Potential Antidiabetic Activities of Probiotic Strains, *L. acidophilus* and *L. bulgaricus* against Fructose-Fed Hyperglycemic Rats

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**Abstract**

**Background:** Deregulation of the gut microbiota results in various pathological disorders such as diabetes, inflammation, cancer, dyslipidemia etc. Modulation of intestinal microbiota by probiotics may facilitate the management of a number of clinical conditions of diabetes. **Methods:** The present study was designed to investigate the effect of feeding low-fat probiotic yogurt containing *L. acidophilus* and *L. bulgaricus* on fructose-fed hyperglycemic rats. Yogurt containing *L. acidophilus* or *L. bulgaricus* (9.5 × 10⁹ cfu/rat/day) alone or in combination of both strains was supplied orally for 8 weeks concurrently with 20% fructose solution. Fasting blood glucose (FBG), oral glucose tolerance test, glycosylated hemoglobin (HbA1c), lipid profiles from blood and histopathological study of liver tissues were analyzed to evaluate anti-diabetic effect. Statistical analysis was done by Graph Pad Prism software. Values at *p* < 0.05 were considered statistically significant. **Results:** Administration of *L. acidophilus* or *L. bulgaricus* alone or in combination of both to hyperglycemic rats decreased serum FBG, onset of glucose intolerance, HbA1c, total cholesterol, triglycerides, LDL and VLDL-cholesterol, increased HDL-cholesterol levels significantly and preserved antioxidant pool such as activities of superoxide dismutase, catalase etc. Probiotic administration also prevented/repaired the oxidative damage of liver tissues. **Conclusion:** In conclusion, administration of yogurt containing *L. acidophilus* or *L. bulgaricus* that balanced the intestinal microbiota can prevent or lower risks of type-2 diabetes and its related complications.

**Keywords**

Probiotics, Fructose-Fed Rats, Hyperglycemia, Dyslipidemia
1. Introduction

Diabetes is a condition of multifactorial origin, involving several molecular mechanisms related to the intestinal microbiota for its development [1]. Environmental factors, such as a fat-enriched diet and a sedentary lifestyle, are the causes of the great prevalence of obesity and type 2 diabetes in the population [2]. According to International Diabetes Federation Diabetes Atlas 2017, diabetes affects more than 425 million people worldwide and the number of people with diabetes may rise to 629 million in 2045. At the same time, a further 352 million people with impaired glucose tolerance are at high risk of developing diabetes [3]. It has been estimated that globally as many as 212.4 million people or half (50.0%) of all people 20 - 79 years with diabetes are unaware of their disease. According to WHO—diabetes country profiles 2016 [4], in Bangladesh, the prevalence of diabetes is 8.0%, which is alarming for the population as well as for government. Scientific evidences suggest that increased inflammatory stress is related to molecular mechanisms leading to insulin resistance, and the intestinal microbiota interacts with environmental factors and susceptible genetic factors, contributing to the development of diabetes [5] [6] [7]. Deregulation of these microbiota component results in various pathological disorders such as cancer, diabetes, cardiovascular diseases, dyslipidaemia etc. [8] [9]. Modulation of intestinal microbiota by probiotics may facilitate the management of a number of clinical conditions [10]. Probiotics may be involved in the maintenance of a healthier gut microbiota that would be beneficial for the management of diabetes. The present study aimed to investigate the effect of probiotic, lactobacillus (L) strains, L. acidophilus and L. bulgaricus in the form of functional food, yogurt, against fructose-fed hyperglycemic rats.

2. Materials and Methods

2.1. Reagents and Materials

“Probio” capsule manufactured by Square Pharmaceuticals Ltd., Bangladesh. MRS agar media (Hi Media Laboratories, India), Low fat milk (Pran company ltd., Bangladesh), total cholesterol triglyceride and high density lipoprotein assay kits (Cell Biolabs, Inc., San Diego), blood sugar assay kits (One touch ultra, California, United States) and other reagent grade necessary chemicals were purchased from elsewhere.

2.2. Collection of Commercial Probiotic Sample and Culture in MRS Media

Commercial probiotic sample “Probio” (0.5 gm/capsule) manufactured by Square Pharmaceutical Company Ltd., Bangladesh was collected from the local market. According to manufacturer, Probio contained Lactobacillus acidophilus, Lactobacillus bulgaricus and Bifidobacterium bifidum. Stock solution of probio capsule was cultured in MRS agar media by pour plate culture method as described previously [11]. Briefly, 300 µL of inoculum from $1 \times 10^6$ dilution of
stock solution was mixed with MRS agar medium and transferred into anaerobic jar with an anaerobic kit which provided CO₂ and the plates were then incubated 37°C for 48 hours. The colonies on the MRS plates were then counted and recorded under J2 Colony counter. MRS medium allowed the growth of lactic acid bacteria (LAB) only.

