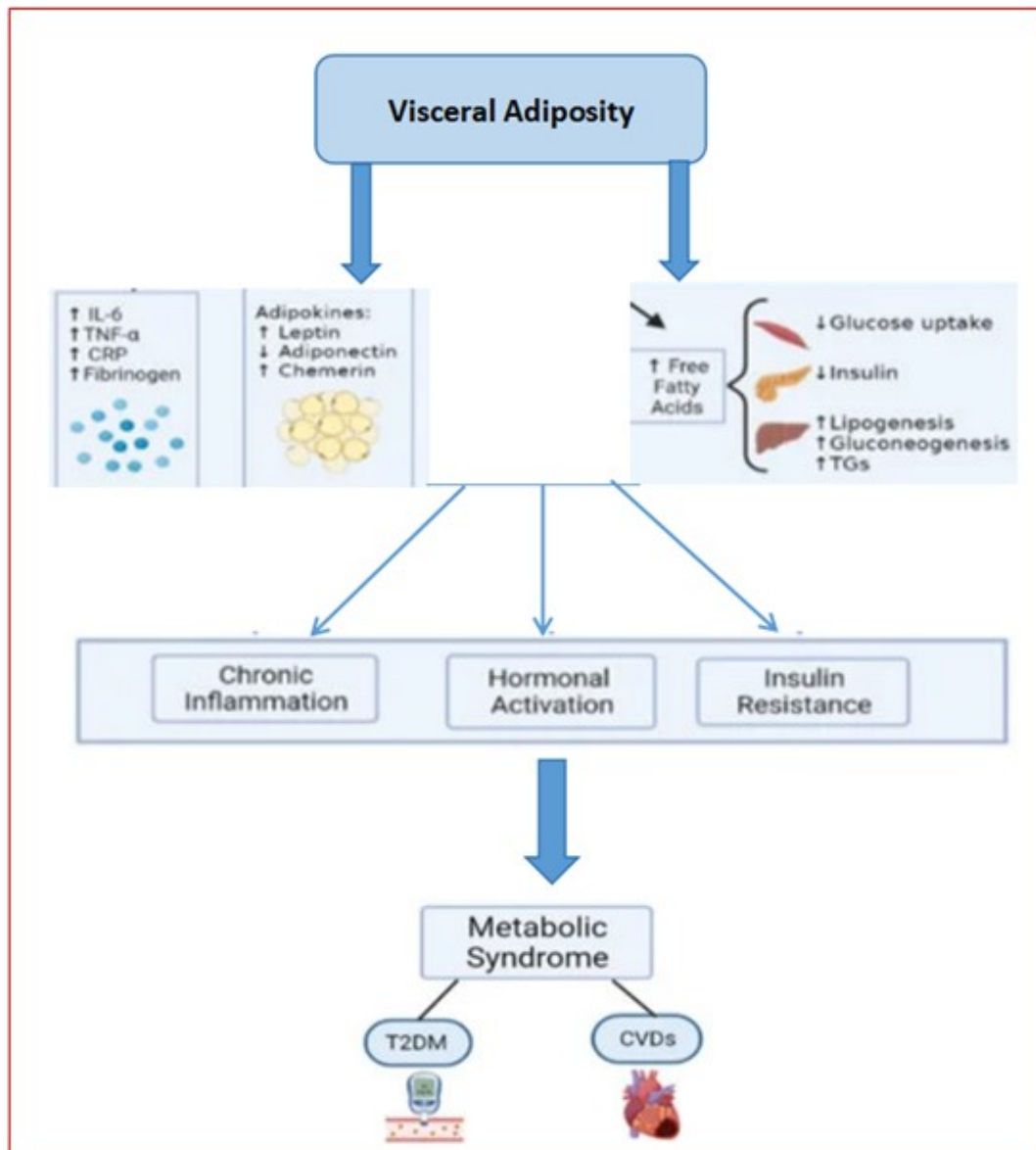


FIGURE 1.1: Pathophysiology of Metabolic Syndrome Explaining its Various Components

is to check probiotics potential of the strain against metabolic syndrome by its structural and functional characterization.[7]

## 1.4 Characteristics of Probiotics

Probiotics bacteria are simply the living bacterial species or strains that helps in the maintenance of our digestive tract because they have the ability to balance the gut microbiome population that may be declined because of any infection in gut or intestine.[8] Probiotic potential on which it can be selected or rejected is

because of this characteristics that they can survive in gut because they have many capabilities like tolerance to acids and bile salt also have antimicrobial activity. They can stimulate immune system also.

TABLE 1.1: Characteristics of Probiotics

Sr.	Characteristics
1.	They have the ability to reside in gut for a long period of time
2.	They get easily attached to epithelial cells present in wall of intestine
3.	They don't show any pathogenic property
4.	They can stimulate the immune system in the body where they are present
5.	They also have capability to decrease the chance of mutations.

## 1.5 Mode of Action of Probiotics

The colon and intestine of human is house of many probiotics like strains of *Bifidobacterium* and *Lactobacillus*, which effects environment of intestinal microbes, immune responses, produce different products, which have role in enhancement of health of host. Benefits of probiotics are in preventing body to get infection and cancer , lowering the level of cholesterol. [9]

- 1.For nutritional source it do competition.
- 2.It produces substances against certain microbes to compete with them in gut.
- 3.They got adhere to host cell wall 4. Or do co-aggregation.

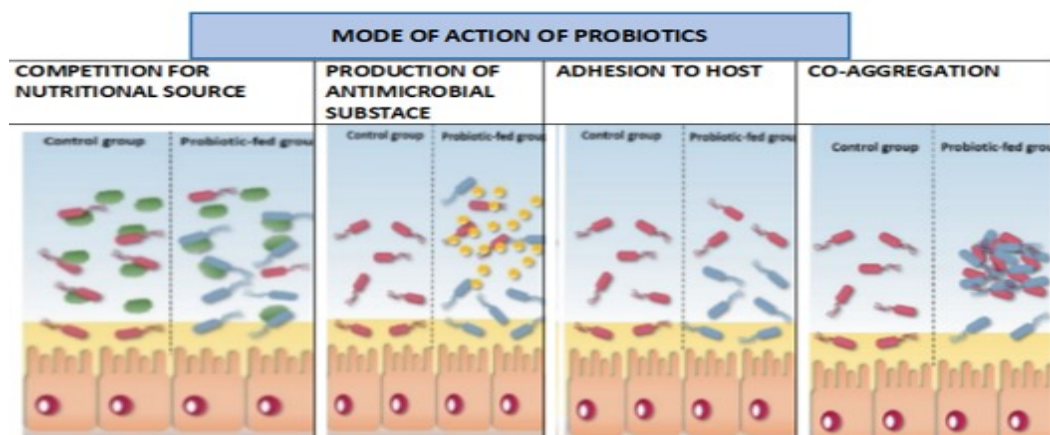


FIGURE 1.2: Summarizes Mechanism of Action of Probiotics

## 1.6 Aims

Metabolic syndrome and its associated diseases are increasing enormously from past few years. Lot of management strategies are being used to treat MetS but best one is the probiotics strains. Variety of probiotics strains has been discovered. The requirement is to identify strains that have greatest probiotics potential against MetS. This study is being designed with the aim to explore the effective probiotics strains which have potential to reduce risk of MetS. We have also tried to explore their genes that are letting them being best as probiotics strains.

## 1.7 Objectives

Lot of management strategies are present to treat MetS but all are not effective against it and the best one is use of the probiotics strains. Variety of probiotics strains has been discovered. We have also tried to explore their genes that are letting them being best as probiotics strains.

The study is designed with given objectives.

1. Identification and prioritization of probiotics strains against MetS.
2. Characterization of prioritized strains for probiotics and probiotics potential.
3. Effectiveness of probiotics strains through their activity, metabolic and functional characterization.

# Chapter 2

## Literature Review

### 2.1 Metabolic Syndrome

Despite of a fact that NCEP:ATPIII introduced the term MetS in 2001, from many years in the review of literature this idea is being given to group metabolic syndrome and cardiovascular disorders together.in reality , current assessments have shown out that observations about connection between the disease diabetes mellitus II and disease hypertension were published by independent experts as early as the 1920s.[10] [11].

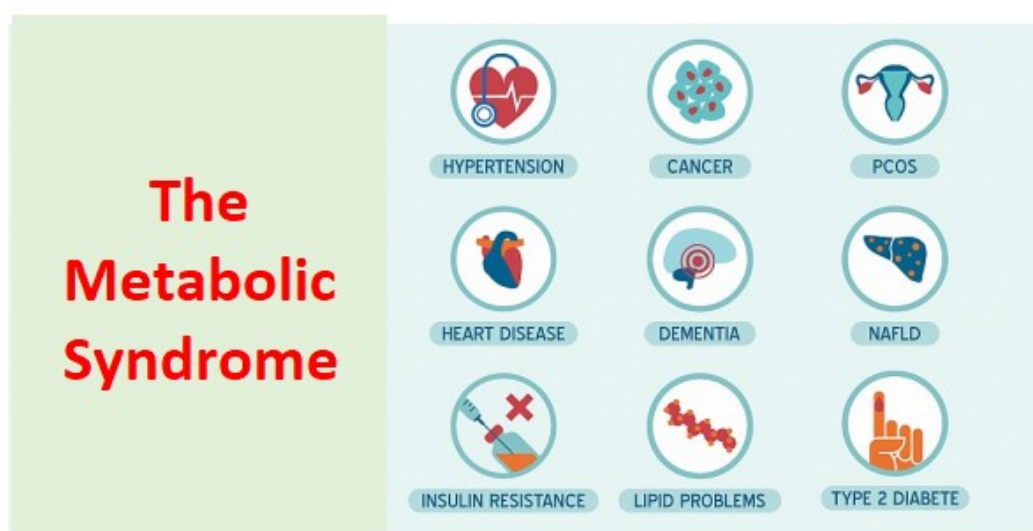


FIGURE 2.1: Metabolic Syndrome is Not a Disease but it's a Cluster of Risk Factors

Even while many criteria are used to identify the MetS, they all seem to concur that its primary indicators—obesity, insulin resistance, dyslipidemia, and hypertension—are present. Type II diabetes, glucose intolerance can cause insulin resistance that combine to be important risk factor of metabolic syndrome in many people. High level of triglycerides or lower level of high density lipids , disease like hypertension are being required to increase insulin resistance that is major factor contributing to it. [12]

## 2.2 Prevalence and Incidence

The frequent occurrence of metabolic syndrome in people coincides with the wide spread of disease like obesity and diabetes type II. According to statistics, of year 1988 up to year 2009, the average disease in the USA increased by 0.37% each year for both genders , while the average rise in waist circumference was 0.37 and 0.27% for women. About 30.2 million persons in the USA, or 12.7% of all adults, have diabetes, according to CDC data released in 2017. Twenty-four percent of them (23.8%) were unaware that they had diabetes. T2DM prevalence rose with age, peaking at 25% among seniors (60 age or more). MetS or diabetes prevalence was triple the amount. In the US, metabolic syndrome affects around one third of individuals [6][10]

According to WHO criteria, the widespread of obesity from 14 to 21% in China between 1992 and 2002. The rise was from 20 to 29% when using the lower BMI cutoff in the Chinese definition of obesity. In urban regions, MetS rose from 10 to 20%, and in rural areas, it rose from 4 to 5%. The widespread of MetS in country China from 2016-17 would be approximately 15.5% assuming the same pace of rise.[13][14]

## 2.3 Prevalence in Pakistan

In Pakistan, 46–68% of the population is obese as measured by waist circumference, and there is a clear link between arm fat and insulin sensitivity. 68–81% of

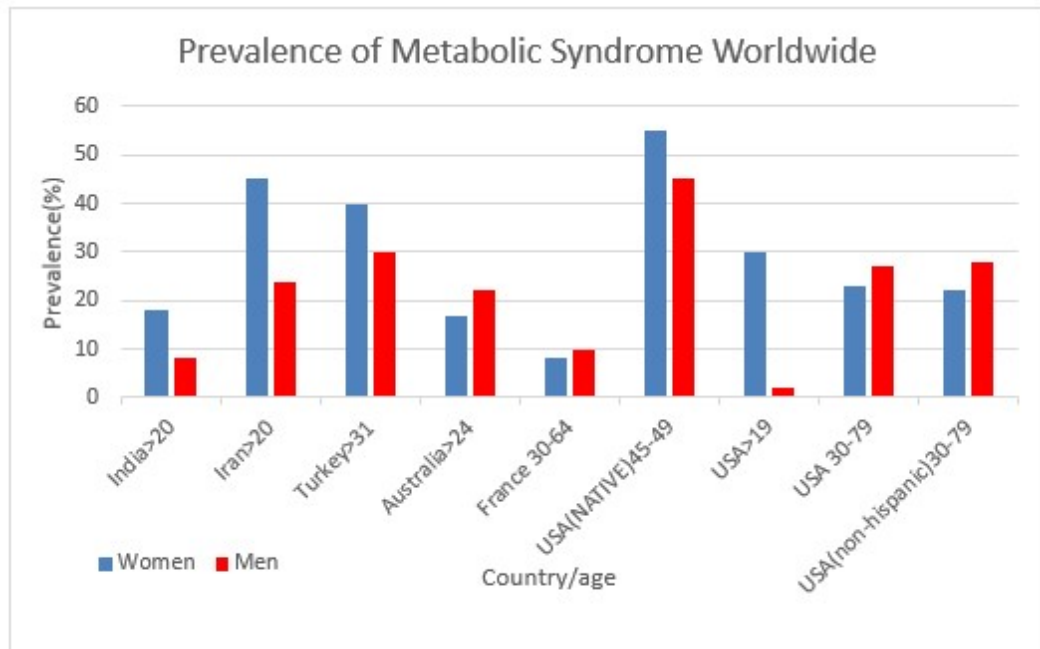


FIGURE 2.2: Worldwide Prevalence of Metabolic Syndrome

those with dyslipidemia have low levels of high-density lipoprotein (HDL), whereas 27–54% have hypertriglyceridemia. It was discovered that 50% of them had hypertension. Given the high prevalence of all of these metabolic risk factors, metabolic syndrome prevalence in Pakistan has been estimated to range from 18% to 46% depending on the definition used, which is comparable to statistics from other South Asian nations[6]

## 2.4 Components of the Metabolic Syndrome

Metabolic syndrome is itself not a disease but a combination of various components that together cause this syndrome to work in body. Insulin resistance ,dyslipidemia,dysglycemia, abdominal obesity known as belly fat, high triglycerides , low or high levels lipids (HDL ,LDL),high levels of cholesterol and hypertension. Overweight and obesity are the main key factors that lead to these various conditions. Because of all these risk factors metabolic syndrome is effecting millions of people.

