Title: Probiotics as the potential biotherapeutics in the management of Type 2 Diabetes – Prospects and Perspectives

Authors: Harsh Panwar*, Hogarehalli Mallappa Rashmi*, Virender Kumar Batish and Sunita Grover

Molecular Biology Unit, Dairy Microbiology Division, National Dairy Research Institute, Karnal - 132001, Haryana, INDIA

Correspondence:
Sunita Grover, Ph.D
Principal Scientist,
Molecular Biology Unit, Dairy Microbiology Division,
National Dairy Research Institute, Karnal -132001, Haryana, India.
E-mail: sungro@gmail.com,
Tel No.:+91-184-2259100, Fax: +91-184-2250042

*First and second authors contributed equally

Abbreviations: T2D, Type 2 Diabetes; CVD, Cardio Vascular Diseases; GLP-1, Glucagon Like Peptide-1; HFD, High Fat Diet; NF-kB, Nuclear Factor kappa-B; LPS, Lipopolysaccharides; NGF, Nerve growth factor; TNF-α, Tumour Necrosis Factor-α; NIDDM, Non-insulin Dependent Diabetes Mellitus” NOD, Non-obese Diabetic; STZ, Streptozotocin, DCs, Dendritic cells; NK, Natural Killer.

Running Title: Probiotics for diabetes management

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Abstract:

Diabetes Mellitus is a looming epidemic worldwide, affecting almost all the major sections of the society causing burden on global health and economy. A large number of studies have identified a series of multiple risk factors such as genetic predisposition, epigenetic changes, unhealthy life style, altered gut microbiota that causes increased adiposity, β cell dysfunction, hyperglycemia, hypercholesterolemia, adiposity, dyslipidaemia, metabolic endotoxemia, systemic inflammation, intestinal permeability (leaky gut), defective incretins secretion, oxidative stress associated with T2D as a multifactorial disorder. Recent studies have proposed multi-factorial interventions including dietary manipulation in the management of T2D and are also recommended by many national and international diabetes associations. These studies are aimed at deciphering the Gut microbial influence on health and disease. Interestingly, results from several genomic, metagenomic and metabolomic studies have provided substantial information to target gut microbiota by dietary interventions for the management of T2D. Probiotics particularly lactobacilli and bifidobacteria have recently emerged as the prospective biotherapeutics with proven efficacy demonstrated in various in vitro and in vivo animal models adequately supported with their established multifunctional roles and mechanism of action for the prevention and disease treatment. The dietary interventions in conjunction with probiotics - a novel multifactorial strategy to abrogate progression and development of diabetes holds considerable promise through improving the altered gut microbial composition and by targeting all the possible risk factors. This review will highlight the new developments in probiotic interventions and future prospects for exploring probiotic therapy in the prevention and control of life style diseases like T2D.

Key Words: Type 2 diabetes, Multifactorial approach, Gut Microbiota, Probiotics
Introduction:

Over the past two decades, “Type 2 diabetes” (T2D) has become truly a larger public health issue with significant socio-economic burden on global health. The prevalence of type 2 diabetes continues to increase worldwide and by 2030, the number of people with type 2 diabetes is expected to reach 552 million [1]. Numerous patho-physiological studies identified several risk factors of type 2 diabetes, a multifactorial disorder caused by genetic, epigenetic, environmental and lifestyle related factors resulting in development of pathophysiological manifestations such as β cell dysfunction, hyperglycemia, hypercholesterolemia, adiposity, dyslipidaemia, metabolic endotoxemia, systemic inflammation, intestinal permeability, defective incretin secretion, ectopic fat storage and oxidative stress in the development of T2D [2,3] (Fig. 1). As a multifactorial disorder, T2D calls for a multifactorial treatment approach targeting multiple risk factors. Yet, less efforts have been made on integrated approaches targeting multiple risk factors.