2.3. Characterization of Pure Cultures and Preparation of Yogurt

Two different colonies were selected from previously cultured petri dish and transferred into two new MRS plates and incubated at 37°C for 48 hrs. After the incubation period, the plates with no contamination were checked for their growth patterns, morphology. The plates with no contamination were selected as pure cultures and designated as LAB A and LAB B. Morphological, physiological and biochemical examination of LAB A and LAB B were performed according to the previous report [11] in order to characterize the strains. Yogurt was prepared by inoculating probiotic strains LAB A and LAB B separately in low fat UHT liquid milk at 37°C for 48 hours.

2.4. Experimental Animal

Thirty, white male Wistar rats, 6 weeks of age were purchased from animal centre, department of pharmacy, Jahangirnagar University, Dhaka, Bangladesh. The rats were kept in polypropylene rat cages throughout the study. They were housed in a temperature-controlled (24°C ± 1°C) room and standardized light/dark (12/12 hour) cycles. They were acclimated for 1 week and fed with standard rat diet and tap water ad libitum. The experimental protocols were approved by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) at the institute of biological sciences, University of Rajshahi, Bangladesh.

2.5. Doses and Treatment

Hyperglycemia model was developed in animals by giving 20% fructose solution in drinking water ad libitum for a period of 8 weeks [12] [13] [14] with concurrent administration of yogurt containing lactobacillus strains (ca 9.5 × 10⁹ cfu/rat/day).

2.6. Experimental Design

Healthy rats with a mean body weight of 100 - 130 g were subdivided into six groups of five animals in each group.

Group 1 (Normal control) received standard diet and normal drinking water ad libitum.

Group 2 (Diabetic control) received standard diet and 20% fructose solution ad libitum.

Group 3 (Positive control) received standard diet, 20% fructose solution and metformin 150 mg/kg body weight/day [15] [16].
Group 4, 5 and 6 (Treatment group) received standard diet, 20% fructose solution and yogurt, containing LAB A, LAB B, and LAB A + B, respectively.

2.7. Monitoring Blood Glucose Level during Experimental Period

The blood glucose level of the rats was observed weekly. Blood samples were collected from the tail vein and glucose concentration was determined with a glucometer (One touch ultra, California, United States).

2.8. Oral Glucose Tolerance Test (OGTT)

OGTT was performed after the eight weeks of the experimental period according to the protocol described in a previous report [17]. After an overnight fast (12 h), rats were orally dosed with a D-glucose solution (2.0 g/kg of body weight) by orogastric gavage and blood glucose levels were measured at 0, 30, 60, 90, and 120 minutes after the oral dosing of glucose by glucometer.

2.9. Blood and Liver Tissue Sampling

At the end of the experimental period, rats were euthanized by chloroform anesthesia and then cervical decapitation and dissection. Blood samples were collected and liver was excised for biochemical as well as for histopathological studies. Blood sample was collected from jugular vein of each animal (5 ml) in a centrifuge tube and left to clot at room temperature for 45 min. Sera were separated by centrifugation at 4000 r.p.m. at 4°C for 10 min and kept frozen at −20°C for various physiological and biochemical analyses. Liver from each animal was excised after dissection. One part was fixed in buffered formalin for 24 h, trimmed and then transferred into 70% alcohol for histopathological examination. 0.5 g was homogenized in 5 ml phosphate buffered solution (PBS) using polytron homogenizer (IKA, Japan).

2.10. Biochemical Analyses

Serum total cholesterol, triglyceride, and high-density lipoprotein (HDL) levels were determined colorimetrically by using assay kits according to the manufacturer protocol (Cell Biolabs, Inc., San Diego). Low-density lipoprotein (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels were calculated by using Friedewald’s equation [18]. Liver catalase activity was assayed following the method of Kar and Mishra, 1976 [19]. Liver superoxide dismutase (SOD) activity is assayed according to the method of Marklund and Marklund [20].

2.11. Histopathological Studies of Liver Tissues

Liver tissues preserved in 10% neutral buffered formalin were treated according to a standard laboratory protocol. Embedded sections were cut at a size of 5 μm. Then, slides were deparaffinized in p-xylene and rehydrated in changes of ethanol (100%, 90%, 80%, 70%, and 50%) and rinsed under tap water. Slides were stained by hematoxylin and counterstained by eosin, mounted in DPX, cov-
er-slipped and viewed under a light microscope (Olympus IX71, Japan) connected to a computer.

