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Improving the Gut Microbiota with Probiotics and Faecal Microbiota Transplantation

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Abstract

Probiotics are "live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host". After birth, our intestine is colonized by microbes like *Escherichia coli, Clostridium* spp., *Streptococcus* spp., *Lactobacillus* spp., *Bacteroides* spp., and *Bifidobacterium* spp. Our intestine is an extremely complex living system that participates in the protection of host through a strong defence against external aggregations. The microbial ecosystem of the intestine includes many native species of Bacteroides and Firmicutes that permanently colonize the gastrointestinal tract. The composition of flora changes over time depending upon diet and medical emergencies which leads to the diseased condition. Probiotics exert their mode of action by altering the local environment of the gut by competing with the pathogens, bacteriocins production, H_2O_2 production etc. Obesity is one of the major health problems and is considered as the most prevalent form of inappropriate nutrition. Probiotics like *Lactobacillus* Sp., *Bifidobacterium* Sp., *Streptococcus* Sp. are successfully used in the treatment of obesity proved in clinical trials. Faecal microbiota transplant (FMT), also known as a stool transplant, is the process of transplantation of Faecal bacteria from a healthy donor into a recipient's gut to restore normal flora in the recipient. The therapeutic principle on which FMT works is microbes and their functions and metabolites produced by them which are used to treat a variety of diseases. The present review focuses on the role of gastrointestinal microbiome, probiotic selection criteria, their applications and FMT to treat diseases.

Keywords: Probiotics, Obesity, Microbial ecosystem, Faecal microbiota transplantation

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INTRODUCTION

In today's era focus is on functional foods for health enrichment with minimum efforts. Interest in self-care and integrative medicine, coupled with foods that promote health, apart from providing basic nutrition has emerged in recent times. Historically, yoghurt is probably the oldest example of fermented food which was used to support intestinal and overall health. In ancient Indian societies, it was a common practice to consume a yogurt drink called Lassi before-dinner and a small serving of curd at the end of meal. These Indian traditions assumed of using sour milk are perhaps the earliest examples of a probiotic delivery system to our body. The origin of word 'Probiotic' is traced to Greek language, 'pro bios' which means 'for life'. Its opposite 'antibiotic' is more familiar meaning 'against life'. Probiotics were first described in 2002 by the authorities from the Food and Agriculture Organization of the United Nations and the World Health Organization. The definition was revised by the International Scientific Association in 2013[1]. The definition reveals that, "probiotics are live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host." The intestinal tract of new-born is sterile at birth, with time and age it gets colonized by number of organisms from breast milk, formula fed milk, mother's vagina, diet, environment etc. A vast number of factors affect colonization of intestine by bacteria. The very first organisms to colonize in the new-born are *Escherichia coli, Clostridium* spp., *Streptococcus* spp., *Lactobacillus* spp., *Bacteroides* spp., and *Bifidobacterium* spp. Bifidobacterium which colonizes in highest numbers gets excreted in faeces of breast-fed infants[2]. The composition of flora changes overtime depending upon diet and medical emergencies. The number of bacteria present in large intestine is upto 10^{11} colony-forming units/g. The healthful human gut is conquered by *Bacteroides, Firmicutes* and *Actinobacteria*, each comprising a unique bacterial composition of the stool[3]. The gut microflora plays a significant role in human well-being, not only due to its contribution in the digestion progression but also for its critical role in the maturity of the gut and the immune system. The human gastrointestinal tract (GIT) comprises an extraordinarily complex and active microbial community which in particular for every individual depends on environmental and genetic factors[4]. The gastrointestinal flora is increasingly being used for prevention and treatment of several disorders like lactose intolerance, allergies, colon cancer, Irritable bowel syndrome (IBS), Osteoarthritis and diseases like *Clostridium difficile* (CD) infection and diarrhoea[5-7]. Knight and his coworkers showed presence of probiotics in faeces after three days of oral administration therapy with Synbiotic 2000 Forte which is a mixture of probiotics able to survive in the stomach and gut. Synbiotic 2000 Forte consists of combination of 10^{11} colony forming units of each of four probiotics, *Lactobacillus paracasei* 25%, *L. plantarum* 25%, *Leuconostoc mesenteroides* 25%, *Pediococcus pentosaceus* 25% with bioactive fibres, oat bran, 2.5 g, pectin, 2.5 g, resistant starch, 2.5 g, inulin, 2.5 g (total weight, 10 g)[8].