The normal commensal gut microflora by and large is stably maintained in individuals and represent their distinct microbial fingerprints for optimal gut functionality. However, any disturbance in the structure, composition and proportion of the normal gut microbiota is indicative of some breach in the individual’s health status due to changes in the life style, food habits, exposure to stress (hostile environmental conditions, indiscriminate use of drugs), genetic predisposition and aberration in the immunity etc. resulting into onset of some medical conditions. Under these situations, restoring the disturbed gut microflora back to normal by applying innovative dietary strategies seems to be the viable proposition to reduce the severity of these medical conditions. In this context, probiotic interventions in the form of novel food formulations enriched with proven strains of probiotics could be very effective dietary strategy to manage chronic intestinal diseases and lifestyle metabolic inflammatory disorders like T2D, obesity and “Cardio Vascular Diseases” (CVD) etc. Probiotics are the “live micro-organisms which when administered in adequate amounts confer a specific health benefit on the host” [4]. They are currently the major focus of attention across the world due to their enormous health promoting functions which are highly strain specific. Lactobacilli and Bifidobacteria - the two key members of this group used extensively in the development of novel functional and health foods and other formulations, are now being explored as biotherapeutics in the management of several diseases. The efficacy of probiotic strains and their formulations against several gut related diseases such as diarrhoea, ulcerative colitis.
(UC), peptic ulcers, Inflammatory Bowel Diseases (IBD), Inflammatory Bowel Syndrome (IBS), Crohn’s Disease (CD), Colon cancer, atopic dermatitis and topical allergies etc. has been thoroughly investigated and well documented and proven through well designed in vitro, in vivo and human clinical trials [5]. However, the prospects of probiotic therapy in the management of life style diseases particularly T2D, obesity and CVD have not been explored fully as yet. There are only a handful of studies initiated in this regard that too with mixed responses. Nonetheless, some recent studies clearly point towards the antidiabetic prospects of probiotic based interventions, thereby, leaving ample scope to pursue more indepth studies to reach some consensus on the efficacy and effectiveness of probiotic therapy in T2D. This review is specifically aimed to showcase the status of recent developments in probiotic research and leads that emerged from the outcome of various studies on probiotic interventions against life style diseases particularly with regard to T2D and future strategies to be initiated on these lines.

**Diabetes Mellitus: The silent killer**

Diabetes Mellitus, the looming epidemic of the 21st century poses major threat to global health and economic development. The recent study by International Diabetes Federation has provided the global map on diabetes after analyzing 170 appropriate data sources from 110 countries for 2011 and 2030 [1]. As per this study, the current figure of 366 million people with diabetes recorded in 2011 is expected to rise to 552 million by 2030. This increasing trend in diabetic population is being witnessed in almost every country and more than 70% of people with diabetes live in low and middle-income countries. Amongst the middle income countries, the prevalence of diabetes is higher in upper middle-income countries (10.1%) than in lower middle-income countries (8.6%) driven by an increase in the adult population, urbanization and economic development. China and India have emerged as the leading countries afflicted with diabetes. T2D is the most common form of diabetes and accounts for almost 90% of all diabetes in high-income group and may account for an even higher percentage in low and middle-income countries and put a huge burden on healthcare agencies and governments [6,7]

Both type 1 and T2D are closely linked to CVD - the main cause of death in people with diabetes. The mortality rate of diabetes varies sharply with the prosperity of the country. In 2011, the disease caused more than 3.5 million deaths in middle-income countries, of
which more than 1 million were in China and just less than a million were in India. Approximately 1.2 adults die of a diabetes-associated illness per 1,000 cases in 2011 in low- and middle-income countries.

Keeping in view the alarming increase in the incidence and prevalence of diabetics in India, the World Health Organization has declared India as the Diabetic Capital of the World [8]. Indians (40-59 yrs age groups) are particularly prone to diabetes due to several environmental factors viz. psychological stress, socio-economic status, life style changes, acquired obesity along with their genetic and phenotypic architecture and poor immunity. As a result of alarming socio-economic impact of this disease and its other related health implications on a vast majority of Indian population, diabetes has now become a major challenge from the Indian perspective and requires immediate attention for taking appropriate measures immediately for its eradication at National level on priority before it becomes a catastrophe beyond control.

**Type 2 diabetes: A Multifactorial catastrophe**

As mentioned above, T2D is a polygenic disorder. Advances in genetic epidemiology have increased our understanding of T2D. The recent developments in genome-wide association studies (GWAS) in many thousands of samples from different populations and meta-analyses across many studies have identified genes responsible for T2D susceptibility. A recent large and comprehensive analysis of 50,000 genetic variants across 2,000 genes linked to cardiovascular and metabolic function has identified the related genes and genetic variants associated with T2D among multiple ethnic groups [9]. This decoding of genetic background of T2D serves multiple goals ranging from expanding our knowledge on the disease pathogenesis and identifying future targets for personalized drug development.

In addition to genetic predisposition, environmental and lifestyle factors also contribute to the pathogenesis of T2D. Epigenetic changes may provide the link for translating environmental exposures into pathological mechanisms. Studies from the last five years explored the epigenetic mechanisms in the development of T2D. The recent comprehensive DNA methylation profiling of pancreatic islets from T2D uncovered 276 CpG loci affiliated to promoters of 254 genes displaying significant differential DNA methylation in diabetic islets [10].
Like genetic and epigenetic changes, gut microbiota is also considered as one of the most important environmental factor in the development and management of T2D by impacting host physiology and metabolism.