2.12. Statistical Analysis

Statistical analysis was carried out by using a statistical software package GraphPad Prism 7.0 (San Diego, CA, USA). All data are presented as the mean ± SEM (standard error of the mean). Differences among groups were assessed by one-way analysis of variance (ANOVA). Student t-test was used for comparison between two groups. Values at $p < 0.05$ were considered statistically significant.

3. Results

3.1. Characterization of Probiotic Strains for the Preparation of Yogurt and Subsequent Colony Count

Morphological, physiological and biochemical examination of probiotics (Table 1), suggested that the isolated strains from the commercial product “Probio” were *Lactobacillus (L) acidophilus* (LAB-A) and *L. bulgaricus* (LAB-B) that all features consistent to previous reports [21] [22]. After preparation of yogurt by inoculating probiotic strain *L. acidophilus* or *L. bulgaricus* in low fat UHT liquid milk at 37°C for 48 hours, colonies were counted. The yogurt inoculated by *L. acidophilus* was contained 9.73 billion ($9.73 \times 10^9$) viable *L. acidophilus* cells per gram and the yogurt inoculated by *L. bulgaricus* contained 9.57 billion ($9.57 \times 10^9$) viable *L. bulgaricus* cells per gram.

**Table 1.** Comparison of characteristics of isolated LAB strains.

| Characteristics            | LAB-A          | LAB-B          |
|----------------------------|----------------|----------------|
| **Colony Color**           | White          | Creamy White   |
| **Colony Shape**           | As a surface colony, they are Small, irregular, Cottony-fluffy colonies | As a surface colony, they are Large, Circular, Lenticular colonies in middle of the medium |
| **Surface**                | Rough          | Smooth         |
| **Elevation**              | Convex to umbonate | Raised        |
| **Margin**                 | Undulate       | Entire         |
| **Gram staining**          | +              | +              |
| **Catalase test**          | −              | −              |
| **Milk coagulation test**  | +              | +              |
| **NaCl tolerance test**    |                |                |
| 1.5%                       | +              | +              |
| 2.5%                       | +              | −              |
| 3.5%                       | +              | −              |
| 4.5%                       | −              | −              |
| **Possibility of strains** | *L. acidophilus* | *L. bulgaricus* |

Here, (+) positive response, (−) negative response, (++) good positive response, (+++) very good positive response.
3.2. Evaluation of Antidiabetic Activity of Probiotics

The parameters of fasting blood glucose (FBG) level, oral glucose tolerance test (OGTT), and glycosylated hemoglobin (HbA1c) were estimated to investigate the effect of probiotics in fructose-fed hyperglycemic rats. As shown in Figure 1(A), oral administration of 20% (w/v) fructose for a period of 8 weeks caused progressive and stable hyperglycemia. Simultaneous administration of yogurt containing the probiotic *L. acidophilus*, AF or *L. bulgaricus*, BF or in a combination of both *L. acidophilus* and *L. bulgaricus*, ABF (ca 9.5 × 10⁹ colonies/rat/day) significantly decreased the fructose-fed excessive load of blood glucose level. In the present study, oral glucose tolerance test (OGTT) has been done to investigate the glucose intolerance in the experimental rats. As shown in Figure 1(B), 30 min after oral administration of glucose, there was a significant elevation of blood glucose level. The rise of blood glucose level in diabetic control (DC) group at 30 min period was 2.20 fold higher than the initial 0 min value. However, it was only 1.81, 1.60 and 1.58 fold higher than the 0 min value for AF, BF and ABF, respectively, indicating that rats treated with *L. acidophilus* or *L. bulgaricus* individually or in combination form (ABF) protected against the development of fructose-induced carbohydrate intolerance. The increase in the level of HbA1c in the diabetic rats was observed in the present study. Administration of *lactobacillus* strains, AF, BF or ABF significantly attenuated the fructose-induced elevation of HbA1c levels (Figure 1(C)). The effect of ABF (combination of both *L. acidophilus* and *L. bulgaricus*) was more prominent than the strain individually. These results strongly suggest that those probiotics have potential antidiabetic effects. During experimental period, we investigated rat’s weight. Our findings showed a slight increase in rat’s weight after treatment in all groups. However, these variations did not reveal a significant difference in the mean body weight between the studied groups of rats at the end of the experimental period.