Metabolic syndrome increases risk of various diseases like diabetes T2, cardiovascular disease, liver disease, stress disorders, PCOS in young women, non-alcoholic

fatty liver disease (NAFLD). It increases risk for heart diseases and diabetes that ultimately lead to organ damage and mortality. When obesity increases above the certain limits fatty acids accumulation also rises that results in excessive deposition of adipokines and cytokines that result in HDL or LDL and organ damage like liver, kidneys pancreas due to fluctuation in blood pressure. Type 2 diabetes and cardiovascular diseases are the main powerful diseases associated with metabolic syndrome. [13][15]

## 2.5 Risk Factors

Risk factors are those characteristics that lead to the arousal of a particular disease or syndrome. They play key role in the prevalence of syndrome. Body mass index, family history, physical activity, dietary habits, stress levels, sleep smoking, alcohol, lack of knowledge, malnutrition, middle class status, all are the major risk factors that are leading in prevalence of metabolic syndrome among people. Obesity is the major contributing factor toward metabolic syndrome. [14] People who take diet rich in fatty acids, lipids become obese and it lead to cardiovascular diseases, arteriosclerosis, heart tissue damage that ultimately lead to severe complication. Nutritional imbalance or malnutrition lead to the condition like diabetes that effect pancreas and body functioning. Alcohol and smoking also lead to the metabolic syndrome by increase carcinogens and toxins inside blood that effects blood pressure and circulation of blood to body parts. [10][16]

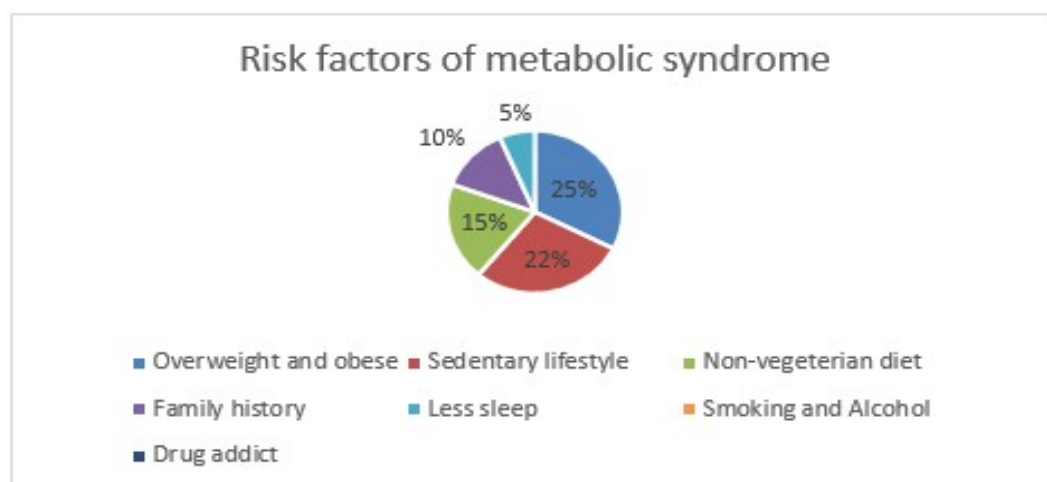


FIGURE 2.3: Graphical Representation of Risk Factors

## 2.6 Contribution of Sedentary Lifestyle

Sedentary lifestyle refers to inactivity of individuals more than 6 hours per day without having any physical activity. Sedentary behaviors affect the body because of inactivity and cause metabolic disorder by fluctuating various mechanisms like it decreases activity of lipase that metabolizes lipoprotein, reduces protein transporter activities, lipid metabolism can't be done properly and carbohydrate metabolism is also affected. It affects circulation of blood and hormones, insulin sensitivity is lessened, weight is gained because of accumulation of adipose tissue, cancer risk is increased. Depression and mental abilities are retarded. All these factors together result in metabolic syndrome that eventually leads to death [17]. It is believed that prolonged inactivity causes an increase in risk of metabolic syndrome among people. To eradicate metabolic syndrome we have to omit the prolonged inactivity period of the individuals [11] [18].

## 2.7 Dietary Habits

Unhealthy food and meals contribute to diseases. More westernized dietary habits are increasing the risk of cardiovascular diseases and metabolic syndrome. Eating of fast food, overeating, more salty food, sweeter food, high meat consumption, highly fried foods that contain lipid in rich amount increases risk of diabetes, heart failure, high blood pressure that ultimately combined as metabolic syndrome. [19] Over nutrition leads to gut inflammation that ultimately affects your body metabolism. Some people live in malnutrition areas where lack of food is available. Malnutrition results in metabolic syndrome when there is not enough diet imbalance of protein and nutrients would affect homeostasis of body. Patients with malnutrition can develop cardiovascular diseases, renal failure results in mortality. Children with malnutrition develop glucose intolerance, poor growth and poor height during early life. In adulthood they likely to develop metabolic disorders due to poor growth in early life [10]. Gut dysbiosis is the imbalance of bacteria present in gut also contribute toward metabolic syndrome. Westernized diet containing high sweet or salt in food disturbs gut microbiota that lead gut

dysentery and dysbiosis that ultimately lead to metabolic syndrome by causing insulin resistance, inflammation, dyslipidemia and obesity. With use of antibiotics, probiotics and prebiotics it can be cured otherwise it causes severe metabolic disorders leading to death. [18]. Changing dietary habits and routine of exercise helps in improving of cholesterol levels, body weight, blood pressure and glucose level. The best therapy to treat metabolic syndrome is long term change in lifestyle and diet [20].

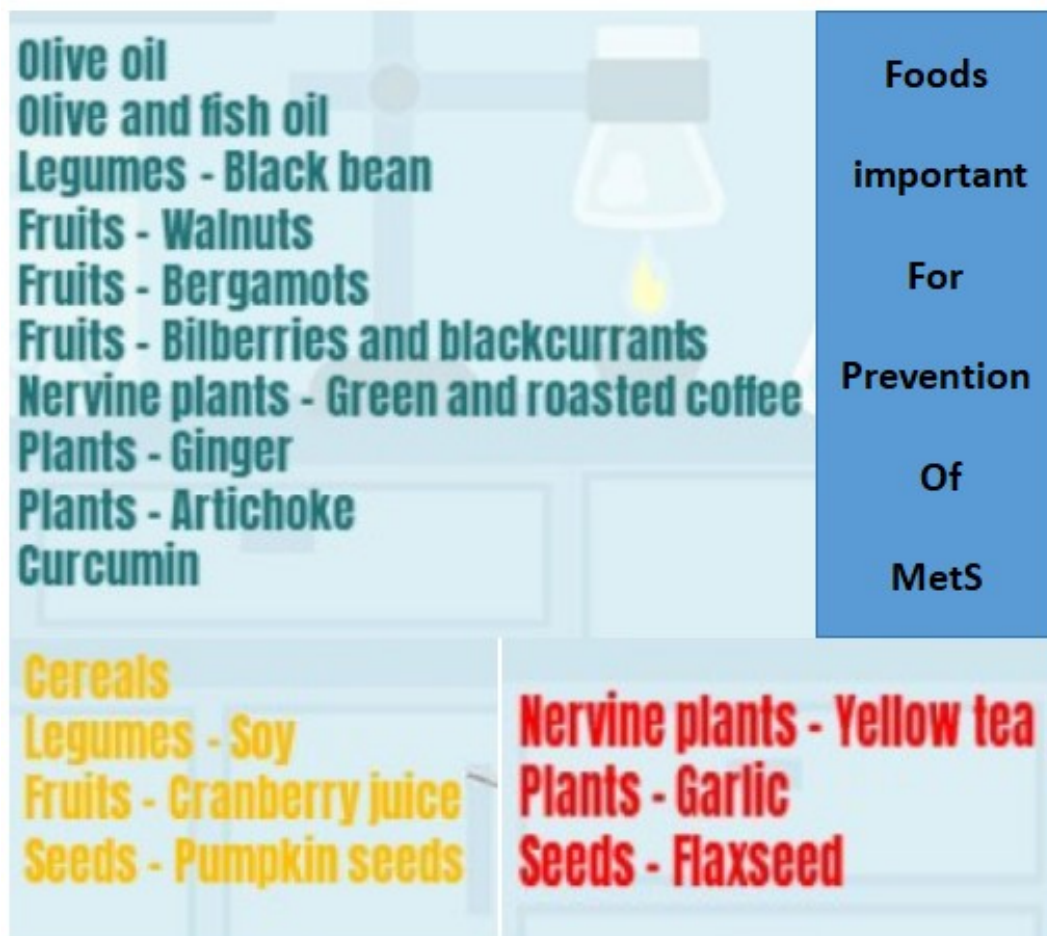


FIGURE 2.4: Foods that are playing role in prevention of Metabolic syndrome

## 2.8 Disease Burden

Metabolic syndrome is a non-communicable disease that is responsible for 41 million deaths in whole world yearly [11]. The more spread of thgis syndrome is causing in increased used of healthcare centers by patients resulting in increased burden on economy of a country. With metabolic syndrome people need more

expenditure to buy drugs, medicines, more hospitalization, and more physician's services. It causes economic burden to patients, their family as a whole society would suffer [21]. The economic loss is much more if we consider how the agriculture industry is suffering because of health issues related to metabolic syndrome. This results in economic burden that effects whole country. The health sector also become burdened because of high demands of patient's regular test, reports, plans and procedure. Budget for health sector become more that creates imbalance in economy. Moreover diagnosis is not done in early stages so that disease can be treated easily that also contribute to disease burden. Lack of systematic research on metabolic disorder, data unavailability, proper screening, guidance and lack of early diagnosis result in disease burden and loss of economy [12].

## **2.9 Treatment and Management**

Treatment and management include mostly lifestyle based or drug based strategies to lower main causes of metabolic syndrome like obesity, insulin resistance, cholesterol level.[18]

### **2.9.1 Healthy Lifestyle and Diet**

Initial treatment of metabolic syndrome is including changes in diet, change lifestyle habits, do more exercise, and have regular medical checkup. Healthy lifestyle is the first key to manage metabolic syndrome. Nutrition must be considered to lessen the prevalence of metabolic syndrome. Take healthy diet with less carbs and lipids to reduce cholesterol and sugar level. Avoid stress to maintain your blood pressure and reduce stress on heart tissues.[7].

### **2.9.2 Pharmacological Management**

It includes multiple drugs to reduce the chance of metabolic syndrome. Metabolic syndrome include many components because of that poly pharmacy uses combined

effect of drugs to treat it. Anti-hypertensive medicines, anti-inflammatory, anti-obesity, anti-diabetic, cholesterol lowering drug. All are used to treat metabolic syndrome.[22]

TABLE 2.1: Drugs Involved in Treatment of Metabolic Syndrome

Drug family	Name	Functions	References
Statins	Simvastatin	Lower low density lipid cholesterol level	[18]
	Atorvastatin		
	Rosuvastatin		
Cholesterol absorption inhibitors	Ezetimibe	Lower the LDL-C level	[18]
Antidiabetics	Biguanides Thiazolidinediones	Lower sugar level	[22]
Insulin sensitizing agent	Metformin Actos	Treat hyperglycemic patient	[23]
ACE inhibitors	Capotene	To maintain blood pressure	[24]
Aspirin	Low dose aspirin	Treat prothrombotic patient	[8]

### 2.9.3 Treatment Using Probiotics

The colon and intestine of human is house of many probiotics like strains of *Bifidobacterium* and *Lactobacillus*, which effects environment of intestinal microbes, immune responses, produce different products, which have role in enhancement of health of host. Benefits of probiotics are in preventing body to get infection and cancer , lowering the level of cholesterol.

A strain's safety and effectiveness in relation to these characteristics must be scientifically shown before it can be categorized as a probiotic. Prebiotics are non-digestible poly or oligosaccharides (OS), according to Gibson and Roberfroid, that have a positive effect on the organism in which they are residing by choosing

effectively and promoting the proliferation or activity of many or little number of advantageous bacteria living in the colon [9].

### 2.9.4 Metabolic Syndrome and Gut Microbiota

The development and course of MetS are significantly influenced by gut bacteria. The gut microbiota has been shown to influence oxidative stress, pro-inflammation, hunger, serum lipids, and plasma glucose. The gut microbiome produces bioactive metabolites and signalling molecules that enable the intricate and coordinated molecular cross-talk between the host and the microbiota.

These metabolites have a big impact on how the human body regulates health in various ways. The gut microbiota may therefore represent a viable therapeutic target for metabolic illnesses based on these recent advances.[25]

### 2.9.5 Probiotic Bacterial strains

TABLE 2.2: Effective Probiotic Bacterial Strains for Metabolic Syndrome

Isolated from	Strains	Functions	References
	<i>Apilactobacillus</i> kunkei		
Isolated from honey bee gut	<i>lactobacillus</i> kimbladii	They help in lowering the cholestrol level	[26]
	<i>Fructobacillus</i> fructus		
Isolated from feces of humans	<i>Faecalibacterium</i> prausnitzii	Help to treat Inflammatory bowl disease because of anti-inflammation properties	[15]
Isolated from a food in turkey known as kimchi	<i>Weissella cibaria</i>	In upper respiratory tract infection they play role as antimicrobial and have antibiofilm properties	[27]
From sheep rumen	<i>Pichia</i> kudriavzevii	Anticancer activity	[28]

## 2.10 Clinical Significance of Probiotics

Probiotics are now very important in medical research because they have very useful impact on life of humans. They have been proven beneficial as they are specific for specific host, their location is specific, and their composition is also specific. They have lot of important characteristics that help to cure many diseases and improve health of gut [29].

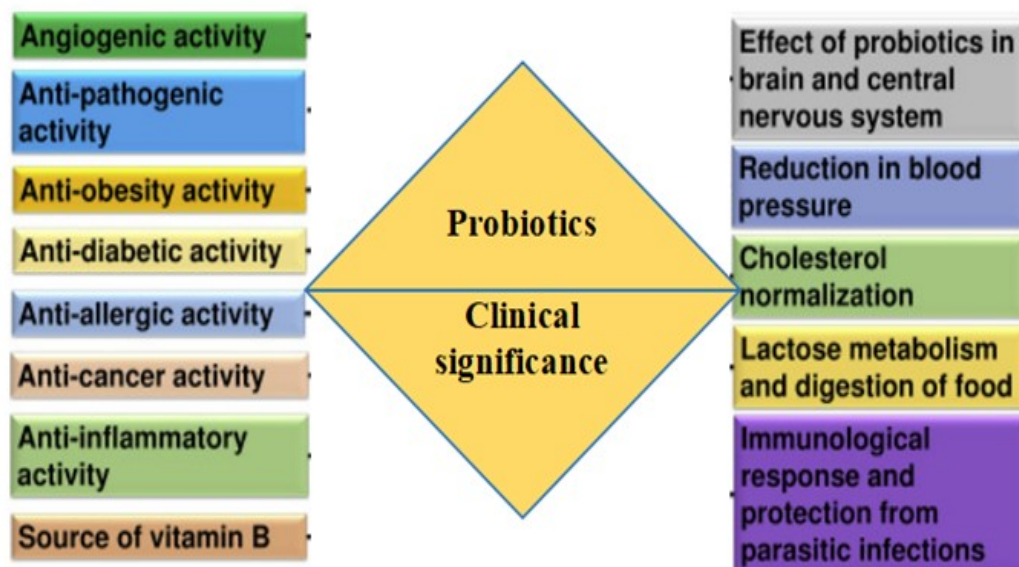


FIGURE 2.5: Role of Probiotics in Human Health

Help balance of digestive system Probiotics contain many healthy bacteria that play their role in maintaining health of host. Restoring of gut bacterial balance is the natural characteristics of a probiotics that is helping them. This imbalance can be happened due to poor diet, intake of antibiotics and various diseases [30].

The consequences include many health problems like mental disturbance, being fat, allergies etc.

They help in improving mental health conditions Research has found that strains of bacteria *Bifidobacterium* and *Lactobacillus* help in the improvement of mental diseases like anxiety, memory disorders, and depression like diseases.