**Gut microbiota and Diabetes**

The gut microbiota is now virtually recognised as a complex whole organ consisting of incredible amount of at least \(10^{14}\) bacteria of thousands of species. The collective genome of the entire gut micorbiota designated as ‘microbiome’ exceeds the human nuclear genome by at least 100 times [11]. Over the last five years, an intense research effort has been made to understand the crucial role of gut microbiota in health and disease by various metagenomic and metabolomic studies using with high-throughput sequencing technologies, mass and nuclear magnetic resonance (NMR) spectroscopy that enabled in-depth analysis of gut microbial compositional changes, structural elements and metabolites in host physiology and metabolism under different conditions [12,13,14]. Gut microbiota has also been reported to play a pivotal role in pathogenesis of T2D, obesity and related inflammatory metabolic disorders. Accumulating evidence supports the new hypothesis that metabolic diseases like obesity and T2D develop due to low grade, systemic and chronic inflammation by disruption of the normal gut microflora induced by dietary intake of high fat and fructose diet. In this context, nutrition can play a significant role in directly modulating our microbiomes and health phenotypes. Poorly balanced diets can shift the gut microbiome from healthy microflora towards unhealthy ones with the predominance of pathogenic microflora in chronic disease [15]. Hence, the microbiome data of human gut under different conditions could serve as the important resource material for exploring customized novel dietary based strategies in the management of specific gut related diseases.

The gut microbiota of human adults with T2D studied by high-throughput, tag-encoded amplicon pyrosequencing of the V4 region of the 16S rRNA gene showed decreased proportions of phylum *Firmicutes* and *Clostridia*, increased proportions of *Betaproteobacteria* and increased ratios of *Bacteroidetes* to *Firmicutes* and *Bacteroides-Prevotellagroup* to *C. coccoides - E. rectale* group in correlation with plasma glucose concentration [16] (Fig. 2.) In addition, numerous metagenomic studies also investigated the gut microbial changes in obesity - the main precursor in the development of T2D in various animal and human studies [17]. In these human studies, the gut microbiota showed variable
profiles where bacteroidetes-related taxa have been reported to increase, remain neutral or decrease after weight loss [18,19,20].

Interestingly, the recent studies have revealed the compositional changes in the group of bifidobacteria and lactobacilli during the onset of insulin resistance in “High Fat Diet” (HFD) mice. The gut microbial profile of these studies recorded reductions in *Bifidobacterium* spp. and *Lactobacillus* spp. with increased plasma “Lipopolysaccharides” (LPS) that caused metabolic endotoxemia via NF-κB “Nuclear Factor kappa B” activation on the molecular onset of Insulin resistance [21,22]. In addition, relationship among gut microbial changes, gut permeability and metabolic endotoxemia was also studied in HFD mice which revealed reduced proportions of lactobacilli in gut microbial profile, reduced transepithelial resistance and increased metabolic edotoxemia in HFD mice [23].

Currently, lot of attention is duly being paid to elucidate the role of gut microbiota in energy homeostasis through microbe-gut-brain axis. Changes in gut microbiota that sends altered enteroendocrine signals through the secretion of gut hormones to the Central Nervoos System (CNS) have been reported by several investigators. The gut hormones such as GIP (Glucose Dependent Insulinotropic Peptide), GLP-1 “Glucagon Like Peptide -1”, GLP-2 (Glucagon Like Peptide -2), PYY that regulates energy homeostasis through insulinotropic, satietogenic properties that affect β-cell mass and function, energy intake, nutrients absorption and energy storage, insulin secretion have been investigated in relation to food intake. In this context, recent studies proposed prebiotics as a safe and cost-effective dietary strategies for modulating the gut microbiota to promote improved host:bacterial interactions in obesity and insulin resistance [24,25,26].