Faecal microbiota transplant (FMT) is a technique in which Faecal matter from a healthful donor is mixed with saline solution and is placed into a recipient with chronic, serious, or complicated *Clostridium difficile* (CD) infection. The primary purpose of FMT is to re-establish a healthy microbiota balance that facilitates the resistance to growth of pathogenic bacteria, thus reducing the chance of recurrent colitis and dysentery.

Criteria for selection of Probiotics

The source of probiotic culture often is of human origin like human faces or breast milk, animal source like fresh raw milk or fermented food. As per the guidelines of FAO/WHO 2002 probiotic tests which are currently used *in vitro* for screening of cultures are

i. Resistance to gastric acidity
ii. Bile acid resistance
iii. Adherence to mucus and/or human epithelial cells and cell lines
iv. Antimicrobial activity against potentially pathogenic bacteria
v. Capability to reduce pathogen adhesion to surfaces
vi. Bile salt hydrolase activity
vii. Resistance to spermicides (applicable to probiotics for vaginal use)

Probiotics commonly provided through dairy products improve the transit tolerance of bacteria. Other delivery systems for probiotics

are meat and fish-based products, confectionery, table olives, soya and cereal-containing products, edible spreads, artichokes (European plant) etc.[9] Artichokes or olives furnish nutrients to enable bacterial survival throughout shelf storage. The mode of action Probiotics is by altering the local environment of the gut, producing antimicrobial compounds like bacteriocins to be used against pathogens. Hence a strict selection criterion is employed to obtain functional probiotic strains. Most of the probiotics are a mixture of strains of Lactobacilli and (or) Streptococci and *Bifidobacterium.* Some commercial products contain strains in combination with others leading to enhanced effect. The probiotic strain should be of host origin, well characterized, able to colonize by surviving the rigors of the digestive tract and, biologically active against the target as well as stable and flexible for commercial production and distribution[10].

Desirable Characteristics for Potential Probiotic Strain

To be defined as probiotic, a microbe must possess a following set of criteria[11]

- Exert a beneficial effect on host
- Nontoxic and non-infective
- Precise taxonomic classification
- Normal inhabitant of species
- Able to survive, proliferate, and having metabolic activity in the target site, which include
    - Resistance to gastric and bile salts
    - Adherence to intestinal epithelial cell lining
- Capacity to compete with resistant flora
- Produce antimicrobial substances
- Stabilize intestinal microflora
- Acceptable to host's immune response
- In vivo antagonism towards pathogenic bacteria
- Genetically stable
- Amenability of strain
- Stability of desired characteristics during processing, storage, and delivery
- Feasibility at highest population
- Good shelf life in food
- Biosafety, the strain used should belong to, Generally Regarded as Safe (GRAS) category

Bile Tolerance

Survival in gastrointestinal tract requires the probiotic strain to be able to resist low pH due to gastric acid, bile and gastric enzymes. Acid and bile stability are significant among the other criteria used in selection probiotic microbes[12]. The bile salts act as biological detergent which emulsifies and solubilizes fats. This confers powerful antimicrobial properties to bile and plays an significant role in the body's physico-chemical defence system[13]. Probiotics like Lactobacilli[14], Bifidobacteria[15], Enterococcus[16], Saccharomyces[17] assimilate cholesterol and deconjugate bile acids, leading to a decrease in serum cholesterol. Bile salts are produced in liver from cholesterol and secreted as amino acid conjugates into the duodenum. The exact mechanism by which probiotics lower serum cholesterol is unclear but it is thought that cholesterol elimination from culture media is a result of formation of cholesterol with free bile acids in the media due to activity of bile salt hydrolase enzyme. *Enterococcus faecium* WEFA23 isolated from human infants has Glycodeoxycholic acid (GDCA) hydrolase activity and cholesterol-removal ability in an environment containing 3% bile salt[18].