More absorption of fats in intestine is induced by some bacterial strains that help in lowering fat in your body results in person become less obese. They help in

reduction of infections like urinary infections and respiratory infections and helps us in boosting of host immune system. They make host able to fight against diseases and infections.[31] [32]

They are important in lowering the level of cholesterol and blood pressure that in return important for healthy heart function. Bacterial strains that produce lactic acid bacteria breakdown bile in the gut of host so that it can not be absorbed back to body as cholesterol that reduces cholesterol level in our body and help in treatment of cardiac diseases.

## 2.11 Mechanisms of Probiotic Actions

The role of probiotics is not directly related to the microflora that is present in gut. But it can be different like sometime they become the member of microflora present in gut but sometimes they play role in changing of influencing the bacteria already present. Like species of *Bifidobacterium* reside in intestine but *lactobacillus* species play role in influence of that microflora. [33] The major role they play is discussed below.

## 2.12 Probiotics Acting as Barrier

By reducing the Process of apoptosis in cells of intestines or with the help of regulation of production of mucin component ,it changes epithelial intestinal function. Those bacterial strains that release acetic acid or lactic acid help as Probiotics by playing role in lowering the levels of pH that stops growth of pathogens. [34]

## 2.13 Production of Antimicrobial Substances

Probiotic strains make changes inside the cells of host by releasing various chemical that directly bind to cells of epithelium of gut stopping the entry of pathogen or binding of pathogen to the epithelium cell wall.Chemicals like defensins are

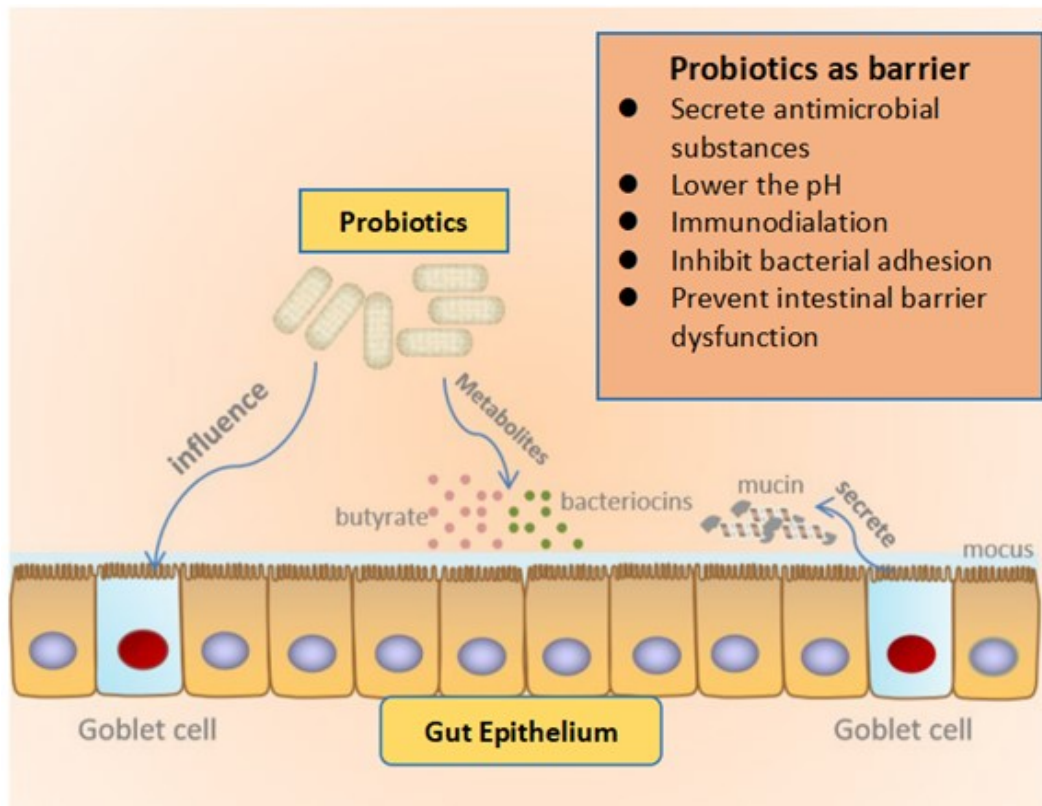


FIGURE 2.6: Summarizes Role of Probiotics as Barrier in Intestine

released by some strains like *lactobacillus* that show antimicrobial properties. By releasing chemicals like defensins, acetic acid and lactic acid these probiotic strains make a tough non-living environment for bacteria and pathogens to grow that reduces infections in the body. [35]

Some short fatty acids are also released by some strains that cause changes in the outer wall of the cell to inhibit pathogen growth. Microcins are the chemicals that directly act on the DNA or protein structure of bacteria and destroy them. Chemicals like bacteriocins help in disruption so that the growth of pathogens may stop. [36]

## 2.14 Modulation of Immune System

Probiotics help in the regulation of the immune system. It involves modulation of the innate and adaptive immune systems. By inducing the functions of dendritic cells, white blood cells like T lymphocytes and B lymphocytes. By activating the receptors like toll receptors, the immune system can also be enhanced by probiotic strains of

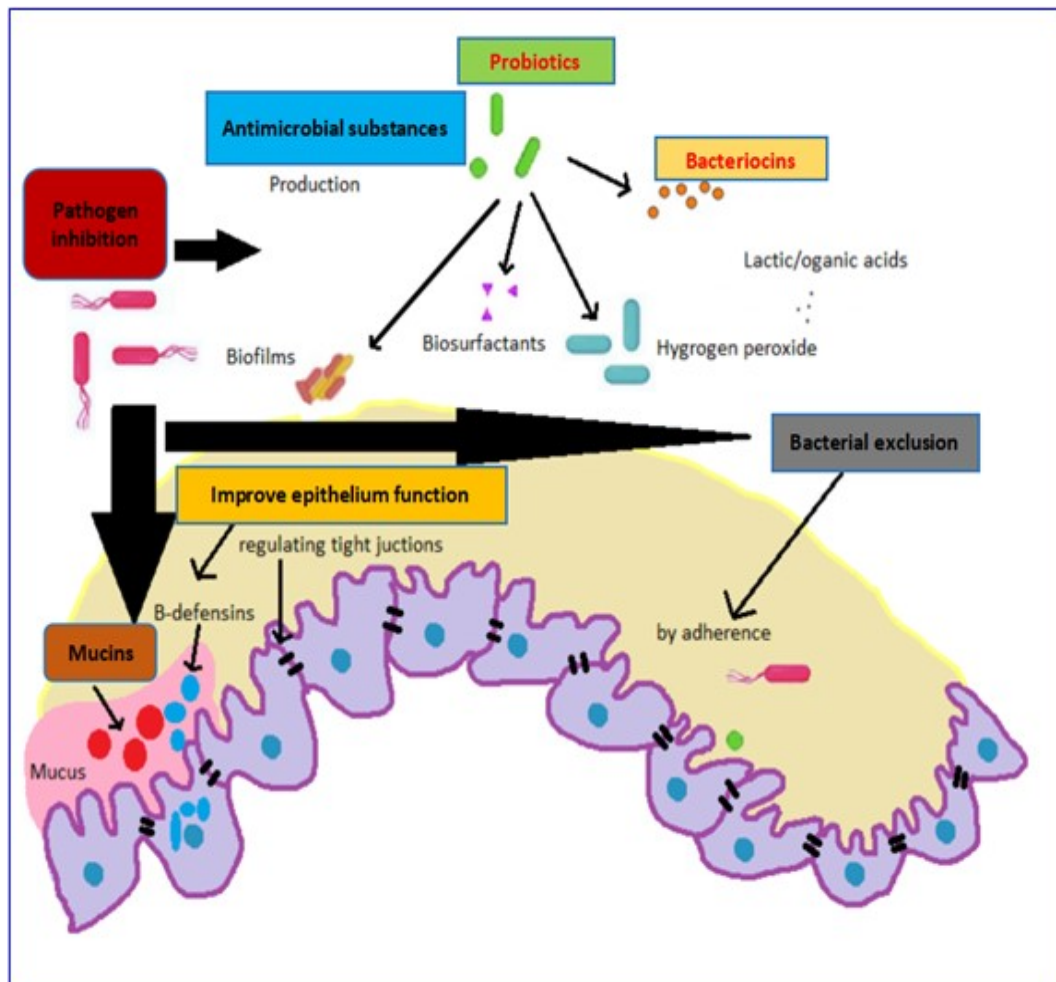


FIGURE 2.7: Summarizes the Process of Release of Antimicrobial Substances by Probiotic Bacterial Strains and their Effect on Pathogens

bacteria .they also regulate immune function of epithelium pf intestine to prevent body against pathogens and microbes .They show different effects like gene expression, signaling of immune cells, expression of gene in modulation of immune response.[37] [31]

## 2.15 Effective Role of Probiotics Against Different Gastrointestinal Diseases

Different bacterial probiotic strains are being identified and are now being used in treatment of various gastrointestinal diseases. By checking the probiotic potential of certain strain it is subjected to clinical trials and than used for treatment in medical science .

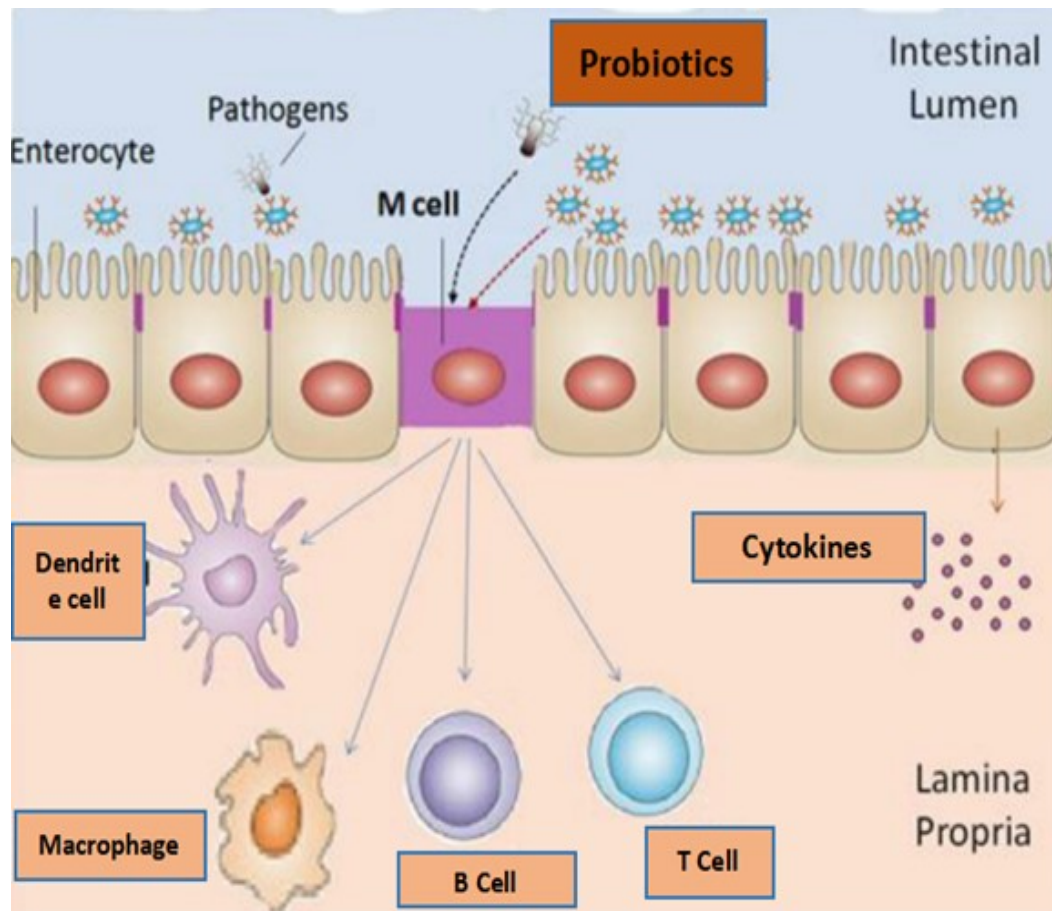


FIGURE 2.8: Role of probiotic Strains in Modulation of Immune System

### 2.15.1 Ventilator Associated Pneumonia

The most adverse type of pneumonia is Ventilator associated Pneumonia. The people who suffered from this pneumonia have greater risk of death because lungs completely lost their functions due to high rate of infection in tubes.[38] The pathogens associated with VAP are those who make biofilms and release contaminated secretions. Resistance against antibiotics have lead to make new methods to treat such type of pathogens.[39]

Probiotics like *lactobacillus casei* is effective against VAP. They decrease the rate of colonization in gastric as well as tract of respiratory system. [40] [38]

### 2.15.2 Antibiotics Associated Diseases

The percentage of antibiotic associated diseases ranges from 5-30% in body of host. By taking excessive amount of broad spectrum antibiotics you likely to get

these diseases. Like clindamycin is the major cause of antibiotic associated diarrhea. [41] Probiotics can help in treatment of diarrhea by reducing the pH of

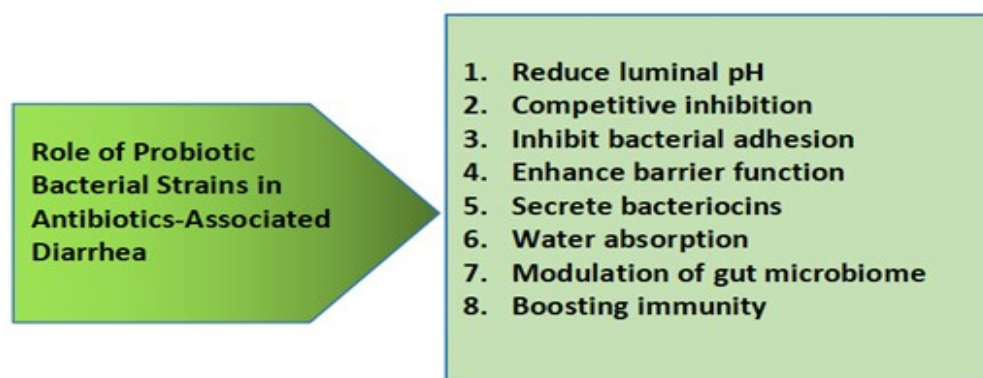


FIGURE 2.9: Enlisting the Role of Probiotics in Treatment Antibiotic Associated Diarrhea

lumina . By secreting chemicals like bacteriocins, or by balancing the amount of microflora that resides inside gut and had disturbed because of antibiotic associated diseases. [42]

### 2.15.3 Infection by *Helicobacter pylori*

This bacteria has strong relation with a peptic ulcer of duodenum and it causes severe gastrointestinal infections . Recent therapies have been used to remove this bacterial infection. Studies about probiotics showed effect against *Helicobacter pylori* because probiotics increase the antimicrobial substance production and production of metabolites.[43]. By using *Saccharomyces boulardi* and *Bifidobacterium* probiotic strains in correct proportion have shown significant reduction in infection caused by *Helicobacter* by reducing rate of nausea and diarrhea.[36]

### 2.15.4 *Clostridium Difficile* Infections

In recent time *Clostridium* infections have been reported with severity. It causes diarrhea, and disease like colitis . Treatment of CDI have been done by vanomycin but symptoms again reappear. Due to sudden severity of this disease and reporting of death lead to formulation of new drugs or treatments [32]. *Saccharomyces*

boulardi is proved defensive against Clostridium infection because it kills toxins A and B produced by it and increased the level of IgA that causes an antitoxic activity and relief from infections. [44]. Now the tablets of S.boulardi is being available because it is only effective probiotic being used against Clostridium infection. [38]. Probiotics align capsule , nutrition bars and adult formula capsules are also present in combination form of *Lactobacillus* species and *Bifidobacterium* species. They have been used with combination of vanomycine to prevent this repeating infection in body.[45]