Furthermore, the role of gut microbial metabolites in glucose homeostasis is of considerable research interest in the field of metabolomics. In this context, the role of gut microbial metabolites such as Short Chain Fatty Acides (SCFA) in the down regulation of inflammatory markers and upregulation of gut hormone secretion via FFAR2 (Free Fatty Acid Receptor 2) activation were studied in relation to glucose homeostasis [27,28]. Other gut flora associated metabolites such as hippuric acid, methylxanthine, methyluria acid and 3-hydroxyhippuric acid were reduced considerably in individuals with impaired glucose tolerance. In this study, UPLC-qTOF-MS approach was established and validated by metabonomic analysis of clinical samples like serum and urine [29,30]. These metabonomic
studies further supported the role of gut microflora in pathogenesis of diabetic and pre-diabetic states. Gut microbiota associated metabolite biomarkers were decreased in urine samples of individuals with impaired glucose tolerance indicating important role of gut microbiota in energy metabolism and immune function of host [31].

In another study that included 40 diabetic and 60 control individuals, diabetic individuals could be differentiated from healthy individuals on the basis of presence of secondary metabolites of bile acids - the products of gut microflora in diabetic individuals. Cholate was more frequently detected in control or healthy individuals while its secondary product, deoxycholate was found to be more prominent in diabetic group. The outcome of the study clearly indicated alterations in bile acid pool and their biosynthetic pathway in diabetic patients and ascribed the same to the role of altered gut microflora as a result of higher rate of conversion of primary bile acids to secondary bile acids [32].

Hence, the data that emerged from various studies by different investigators have provided sufficient evidence that modulating the gut microbiota through dietary interventions may contribute in the prevention and control of inflammatory metabolic disorders including T2D and obesity etc.

**Probiotics as multifactorial biotherapeutics**

Probiotics are currently the subject of intensive investigation as the prospective natural biotherapeutics due to their enormous health promoting potentials and inbuilt ability to fight specific diseases including metabolic syndrome such as T2D. Since, many of the novel multifactorial physiological functions of putative probiotics are highly strain specific, judicious pre selection of appropriate probiotic strains based on the expression of functional biomarkers associated for a particular medical condition is extremely crucial to demonstrate their functional efficacy. The interest and scope for taking new R&D initiatives on probiotics to fully explore their biotherapeutic potentials in the management of T2D and other life style diseases have dramatically increased across the world over the recent years. The antidiabetic efficacy of probiotics and probiotic preparations has been investigated in different independent studies in established *in vitro* cell lines and animal models and validated by double blind placebo controlled randomized clinical trials in target human population with mixed responses. The major significant findings of these studies on exploring the efficacy of
probiotics in the management of T2D both under *in vitro* and *in vivo* conditions are briefly reviewed below.

**Probiotic efficacy in *in-vitro* cell line models**

The multigenic approach of using probiotics as biotherapeutics in the management of type T2D has been evaluated in various cell line models for their hypoglycemic, anti-inflammatory, antioxidative, insulinotropic and satietogenic effects (Fig. 3.). In one of the studies carried out by Ma *et al.*, 2004, the human colonic adenocarcinoma T84 and HT29 were challenged with live and killed *Lactobacillus reuteri* cells and the expression of “Nerve growth factor” (NGF) and anti-inflammatory molecules were analysed. The live probiotic bacterial cells were able to upregulate the expression of NGF, inhibited the cellular accumulation and secretion of IL-8 induced by “Tumour Necrosis Factor-α” (TNF-α). However, expression of IL-10 remained unaffected in both T84 and HT29 cells [33]. In another study, HeLa cell line was explored as a model to investigate the activation of NF-kB signalling in the progression of inflammation [34]. In this study, preincubation of the HeLa cells with live *Lactobacillus reuteri* cells for 1-2 hrs prevented translocation of NF-kB to the nucleus, inhibited degradation of IkB, prevented expression of various pro-inflammatory cytokines under NF-kB regulation. In a previous study, Neish *et al.*, 2000 [35], reported that heat inactivated and conditioned culture media lacked this NF-kB attenuating activity, indicating that bacterial-epithelial cell contact was required to bring about the positive effect.

Since, gut hormones play an important role in glucose and energy homeostasis through their insulinotropic and satietogenic mechanisms, the insulinotropic effect of genetically engineered *Escherichia coli* Nissle 1917 for GLP-1 and transcriptional activator PDX-1 secretion was examined in Caco-2 cell line. The cell free culture supernatant of engineered strain stimulated the epithelial cells and caused the secretion of insulin corresponding to blood insulin concentration of 164 pmol/ml to 164 nmol/ml respectively for $10^6$ to $10^9$ cfu/ml survivability of engineered strain under unoptimized conditions. They reported that optimized conditions may further improve the ability of engineered strain to increase insulin secretion to the magnitude as would be required for normal metabolism [36].