3.3. Effect of Probiotics on Serum Lipid Profiles in Fructose-Fed Hyperglycemic Rats

Lipid profiles are highly variable in type 2 diabetes. After the experimental period, rats blood serum were collected and investigated the serum total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), VLDL and HDL levels according to the manufacturer’s protocol as described in the Methods section. As shown in Table 2, administration of *lactobacillus* strains, AF, BF or ABF significantly reduced the fructose-induced elevation of TC, TG, LDL, and VLDL levels and increased HDL levels.

3.4. Effects of Probiotics on Liver Histopathological Changes in Fructose-Fed Hyperglycemic Rats

The histopathological studies of liver tissues showed varying degree of cellular changes in fructose-fed (DC) diabetic group’s rats as compared to the normal
Figure 1. Effect of probiotics in normal control and fructose-fed hyperglycemic rats. (A) Measurement of anti-hyperglycemic effect, (B) measurement of impaired glucose tolerance, (C) Measurement of HbA1c levels. Data are expressed as mean ± SEM of measurements from 5 rats. Groups are labeled as: NC: normal control, DC: fructose-fed diabetic control, MF: diabetic rats treated with metformin, AF: diabetic rats treated with yogurt, containing *L. acidophilus* (ca 9.5 × 10⁹ cfu/rats/day), BF: diabetic rats treated with yogurt, containing *L. bulgaricus* (ca 9.5 × 10⁹ cfu/rats/day), ABF: diabetic rats treated with yogurt, containing *L. acidophilus* and *L. bulgaricus* in a combination (ca 9.5 × 10⁹ cfu/rats/day). * indicates significant differences (p < 0.05) DC vs NC. # indicates significant differences (p < 0.05) between DC vs lactobacillus strain treatment group (AF, BF or ABF).
Table 2. Effect of probiotics on serum lipid profiles.

| Treatment (Group) | TG (mg/dl) | TG (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | HDL (mg/dl) |
|-------------------|------------|------------|-------------|--------------|-------------|
| NC                | 101.33 ± 6.94 | 55.33 ± 6.98 | 57.60 ± 8.35 | 11.07 ± 1.40 | 32.67 ± 2.33 |
| DC                | 139.00 ± 8.74* | 92.67 ± 4.98* | 93.80 ± 11.50* | 18.53 ± 1.00* | 23.33 ± 2.19* |
| MF                | 113.67 ± 4.91* | 66.33 ± 3.93* | 69.73 ± 5.71* | 13.27 ± 0.79* | 30.67 ± 0.88* |
| AF                | 107.33 ± 5.55* | 63.67 ± 3.28* | 63.27 ± 8.10* | 12.73 ± 0.66* | 31.33 ± 2.03* |
| BF                | 108.33 ± 6.89* | 61.67 ± 4.70* | 65.33 ± 5.77* | 12.33 ± 0.94* | 30.67 ± 1.20* |
| ABF               | 105.67 ± 6.01* | 60.67 ± 4.06* | 62.87 ± 6.00* | 12.13 ± 0.81* | 30.67 ± 0.33* |

Data are expressed as mean ± SEM of measurements from 5 rats. Groups are labeled as: NC: normal control, DC: fructose-fed diabetic control, MF: diabetic rats treated with metformin, AF: diabetic rats treated with yogurt, containing L. acidophilus (ca 9.5 × 10⁹ cfu/rats/day), BF: diabetic rats treated with yogurt, containing L. bulgaricus (ca 9.5 × 10⁹ cfu/rats/day), ABF: diabetic rats treated with yogurt, containing L. acidophilus and L. bulgaricus in a combination (ca 9.5 × 10⁹ cfu/rats/day). * indicates significant differences (p < 0.05) DC vs NC. # indicates significant differences (p < 0.05) between DC vs lactobacillus strain treatment group (AF, BF or ABF).

3.5. Effect of Probiotics on Liver Catalase and Superoxide Dismutase Activity

The activity of endogenous antioxidative liver enzymes, catalase (CAT) and superoxide dismutase (SOD) was measured from the homogenized liver tissues as described in “Methods”. Fructose-fed hyperglycemia caused the decrease of liver CAT and SOD activity as compared to the normal control (NC) group suggested that hyperglycemia resulted the excess load of oxidative stress. Administration of probiotic yogurt, containing L. acidophilus or L. bulgaricus alone or both strains, AF, BF or ABF significantly showed high CAT and SOD activity as compared to diabetic control group (Table 3).