### 2.15.5 Irritable Bowl Syndrome

Studies suggest that disease gastroenteritis is the main reason behind bowl syndrome. It raises the risk of bowl syndrome in many people, having pain and bloating in abdomen . Alteration of microbes and environment of gut have serious effect on bowl syndrome and gastroenteritis.[37]. Evaluation of probiotics as treatments have resulted in conclusion that bacterial strains that have potential can be used as treatment of bowl syndrome. [46] *Bifidobacterium* infantis probiotics is the most effective strain that plays role in treatment of bowl syndrome. It is present in form of align capsules that reduces abdominal pain and reduction in bowl syndrome symptoms. [47]

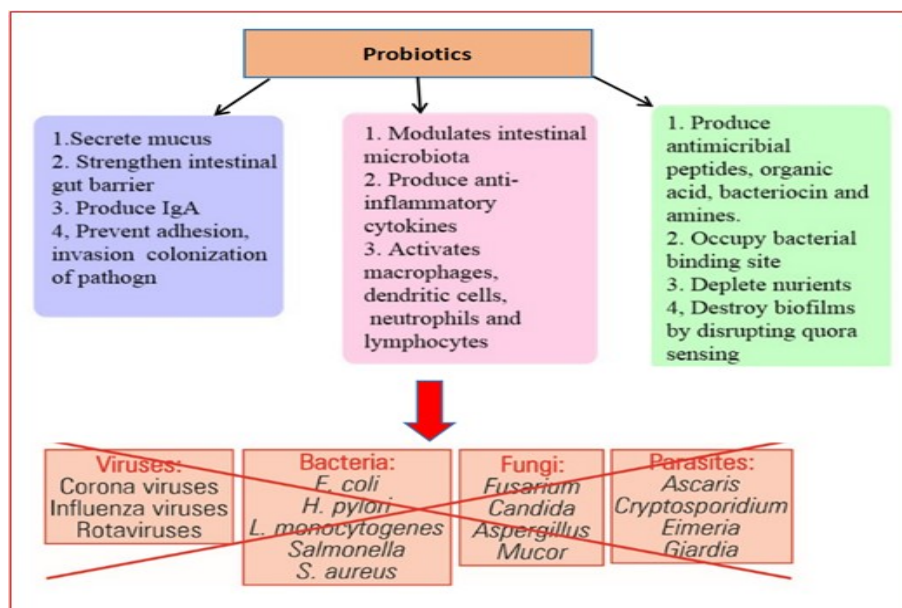


FIGURE 2.10: Summary of Probiotics Role in Treatment of Gastrointestinal Diseases by Effecting Growth of Pathogens

## 2.16 Probiotic *Bifidobacterium* Strains

*Bifidobacterium* genus is a major group found in intestines , mouth , vagina and gut of humans and many mammals. They are lactic acid bacteria involved in metabolism of carbohydrate and also have probiotic properties. Recent researchers have used them as treatment for many diseases because they have such properties [48] [49] .table 2.2 shows many strains of *Bifidobacterium* genus involved in probiotic activity.

TABLE 2.3: Strains of *Bifidobacterium* Genus Involved in Probiotic Activity

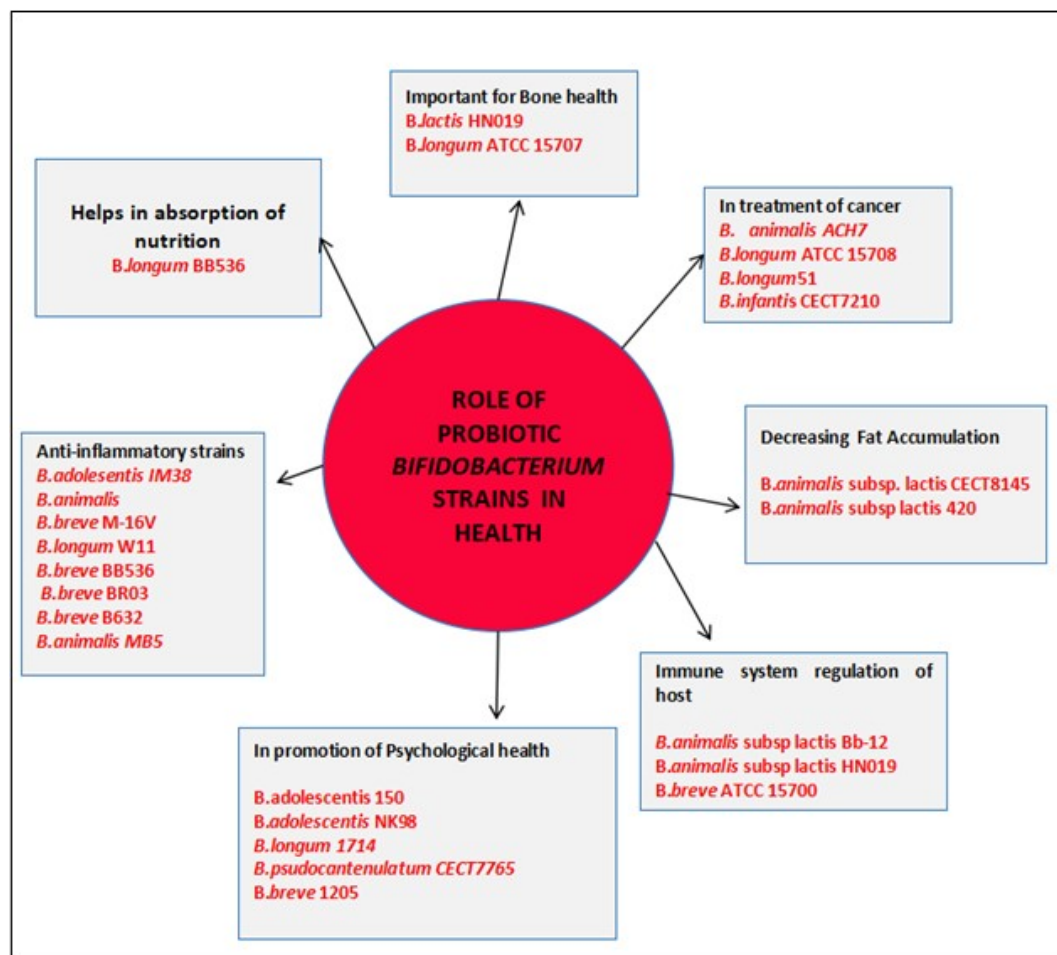
Strains	Strains
<i>Bifidobacterium adolescentis</i>	<i>Bifidobacterium bombi</i>
<i>Bifidobacterium animalis</i>	<i>Bifidobacterium canis</i>
<i>Bifidobacterium bifidum</i>	<i>Bifidobacterium castories</i>
<i>Bifidobacterium breve</i>	<i>Bifidobacterium gallicum</i>
<i>Bifidobacterium infantis</i>	<i>Bifidobacterium indicum</i>
<i>Bifidobacterium longum</i>	<i>Bifidobacterium longum</i>
<i>Bifidobacterium aerophilum</i>	<i>Bifidobacterium miconis</i>
<i>Bifidobacterium angulatum</i>	<i>Bifidobacterium myosotis</i>
<i>Bifidobacterium anseris</i>	<i>Bifidobacterium panos</i>
<i>Bifidobacterium apri</i>	<i>Bifidobacterium parmae</i>
<i>Bifidobacterium saguini</i>	<i>Bifidobacterium porcinum</i>
<i>Bifidobacterium samiri</i>	<i>Bifidobacterium ruteri</i>
<i>Bifidobacterium subtile</i>	<i>Bifidobacterium urinalis</i>

## 2.17 Role of *Bifidobacterium* Genus as Probiotics

It is being used in clinics and medical science in prevention and cure of many diseases. Due to its probiotic potential it is more helpful. [50] [51]

TABLE 2.4: Role of *Bifidobacterium* in Treatment of Diseases

Sr.	Role of <i>Bifidobacterium</i> in Treatment of diseases
1	They help in regulation of internal microbial population whenever body gets infection.
2	It represses carcinogenic activity inside the microbes of body
3	It can also help in formation of bioactive compounds from different vitamins.
4	Can also help in reduce pain in abdomen.
5	It decreases growth of gram-negative infectious organisms in children
6	It lowers the pH of gut so that infections can be decreased by lowering growth rate of pathogens.

FIGURE 2.11: Summarizes the Role of *Bifidobacterium* as Probiotics in Field of Health

## 2.18 Anti-obesity Effect of *Bifidobacterium* Strains

Researchers suggested that these bacterial species can reduce obesity which in turn helps in controlling metabolic disorders. When level of lipids became lower in the body they ultimately result in reduction of coronary artery diseases[20]. *Bifidobacterium* probiotic strains helps in stopping the synthesis of enzymes that are used in synthesis of cholesterol .When these enzymes are not produced they will lower its level[.They also help in the removal of feces with these extra compounds of cholesterol to control its level in body. They also get involve in recycling of bile salts present in body so that cholesterol levels may not get high. [52] [53]. They are helpful in lowering level of triglycerides. A hormone is being produced by the adipose tissues of our body to regulate the weight and energy of our body that is leptin. Its production rate is directly proportional to amount of fat produced in body . If it is lower it will not produce in large amount so it helps in management of obesity. [54]

## 2.19 Use of Bacteria as Probiotics and their Safety Concerns

As bacteria and microorganism can modify them during any harsh activity in body of host. Genomic stability is major concern while using bacteria as probiotic strain and they can be detected by Genomic analysis. If genomic analysis is done and result shows the stability in genes that's means the strain is non virulent and can be used against any disease. So before using bacterial strains genome stability must be considered.[55].

Their adaptations of genes in any environment results in their genome plasticity . As a probiotic strain they must not have any pathogenic gene that is harmful and genes of resistance against antibiotics must be present to be safely used against treatment of any disease. [48].

## 2.20 Competitive Against Pathogens

To be a good probiotic strain a bacteria must have ability to compete against pathogens . They must be able to overcome the situation where they meet pathogenicity. For this they must possess some genes that can produce anti-pathogenic compounds, antimicrobial substances that help them to be competitive against it. If they have ability to produce adhesive compounds they become more powerful and potentially ready to be used against diseases safely.[50]

## 2.21 Tolerance from Acids and Bile Salts

As probiotic are taken by mouth as an oral suspension so they must meet and survive harsh conditions of environment in gastrointestinal tract. Low pH of this tract, make the tract more acidic which is difficult environment for a bacteria to live in . Bile salts can also make their living impossible in gut tract . So for being used as potential probiotic strain they must possess genes that can tolerate high/low acid ranges, salt imbalance , oxidative stresses , heat and cold environment of the tracts. [56]

## 2.22 Metabolites Produced by *Bifidobacterium*

They produce metabolites that are very useful for increasing immune function and maintaining barrier of intestines. Acetic acid is being produced by many *Bifidobacterium* strains that are helpful in being used as probiotics. Production of metabolites is a major concern now if we select any bacterial strain to be used against any metabolic disease. Metabolites produced by various bacterial species help in regulation of metabolic pathways. Genes must be analysed to get possible data of metabolites to be produced by various species. They use metabolic pathway to synthesis a compound of aromatic lactic acid which help in anti-inflammatory action during their mode of action. Some strains also produce various vitamins which helps them in signaling and effect the host by effecting its physiological state.[20]

## **2.23 Co-Culturing of Bacterial Strains to be Used as Probiotics**

As different strains of bacteria behave differently in intestines and gut. Some produce one metabolite like acetic acid that perform a function and other produces the different one linoloic acid that has a different function. One can tolerate a condition but other can not. If we co-culture strains of our desired functions we can use them as probiotics against a certain disease.[57]

# Chapter 3

## Materials and Methods

Metabolic syndrome is a disease which is a combination of many risk factors causing it to be most common these days. Probiotics strains are being used in treatment of these factors that are causing metabolic syndrome. The property of probiotics potential of any strain made it possible for us to identify it and be used against any treatment. Tolerance against acid and lysozyme, resistance against antibiotics, production of antioxidants and activity against any microbe are some characterizations of being a probiotics strain.

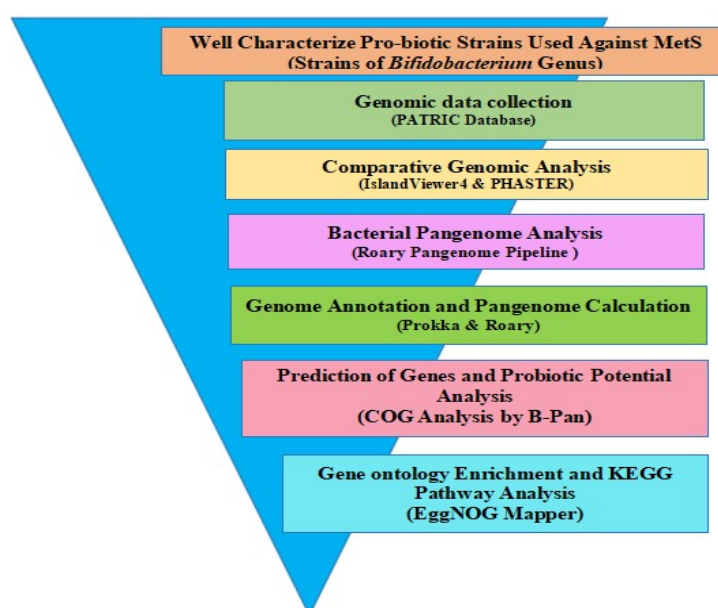


FIGURE 3.1: Summarizes the Methodological Steps Used for Functional Analysis of Probiotic Strains Against Metabolic Syndrome

In this project we have used insilco approach to use well characterize probiotics strain against metabolic syndrome. *Bifidobacterium* genus strains are being analysed associated with metabolic syndrome by using methodology presented in Fig 3.1.

## 3.1 Genomic Data Collection

First step of methodology was to collect genomic data for further analysis. This step comprises three sections, selection of strain based on literature review, retrieval of bacterial strain genome from any database and than by using human host genomic analysis would be done.

### 3.1.1 Selection of Strains

Selection of strains was done using the survey based on literature review. *Bifidobacterium* genus strains were selected as being mostly associated with metabolic syndrome.

### 3.1.2 Retrieval of Bacterial Sequences

Retrieved the 157 Genomes of well characterized probiotics strain of *Bifidobacterium* genus associated against metabolic syndrome. PATRIC database was used for collection of bacterial strains. The PATRIC(Pathosystems Resource Integration Centre ) database(<https://www.patricbrc.org/>) is a bacterial genomic database where you found variety of viral genomes of pathogens and bacteria and tools for comparative analysis. The human host were selected to filter out genomes.

## 3.2 Comparative Genomic Analysis

The comparative analysis of 11 pre-identified strains against human host was conducted by examining genomic islands and prophages using two databases. 1.

IslandViewer 4 (<http://www.pathogenomics.sfu.ca/islandviewer/>) which is used to visualize bacterial genome to know about virulence factors and their adaptations in detail . 2. PHASTER(PHAge Search Tool Enhanced Release)database (<https://phaster.ca/>) used for the annotation of the bacterial genes and sequences of plasmids.

### 3.3 Pangenome Analysis

To analyze the Pangenome of collected *Bifidobacterium* strains , we used the Roary Pangenome pipeline(<https://www.sanger.ac.uk/tool/roary/>). Roary is a tool designed for rapid , large scale comparative genomics which identifies orthologous genes across multiple genomes. The steps followed in Pangenome analysis were genome annotation and Pangenome calculation.