Furthermore, in a recent study conducted by Paszti-Gere *et al.* [37], it was reported that oxidative stress that imposed damage to insulin-secreting β-cells was prevented by metabolites of *Lactobacillus plantarum* 2142. In this study, the spent culture supernatant
[SCS] of *Lactobacillus plantarum* 2142 lowered the oxidative stress-induced overexpression of proinflammatory cytokines IL-8 and TNF-α in IPEC-J2 cell line.

**Probiotic efficacy in animal models**

Different animal models have been extensively explored to study the multiple mechanisms of probiotics in the management of diabetes. Initially, antidiabetic effects of oral administration or diet supplementation of heat killed *Lactobacillus casei* were studied in three different mice models such as: 1) “non-insulin-dependent diabetes mellitus” (NIDDM) mice model using KK-Ay mice 2) insulin-dependent diabetes mellitus model i.e NOD “Non-obese diabetic mice” 3) Alloxan-induced diabetes in mice. In all these studies, the oral administration (0.05%) or diet supplementation (0.1%) of heat killed cell of *Lactobacillus casei* reduced the plasma glucose level and occurrence of diabetes [38,39,40].

In another study, using neonatal STZ “streptozotocin”-induced diabetic (n-STZ) rats, feeding of diet containing *Lactobacillus rhamnosus* GG for a period of 9 weeks (from the age of 9 weeks to 18 weeks) lowered the blood hemoglobin level and improved glucose tolerance in comparison to control group fed with common diet. In the *Lactobacillus rhamnosus* GG treatment group, the serum insulin level at 30 min after glucose loading was significantly higher than in the control group (p<0.05) [41]. Working on similar lines, Calcinaro *et al.* [42] reported that the feeding of VSL#3 decreased β-cell destruction and inflammation in NOD mice. This prevention was associated with increased IL-10 secretion in pancreas, Peyer’s patches and spleen that improved inflammation and prevented β cell destruction in the treated group.

In a different study, the feeding of probiotic dahi containing $10^8$ cfu/g of *Lactobacillus acidophilus* NCDC14 and *Lactobacillus casei* NCDC19 significantly decreased the blood glucose and glycosylated hemoglobin, free fatty acids and triglycerides in fructose-induced diabetic rats [43]. The feeding of the same probiotic dahi to the STZ-induced rats significantly suppressed STZ-induced oxidative damage in pancreatic tissues by inhibiting the lipid peroxidation, formation of nitric oxide and improved the antioxidant potential glutathione, superoxide dismutase, catalase and glutathione peroxidase. These results suggest that the oral administration of probiotic dahi improved the risk factors such as hyperglycemia, dyslipidemia and oxidative stress in diabetic rats [44]. Similarly, in a parallel study, it was revealed that probiotic pre-treatment with a mixture of *Lactobacillus acidophilus*,

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*Bifidobacterium lactis* and *Lactobacillus rhamnosus* reduced the blood glucose and improved the bioavailability of gliclazide, a second generation sulphonylurea used to treat non-insulin dependent diabetes mellitus in alloxan induced diabetic rats [45].

Probiotics have also been reported to exert their antidiabetic effects against insulin resistance by increasing liver “Natural Killer” T (NKT) cells. Liver is the principal target organ responsible for inflammation mediated insulin resistance, where inflammation is regulated through NKT cells by balancing the production of pro-inflammatory and anti-inflammatory cytokines. Depletion of NKT cells in liver led to over production of pro-inflammatory cytokines and HFD is known to induce depletion of hepatic NKT cells resulting into development of insulin resistance. However, HFD induced depletion of NKT cells in male wild type C57BBL-6 mice was found to be significantly improved on oral administration of VSL#3 probiotic preparation for 4 weeks after mice had been on HFD for 8 weeks. This probiotic treatment also reduced the weight, improved insulin resistance and inflammation by modulating TNF-α expression and reducing NF-kB binding activity [46].

In two subsequent studies carried out at the same time, the probiotic treatment with *L. plantarum* DSM 15313 and *Lactobacillus reuteri* GMNL-263 was found to lower the blood glucose and glycosylated hemoglobin respectively in HFD C57BL/6J mice and STZ-induced diabetic rats [47,48].