4. Discussion

A well balanced diversity of gut microbiota is an important aspect of health. In the healthy state, potentially pathogenic bacteria are kept under control by the non-pathogenic flora, so called colonization resistance. On the other hand, altered intestinal microbiota referred to as dysbiosis leads to increased intestinal permeability and mucosal immune response, contributing to the development of diabetes [23].

The present study were concentrated to investigate the effect of regular
Figure 2. Effects of probiotics on liver histopathological changes in fructose-fed hyperglycemic rats. Tissues were stained with hematoxylin and eosin, viewed under a light microscope (Olympus IX71, Japan) with 40× magnification connected to a computer. The representative photographs shown are (A) normal control, (B) fructose-fed diabetic control, (C) diabetic rats treated with yogurt, containing *L. acidophilus* (ca 9.5 × 10⁹ cfu/rats/day), (D) diabetic rats treated with yogurt, containing *L. bulgaricus* (ca 9.5 × 10⁹ cfu/rats/day), (E) diabetic rats treated with yogurt, containing *L. acidophilus* and *L. bulgaricus* in a combination, (F) diabetic rats treated with metformin standard. Scale bars, 50 µm.

Administration of *L. acidophilus* or *L. bulgaricus* alone or in combination (ca 9.5 × 10⁹ colonies/rat/day) in a milk vehicle as yogurt in the form of probiotic supplement for the prevention and/or delaying the onset of type 2 diabetes and related complications. The viability of probiotic microorganisms in the final product until the time of consumption is important to exert health benefits. It is highly desirable that the viable number of probiotic bacteria in the final product to be at least 10⁶ - 10⁷ CFU/g to be accepted as the therapeutic minimum [24]. This adequate amount varies from country to country; in Japan a product should
Table 3. Effect of probiotics on liver CAT and SOD activity.

| Treatment (Group) | CAT activity Units/ml | SOD activity Units/ml |
|-------------------|------------------------|------------------------|
| NC                | 70.73 ± 3.41           | 7.23 ± 0.19            |
| DC                | 51.77 ± 2.62*          | 4.31 ± 0.17*           |
| MF                | 62.70 ± 1.42*          | 6.12 ± 0.14*           |
| AF                | 59.98 ± 1.23*          | 6.03 ± 0.06*           |
| BF                | 58.85 ± 1.31*          | 5.86 ± 0.08*           |
| ABF               | 62.73 ± 1.8*           | 6.13 ± 0.08*           |

Data are expressed as mean ± SEM of measurements from 5 rats. * indicates significant differences (p < 0.05) between fructose-fed diabetic control group (DC) vs normal control group (NC). # indicates significant differences (p < 0.05) between fructose-fed diabetic control group (DC) vs lactobacillus strain treatment group (L. acidophilus, AF, L. bulgaricus, BF or L. acidophilus + L. bulgaricus, ABF).

contain a minimum of 10^7 colony-forming unit (CFU)/g of probiotic bacteria to be considered a probiotic one, while the USA has developed a standard which requires at least 10^8 CFU/g of the product to be labeled as probiotic [25]. The adequate amount should not be less than 10^6 - 10^9 CFU/g of the probiotic [26][27]. After counting of viable bacterial cell, we confirmed that the CFU of probiotic into prepared yogurt for treatment of diabetics was more than the minimum value. Besides, we cultured rat stoolson MRS media and found the presence of probiotic bacterial species in the specimens. The presence of lactobacilli was much higher in the yogurt treatment group (data not shown) of rat’s stools as compared to the normal control group, suggesting the viability of LAB strains in the gut of rats.

Recent studies have concentrated on various strategies to prevent and/or delay the onset of type 2 diabetes and its complications [28]. One of these strategies is the consumption of foods low in the glycemic index with bioactive agents that have been adopted to prevent or delay the onset of disease. Our results indicated that balancing microbiota by L. acidophilus and/or L. bulgaricus significantly prevented or lowered risks of diabetes. High fructose diets have been used in animal models to induce metabolic changes similar to type 2 diabetes. Impaired glucose tolerance testing is an important predictor of type 2 diabetes [29]. Rats treated with Lactobacillus probiotics protected against the development of fructose-induced carbohydrate intolerance. High amount of blood glucose in diabetess mellitus reacts with other biomolecules to form advanced glycated end-products (AGEs), chiefly HbA1c. The formation/activation of AGEs, transcription factors, and protein kinase C results in an increase in oxidative stress [30][31]. The increase in the level of HbA1c in the fructose-fed diabetic rats observed in the present study might be due to increase in blood glucose level that was significantly suppressed by Lactobacillus probiotics.