Acid Tolerance

The initial screening of probiotics includes tolerance of cultures to gastric acid. Acid tolerance of probiotics is also essential for production of probiotic products like fermented foods. Hence acid tolerance is primarily important in screening for probiotic cultures[12]. A healthy adult human stomach secretes about 1.5 litres of gastric acid daily. To survive in the stomach, probiotic strains must bear the acidic conditions of the stomach pH 1.5 to 3.5. The presence of prebiotic substances carried by artichokes or olives such as inulin, contribute efficiently to favour the growth of indigenous lactobacilli and/or bifidobacteria[9]. Artichokes when preserved in brine of pH 3.5, enriched with *L. plantarum* ITM21B or *L. paracasei* IMPC2, no considerable decrease in the bacterial population was observed for either strain after 30 days of storage[9]. In order to survive in the stomach, probiotic strains must tolerate the acidic pH of the stomach i.e. about 1.5 to 3.5. It is assumed that acid tolerance of *Bifidobacteria* depends on H^+-ATPase activity[19]. *B. animalis* and *B. lactis* are more acid tolerant among the Bifidobacteria[20] having higher H^+-ATPase activity[21].

Antipathogenic activity

One selection criterion for probiotics is the production of antimicrobial substances, which exert a beneficial effect on host. Lactic acid and acetic acid producing probiotics decrease the pH of the lumen creating unfavorable conditions for pathogens[22]. Along with bacteriocins, LAB produce organic acids, such as lactic and acetic acids, hydrogen peroxide, or diacetyl having antimicrobial potential[23]. Probable mechanism by which probiotics exhibit antipathogenic effect is shown in Fig. 1. Other antimicrobial compounds secreted by LAB and *Bifidobacteria* against dermal pathogen *Staphylococcus aureus* are lactic acid, acetic acid, hydrogen peroxide, and diacetyl[24]. Thus, probiotic strains that are able to colonize in the digestive tract play a significant role in the release of antimicrobial compounds which, by their highly inhibitory action, regulates the composition of normal flora in the digestive tract. An imbalance in normal intestinal flora is an obvious reason of establishment and growth of enteropathogens like *Salmonella, Campylobacter, Escherichia coli* and others. A commercially available bifidus yoghurt and oral preparation of *Bifidobacteria* is used to eliminate *Campylobacterium jejuni* from stools of children suffering from enteritis[25]. *Bifidobacterium animalis lactis* BB-12 and *Enterococcus faecium* used as adjuvant in therapy, are clinically proven to eradicate *Helicobacter pylori* (Hp) infection with reduction in incidences side effects, mainly diarrhea[26]. Probiotics also help us in fighting against common food borne pathogens like *Salmonella* spp., *S. aureus, Listeria monocytogenes, E. coli, Shigella* spp., *Campylobacter* spp. along with, species of *Pseudomonas, Bacillus* and *Serratia.*[27-29].