Differential stimulation of dendritic cells by probiotics has been the subject of intensive discussion to understand the exact mechanism involved in conferring the therapeutic effect of probiotics against T2D. In this context, “dendritic cells” (DCs) from NOD were stimulated with three different strains of lactobacilli including *Lactobacillus casei*, *Lactobacillus reuteri* and *Lactobacillus plantarum* for 24hrs. Out of the three strains tested, *Lactobacillus casei* was found to induce DCs to produce highest level of IL-10 and lowest level of IL-12 expression. When the *Lactobacillus casei* stimulated dendritic cells were transferred again to NOD mice, they demonstrated a significant delay in diabetes incidence [49]. In a related study, *Bifidobacterium longum* CGMCC NO.2107 supplementation in HFD was reported to reduce the metabolic endotoxin (LPS) concentrations of plasma, improved intestinal inflammation and increased expression of intestinal Reg I as a regulatory growth factor in a HFD rats [50].
Interestingly, a recent study by Amar et al. [51] investigated the effect of probiotic treatment on mucosal dysbiosis, bacterial translocation and glucose metabolism using various mouse models. The results showed that, increased bacterial translocation was prevented in mice lacking the microbial pattern recognition receptors Nod1 or CD14. However, it was increased in Myd88 knockout and ob/ob mouse under the same conditions. The probiotic treatment with *Bifidobacterium animalis* subsp. *lactis* 420 reduced bacterial translocation to mesenteric adipose tissue, decreased the expression of major pro-inflammatory cytokines TNF-α, IL-1β, PAI-1 and IL-6 in mesenteric adipose tissue, liver and muscle, improved the insulin sensitivity and fasting hyperinsulinemia in treated HFD mice when compared with untreated HFD control mice [51]. The major outcome of these studies have been recorded in Table 1.

**Probiotic efficacy in human clinical trials**

Although, the efficacy of probiotics as such or other formulations in food formats against diabetes has been extensively studied and demonstrated in appropriate human epithelial cell lines and animal models, the purported antidiabetic effects of probiotics have not been adequately validated in the target human population. This is precisely because very few attempts have been made in this regard to explore efficacy of probiotic therapy in diabetes on the affected human subjects. There are only a handful of published reports available in this context. Nonetheless, the leads that emerged from *in vitro* cell culture and *in vivo* animal studies described in the previous sections clearly demonstrate that probiotics do have the potential to control diabetes and hence their antidiabetic effects can be exploited in the treatment of T2D effectively. Hence, in the backdrop of the present scenario whatever information is available in this particular context is reviewed here.

As oxidative stress, hypercholesterolemia and altered lipid profile play a major role in the pathogenesis and progression of diabetes, randomized, double-blind, placebo controlled clinical trials were conducted in separate studies by the same investigators to assess the effects of probiotic on blood glucose, antioxidant status and lipid profile in T2D. In these studies, patients with T2D mellitus were assigned into two groups. The patients in the probiotic intervention group consumed 300 g/d of probiotic yogurt containing $10^6$ cfu/ml *Lactobacillus acidophilus* La5 and $10^6$ cfu/ml *Bifidobacterium lactis* Bb12 and those in the control group consumed 300 g/d of conventional yogurt for 6 wk. The probiotic intervention
groups in these studies showed significant decrease in fasting blood glucose and hemoglobin A1c (P < 0.05), increased the erythrocyte superoxide dismutase and glutathione peroxidase activities and total antioxidant status (P < 0.05) compared to control group. The probiotic yoghurt consumption also decreased the total cholesterol by 4.54%, LDL-C by 7.45% in intervention group. The total cholesterol:HDL-C ratio and LDL-C:HDL-C ratio as atherogenic indices were significantly decreased in the probiotic group compared with the control group [52,53]. From the above two studies, it is quite evident that consumption of probiotic yoghurt significantly improved the antioxidant status and lipid profile in the intervention group and presented multigenic approach for the management of T2D.

Recently, a randomized, double-blind, placebo-controlled study conducted on twenty volunteers (ten for placebo group and ten for symbiotic group), aged 50 to 60 years, over a total test period of 30 days to study the effect of a symbiotic drink (a preparation with a combination of both probiotics and prebiotics) on glycemia and cholesterol levels in elderly people with T2D mellitus. The results of the symbiotic group that consumed $10^8$ cfu/ml of *Lactobacillus acidophilus*, $10^8$ cfu/mL *Bifidobacterium bifidum* and 2 g oligofructose showed a significant increase (P < 0.05) in HDL (High Density Lipoprotein) cholesterol, non-significant reduction (P > 0.05) in total cholesterol and triglycerides and a significant reduction (P < 0.05) in fasting glycemia. However, no significant changes were recorded in the placebo group [54].