#### 3.3.1 Genome Annotation

All annotation of the genomes was done using a software called Prokka (<https://github.com/tseem/Prokka>) a rapid prokaryotic genome annotation tool that quickly annotates bacterial genes and produce standard output files as a result.

#### 3.3.2 Pangenome Calculation

The annotated genomes were processed through Roary to determine the presence and distribution of genes across strains.

### 3.4 Prediction of Genes and Probiotics Potential Analysis

Cluster of Orthologous Groups (COG) analysis was performed using B-pan, a bioinformatics tool designed for the Pangenome analysis of bacterial genomes. To

predict the function of genes the core genome identified in Pangenome analysis was subjected to COG analysis.

### **3.5 Gene Ontology (GO) Enrichment Analysis and Kyoto Encyclopedia of Genes and Genomes(KEGG )Pathway Analysis**

Next to characterize the functional potential of identified probiotics strains, we perform we will do Gene Ontology (GO) enrichment analysis and than after that KEGG pathway analysis by using by using a software known as EggNOG Mapper (<http://eggnog-mapper.embl.de/>) and KEGG ( <https://www.genome.jp/kegg/>).

# Chapter 4

## Results and Discussion

*Bifidobacterium* genus is a probiotics strain having potential to treat metabolic syndrome. It can act as anti-obese strain that effectively decrease lipid level which in turn is helpful for metabolic syndrome treatment. Although it is not directly involved in any infectious activity. So this thesis is designed to check well characterized strains that are being potentially able to be used as probiotic against metabolic syndrome treatment.

### 4.1 Genomic Data Collection

Retrieved the 165 Genomes of well-characterized probiotic strains of *Bifidobacterium* genus associated against metabolic syndrome were collected from the PATRIC database. The PATRIC (Pathosystems Resource Integration Center) database is a comprehensive resource for bacterial genome data, providing a variety of tools for comparative analysis. The human host were selected to filter out genomes.

TABLE 4.1: Genomes of Well-Characterized Probiotic Strains of *Bifidobacterium* Genus Associated Against Metabolic Syndrome Were Collected from the PATRIC Database

Genome ID	Genome Name
1168290.3	<i>Bifidobacterium animalis</i> subsp. lactis B420
1207542.9	<i>Bifidobacterium bifidum</i> LMG 13195 strain JCM 7004

TABLE 4.1: Genomes of Well-Characterized Probiotic Strains of *Bifidobacterium* Genus Associated Against Metabolic Syndrome Were Collected from the PATRIC Database

Genome ID	Genome Name
1254439.12	<i>Bifidobacterium thermophilum</i> RBL67
1322347.4	<i>Bifidobacterium longum</i> E18
1385938.3	<i>Bifidobacterium breve</i> 12L
1385939.3	<i>Bifidobacterium breve</i> STRAIN JCM 7017
1385940.3	B. Brevestrain JCM 7019
1385941.3	<i>Bifidobacterium breve</i> subsp. NCFB 2258
1385942.3	<i>Bifidobacterium breve</i> 689b
1447715.5	<i>Bifidobacterium pseudolongum</i> PV8-2
1447716.4	<i>Bifidobacterium kashiwanohense</i> PV20-2
1454229.19	<i>Bifidobacterium faecale</i> JCM 19861
1679.1	<i>Bifidobacterium longum</i> (CCUG30698)subsp. longum strain
1679.241	<i>Bifidobacterium longum</i> YS108R subsp. longum strain
1679.26	<i>Bifidobacterium longum</i> subsp. (AH1206) longum strain
1679.263	<i>Bifidobacterium longum</i> ( 5914)subsp. longum strain KCTC
1679.264	<i>Bifidobacterium longum</i> strain JCM 7050subsp. longum
1679.265	<i>Bifidobacterium longum</i> subsp. longum strain JCM 11341
1679.267	<i>Bifidobacterium longum</i> JCM 11343subsp. longum strain
1679.27	<i>Bifidobacterium longum</i> subsp. longum strain JCM 7052
1679.271	<i>Bifidobacterium longum</i> 11340( subsp. longum strain JCM )
1679.313	<i>Bifidobacterium longum</i> subsp. longum strain NBRC 114494
1679.326	<i>Bifidobacterium longum</i> subsp. longum BB-46
1679.4	<i>Bifidobacterium longum</i> subsp. longum BG-L47
1679.448	<i>Bifidobacterium longum</i> subsp. longum DS1566
1679.51	<i>Bifidobacterium longum</i> BORIs subsp. longum strain
1679.9	<i>Bifidobacterium longum</i> NCIMB8809subsp. longum strain
1680.104	<i>Bifidobacterium adolescentis</i> strain 1-11
1680.142	<i>Bifidobacterium adolescentis</i> strain 6
1680.1909	<i>Bifidobacterium adolescentis</i> strain ZJ2
1680.2061	<i>Bifidobacterium adolescentis</i> strain PRL2019

TABLE 4.1: Genomes of Well-Characterized Probiotic Strains of *Bifidobacterium* Genus Associated Against Metabolic Syndrome Were Collected from the PATRIC Database

Genome ID	Genome Name
1680.2161	<i>Bifidobacterium adolescentis</i> NB2B-16-TSAB
1680.2363	<i>Bifidobacterium adolescentis</i> LMG 10502
1680.2411	<i>Bifidobacterium adolescentis</i> iVS-1
1680.4	<i>Bifidobacterium adolescentis</i> strain 22L
1680.5	<i>Bifidobacterium adolescentis</i> strain BBMN23
1681.114	<i>Bifidobacterium bifidum</i> strain S6
1681.1291	<i>Bifidobacterium bifidum</i> VSI20
1681.137	<i>Bifidobacterium bifidum</i> strain TMC 3115
1681.42	<i>Bifidobacterium bifidum</i> strain BF3
1681.94	<i>Bifidobacterium bifidum</i> strain PRI 1
1681.973	<i>Bifidobacterium bifidum</i> strain HN002
1681.979	<i>Bifidobacterium bifidum</i> strain CNCM I-4319
1682.16	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> strain KCTC 5934
1682.161	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> strain
1682.162	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> strain JCM 11347
1682.17	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> strain BINF
1682.195	<i>Bifidobacterium longum</i> JRPTsubsp. <i>infantis</i>
1682.213	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_003
1682.214	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_002
1682.215	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> BB-02
1682.216	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_004
1682.217	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_010
1682.218	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_009
1682.219	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_008
1682.22	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_007
1682.221	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_006
1682.222	<i>Bifidobacterium longum</i> subsp. NLS <i>infantis</i>
1682.223	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> PI_005
1682.24	<i>Bifidobacterium longum</i> 9 <i>infantis</i> strain )BT1subsp.

TABLE 4.1: Genomes of Well-Characterized Probiotic Strains of *Bifidobacterium* Genus Associated Against Metabolic Syndrome Were Collected from the PATRIC Database

Genome ID	Genome Name
1682.258	<i>Bifidobacterium longum</i> subsp. infantis BB-02
1682.48	<i>Bifidobacterium longum</i> subsp. infantis strain NCTC11817
1685.1	<i>Bifidobacterium breve</i> strain BR3
1685.1062	<i>Bifidobacterium breve</i> VSI11
1685.1344	<i>Bifidobacterium breve</i> BS2-PB3
1685.1494	<i>Bifidobacterium breve</i> TCI761
1685.191	<i>Bifidobacterium breve</i> (FDAARGOS_561 strain)
1685.199	<i>Bifidobacterium breve</i> strain lw01
1685.2	<i>Bifidobacterium breve</i> strain NCTC11815
1685.52	<i>Bifidobacterium breve</i> strain IDCC4401
1685.526	<i>Bifidobacterium breve</i> strain JR01
1685.568	<i>Bifidobacterium breve</i> strain JTL
1685.73	<i>Bifidobacterium breve</i> strain DRBB26
1685.74	<i>Bifidobacterium breve</i> strain NRBB01
1685.75	<i>Bifidobacterium breve</i> strain NRBB02
1685.76	<i>Bifidobacterium breve</i> strain NRBB04
1685.77	<i>Bifidobacterium breve</i> strain NRBB11
1685.774	<i>Bifidobacterium breve</i> BIF195
1685.775	<i>Bifidobacterium breve</i> 1101A
1685.78	<i>Bifidobacterium breve</i> strain NRBB09
1685.79	<i>Bifidobacterium breve</i> strain NRBB57
1685.8	<i>Bifidobacterium breve</i> strain NRBB50
1685.81	<i>Bifidobacterium breve</i> strain NRBB52
1685.82	<i>Bifidobacterium breve</i> strain NRBB51
1685.83	<i>Bifidobacterium breve</i> strain NRBB56
1685.84	<i>Bifidobacterium breve</i> strain DRBB27
1685.85	<i>Bifidobacterium breve</i> strain 017W439
1685.86	<i>Bifidobacterium breve</i> strain 215W447a
1685.87	<i>Bifidobacterium breve</i> strain DRBB28

TABLE 4.1: Genomes of Well-Characterized Probiotic Strains of *Bifidobacterium* Genus Associated Against Metabolic Syndrome Were Collected from the PATRIC Database

Genome ID	Genome Name
1685.88	<i>Bifidobacterium breve</i> strain 180W83
1685.89	<i>Bifidobacterium breve</i> strain 082W48
1685.9	<i>Bifidobacterium breve</i> strain 139W423
1685.91	<i>Bifidobacterium breve</i> strain CNCM I-4321
1685.92	<i>Bifidobacterium breve</i> strain NRBB18 strain infant
1685.93	<i>Bifidobacterium breve</i> strain NRBB19 strain infant
1685.94	<i>Bifidobacterium breve</i> strain NRBB20 strain infant
1685.95	<i>Bifidobacterium breve</i> strain NRBB27 strain infant
1685.96	<i>Bifidobacterium breve</i> strain NRBB49 strain infant
1685.97	<i>Bifidobacterium breve</i> strain DRBB29 strain infant
1685.98	<i>Bifidobacterium breve</i> strain DRBB30 strain infant
1685.99	<i>Bifidobacterium breve</i> strain NRBB08 strain infant
1686.285	<i>Bifidobacterium catenulatum</i> IMAU FB087
1689.18	<i>Bifidobacterium dentium</i> strain NCTC11816
206672.9	<i>Bifidobacterium longum</i> NCC2705
216816.111	<i>Bifidobacterium longum</i> strain BXY01
216816.114	<i>Bifidobacterium longum</i> strain BG7
216816.126	<i>Bifidobacterium longum</i> strain 35624
216816.1974	<i>Bifidobacterium longum</i> strain 51A
216816.2033	<i>Bifidobacterium longum</i> strain BAMA-B05
216816.2087	<i>Bifidobacterium longum</i> strain LTBL16
216816.211	<i>Bifidobacterium longum</i> strain Jih1
216816.2315	<i>Bifidobacterium longum</i> strain LC67
216816.2362	<i>Bifidobacterium longum</i> strain HN001
216816.2457	<i>Bifidobacterium longum</i> strain NBRC 114370
216816.2506	<i>Bifidobacterium longum</i> T1
216816.2621	<i>Bifidobacterium longum</i> W13
216816.273	<i>Bifidobacterium longum</i> LBUX23
216816.2874	<i>Bifidobacterium longum</i> XZM1

TABLE 4.1: Genomes of Well-Characterized Probiotic Strains of *Bifidobacterium* Genus Associated Against Metabolic Syndrome Were Collected from the PATRIC Database

Genome ID	Genome Name
216816.2973	<i>Bifidobacterium longum</i> YGMCC0008
216816.3015	<i>Bifidobacterium longum</i> YGMCC0410
216816.3139	<i>Bifidobacterium longum</i> YGMCC0297
216816.314	<i>Bifidobacterium longum</i> YGMCC0230
216816.3141	<i>Bifidobacterium longum</i> YGMCC0228
216816.3142	<i>Bifidobacterium longum</i> YGMCC0209
28025.1	<i>Bifidobacterium animalis</i> strain A6
28025.127	strain 01 <i>Bifidobacterium animalis</i>
28025.133	strain B06 <i>Bifidobacterium animalis</i>
28025.19	<i>Bifidobacterium animalis</i> strain BL3
28025.247	HY8002 <i>Bifidobacterium animalis</i>
28025.374	<i>Bifidobacterium animalis</i> B01
28026.1094	<i>Bifidobacterium pseudocatenulatum</i> strain YIT12820
28026.1095	<i>Bifidobacterium pseudocatenulatum</i> strain YIT11956
28026.1096	<i>Bifidobacterium pseudocatenulatum</i> strain YIT11952
28026.1097	<i>Bifidobacterium pseudocatenulatum</i> strain YIT11953
28026.1098	<i>Bifidobacterium pseudocatenulatum</i> strain YIT11027
28026.1206	<i>Bifidobacterium pseudocatenulatum</i> JCLA3
28026.1633	<i>Bifidobacterium pseudocatenulatum</i> Bi-OTA128
302911.167	<i>Bifidobacterium animalis</i> strain H1 subsp. lactis
302911.168	<i>Bifidobacterium animalis</i> subsp.strain H3( lactis )
302911.225	<i>Bifidobacterium animalis</i> BLa80(subsp. Lactis)
302911.25	<i>Bifidobacterium animalis</i> subspecie lactis BF052
302911.269	<i>Bifidobacterium animalis</i> subsp. lactis BP026
302911.294	<i>Bifidobacterium animalis</i> subsp. lactis BAMA-B06/BAu-B0111
302911.306	<i>Bifidobacterium animalis</i> lactis MH-02 subsp.
302911.337	<i>Bifidobacterium animalis</i> subsp. lactis LPL-RH
302911.433	<i>Bifidobacterium animalis</i> subsp. lactis GOLDGUT-BB18
302911.44	<i>Bifidobacterium animalis</i> subsp. lactis TCI604

TABLE 4.1: Genomes of Well-Characterized Probiotic Strains of *Bifidobacterium* Genus Associated Against Metabolic Syndrome Were Collected from the PATRIC Database

Genome ID	Genome Name
302911.449	<i>Bifidobacterium animalis</i> lactis BI040subsp.
302911.62	<i>Bifidobacterium animalis</i> lactis strain S7subsp.
302911.63	<i>Bifidobacterium animalis</i> subsp. IDCC4301 lactis strain
302912.1	<i>Bifidobacterium animalis</i>
326426.4	<i>Bifidobacterium breve</i> UCC2003
391904.5	<i>Bifidobacterium longum</i> infantis subsp.
398513.13	<i>Bifidobacterium bifidum</i> NCIMB 41171
484020.3	<i>Bifidobacterium bifidum</i> BGN4
565040.3	<i>Bifidobacterium longum</i> subsp. infantis 157F strain 157F-NC
565042.3	<i>Bifidobacterium longum</i> subsp. longum JCM 1217
573236.3	<i>Bifidobacterium animalis</i> subsp. lactis V9
630129.12	<i>Bifidobacterium catenulatum</i> ( subspecie)
702459.3	<i>Bifidobacterium bifidum</i> PRL2010
722911.3	<i>Bifidobacterium longum</i> subsp. longum F8
742729.3	<i>Bifidobacterium animalis</i> . lactis Bi-07(subsp)
866777.3	<i>Bifidobacterium breve</i> ACS-071-V-Sch8b
890402.3	<i>Bifidobacterium longum</i> - longum BBMN68(subsp)
936351.3	<i>Bifidobacterium breve</i> S27

## 4.2 Selection of Strains

For this all strains are available with their complete sequences along with source of isolation. 11 pre-identified probiotic strains against the human host were chosen for the further comparative analysis. Table 4.1 shows the selected *Bifidobacterium* genus strains used in analysis. These 11 strains are selected for comparative analysis after rigorous research because of their less pathogenicity and toxicity. Because they are most suitable to be used as probiotics against metabolic syndrome they

would be further used to check their genes and functions that may help in combating it.