In the present study, we observed that the concentrations of lipids, such as TC, TG, LDL and VLDL were significantly high, and HDL was low in diabetic rats compared to control group. The variation of lipid levels in type 2 diabetics is...
due to derangements in metabolic and regulatory mechanisms [32]. Significant decrease in the level of serum TC, TG, LDL, and VLDL and increase in level of HDL were observed in diabetic rats after administration of L. acidophilus or L. bulgaricus alone or in combination of both. Several mechanisms for the decrease in cholesterol concentration by probiotics have been proposed. It may be due to decrease in cholesterol absorption from intestine [33] [34] [35] or by enzymatic deconjugation of bile acids by bile salt hydrolase, interfering with the enterohepatic circulation of bile salts.

Liver is the primary organ susceptible to the effects of hyperglycemia-induced oxidative stress, which may lead to liver tissue injury [36] [37], and thus further aggravating diabetes by decreasing insulin sensitivity and/or increasing insulin resistances [38]. Histopathological analyzes have shown that probiotic supplementation protected high fructose-induced liver changes, suggesting that probiotic supplementation kept the normal functions of liver for carbohydrate metabolism. Living organisms possess endogenous enzymatic antioxidative defenses such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) etc., that repair systems to protect them against oxidative stress [39]. These endogenous antioxidants play the first line of defense against oxidative stresses. Administration of L. acidophilus or L. bulgaricus alone or in combination of both attenuated the hyperglycemia-induced reduction of SOD and CAT. The probiotic-mediated high liver CAT and SOD activity might be the proposed mechanism of how probiotic strains are involved in the prevention and/or repair of oxidative damage of the liver tissues and keeping insulin sensitivity towards the fructose-fed rats.

5. Conclusion

In conclusion, administration of low fat yogurt containing L. acidophilus or L. bulgaricus to high fructose-fed hyperglycemic rats decreased fructose-induced elevated serum FBG, HbA1c, total cholesterol, triglycerides, LDL and VLDL-cholesterol, and increased HDL-cholesterol levels significantly and preserved antioxidant pool such as activities of superoxide dismutase, catalase etc., which prevented/repaired the oxidative damage of liver tissues. So, our findings indicate that balanced microbiota by L. acidophilus and L. bulgaricus can prevent or lower risks of type-2 diabetes and its related complications. However, further studies necessitate developing functional foods and their clinical trials in human.

Declarations

Ethical approval and the consent of animal experiment were approved and handling guidelines were followed by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) at the institute of biological sciences, University of Rajshahi, Bangladesh. This work was supported in part by the Ministry of Science and Technology, People’s Republic of Bangladesh and Department of Pharmacy, University of Rajshahi. The authors acknowledge to
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Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Gomes, A., Bueno, A. and de-Souza, R. (2014) Gut Microbiota, Probiotics and Diabetes. Nutrition Journal, 13, 60. https://doi.org/10.1186/1475-2891-13-60

[2] Kahn, S., Hull, R. and Utzschneider, K. (2006) Mechanisms Linking Obesity to Insulin Resistance and Type 2 Diabetes. Nature, 444, 840-846. https://doi.org/10.1038/nature05482

[3] IDF (2017) International Diabetes Federation Diabetes Atlas 2017. 8th Edition, Brussels.

[4] WHO (2016) Diabetes Country Profiles.

[5] Cani, P., Daubioul, C., Reusens, B., Remacle, C., Catillon, G. and Delzenne, N. (2005) Involvement of Endogenous Glucagon-Like Peptide-1 Amide on Glycaemia-Lowering Effect of Oligofructose in Streptozotocin-Treated Rats. Journal of Endocrinology, 185, 457-465. https://doi.org/10.1677/joe.1.06100

[6] Rehman, K. and Akash, M. (2016) Mechanisms of Inflammatory Responses and Development of Insulin Resistance: How Are They Interlinked? Journal of Biomedical Science, 23, 87. https://doi.org/10.1186/s12929-016-0303-y

[7] Chen, L., Chen, R., Wang, H. and Liang, F. (2015) Mechanisms Linking Inflammation to Insulin Resistance. International Journal of Endocrinology, 2015, Article ID: 508409. https://doi.org/10.1155/2015/508409

[8] Natividad, J. and Verdu, E. (2013) Modulation of Intestinal Barrier by Intestinal Microbiota: Pathological and Therapeutic Implications. Pharmacological Research, 69, 42-51. https://doi.org/10.1016/j.phrs.2012.10.007

[9] Silva, H.D., Millard, P., Soper, N., Kettlewell, M., Mortensen, N. and Jewell, D. (1991) Effects of the Faecal Stream and Stasis on the Ileal Pouch Mucosa. Gut, 32, 1166-1169. https://doi.org/10.1136/gut.32.10.1166

[10] Floch, M. and Montros, D. (2005) Use of Probiotics in Humans: An Analysis of the Literature. Gastroenterology Clinics of North America, 34, 547-570. https://doi.org/10.1016/j.gtc.2005.05.004

[11] Kumar, A. and Kumar, D. (2014) Isolation and Characterization of Bacteria from Dairy Samples of Solan in Himachal Pradesh for Identification of Lactobacillus spp. International Journal of Pharmaceutical Sciences Review and Research, 25, 110-114.