In another recent study conducted on similar lines to assess by Shao et al. [55], the effect of microbiological and immunological enteral nutrition in patients with gastrointestinal cancer complicated with diabetes mellitus was investigated. In this study, 67 patients with gastrointestinal cancer complicated with diabetes mellitus were randomized into the treatment group (n=33, enteral nutrition with probiotics, glutamine, and fish oil) and the routine group (n=34, regular enteral nutrition). Fasting blood glucose (FBG), insulin (FINS), number of lymphocytes including CD3(+)T cell, CD4(+)T cell, CD8(+)T cell, CD4(+)/CD8(+) and NK cells of treated and routine groups were measured on the day before surgery and postoperative day 3 and 7. Insulin resistance index (InHOMA-IR) was also calculated by using the homeostasis model assessment (HOMA) for both groups and the supplementary data on incidence of nosocomial infections, intestinal function recovery time and length of hospital stay were also collected during the study. The enteral intervention with probiotics, glutathione and fish oil lowered fasting insulin and Insulin resistance index, increased the
number of CD4 and NK cells compared to routine group. There were however no significant differences in nosocomial infection and intestinal function recovery between the two groups. Moreover, the length of hospital stay [(17 ± 3.8) d vs (21 ± 4.2) d] was significantly reduced from 21 to 17 shorter in the treatment group.

In a different but related study carried out by Luoto et al. [56], the impact of maternal probiotic-supplemented dietary counseling during pregnancy on colostrum adiponectin concentration in neonatal metabolism, nutrition, and immune function was recorded in a randomized, placebo-controlled study. In this study, 256 pregnant women were randomized into three study groups: dietary intervention with probiotics (diet/probiotics), with placebo (diet/placebo) and a control group (control/placebo). The combination of Lactobacillus rhamnosus GG and Bifidobacterium lactis were used in probiotic intervention group. Dietary intake was evaluated by food records at every trimester of pregnancy. Breast milk samples were collected after birth (colostrum) for adiponectin concentration analysis. As a result, the dietary intervention with probiotics significantly increased the colostrum adiponectin concentration when compared with control (12.7 ng/ml vs. 10.2 ng/ml, P=0.024). This improved adiponectin concentration is a measure of neonatal metabolic homeostasis and is also indicator of reduced chances of gestational diabetes.

Notwithstanding the positive efficacy of probiotics on diabetic patients as obtained form the aforesaid studies, conflicting results were also recorded in one clinical trial while assessing the effects of probiotic on attenuating systemic inflammation and improving insulin sensitivity. In this randomized, double-blinded, clinical trial, the commercial probiotic Lactobacillus acidophilus NCFM was used as intervention together with placebo in a group of forty-five males for a period of 4 weeks. In this case, even after 4 weeks of intervention, no changes in the expression of baseline inflammatory markers and the systemic inflammatory response were observed, thereby, indicating ineffectiveness of the probiotic therapy in diabetic patients [57]. However, this inconsistency in results could be attributed to heterogeneity with regard to probiotic strain and population demographics. As we mentioned earlier, biotherapeutic effects of probiotics are both strain and host specific and thus should be designed for a specific group with a specific strain which is the hallmark of probiotic functionality.
Conclusion and Future Perspectives

Hence, based on the outcome of these limited studies, it would not be proper at the moment to draw a clear cut demarcation on probiotic functionality to claim efficacy of probiotic therapy in diabetes in the target human population with conviction due to lack of sufficient scientific evidence in support of the health claims to prove this hypothesis. Nevertheless, there is a good reason to believe from the interesting leads that emerged from extensive in vitro cell line and animal studies that probiotics do have the potential to prevent and reduce the severity of T2D diabetes and other metabolic syndromes possibly through modulating gut microbiota, immune response and other mechanisms. However, since T2D has a multigenic aetiology associated with multiple risk factors, the exact mechanism of specific target based action of the antidiabetic effects of probiotics at molecular level have still to be worked out and thus better understanding of probiotic action is important to exploit their biotherapeutic potential maximally in the management of this chronic disease. Thus, future research should be directed to move beyond profiling human gut microbial species and focus on functional properties of probiotics to ensure optimal health benefits for the host. Furthermore, rapid advancements made during the last few years in genomics, transcriptomics, proteomics, metabolomics, metabonomics and nutrigenomics in the backdrop of conclusion of human whole genome project, progression of ongoing human microbiome projects and the availability of whole genome sequences of several proven commercial probiotic strains as public domain are likely to generate very useful information that could further widen the scope and future prospects of probiotics as natural food supplements and biotreatments specifically targeted against diabetes mellitus. In this context, concept of designer probiotics suitably manipulated by applying advanced genetic engineering and PCR techniques specifically for diabetic population is already on the cards and it would soon emerge as the future therapy for the management of diabetes. The evaluation of probiotic efficacy in free living target human population is however far more complex than under controlled experimental conditions since many of the confounding factors such as use of antibiotics, diet, endotoxin content of ingested food and frequency of meals along with physical activity may also affect gut microbiota, energy balance, glucose metabolism, insulin secretion and other gut hormones like incretins. Hence, understanding these factors may enable the researchers to design future trials and better understand the relative effect of probiotics on diabetes. It is, therefore, imperative to carry out more
extensive well designed metabolic clinical studies involving a wide range of target human population with clearly defined proven probiotic strains or their formulations to reach some meaningful conclusion as far as their efficacy against T2D is concerned. Thus, the prospects of exploring probiotics and their formulations as antidiabetic therapy are quite bright and could prove to be an asset in the near future by providing adequate relief to the mass diabetic population across the world.