TABLE 4.2: *Bifidobacterium* Strains Used for Comparative Analysis

Genome Name
<i>Bifidobacterium animalis</i> subsp. lactis strain H3
<i>Bifidobacterium animalis</i> subsp. lactis strain H1
<i>Bifidobacterium breve</i> strain JTL
<i>Bifidobacterium animalis</i> strain 01
<i>Bifidobacterium longum</i> strain 51A
<i>Bifidobacterium breve</i> strain lw01
<i>Bifidobacterium thermophilum</i> RBL67
<i>Bifidobacterium bifidum</i> BGN4
<i>Bifidobacterium animalis</i> subsp. lactis V9
<i>Bifidobacterium bifidum</i> PRL2010
<i>Bifidobacterium animalis</i> subsp. lactis BF052

### 4.3 Comparative Genomic Analysis

The comparative analysis of 11 pre-identified probiotic strains against the human host was conducted by examining genomic islands and prophages using IslandViewer 4 and PHASTER (PHAge Search Tool Enhanced Release). These results highlight the existence of many genomic islands in each strain, which suggests a significant level of gene transfer horizontally contributing to the genetic diversity of these probiotics.

Additionally, the presence of prophages in certain strains (e.g., *Bifidobacterium longum* strain 51A, *Bifidobacterium breve* strain lw01, *Bifidobacterium bifidum* BGN4, and *Bifidobacterium bifidum* PRL2010) in Table 4.1 indicates that bacteriophages may play a role in shaping the genomes of these probiotic strains, potentially impacting their functionality and interactions with the host. Genomic islands and phages are being listed in the table to show that how these strains interact with the host organism and their capability to be used in the body as a probiotic.

TABLE 4.3: Prophages in Certain Strains Shaping the Genomes of these Probiotic Strains, Potentially Impacting their Functionality and Interactions With the Host

Genome Name	Genome Island	Phages
<i>Bifidobacterium animalis</i> subspecie strainlactis H3	19	0
<i>Bifidobacterium animalis</i> subspecie 0strain lactis H1	21	0
<i>Bifidobacterium breve</i> strain JTL	21	0
<i>Bifidobacterium animalis</i> strain 01	5	0
<i>Bifidobacterium longum</i> strain 51A	27	1
<i>Bifidobacterium breve</i> strain lw01	19	1
<i>Bifidobacterium thermophilum</i> RBL67	11	0
<i>Bifidobacterium bifidum</i> BGN4	10	1
<i>Bifidobacterium animalis</i> subspecie strain of lactis V9	5	0
<i>Bifidobacterium bifidum</i> PRL2010	9	1
<i>Bifidobacterium animalis</i> subspecie lactis strain BF052	5	0

## 4.4 Pangenome Analysis

As technologies are progressing the analysis has also been standardized to use Pan genome analysis to get the entire picture of all the bacterial genome that is being reported. The Pangenome analysis is done to get all the possible genes present in the strains being selected to check their functionality.

All the strains are compared and different gene types are identified. Conserved genes identified in strains of *Bifidobacterium* that were analyzed. These genes are present in all organisms from the last common ancestor they have. These conserved genes contain all the RNAs and protein required for transcription and translation to perform their functions. Fig 4.2 indicates all the total and conserved genes of *Bifidobacterium* strains.

When subjected to Pangenome analysis mostly conserved genes are found in the per-identified bacterial strains which shows their relationship with their ancestors and also indicates that many functions are in common between all these species and they can be used as probiotics easily.

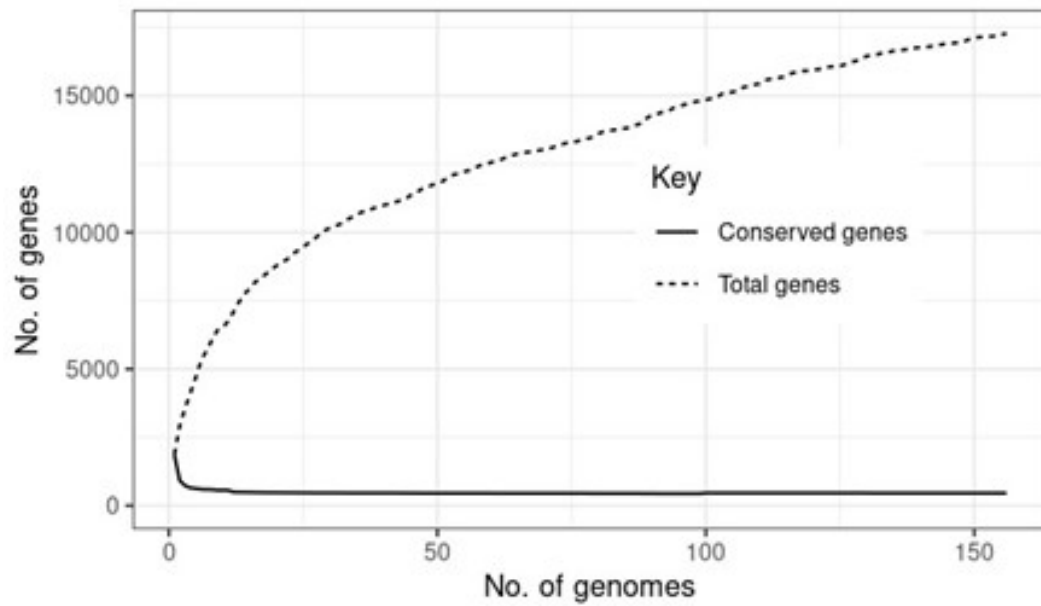


FIGURE 4.1: Total Conserved Genes in *Bifidobacterium* Probiotic Strains that are Subjected to Pangenome Analysis

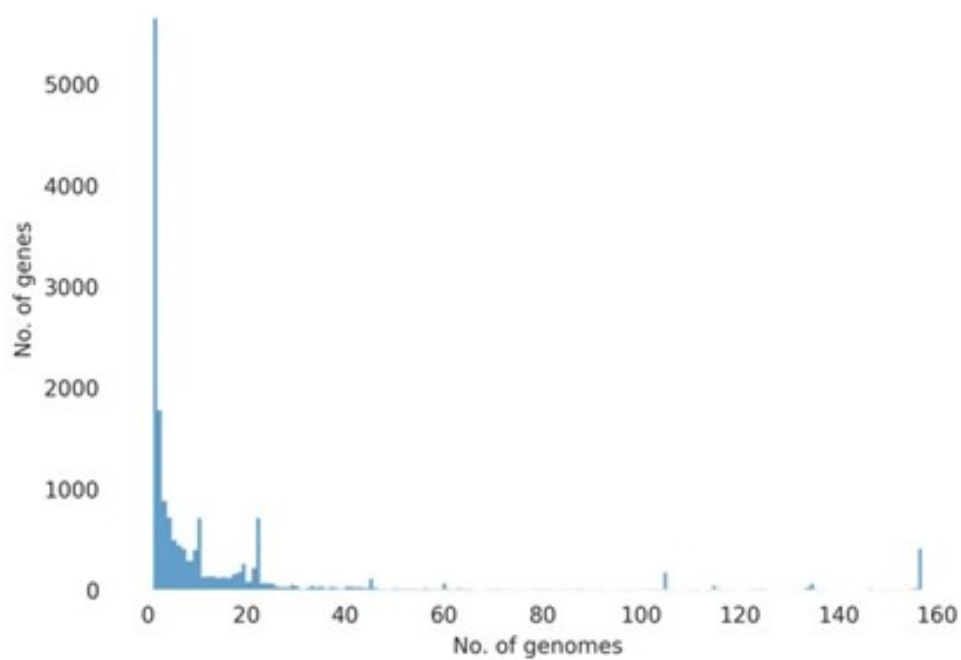


FIGURE 4.2: Frequency of Genome of *Bifidobacterium* Probiotic Strains being Analysed in Pangenome Analysis

Fig 4.3 showing frequency of genome of total bacterial population/strains that are being analyzed for Pan Genome. In Pan genome analysis genes present in all the bacterial strains are referred to as core genes, those which are present in only one

two or more strains are shell genes and those present only in single specie / strain are cloud genes.

**Core genes :** Those genes that are found in every individuals

**Genes(Soft core):** Genes which are found in some of the bacterial strains

**Genes(Shell) ;** Genes found in one or more than one two bacterial strains

**Genes( Cloud ):** Genes found in only one of the strains The pangenome analysis of the collected *Bifidobacterium* strains yielded the following results:

The pangenome analysis of the collected *Bifidobacterium* strains yielded the following results:

TABLE 4.4: Results from Roary for Pangenome Analysis

1	Core genes	(more than 99% and less than 100%):	444 genes
2	Soft core genes	(more or equal to 95% or less or equal to 99%	26 genes
3	Shell genes		2,238 genes
4	Cloud genes	(0% <= strains <= 15%):	14,413 genes
5	Total genes	(0% <= strains <= 100%):	17,121 genes

Table 4.2 shows Pan genome results .These results indicate a large and open pangenome, with a significant number of genes (14,413) classified as cloud genes, suggesting high genetic variability among the *Bifidobacterium* strains. Fig 4.3The presence of 444 core genes highlights a conserved set of genes essential for the basic functions of these probiotic strains.

Conserved genes are present in bacterial strains that common from the ancestors and that are 444 total in number. These will be subjected to COG analysis to check out all possible functions they perform. The functions of these genes would be helpful in predicting whether they can be used as probiotics or not. These core genes are really helpful in prediction of functional genes in them.

All the genes that are performing any function in metabolic pathways would be helpful for regarding them as potential for used as probiotics.

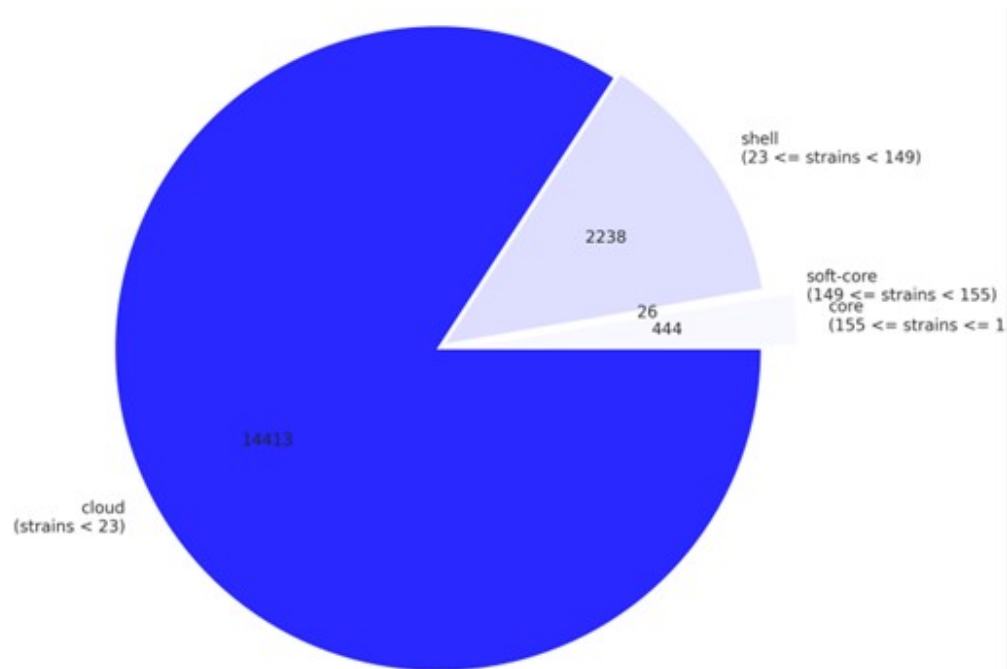


FIGURE 4.3: Information about Unique Core , Shell and Cloud Genes from Roary

## 4.5 Prediction of Genes

Cluster of Orthologous Groups (COG) analysis was performed using B-pan, a bioinformatics tool designed for the pangenome analysis of bacterial genomes. The core genome, identified in the previous pangenome analysis, was subjected to COG analysis to predict the functions of these genes.

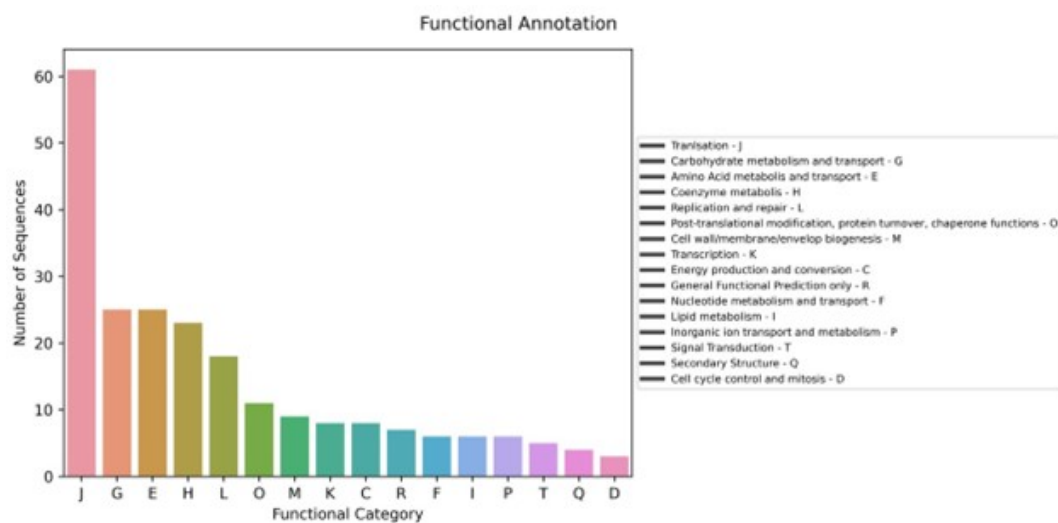


FIGURE 4.4: Clusters of Orthologous Groups(COG) and Distribution of Core Genes in *Bifidobacterium* Strains

The distribution pattern of COG shows that these core genes are involved in translation, ‘Carbohydrate Metabolism’, ‘Coenzyme Metabolite’, ‘Cell Envelop Biogenesis’, ‘Cell Cycle Mitosis’, ‘Energy Production’, ‘Lipid Metabolism’. In fig 4.5 the functional annotation is shown with all the percentages that which category is present in less amount an which id present more.