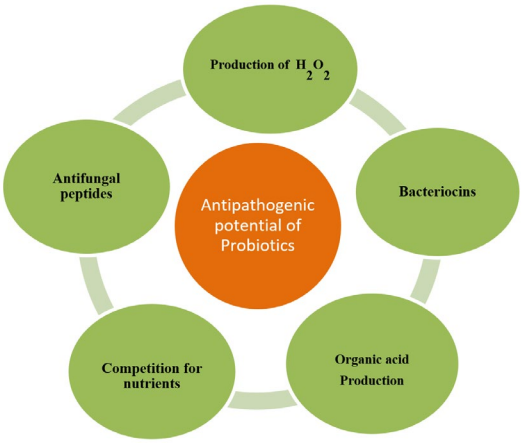

Fig. 1. Antipathogenic Potential of Probiotics

Adherence to mucus and human epithelial cells and cell lines

Capability to adhere to human intestinal surfaces is one of the key selection criteria for probiotic bacteria. Adhesion of probiotics to intestinal wall helps to maintain normal flora in the gut thus influencing the host's microbial balance and gastrointestinal system. To study in vitro adhesive properties of probiotics enterocyte like Caco-2 tissue culture cells[30], intestinal mucus and human ileostomy glycoproteins are currently used[31]. Intestinal mucus protects mucosal lining from microbes, provides initial binding site and matrix on which microbes adhere, also serving as a nutrient source to them. *B. longum* and *B. catenulatum* adhere to human intestinal mucus and inhibit competitively pathogens like *Salmonella enterica serovar typhimurium, E. coli, L. monocytogenes, Enterobacter sakazakii,* and *Clostridium difficile* from adhering to human intestinal mucus[32]. *L. acidophilus* CRL1259L, *L. crispatus* CRL 1266, *L. paracasei* CRL 1289 adhere competitively to vaginal epithelial cells (VEC) and exclude urogenital pathogens like *S. aureus. L. acidophilus* 1259 and *L. paracasei* CRL 1289 competitively excluding Group B Streptococci[33]. *L. delbrueckii* spp. *bulgaricus* (LDB B-30892) substantially lowers the adhesion of *C. difficile* to the human colonic Caco-2 cells[34]. Live and heat killed strain of *L. rhamnosus* CNCM-I-3698 and *L. farciminis* CNCMI-3699 respectively, are adherent to intestinal matrix models and their high co-aggregation property has been proved in vitro which eliminates *Campylobacter* spp.[35]

In addition to adhesion, another important mechanism for pathogen exclusion by probiotics is aggregation. Probiotics also exert capacity to aggregate among themselves (auto-aggregation), with other probiotics and with pathogens (co-aggregation). Co-aggregation leads to the formation of a protective barrier for intestinal epithelium, inhibiting colonization by harmful pathogens. Measuring co-aggregation of pathogens serves as a preliminary screening to identify potentially probiotic organisms[36].

Biosafety

Probiotics are not under regulation of the US Food and Drug Administration (FDA) as they are considered to be dietary rather than a therapeutic product. They are easily available in market and can be bought without prescription. Probiotics are consumed as health supplements rather than therapy. Some may experience gas and bloating after consumption[37]. With respect to safety consideration of probiotic microorganisms, it is recommended that probiotic microorganisms are strictly free of genetic resistance determinants like plasmid, which encode resistance against clinically used antibiotics[38]. Such probiotic are generally regarded as safe (GRAS) and can be used safely[39]. Available safety data regarding the broad range of probiotic strains added to food or feed are periodically compiled by the European Food Safety Authority (EFSA)[40].

Health Benefits

There are numerous health benefits of probiotic organisms. Most probiotic strains of human origin are safe to use because they are commensals and exert rapid effects.

Gut microbiota

Gut microbiota (GM) is also called as "forgotten organ" which harbours 100 trillion bacteria, (fungi, archaea, and viruses) which are 10 times greater than the number of cells present in human body[41]. Intestine is an enormously multifaceted living system that protects the host strongly against external aggregations. The small intestine harbours a single dense mucus layer. In the colon, mucus is organized into two distinct layers, an outer loose layer, and an inner dense layer that is connected to the epithelium. The fast-increasing facultative anaerobes overgrow microbial community of the small intestine that tolerates the influences of bile acids and antimicrobials. Small intestine of humans contains *Proteobacteria* and *Clostridium* species. The cecum and colon of large intestine contains anaerobic fermentative polysaccharide catabolizing anaerobes belonging to families *Bacteroidaceae* and *Clostridiaceae*. Dominant organisms in the colon belong to Firmicute families, *Lachnospiraceae* and *Ruminococcaceae*. In stomach dominant organisms are Bacteroidetes families, *Prevotellaceae, Bacteroidaceae,* and *Rikenellaceae*[42]. As stated above, bacterial population is much higher in the colon as compared to the small intestine. The colon is colonized by bacterial families including *Bacteriodaceae, Ruminococcaceae, Prevotellaceae,* and *Clostridiaceae* (10^{11}–10^{12}CFU/ml). Indigenous flora of stomach consist of species from the families *Lactobacillaceae* and *Streptococcaceae* in the epithelial layer. The gut microflora plays a key role against extrinsic bacteria through resistance to colonization. The homeostasis in gut can be due to dietary changes, antibiotic exposure, infections, disease states, and maturing. Aging was complemented with growing proportion of Bacteroides, Eubacterium and Clostridiaceae. Enterobacteriaceae was augmented in infant and aged people. There is age-related decrease in the performance of gut microbiota, as well as rise of inflammation and diseases, particularly for the aged people older than 90s[43].