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Table 1: Antidiabetic efficacy of probiotic interventions in animal models

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<td><em>L. rhamnosus</em> GG</td>
<td>Neonatal streptozotocin induced diabetic rats</td>
<td>Lowered blood HbA1, suppressed oxidative stress, improved glucose tolerance and enhanced insulin secretion</td>
<td>41</td>
</tr>
<tr>
<td>VSL#3</td>
<td>NOD mice</td>
<td>Decreased rate of β-cell destruction with increased production of IL-10</td>
<td>42</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em> NCDC14 and <em>Lactobacillus casei</em> NCDC19</td>
<td>Fructose-induced diabetic rats</td>
<td>Significantly lowered the blood glucose and HbA1c levels and free fatty acids and triglycerides</td>
<td>43</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em> NCDC14 and <em>Lactobacillus casei</em> NCDC19</td>
<td>STZ-induced diabetic rats</td>
<td>Improved diabetic dyslipidemia, inhibited lipid peroxidation and nitrite formation</td>
<td>44</td>
</tr>
<tr>
<td>Probiotic mixture <em>Lactobacillus acidophilus</em>, Lactobacillus casei NCDC19</td>
<td>Alloxan induced diabetic rats</td>
<td>Reduced blood glucose by improving gliclazide bioavailability in diabetic rats</td>
<td>45</td>
</tr>
<tr>
<td><strong>Bifidobacterium lactis</strong> and <strong>Lactobacillus rhamnosus</strong></td>
<td>HFD C57BBL-6 mice</td>
<td>Increased NKT cells, improved insulin resistance, reduced inflammation</td>
<td>46</td>
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<tr>
<td><strong>L. plantarum DSM 15313</strong></td>
<td>HFD C57BL/6J mice</td>
<td>Lowered plasma glucose levels</td>
<td>47</td>
</tr>
<tr>
<td><strong>Lactobacillus reuteri GMNL-263</strong></td>
<td>STZ-induced diabetic rats</td>
<td>Reduced glycated hemoglobin and blood glucose</td>
<td>48</td>
</tr>
<tr>
<td><strong>L. casei, L. reuterii, L. plantarum</strong></td>
<td>Dendritic cells from NOD mice stimulated with probiotic lactobacilli for 24hrs</td>
<td><em>L. casei</em> induced DC to produce high level of IL-10, delay in diabetes incidence</td>
<td>49</td>
</tr>
<tr>
<td><strong>Bifidobacterium longum</strong> (BIF CGMCC NO. 2107)</td>
<td>HFD rats</td>
<td>Reduced metabolic endotoxin (LPS) concentrations and intestinal inflammation and increased the expression of intestinal Reg I as a regulator of growth factor</td>
<td>50</td>
</tr>
<tr>
<td><strong>Bifidobacterium animalis subsp. lactis 420</strong></td>
<td>C57bl6, ob/ob, CD14&lt;sup&gt;−/−&lt;/sup&gt;, ob/obxCD14&lt;sup&gt;−/−&lt;/sup&gt;, Myd88&lt;sup&gt;−/−&lt;/sup&gt;, Nod1&lt;sup&gt;−/−&lt;/sup&gt;, Nod2&lt;sup&gt;−/−&lt;/sup&gt; with normal chaw diet and HFD</td>
<td>Reversed bacterial translocation process, improved animals inflammatory and metabolic status</td>
<td>51</td>
</tr>
</tbody>
</table>
Figure 1. Type 2 Diabetes - Multifactorial Catastrophe
Figure 2. Role of Gut Microbiota in the Development and Control of Type 2 Diabetes
Figure 3. Probable Mechanisms of Probiotics Action in the Management of Type 2 Diabetes