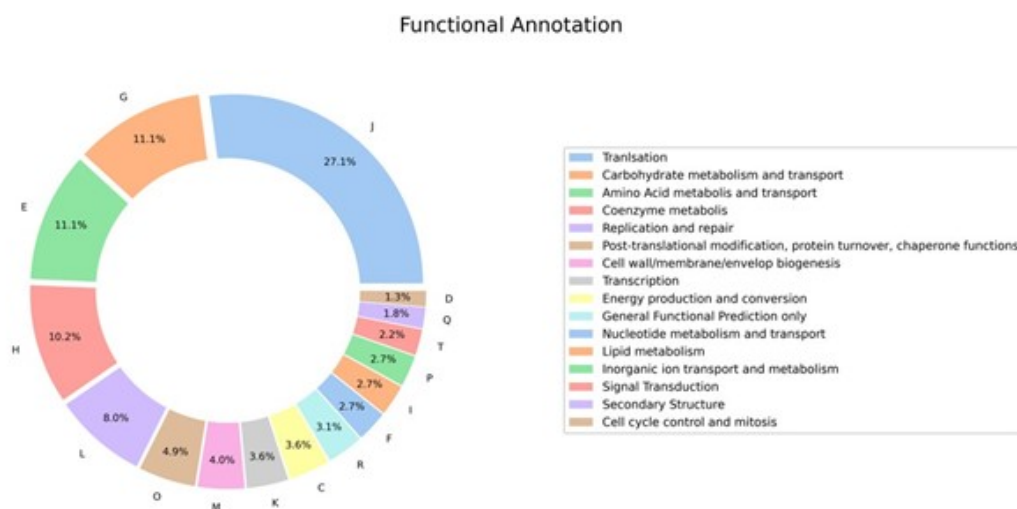


FIGURE 4.5: Distribution of Core Genes , Shell Genes , Cloud Genes Involve in Different Processes in *Bifidobacterium* Strains

The head known as J ( biogenesis of compounds, translation of proteins, synthesis of ribosome ) constituted the majority, with 64.81% of the core genes. This indicates a significant role in protein synthesis and maintenance, which is crucial for the probiotic function of *Bifidobacterium* strains. Percentages with respect to category given in Table 4.2

TABLE 4.5: Functional Categories of Gene with their Percentages

Sr.	Functional Category	Number of Sequences	Percentage
1	J	35	64.81481481
2	E	3	5.555555556
3	K	2	3.703703704
4	G	2	3.703703704
5	EF	2	3.703703704
6	F	2	3.703703704
7	I	1	1.851851852
8	C	1	1.851851852
9	EH	1	1.851851852
10	H	1	1.851851852
11	TE	1	1.851851852
12	O	1	1.851851852
13	R	1	1.851851852
14	L	1	1.851851852

The category J constituted the majority, with 64.81% of the core genes. This indicates a significant role in protein synthesis and maintenance, which is crucial for the probiotic function of *Bifidobacterium* strains. Prediction of J category of core genes suggested that they are mostly involved in protein synthesis, ribosomal synthesis and metabolic pathways related to lipids etc. These genes can be potentially used against MetS to decrease lipid content of body which in result can be helpful in lowering level of cholesterol, obesity, lower risk of cardiovascular disorders.

## 4.6 Gene Ontology (GO) Enrichment Analysis and KEGG Pathway Analysis

To further characterize the functional potential of the identified probiotic strains, we performed Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis by using EggNOG Mapper and KEGG (Kyoto Encyclopedia of Genes and Genomes).

TABLE 4.6: Gene Ontology Showing Genes and Cogs Description Of Each Gene

	LOCUS Tag	Gene/Protein ID	COGs Description
0	KDGFCFMN_01249	WP_012577167.1	isoprenyl transferase [ <i>Bifidobacterium longum</i> ]
			30S ribosomal protein S12
1	KDGFCFMN_00614	YP_003985582.1	[ <i>Gardnerella vaginalis</i> ATCC 14019]
2	KDGFCFMN_00615	WP_012578203.1	30S ribosomal protein S7 [ <i>Bifidobacterium longum</i> ]
			elongation factor for translation Tu
3	KDGFCFMN_00617	YP_003985585.1	[ <i>Gardnerella vaginalis</i> ATCC 14019]

TABLE 4.6: Gene Ontology Showing Genes and Cogs Description Of Each Gene

LOCUS Tag	Gene/Protein ID	COGs Description
		MULTISPECIES:
4	KDGFCFMN_00378	WP_012578474_1
		30S ribosomal protein S10 [ <i>Bifidobacterium</i> ]
5	KDGFCFMN_00378	YP_003986389_1
		ribosomal protein30S, S10 [Gardnerella vaginalis ATCC 14019]
6	KDGFCFMN_01433	WP_012576744_1
		F0F1 ATP synthase subunit beta [ <i>Bifidobacterium longum</i> ]
7	KDGFCFMN_00143	WP_012576586_1
		ketol-acid reductoisomerase [ <i>Bifidobacterium longum</i> ]
8	KDGFCFMN_01347	YP_003986314_1
		DNA-directed RNA polymerase subunit beta [Gardnerella vaginalis ATCC 14019]
9	KDGFCFMN_00379	YP_003986388_1
		50S ribosomal protein L3 [Gardnerella vaginalis ATCC 14019]
10	KDGFCFMN_00382	YP_003986385_1
		ribosomal protein 50S,L2 [Gardnerella vaginalis ATCC 14019]
		MULTISPECIES:
11	KDGFCFMN_00384	WP_007053035_1
		50S ribosomal protein L22 [ <i>Bifidobacterium</i> ]
12	KDGFCFMN_00385	WP_012578471_1
		ribosomal protein 30s S3 [ <i>Bifidobacterium longum</i> ]
13	KDGFCFMN_00389	YP_003986378_1
		ribosomal protein 50s, L14 [Gardnerella vaginalis ATCC 14019]

TABLE 4.6: Gene Ontology Showing Genes and Cogs Description Of Each Gene

	<b>LOCUS Tag</b>	<b>Gene/Protein ID</b>	<b>COGs Description</b>
			MULTISPECIES: 30S
14	KDGFCFMN_00393	WP_003829896.1	ribosomal protein S8 [ <i>Bifidobacterium</i> ]
			ribosomal protein 30S,S13
15	KDGFCFMN_00403	YP_003986365.1	[ <i>Gardnerella</i> vaginalis ATCC 14019]
			MULTISPECIES: 30S
16	KDGFCFMN_00404	WP_003829907.1	ribosomal protein S11 [ <i>Bifidobacterium</i> ]
17	KDGFCFMN_00845	WP_012577436.1	phosphoglycerate kinase [ <i>Bifidobacterium longum</i> ]
18	KDGFCFMN_01012	WP_006292965.1	phosphopyruvate hydratase [ <i>Scardovia inopinata</i> ]
			ammonia ligase-
19	KDGFCFMN_01221	WP_012577205.1	type I glutamate [ <i>Bifidobacterium longum</i> ]
			ammonia ligase-type
20	KDGFCFMN_00695	WP_012578179.1	I glutamate- [ <i>Bifidobacterium longum</i> ]
21	KDGFCFMN_00068	WP_012576516.1	tryptophan-tRNA ligase [ <i>Bifidobacterium longum</i> ]
			ribosomal protein 30S S19
22	KDGFCFMN_00383	YP_003986384.1	[ <i>Gardnerella</i> vaginalis ATCC 14019]
			ribosomal proteiS 50s L16
23	KDGFCFMN_00386	WP_012578470.1	[ <i>Bifidobacterium longum</i> ]
			MULTISPECIES: ribosomal
24	KDGFCFMN_00390	WP_007053803.1	protein 50S, L24 [ <i>Bifidobacterium</i> ]

TABLE 4.6: Gene Ontology Showing Genes and Cogs Description Of Each Gene

	<b>LOCUS Tag</b>	<b>Gene/Protein ID</b>	<b>COGs Description</b>
			MULTISPECIES: ribosomal
25	KDGFCFMN_00392	WP_003814530_1	protein 30S,S14 type Z [ <i>Bifidobacterium</i> ] DNA-directed
26	KDGFCFMN_00405	WP_007053048_1	RNA polymerase subunit alpha [ <i>Bifidobacterium longum</i> ]
27	KDGFCFMN_00406	WP_012578465_1	50S ribosomal protein L17 [ <i>Bifidobacterium longum</i> ]
			MULTISPECIES:
28	KDGFCFMN_00300	WP_007053061_1	50S ribosomal protein 50S, L27 [ <i>Bifidobacterium</i> ]
			MULTISPECIES:
29	KDGFCFMN_01401	WP_007053677_1	pyridoxal 5' -phosphate synthase lyase subunit PdxS [ <i>Bifidobacterium</i> ]
30	KDGFCFMN_01350	WP_012578321_1	tRNA (cytidine(34)-2'-O)- methyltransferase [ <i>Bifidobacterium longum</i> ]
31	KDGFCFMN_00347	WP_012578498_1	ribosomal protein 50S L7/L12 [ <i>Bifidobacterium longum</i> ] P called elongation factor
32	KDGFCFMN_00620	YP_003985860_1	[ <i>Gardnerella</i> vaginalis ATCC 14019] 30S ribosomal protein
33	KDGFCFMN_01497	YP_003986262_1	30S, S18 [ <i>Gardnerella</i> vaginalis ATCC 14019]

TABLE 4.6: Gene Ontology Showing Genes and Cogs Description Of Each Gene

	LOCUS Tag	Gene/Protein ID	COGs Description
34	KDGFCFMN_00402	WP_003808136_1	MULTISPECIES: ribosomal protein OF 50SL36 [ <i>Bifidobacterium</i> ]
35	KDGFCFMN_00402	WP_003842636_1	MULTISPECIES: ribosomal protein OF 50S L36 [Terrabacteria group]
36	KDGFCFMN_00360	YP_003986403_1	ribosomal protein 50S, L33 [Gardnerella vaginalis ATCC 14019]
37	KDGFCFMN_00360	WP_006292704_1	ribosomal protein 50S, L33 [Scardovia inopinata]
38	KDGFCFMN_01160	WP_032682519_1	MULTISPECIES: IF-3 translation initiation factor [ <i>Bifidobacterium</i> ]
39	KDGFCFMN_01158	WP_011068013_1	50S ribosomal protein [ <i>Bifidobacterium</i> longum]L20
40	KDGFCFMN_00291	WP_007051394_1	MULTISPECIES: ribosomal protein of 50S L19 [ <i>Bifidobacterium</i> ]
41	KDGFCFMN_00187	WP_007051565_1	MULTISPECIES: family nitrogen regulator P-II [ <i>Bifidobacterium</i> ]

TABLE 4.6: Gene Ontology Showing Genes and Cogs Description Of Each Gene

LOCUS Tag	Gene/Protein ID	COGs Description
		MULTISPECIES:
42	KDGFCFMN_00401	WP_003808114.1 translation initiation factor IF-1 [ <i>Bifidobacterium</i> ]
43	KDGFCFMN_00272	WP_012576821.1 acetolactate synthase small subunit [ <i>Bifidobacterium longum</i> ]
44	KDGFCFMN_01554	WP_012576591.1 molecular chaperone DnaK [ <i>Bifidobacterium longum</i> ]
45	KDGFCFMN_00623	WP_012577127.1 large subunit carbamoyl-phosphate synthase [ <i>Bifidobacterium longum</i> ]
46	KDGFCFMN_00623	WP_012577127.1 large subunit carbamoyl- phosphate synthase [ <i>Bifidobacterium longum</i> ]
47	KDGFCFMN_00736	WP_077323637.1 ribose-phosphate diphosphokinase [ <i>Bifidobacterium longum</i> ]
48	KDGFCFMN_00616	YP_003985584.1 G -elongation factor [Gardnerella vaginalis ATCC 14019]
49	KDGFCFMN_00889	WP_012577806.1 elongation factor 4 [ <i>Bifidobacterium longum</i> ]
50	KDGFCFMN_00855	WP_012577420.1 ATP-binding cassette domain-containing protein—ABC-F family [ <i>Bifidobacterium longum</i> ]

TABLE 4.6: Gene Ontology Showing Genes and Cogs Description Of Each Gene

	<b>LOCUS Tag</b>	<b>Gene/Protein ID</b>	<b>COGs Description</b>
51	KDGFCFMN_00916	WP_014484975_1	CTP synthase [ <i>Bifidobacterium longum</i> ] MULTISPECIES:
52	KDGFCFMN_00939	WP_007052130_1	[ <i>Bifidobacterium</i> ] contain 30S ribosomal protein S4 excinuclease
53	KDGFCFMN_00981	WP_012578046_1	ABC subunit UvrB [ <i>Bifidobacterium longum</i> ]

Gene I'd and the COG description of all the core genes that has been identified and subjected to COG mapping.

Different genes are involved in synthesis of different type of proteins used to synthesize enzymes or ribosomes. 30S or 50S ribosomal proteins are being coded by theses genes. Subunits of enzymes that are involved in translation and transcription are being coded by these genes.

Initiation factor that are involved in formation of different proteins are being synthesized by these orthologous genes. Regulation factor that are involved in various metabolic reactions are being present. Genes that are involved in receptor binding and interaction are being listed in it that are helpful for a bacteria to act as probiotic.

All these COG description indicates that these genes are involved in mostly the ribosomal or protein synthesis . These genes are involved in regulation of metabolism and their pathways that is clearly predicting the probiotic potential of these bacterial strains. These COG genes in next step would go for their KEGG pathway analysis that would tell us about metabolic pathways and procedures in which they are involved so we can predict how they will helpful in combating diseases like metabolic syndrome.

TABLE 4.7: Description of COG Class their IDs

Gene/Protein ID	Symbol	COGs Class Description	COGs ID
WP_012577167.1	I	Lipid metabolism	COG0020
YP_003985582.1	J	Translation	COG0048
WP_012578203.1	J	Translation	COG0049
YP_003985585.1	J	Translation	COG0050
WP_012578474.1	J	Translation	COG0051
YP_003986389.1	J	Translation	COG0052
WP_012576744.1	C	Energy production and conversion	COG0055
WP_012576586.1	EH	Amino acid transport and metabolism	COG0059
YP_003986314.1	K	Tanscription	COG0085
YP_003986388.1	J	Translation	COG0087
YP_003986385.1	J	Translation	COG0090
WP_007053035.1	J	Translation	COG0091
WP_012578471.1	J	Translation	COG0092
YP_003986378.1	J	Translation	COG0093
WP_003829896.1	J	Translation	COG0096
YP_003986365.1	J	Translation	COG0099
WP_003829907.1	J	Translation	COG0100
WP_012577436.1	G	Carbohydrate transport and metabolism	COG0126
WP_006292965.1	G	Carbohydrate transport and metabolism	COG0148
WP_012577205.1	E	Amino acid transport and metabolism	COG0174
WP_012578179.1	E	Amino acid transport and metabolism	COG0174
WP_012576516.1	J	Translation	COG0180
YP_003986384.1	J	Translation	COG0185
WP_012578470.1	J	Translation	COG0197
WP_007053803.1	J	Translation	COG0198
WP_003814530.1	J	Translation	COG0199
WP_007053048.1	K	Tanscription	COG0202
WP_012578465.1	J	Translation	COG0203
WP_007053061.1	J	Translation	COG0211
WP_007053677.1	H	Coenzyme transport and metabolism	COG0214
WP_012578321.1	J	Translation	COG0219
WP_012578498.1	J	Translation	COG0222
YP_003985860.1	J	Translation	COG0231

Gene/Protein ID	Symbol	COGs Class	Description	COGs ID
YP_003986262.1	J	Translation		COG0238
WP_003808136.1	J	Translation		COG0257
WP_003842636.1	J	Translation		COG0257
YP_003986403.1	J	Translation		COG0267
WP_006292704.1	J	Translation		COG0267
WP_032682519.1	J	Translation		COG0290
WP_011068013.1	J	Translation		COG0292
WP_007051394.1	J	Translation		COG0335
WP_007051565.1	TE	Signal transduction mechanisms		COG0347
WP_003808114.1	J	Translation		COG0361
WP_012576821.1	E	Amino acid transport and metabolism		COG0440
WP_012576591.1	O	Posttranslational modification, protein turnover, chaperones		COG0443
WP_012577127.1	EF	Amino acid transport and metabolism		COG0458
WP_012577127.1	EF	Amino acid transport and metabolism		COG0459
WP_077323637.1	F	Nucleotide transport and metabolism		COG0462
YP_003985584.1	J	Translation		COG0480
WP_012577420.1	R	To tell the general functions		COG0481
WP_014484975.1	F	Transport of nucleotide and metabolism		COG0504