FMT (Faecal microbiota transplantation): Faecal microbiota transplant (FMT), also well-known as stool transplant, is the practice of transplantation of faecal bacteria from a healthful donor into a recipient to reestablish normal flora in recipient[44]. FMT is the known terminology which defines the infusion of distal faecal matter from a healthy donor into the Gastrointestinal tract of a recipient to re-establish healthy intestinal microflora[45]. The therapeutic principle on which FMT works is microbes, their roles and the metabolites formed by them[46]. The criterion of FMT is to improve intestinal imbalance by transporting stool comprising a stable, live, varied, and normal microbial community from a healthy donor or from well-defined colonic bacterial strains[46]. FMT requires restoration of the microflora by establishing healthy bacterial flora via infusion of stool, e.g. via colonoscopy, enema, orogastric tube, or by mouth in the form of a capsule containing freeze-dried material, gained from a healthy donor. The constant antibiotic medication in patients leads to acute damage of normal intestinal microflora. Replacing the entire intestinal flora of recipient by healthy donor flora gets a donor-like flora composition with stabilization of its usefulness in the recipient. With a level of complexity like an organ, the gut is home to a intricate mixture of more than 1000 microbial varieties, which jointly harbour 100 times more genes than the human genome.

A healthy human gut microbiota comprises of a higher ratio of Bacteroides to Firmicutes and overall higher bacterial diversity[47]. The gastrointestinal microbiome (GIM) may be subject to progressive or dietary compositional variations, antibiotic damage and super infections, like the *C. diffcile* infection (CDI). Changes in diet, administration of particular antibiotics, probiotics, or FMT may be utilized to rebuild dysbiosis or eradicate contagious pathogens, such as *C. diffcile*[45]. Due to the increase in cases of community and hospital acquired CDI, elevated rates of recurrent CDI (projected 20–30% after a first and 50–60% after a third infection), high mortality (~29,000 deaths annually) in the USA, and the emergency for treatments not involving antibiotics gave birth to microbiome-based remedies[48]. Pseudomembranous colitis (PMC) is a disease of the colon caused due to antibiotics or other agents upsetting the normal intestinal flora. *Bacteroides* sp. are absent in patient's stool and are present after FMT when PMC is treated with FMT, indicating that colonization with *Bacteroides* sp. seems to be particularly crucial in maintaining normal bowel movement and increased resistance to Gastrointestinal infections[49]. FMT was first used in 1950s by Mr. Ben Eiseman to treat his patients with antibiotic associated diarrhoea. Since then, it has been a highly effective strategy for treating CDI[50]. After being successfully used in treatment of *C. difficile*, FMT can be used in cure of inflammatory bowel disease (IBD) which is a medical condition accounted as abdominal discomfort or pain and gut dysbiosis without pathological condition[51]. IBD is also a group of immune-mediated diseases mainly denoted by Ulcerative colitis (UC) and Crohn's disease (CD). Traditional medication for IBD treatment involves the use of amino-salicylates, corticosteroids, thiopurines, folic acid antagonists, or biological treatments which only controls inflammation besides healing disease so that patients become intolerant to these remedies. Fecal microbiota transplantation (FMT) and probiotics have been discovered as promising candidates to re-create microbial balance in several immune-mediated illnesses such as IBD[52]. An analysis involving the treatment consequence of FMT and probiotic mixture VSL#3 the clinical amnesty and medical response did not fluctuate much[51]. The exact mechanism of effectiveness of FMT and the specific strains that confer this advantage remain undecided. It is stated that endoscopic FMT might be the first line to cure patients with severe CDI and its complications[53].

