

Study of Effect of Anti -diabetic Drug on Lactobacilli Growth

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Abstract—Diabetes is a metabolic disease, predominantly associated with stress, lifestyle, obesity and hereditary factors characterized by chronic hyperglycemia due to defects either in insulin secretion or action, or both. Diabetes represents one of the leading causes of morbidity and mortality of humans worldwide. The permanent termination of diabetes eludes patients and it is a burden on developing countries. It has been reported that probiotics show beneficial effects in the treatment of diabetes.

The aim of this study is to evaluate the effect of glucose with or without the presence of antidiabetic drug (metformin) on the growth of probiotic *Lactobacillus* as well as the role of probiotic lactobacilli in the glucose metabolism. The study investigates the growth of natural microbes – *Lactobacillus*, found in human intestine as it is an important probiotic that gives health benefits.

Methods: *Lactobacillus* was isolated from probiotic yakult and curd. Estimation of the growth of *Lactobacillus* in the presence of different concentration range of glucose in simulated gastrointestinal fluid was completed by taking absorbance of culture. Comparison was done for the growth of lactobacilli in the presence or absence of antidiabetic drug (metformin) in the MRS agar by colony counting. Similarly comparison was done between the growth of isolates of lactobacilli in the presence of glucose with metformin and independently glucose in the MRS agar by colony counting. Growth of different isolates of *Lactobacillus* was estimated in the presence of metformin with glucose. The effect of combination of drugs metformin and glyclazide and the single drug Glimperide was also observed. Protein profiling was done for comparing the protein pattern of *Lactobacillus* grown in the presence or absence of glucose as well as in the presence of glucose with metformin by using SDS-PAGE. Polymerase chain reaction was performed for the gene sequencing.

Results: The growth of *Lactobacillus* increased slightly with the increase in glucose concentration Figure. A significant difference was seen in the number of colonies of the lactobacillus grown in the presence or absence of metformin on MRS agar. No drastic change was observed in the growth of lactobacilli in the presence of glucose with metformin, relative to that on only glucose on MRS agar. Growth of different isolates of lactobacilli was arguably constant in the presence of glucose with metformin in gastric fluid. Protein profiling showed different band pattern for the lactobacillus grown in the presence or absence of glucose as well as in the presence of glucose with metformin. PCR carried out to detect *Lactobacillus rhamnosus* did not give significant results.

Conclusion and Discussion: This study brings a conclusion that though glucose increases the growth of lactobacillus, metformin barely impedes the growth when present with high glucose. Antidiabetic drug in this study does not seem to inhibit to a significant extent the growth of probiotic, but probiotic bacteria could be useful for the complementary treatment strategies of diabetes and its associated complications.

Keywords: *Lactobacillus*, simulated gastrointestinal fluid.

Introduction: *Lactobacillus* is an important probiotic that is Gram positive and has beneficial effects on human health. It can improve immunity and prevent the growth of other pathogenic bacteria, like *Salmonella*. The role of luxS has been studied in the adhesion of *Lactobacillus plantarum*. The lactobacilli can survive harsh environment like low pH and bile. The ability of lactobacilli in alleviation of diabetes in rats is well reported.

Methods: *Lactobacillus* was isolated from probiotic yakult and curd. Estimation of the growth of *Lactobacillus* in the presence of different concentration range of glucose in simulated gastrointestinal fluid was completed by taking absorbance of culture. Comparison was done for the growth of lactobacilli in the presence or absence of antidiabetic drug (metformin) in the MRS agar by colony counting. Similarly comparison was done between the growth of isolates of lactobacilli in the presence of glucose with metformin and independently glucose in the MRS agar by colony counting. Growth of different isolates of *Lactobacillus* was estimated in the presence of metformin with glucose. The effect of combination of drugs Metformin and Glyclazide and the single drug Glymepiride was also observed. Protein profiling was done for comparing the protein pattern of *Lactobacillus* grown in the presence or absence of glucose as well as in the presence of glucose with metformin by using SDS-PAGE. Polymerase chain reaction was performed for the gene sequencing.

1 ml of curd or probiotic beverage Yakult was serially diluted in 0.9% saline and plated on MRS agar (HiMedia) to get *Lactobacillus*. The genus was confirmed by Gram positive

stain and lack of catalyst in bacterium for hydrogen peroxide degradation. There was inoculation carried out of the isolate in LB broth. 10 µl of the culture was spread plated on MRS agar, in addition to glucose at a concentration of 200mg/100 ml and or antidiabetic drug in petriplates and incubated at 37°C. Centrifugation was carried out of the remaining culture at 7000 rpm for 10 minutes and the pellet resuspended in 8 ml simulated gastric fluid. The gastric fluid had the composition NaCl(3.5 g/L), glucose (2.05 g/L), KH₂PO₄ (0.60 g/L), CaCl₂ (0.11g/L) and KCl (0.37 g/L) and the pH was set to 2 and autoclaved at 121°C for 15 minutes. Porcine bile (0.05 g/L), lysozyme (0.1 g/L) and pepsin (13.3 mg/L). Growth was carried out at 37°C in shaker incubator and absorbance was measured at 600 nm every thirty minutes.