TABLE 4.8: Genes K0 IDs with their description

Gene/Protein ID	GO Term	K0 ID	K0 Description
WP_012577167.1	GO0008834	K00806	uppS; undecaprenyl diphosphate synthase [EC:2.5.1.31] RP-S12, MRPS12, rpsL;
YP_003985582.1	GO0005763	K02950	small subunit ribosomal protein S12 RP-S7, MRPS7,
WP_012578203.1	GO0005763	K02992	rpsG; small subunit ribosomal protein S7

TABLE 4.8: Genes K0 IDs with their description

Gene/Protein ID	GO Term	K0 ID	K0 Description
YP_003985585_1	GO0003746	K02358	tuf, TUFM; elongation factor Tu
WP_012578474_1	GO0005763	K02946	RP-S10, MRPS10, rpsJ; small subunit ribosomal protein S10
YP_003986389_1	GO0005764	K02946	RP-S10, MRPS10, rpsJ; small subunit ribosomal protein S11
WP_012576744_1	GO0046933	K02133	ATPeF1B, ATP5B, ATP2; F-type H <sup>+</sup> -transporting ATPase subunit beta [EC:7.1.2.2]
WP_012576586_1	GO0004455	K00053	ilvC; ketol-acid reductoisomerase [EC:1.1.1.86]
YP_003986314_1	GO0003899	K03043	rpoB; DNA-directed RNA polymerase subunit beta [EC:2.7.7.6]
YP_003986388_1	GO0005762	K02906	RP-L3, MRPL3, rplC; large subunit ribosomal protein L3
YP_003986385_1	GO0005762	K02886	RP-L2, MRPL2, RML2, rplB; large subunit ribosomal protein L2
WP_007053035_1	GO0005762	K02890	RP-L22, MRPL22, rplV; large subunit ribosomal protein L22

TABLE 4.8: Genes K0 IDs with their description

Gene/Protein ID	GO Term	K0 ID	K0 Description
WP_012578471.1	GO0022627	K02982	RP-S3, rpsC; small subunit ribosomal protein S3 RP-L14, MRPL14, rplN;
YP_003986378.1	GO0005762	K02874	large subunit ribosomal protein L14 RP-S8, MRPS8, rpsH;
WP_003829896.1	GO0022627	K02994	small subunit ribosomal protein S8 RP-S13, rpsM; small
YP_003986365.1	GO0022627	K02952	subunit ribosomal protein S13 RP-S11, MRPS11, rpsK;
WP_003829907.1	GO0005763	K02948	small subunit ribosomal protein S11
WP_012577436.1	GO0004618	K00927	PGK, pgk; phosphoglycerate kinase [EC:2.7.2.3]
WP_006292965.1	GO0004634	K01689	ENO1_2_3, eno; enolase 1/2/3 [EC:4.2.1.11]
WP_012578179.1	GO0004356	K01915	glnA, GLUL; glutamine synthetase [EC:6.3.1.2] WARS, trpS;
WP_012576516.1	GO0004830	K01867	tryptophanyl-tRNA synthetase [EC:6.1.1.2] RP-S19, RSM19, rpsS;
YP_003986384.1	GO0022627	K02965	small subunit ribosomal protein S19 RP-L16, MRPL16, rplP;
WP_012578470.1	GO0005762	K02878	large subunit ribosomal protein L16

TABLE 4.8: Genes K0 IDs with their description

Gene/Protein ID	GO Term	K0 ID	K0 Description
WP_007053803.1	GO0005762	K02895	RP-L24, MRPL24, rplX; large subunit ribosomal protein L24
WP_003814530.1	GO0005763	K02954	RP-S14, MRPS14, rpsN; small subunit ribosomal protein S14
WP_007053048.1	GO0003899	K03040	rpoA; DNA-directed RNA polymerase subunit alpha [EC:2.7.7.6]
WP_012578465.1	GO0005762	K02879	RP-L17, MRPL17, rplQ; large subunit ribosomal protein L17
WP_007053061.1	GO0005762	K02899	RP-L27, MRPL27, rpmA; large subunit ribosomal protein L27
WP_007053677.1	GO0036381	K06215	pdxS, pdx1; pyridoxal 5'- phosphate synthase pdxS subunit [EC:4.3.3.6]
WP_012578321.1		K03216	trmL, cspR; tRNA (cytidine/uridine-2'-O-)- methyltransferase [EC:2.1.1.207]
WP_012578498.1	GO0005762	K02935	RP-L7, MRPL12, rplL; large subunit ribosomal protein L7/L12
YP_003985860.1	GO0003746	K02356	efp; elongation factor P RP-S18, MRPS18, rpsR;
YP_003986262.1	GO0005763	K02963	small subunit ribosomal protein S18

TABLE 4.8: Genes K0 IDs with their description

Gene/Protein ID	GO Term	K0 ID	K0 Description
WP_003808136.1	GO0005762	K02919	rpmJ; large subunit ribosomal protein L36
WP_003842636.1	GO0005762	K02919	rpmJ; large subunit ribosomal protein L36
YP_003986403.1	GO0005762	K02913	rpmG; large subunit ribosomal protein L33
WP_006292704.1	GO0005762	K02913	rpmG; large subunit ribosomal protein L33
WP_011068013.1	GO0005762	K02887	rplT; large subunit ribosomal protein L20
WP_007051394.1	GO0005762	K02884	rplS; large subunit ribosomal protein L19
WP_007051565.1		K04751	glnB; nitrogen regulatory protein P-II 1
WP_003808114.1	GO0003743	K02518	infA; translation initiation factor IF-1
WP_012576821.1	GO0003984	K01653	ilvH, ilvN; acetolactate synthase I/III small subunit [EC:2.2.1.6]
WP_012576591.1		K04043	dnaK, HSPA9; molecular chaperone DnaK
WP_012577127.1	GO0004088	K01955	carB, CPA2; carbamoyl-phosphate synthase large subunit [EC:6.3.5.5]
WP_012577127.1	GO0004089	K01955	carB, CPA2; carbamoyl-phosphate synthase large subunit [EC:6.3.5.5]

TABLE 4.8: Genes K0 IDs with their description

Gene/Protein ID	GO Term	K0 ID	K0 Description
WP_077323637.1	GO0004749	K00948	prsA; ribose-phosphate pyrophosphokinase [EC:2.7.6.1]
YP_003985584.1	GO0003746	K02355	fusA, GFM, EFG; elongation factor G GUF1; translation
WP_012577806.1		K21594	factor GUF1, mitochondrial [EC:3.6.5.-]
WP_012577420.1		K03596	lepA; GTP-binding protein LepA
WP_014484975.1	GO0003883	K01937	pyrG, CTPS; CTP synthase [EC:6.3.4.2]
WP_007052130.1	GO0022627	K02986	rpsD; small subunit ribosomal protein S4
WP_012578046.1		K03702	uvrB; excinuclease ABC subunit B

They are mostly the J category genes and their KEGG pathway analysis indicates that they are involved in ribosomal protein synthesis which is helpful in translation and transcription process. Metabolic pathways regulatory substances are also being codes by these genes. They are indicating that these strains are helpful in combating diseases like metabolic syndrome because these genes have potential role in protein formation machinery. Their role in protein synthesis and non virulency make them potential to be used as probiotic.

In table 4.9 KEGG pathway showing that all these genes involved in metabolic pathways can be potentially used against metabolic syndrome as a probiotic. These strains have the ability to combat metabolic syndrome because they are involved in ribosome formation ,making of receptor proteins and regulators and protein assembly which make them effective to be used as a probiotic.

TABLE 4.9: *Bifidobacterium* Strains Genes Showing KEGG Pathway

GENE ID	KO ID	KEGG Pathway
		Biosynthesis of complex
WP_012577167.1	K00806	compounds like peptidoglycan , synthesis of metabolites
YP_003985582.1	K02950	Ribosome
WP_012578203.1	K02992	Ribosome
YP_003985585.1	K02358	Plant-pathogen interactio
WP_012578474.1	K02946	Ribosome
YP_003986389.1	K02946	Ribosome
		Oxidative phosphorylation,
WP_012576744.1	K02133	Metabolic pathways, Thermogenesis, Diabetic cardiomyopathy Biosynthesis of various
WP_012576586.1	K00053	compounds like valine, CoA, amino acids
YP_003986314.1	K03043	RNA polymerase
YP_003986388.1	K02906	Ribosome
YP_003986385.1	K02886	Ribosome
WP_007053035.1	K02890	Ribosome
WP_012578471.1	K02982	Ribosome
YP_003986378.1	K02874	Ribosome
WP_003829896.1	K02994	Ribosome
YP_003986365.1	K02952	Ribosome
WP_003829907.1	K02948	Ribosome
		Breakdown of glucose, metabolism of microbes and production of metabolites
WP_012577436.1	K00927	Glucose catabolism, secondary metabolites are produced,
WP_006292965.1	K01689	

<b>GENE ID</b>	<b>KO ID</b>	<b>KEGG Pathway</b>
WP_012577205_1	K01915	Metabolism of different compounds like aspartate, nitogen and amino acids
WP_012578179_1	K01915	Metabolism of glutamate , metabolism of compunds having nitrogen, amino acids synthesis
WP_012576516_1	K01867	Aminoacyl-tRNA biosynthesis
YP_003986384_1	K02965	Ribosome
WP_012578470_1	K02878	Ribosome
WP_007053803_1	K02895	Ribosome
WP_003814530_1	K02954	Ribosome
WP_007053048_1	K03040	RNA polymerase
WP_012578465_1	K02879	Ribosome
WP_007053061_1	K02899	Ribosome
WP_007053677_1	K06215	Metabolism of vitamins like B6, involved in cofactor synthesis
WP_012578321_1	K03216	
WP_012578498_1	K02935	Ribosome
YP_003985860_1	K02356	
YP_003986262_1	K02963	Ribosome
WP_003808136_1	K02919	Ribosome
WP_003842636_1	K02919	Ribosome
YP_003986403_1	K02913	Ribosome
WP_006292704_1	K02913	Ribosome
WP_032682519_1	K02520	
WP_011068013_1	K02887	Ribosome
WP_007051394_1	K02884	Ribosome
WP_007051565_1	K04751	Two-component system
WP_003808114_1	K02518	

<b>GENE ID</b>	<b>KO ID</b>	<b>KEGG Pathway</b>
WP_012576821_1	K01653	Synthesis of aminoacids like lucin, metabolism of acidic compounds
WP_012576591_1	K04043	RNA degradation, Longevity regulating pathway - worm,
WP_012577127_1	K01955	Arginine biosynthesis, Pyrimidine metabolism, Metabolic pathways
WP_012577127_1	K01955	Arginine biosynthesis, Pyrimidine metabolism, Metabolic pathways
WP_077323637_1	K00948	Metabolism of nitrogen bases like purine , metabolite synthesis
YP_003985584_1	K02355	
WP_012577806_1	K21594	
WP_012577420_1	K03596	Legionellosis Pyrimidine metabolism,
WP_014484975_1	K01937	Metabolic pathways, Nucleotide metabolism,
WP_007052130_1	K02986	Ribosome
WP_012578046_1	K03702	Nucleotide excision repair

# Chapter 5

## Conclusion and Discussion

### 5.1 Conclusion

Many treatments of metabolic syndrome are being encountered in medical science . Due to increase resistance of bacterial strains against the antibiotics lead to a new approach which is use of potential probiotics strains .It is now goal of medical science to use probiotics instead of using harmful chemicals . As probiotics are beneficial for gut and other health related problems so they are widely being used in treatment of metabolic syndrome. As still they don't have any harmful effect.

Obesity is the main factor that is enhancing metabolic syndrome power. Various surgical procedures are now being invented to overcome it. *Bifidobacterium* genus is part of gut microbiome . The project was designed to identify well characterise probiotic strains that can help in treatment of MetS. In silico methods were used to identify powerful probiotic strains of *Bifidobacterium* that ensure safety. Pangenome analysis , COG analysis and KEGG pathway analysis performed was done to get all the core genes that are involved in its probiotic capabilities and check their mode of action that by which pathway they perform various metabolic actions.

Members of genus *Bifidobacterium* have large genome sizes that protein encoding open reading frames . Pangenome analysis of this genus includes 14 They produce

metabolites that are very useful for increasing immune function and maintaining barrier of intestines. Acetic acid is being produced by many *Bifidobacterium* strains that are helpful in being used as probiotics. They use metabolic pathway to synthesis a compound of aromatic lactic acid which help in anti-inflammatory action during their mode of action. Some strains also produce various vitamins which helps them in signaling and effect the host by effecting its physiological state.

Prediction of J category of core genes suggested that they are mostly involved in protein synthesis, ribosomal synthesis and metabolic pathways related to lipids etc. These genes can be potentially used against MetS to decrease lipid content of body which in result can be helpful in lowering level of cholesterol, obesity, lower risk of cardiovascular disorders.

As different strains of bacteria behave differently in intestines and gut. Some produce one metabolite like acetic acid that perform a function and other produces the different one linoleic acid that has a different function. One can tolerate a condition but other can not. If we co-culture strains of our desired functions we can use them as probiotics against a certain disease.

So we can conclude that 11 strains of *Bifidobacterium* have prebiotic potential because they contain the genes that are involved in metabolic pathways. This study was found safe to use them as prebiotic strains against MetS. The limiting factors would be the safety that can be done by obtaining whole genome sequence of strains and find out the virulence factors and pathogenic strains. In vivo studies further can prove that how these probiotic strains may be used against obesity and multifactorial metabolic syndrome.

## 5.2 Future Directions and Scope

*Bifidobacterium* genus strains were identified as major contributors to treat metabolic syndrome by controlling obesity, lipid and cholesterol level. Comparative analysis and Bacterial Pangenome Analysis was used to annotate and functionally analyze the genes of 11 probiotic strains of *Bifidobacterium* genus. Core genes were identified and their potential probiotics characteristics were checked. Gene ontology and

KEGG pathways used to identify the role of genes and their pathway .By getting all the data about COG analysis and pathways of genes we come to know that J category genes of *Bifidobacterium* genus strains are present mostly and involved in ribosomal and protein synthesis , translation and biogenesis.

Prediction of J category of core genes suggested that they are mostly involved in protein synthesis, ribosomal synthesis and metabolic pathways related to lipids etc. These genes can be potentially used against MetS to decrease lipid content of body which in result can be helpful in lowering level of cholesterol, obesity ,lower risk of cardiovascular disorders.

These genes can help in treating metabolic syndrome if these probiotics are used. No pathogenicity and virulence being detected so *Bifidobacterium* genus strains have potential to be used as probiotics against carbohydrate ,lipids metabolism, cholesterol high level and obesity so that metabolic syndrome can be treated.

By using bioinformatics tools we have checked their pathogenicity and virulence and found no virulent strains so they can be used for clinical trials. In future we can use these strains in laboratory and have clinical trials on animal models to check their efficacy whether they have potential to reduce obesity, lipid level or not.

We can also co-culture these strains to be used against metabolic syndrome because it is a combination of risk factors so different probiotic strains with different potential can help us in treatment of various factors together.

If we find the efficacy of these probiotic strains on animal models we can use them or market them for being used by human beings.

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