[12] Yadav, H., Jain, S. and Sinha, P. (2006) Effect of Skim Milk and Dahi (Yogurt) on Blood Glucose, Insulin, and Lipid Profile in Rats Fed with High Fructose Diet. Journal of Medicinal Food, 9, 328-335. https://doi.org/10.1089/jmf.2006.9.328

[13] Dupas, J., Goanvec, C., Feray, A., Guernec, A., Alain, C. and Guerrero, F. (2016) Progressive Induction of Type 2 Diabetes: Effects of a Reality-Like Fructose Enriched Diet in Young Wistar Rats. PLoS ONE, 11, e0146821. https://doi.org/10.1371/journal.pone.0146821

[14] Hokayem, M., Blond, E., Vidal, H., Lambert, K., Meugnier, E., Feillet-Coudray, C., Coudray, C., Pesenti, S., Luyton, C. and Lambert-Porcheron, S. (2013) Grape Polyphenols Prevent Fructose-Induced Oxidative Stress and Insulin Resistance in First-Degree Relatives of Type 2 Diabetic Patients. Diabetes Care, 36, 1454-1461.
Akinola, O., Gabriel, M., Suleiman, A. and Olorunsogbon, F. (2012) Treatment of Alloxan-Induced Diabetic Rats with Metformin or Glitazones Is Associated with Amelioration of Hyperglycaemia and Neuroprotection. *The Open Diabetes Journal*, 5, 8-12. https://doi.org/10.2174/1876524601205010008

Majithiya, J. and Balaraman, R. (2006) Metformin Reduces Blood Pressure and Restores Endothelial Functions in Aorta on Streptozotocin-Induced Diabetic Rats. *Life Sciences*, 78, 2615-2624. https://doi.org/10.1016/j.lfs.2005.10.020

Islam, M., Akhtar, M., Khan, M., Hossain, M., Alam, A., Wahed, M., Amran, M., Rahman, B. and Ahmed, M. (2009) Oral Glucose Tolerance Test (ogtt) in Normal Control and Glucose Induced Hyperglycemic Rats with *Coccinia cordifolia* L. and *Catharanthus roseus* L.. *Pakistan Journal of Pharmaceutical Sciences*, 22, 402-404.

Friedewald, W., Levi, R. and Fredrickson, D. (1972) Estimation of the Concentration of Low Density Lipoprotein Cholesterol in Plasma without Use of the Ultracentrifuge. *Clinical Chemistry*, 18, 449-452.

Kar, M. and Mishra, D. (1976) Catalase, Peroxidase and Polyphenol Oxidase Activities during Rice Leaf Senescence. *Plant Physiology*, 57, 315-319. https://doi.org/10.1104/pp.57.2.315

Marklund, S. and Marklund, G. (1974) Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. *European Journal of Biochemistry*, 47, 469-474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Wheaterd, M. (1955) The Characteristics of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. *Journal of General Microbiology*, 12, 123-132. https://doi.org/10.1099/00221287-12-1-123

Hossain, M., Al-Bari, M., Mahmud, Z. and Wahed, M. (2016) Antibiotic Resistant Microencapsulated Probiotics Synergistically Preserved Orange Juice. *BMC Nutrition*, 2, 59. https://doi.org/10.1186/s40795-016-0098-y

Secondulfo, M., Lafusco, D., Carratu, R., Magistris, L.D., Sapone, A., Generoso, M., Mezzogiomo, A., Sasso, F., Carteni, M. and Rosa, R. (2004) Ultrastructural Mucosal Alterations and Increased Intestinal Permeability in Non-Celiac, Type I Diabetic Patients. *Digestive and Liver Disease*, 36, 35-45. https://doi.org/10.1016/j.dld.2003.09.016

Sherwood, L., Willey, J. and Woolverton, C. (2013) Prescott's Microbiology. 9th Edition, McGraw Hill, New York, 713-721.