Table 1. Diseases treated with FMT

No.	Disorder/ Disease	Symptoms	Ref.
1	Metabolic diseases	Fatness/Obesity	59, 60
		Metabolic syndrome	59
		Type 2 diabetes	60,61
		Insulin resistance	61,62
2	Liver diseases	Alcoholic liver disease &Non-alcoholic fatty liver disease	60,63
		Chronic hepatitis B	64
3	Neuropsychiatric disorders	Parkinson's disease (PD)	41
		Alzheimer's disease (AD)	65
		Depression & Multiple sclerosis (MS)	65
		Autism	65
		Chronic fatigue syndrome (CFS)	66
		Epilepsy	65
4	Autoimmune Disease	Idiopathic thrombocytopenic purpura	67
		Arthritis	65,6
5	Cardiovascular disease	Myocarditis	65,68
6	Kidney diseases		65
7	Intestinal disorders	Inflammatory bowel diseases (IBD)	69
		Crohn's colitis	70
		Irritable Bowel Syndrome (IBS)	71

FMT not only consistently eradicates CDI but also reestablishes the Faecal flora, which is the possible mechanism for the pathogen's colonization and perseverance. In addition to CDI there are many other diseases which are treated by FMT (Table 1). After FMT treatment patients commonly experience symptoms like bloating, cramping, and/or constipation after few days following transplant. FMTs performed via colonoscopy have certain consequences associated with colonoscopy, such as unfavorable tranquilizer reaction and bowel perforation after the procedure[54]. Probiotics are commonly administered in conjunction with antibiotics in order to replenish loss of normal flora. Oral administration of probiotics requires more time for normalization as compared to FMTs. There is a metabolic interconnection between the different microbial communities in gut to alleviate host immunity against pathogens. In this regard many physicians use a few individual species of 'excellent microorganisms' to treat antibiotic-induced acute dysbiosis[49]. Probable mechanisms for FMT efficacy in treatment of CDI, comprises direct battle of *C. difficile* with commensal microbiota delivered by FMT, rebuilding of secondary bile acid metabolism in the colon and restoration of the gut barrier by stimulus of the mucosal immune system[55]. In a controlled study an industrial preparation (Bio-25) available in market containing 11 species of common probiotic bacteria (variety of *Lactobacilli, Bifidobacteria, Lactococcus lactis,* and *Streptococcus thermophilus*) was fed daily to healthy human volunteers for 28 days. The fed probiotic bacteria were discovered in stool samples of all members until they were using the product[56] which led to a assumption that probiotics can be hypothetical target in FMT to boost the effectiveness of therapy. Oral administration of probiotic species *Lactobacillus* and *Bifidobacterium* for four to twelve weeks showed to be effective in treating constipation in Parkinsons Disease (PD)[57].

Analysis of microbes in FMT showed abundant number of genera, *Bacteroides, Prevotella, Bifidobacterium, Faecalibacterium,* and *Lachnospiraceae* present along with *Escherichia, Shigella* and *Alistipes*[58,46]. Gut mucosal establishment of probiotics depends on the capability of probiotics to interact with local microbiome niches, which differ in their physiological properties along the gastrointestinal tract of the individual hence probiotics might solely be used in FMT. FMT provides number of microbes. Further research is needed on microbes and their products responsible for its health benefits. In addition to CDI there are many diseases which are treated by FMT.

Vitamin production

Probiotic bacteria have a positive effect on the immune system and the composition and operation of the gut microbiota[11]. Human beings are unable to make riboflavin or vitamin B2 and fulfill it from dietary intake of milk and dairy products, fish and meat. Riboflavin deficiency is indigenous in populations who are vegetarian, lacking dairy products and meat[72]. The Probiotic lactic acid bacteria *L. lactis* flourish in absence of riboflavin. Based on the genome sequencing of *L. lactis* IL1403, it is shown to harbour all genes required for riboflavin biosynthesis (rib genes)[73]. These micronutrients are used as a cofactor in several enzymatic reactions, as in case of vitamin B12[74]. *L. coryniformis* subsp. *coryniformis* CRL 1001 can manufacture corrinoids with cobalamin action. HPLC and mass spectrometry analysis shows corrinoid produced is pseudo-cobalamin that has adenine like Coα-ligand instead 5, 6-dimethylbenzimidazole. This molecule is one of the forms of coenzyme B12 occurring in atmosphere[75]. Vitamin B1 or Thiamine plays a significant role in amino acid metabolism, and is a rate limiting cofactor in production of neurotransmitter like dopamine, serotonin, gamma amino butyric acid (GABA), noradrenaline, and the hormone melatonin[72,76]. The strains of Lactobacillus used in production of functional foods belong to the species *L. acidophilus, L. casei, L. paracasei, L. plantarum, L. reuteri,* and *L. salivarius* which are powerful folic acid producers[8] . Folic acid plays an important role in purines and pyrimidines metabolism, hence, in DNA formation. With exception to other lactic acid bacteria *L. plantarum* in presence of para-aminobenzoic acid (PABA) produces folic acid *in vivo* in rats.