For protein profiling, growth of lactobacilli was carried with and without glucose. The cell lysate was loaded on SDS PAGE gel.

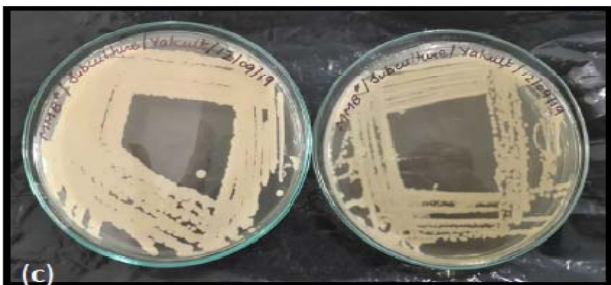


Figure 1: Isolation of Lactobacillus from Yakult and curd

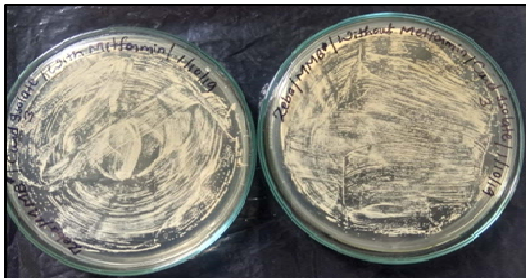


Figure 2. Growth of lactobacilli with and without Metformin - 1 with Metformin 2 without metformin

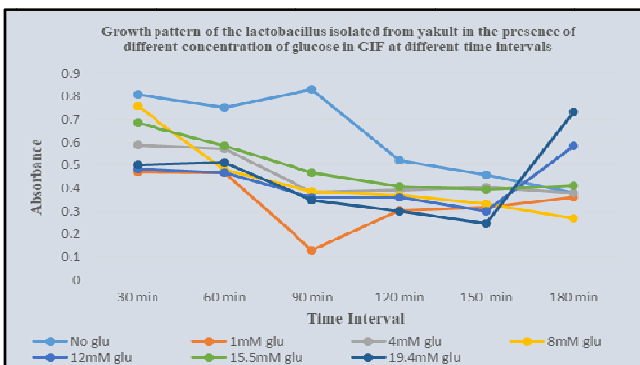


Figure 3: Effect of glucose on growth of lactobacilli in simulated human gastric fluid



Figure 4. Effect of Glimepiride on lactobacilli growth n MRS agar 1- without Glimepiride 2- with Glimepiride

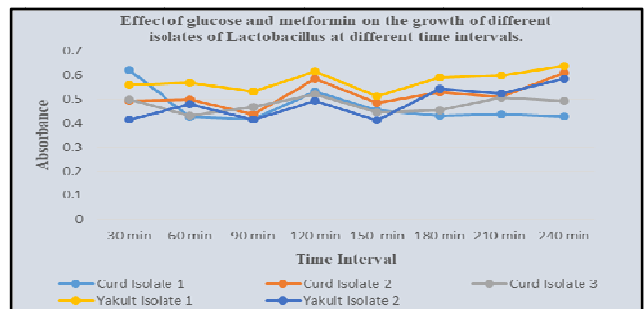


Figure 5. The effect of Metformin and glucose on lactobacilli growth in simulated human gastric fluid

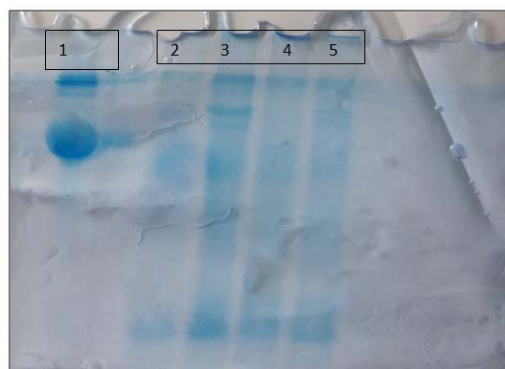


Figure 6. The effect of glucose on protein expression by lactobacilli 1- BSA 2 and 3 – without glucose 4 and 5 – with glucose

Lactobacillus was isolated from Curd and probiotic Yakult (Figure 1). Metformin does increase the growth of lactobacilli when growth is compared of lactobacilli on MRS agar with drug and without it (Figure 2). The effect of glucose was seen on *Lactobacillus* and it showed enhancement of the growth of bacteria in gastric fluid (Figure 3). The other drugs like Glyclazide and Glimepiride do not hamper growth of *Lactobacillus* (Figure 4). Glucose and Metformin did not inhibit growth of lactobacilli in simulated human gastric fluid. The effect of glucose was seen on the expression of protein by lactobacilli and higher expression of certain proteins was observed when glucose was added to culture (Figure 6)

Discussion: Lactobacilli have divergent functions in promoting human health (2) Metformin has been reported to have altered upper intestinal microbiota because of regulation of sodium glucose cotransporter-1. The lactobacillus has an important role to regulate glucose homeostasis (3). In this study the lactobacilli growth was affected by glucose to increase it temporarily. Metformin increased the growth of lactobacilli. Glimepiride has not shown much effect on lactobacilli growth. The protein expression of the bacteria was affected by addition of glucose in culture.

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