Vuyst, L. (2000) Technology Aspects Related to the Application of Functional Starter Cultures. *Food Technology and Biotechnology*, 38, 105-112.

Champagne, C., Ross, R., Saarela, M., Hansen, K. and Charalampopoulos, D. (2011) Recommendations for the Viability Assessment of Probiotics as Concentrated Cultures and in Food Matrices. *International Journal of Food Microbiology*, 149, 185-193. https://doi.org/10.1016/j.ijfoodmicro.2011.07.005

Cruz, A., Faria, J., Saad, S., Bolini, H. and Sant, A. (2010) High Pressure Processing and Pulsed Electric Fields: Potential Use in Probiotic Dairy Foods Processing. *Trends in Food Science & Technology*, 21, 483-493. https://doi.org/10.1016/j.tifs.2010.07.006

Nell, S., Suerbaum, S. and Josenhans, C. (2010) The Impact of the Microbiota on the Pathogenesis of IBD: Lessons from Mouse Infection Models. *Nature Reviews Microbiology*, 8, 564-577. https://doi.org/10.1038/nrmicro2403
[29] Famularo, G., Moretti, S., Marcellini, S. and Simone, C.d. (1997) Stimulation of Immunity by Probiotics. In: Fuller, R., Ed., Probiotics: Therapeutic and Other Beneficial Effects, Chapman and Hall, London, 13361. 
https://doi.org/10.1007/978-94-011-5860-2_6

[30] Fuller, R. (1989) Probiotics in Man and Animals. Journal of Applied Bacteriology, 66, 365-378. https://doi.org/10.1111/j.1365-2672.1989.tb05105.x

[31] Havenaar, R. (1992) Probiotics: A General View. In: Wood, B.J.B., Ed., Lactic Acid Bacteria in Health and Disease, Huis In’t Veld JMJ Elsevier Applied Science Publishers, London, 151-170. https://doi.org/10.1007/978-1-4615-3522-5_6

[32] Salminen, S. (1996) Uniqueness of Probiotic Strains. International Dairy Feeding Sand Nutrition Newsletter, 5, 16-18.

[33] Donohue, D. and Salminen, S. (1996) Safety of Probiotic Bacteria. Asia Pacific Journal of Clinical Nutrition, 5, 25-28.

[34] Guarner, F. and Schaafsma, G. (1998) Probiotics. International Journal of Food Microbiology, 39, 237-238. https://doi.org/10.1016/S0168-1605(97)00136-0

[35] Naidu, A., Bidlack, W. and Clemens, R. (1999) Probiotic Spectra of Lactic Acid Bacteria (LAB). Critical Reviews in Food Science and Nutrition, 39, 13-126. https://doi.org/10.1080/10408699991279187

[36] Bugianesi, E., McCullough, A. and Marchesini, G. (2005) Insulin Resistance: A Metabolic Pathway to Chronic Liver Disease. Hepatology, 42, 987-1000. https://doi.org/10.1002/hep.20920

[37] Palsamy, P., Sivakumar, S. and Subramanian, S. (2010) Resveratrol Attenuates Hyperglycemia-Mediated Oxidative Stress, Proinflammatory Cytokines and Protects Hepatocytes Ultrastructure in Streptozotocin-Nicotinamide-Induced Experimental Diabetic Rats. Chemico-Biological Interactions, 186, 200-210. https://doi.org/10.1016/j.cbi.2010.03.028

[38] Romagnoli, M., Gomez-Cabrera, M., Perrelli, M., Biasi, F., Pallardó, F., Sastre, J., Poli, G. and Viña, J. (2010) Xanthine Oxidase-Induced Oxidative Stress Causes Activation of NF-kappaB and Inflammation in the Liver of Type I Diabetic Rats. Free Radical Biology & Medicine, 49, 171-177. https://doi.org/10.1016/j.freeradbiomed.2010.03.024

[39] Mishra, V., Shah, C., Mokashe, N., Chavan, R., Yadav, H. and Prajapati, J. (2015) Probiotics as Potential Antioxidants: A Systematic Review. Journal of Agricultural and Food Chemistry, 63, 3615-3626. https://doi.org/10.1021/jf506326t

Abbreviations

LAB—lactic acid bacteria; MRS—De Man, Rogosa and Sharpe; PBS—phosphate buffered solution; CFU—colony forming unit; TC—total cholesterol; TG—triglyceride; LDL—low density lipoprotein; VLDL—very low density lipoprotein; FBG—fasting blood glucose; SOD—superoxide dismutase; CAT—catalase; GPx—glutathione peroxidase.