Though abundant source of vitamin K are green leafy vegetables, some individuals are deficient in vitamin K which plays a major role in blood clotting. *L. lactis* ssp. *cremoris* (three strains), *L. lactis* ssp. *lactis* (two strains), and *Leuconostoc*

lactis are high manufacturers of quinone that synthesize higher than 230 nmol of quinones/g of dried cells[77].

Antibiofilm formation

Biofilms are microbially derived immobile communities characteristic of cells that are adjacent to a substratum, an interface, or to each other; are embedded in a self-produced matrix; and display an altered phenotype with regard to growth rate and transcription profile[78]. *Vibrio cholerae* have ability to develop strong biofilms which is vital for its intestinal colonization[79]. This is for the reason that; biofilm forming cells are more efficient in competing for limiting nutrients in the small intestine. Probiotic strains having both antimicrobial and anti-biofilm properties against *V. cholerae* are anticipated to be clinically better. Culture supernatant of Faecal lactobacilli isolates prevent the adherence of both *V. cholerae* and *V. parahaemolyticus* to the colonic epithelial cell line HCT-15 by more than 90%[80]. EPS-producing *L. plantarum* WLPL04 screened from human breast milk acquire anti-adhesion characteristic and ability to displace, and inhibit *E. coli* O157, H7, *P. aeruginosa* CMCC10104, *S. typhimurium* ATCC13311, and *S. aureus* CMCC26003. This indicates that EPS secreted by *L. plantarum* WLPL04 possesses activity against biofilm formed by pathogens[81].

Anti-obesity Properties

The population of overweight people is increasing worldwide. Obesity is one of the defined health troubles and is considered as the most predominant form of malnutrition[82], which increases the frequency of obesity-related complications and the global problem of illness. Obesity is defined as an accumulation of unnecessary fat that weakens health. Excessive intake of calories is responsible for chronic diseases like obesity, gallbladder disease, coronary heart disease (CHD), hypertension, osteoarthritis (OA), type 2 diabetes mellitus, hyperlipidaemia, certain cancers and cardiovascular diseases[83]. The gut microbiota provides a powerful route that influences human health. In particular, polysaccharides and high fibre diet serve as primary modulators of the composition and role of the microbiota. The gut microbiota has been proposed as another factor involved in the onset of obesity. In a cohort study of 87 obese children and 57 healthy children, comparison of overall Faecal microbiota in obese children did not show variability in community diversity and richness[84]. Probiotics exhibiting Bile salt hydrolase (BSH) activity can result in reduced bodyweight, also lower levels of plasma cholesterol and liver triglycerides concentration[85]. The anti-obesity consequence of probiotic dark tea was assessed in high-fat diet fed Sprague-Dawley (SD) rats. Probiotic dark tea lowered the body weight rise, serum triglycerol levels and serum total glycerol levels in extraordinary fat diet SD rats[86]. It is also shown that obesity is linked with increased Firmicutes, while the concentration of Bacteroidetes is reduced in obese patients[87]. Obesity is associated with differentiation of preadipocytes to adipocytes which is examined by Lipid O staining. *L. plantarum* K10 isolated from homemade Kimchi showed 32.61% reduction in lipid accumulation compared to control. Freeze dried cultures of *L. plantarum* K10 fed to mice for 12 weeks demonstrated substantial drop in body weight as contrasted to high fat diet group[88]. One of the approaches of probiotics employing antiobesity activity is inhibition of intestinal lipid absorption by exerting lipase inhibition. *L. casei* Q180 strain of human origin displayed a lipase repressive activity of 83.61±2.31% compared to control and prevention of adipocyte differentiation of 3T3-L1 cells[89]. *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 given alone or in mixture for 9 weeks to mice were found to limit fat buildup in adipose tissue and liver[90]. Many clinical trials proved use of probiotics in controlling obesity as shown in Table 2. Thus, probiotics not only play a positive role in physiology of the host but also plays a modulatory role in obesity. Probiotics have been shown to influence the gut composition, improve gut integrity and restore the normal flora disturbance which leads to obesity.

CONCLUSION

Probiotics are known since from ancient times. The health benefits of probiotics discussed here are vitamin biosynthesis, antiobesity and antibiofilm property. Probiotics are the organisms residing in our intestinal tract. If an imbalance occurs in these microbes it leads to a diseased condition. These gut bacteria are responsible for digestion, absorption of nutrients; thus,

Table 2. Clinical trials of probiotics in treatment of obesity

Type of Study	Probiotic strain	Duration of study	Key findings	Ref.
Randomized, double-blind, placebo-controlled	*L. plantarum* TENSIA; 8.7 log CFU per g	3 weeks	decreased BMI & hypertension	91
Randomized, double-blind, placebo-controlled	*L. gasseri* BNR17 10^9 – 10^{10}CFU/day	12 weeks	decreased Visceral fat, adipose tissue weight	92
placebo controlled, randomized, and double-blind	*L. gasseri* BNR17 10^{10}CFU/day	12 weeks	decreased body weight, waist circumference	92
double-blinded, randomized, controlled	Probiotic yoghurt *L. acidophilus* La5 & *B. lactis* Bb12 300 g/d	8 weeks	decreased mean body weight, BMI, serum ALT and AST, total cholesterol and LDL-C	93
randomized, double-blind, placebo-controlled	*L. urvatus* HY7601 5×10^9CFU/day and *L. plantarum* KY1032 5×10^9CFU/day	12-week	decreased Serum Triglycerol	94
random, double-blind, placebo-controlled	*L. reuteri* JBD301 450 mg/d 10^9 CFU/capsule	12 weeks	decreased body weight, intestinal FFA	95
multicenter, double-blind, randomized, placebo-controlled	*L. gasseri* SBT2055 20g/d	12 weeks	reduced abdominal visceral and subcutaneous fat, body weight, BMI, waist and hip circumferences, body fat mass	96
randomized, double-blind, placebo-controlled	*Bifidobacterium* breve B-3 5 × 10^{10} CFU/ml	12 weeks	decreased body fat mass	97
multicenter, randomized, double-blind, placebo-controlled	fermented milk (FM) containing *B. lactis* GCL2505 8 × 10^{10}CFU/100g	12 weeks	decreased abdominal visceral fat	98
randomized, double-blind, placebo-controlled	Yoghurt NY-YP901 *Streptococcus thermophilus,* *L.* acidophilus, *B.* infantis & *B. breve* (CBG-C2), *E. faecalis* FK-23	8 weeks	decreased body weight and BMI LDL-C	99
a randomized, double-blind, Placebo- crossover	*L. amylovorus* 1.39 × 10^9cfu and *L. fermentum* 1.08 × 10^9	43-day phases	decreased body fat mass	100

ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein; BMI = Body mass index; SCFA, Short chain fatty acids; FFA = Free fatty acids.

maintaining homeostasis. A healthy human gut microbiota contains higher ratio of Bacteroides to Firmicutes which change according to development stage, diet, antibiotic treatment, infections, and age. To replenish such disturbed flora, FMT and use of probiotics can be an option. Probiotic strain used must pass essential criteria as given by WHO/FAO. FMT is used effectively to cure different types of infections. The worldwide problem of obesity can be successfully treated with probiotics; is now clinically proven. Probiotics also boosts our immune system by synthesizing vitamins and antimicrobial compounds. In order to stay healthy a balance of gut microbiota by consuming probiotics needs to be maintained.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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