Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 1 Year 2024 Page 60:69

Characterization Of Probiotic Lactic Acid Bacteria From Honey And Assessment Of Their Effects On Consumption By Type-2- Diabetes Using Wistar Rat

Akhila A[1], Anil Kumar[2], Vinay Kumar Gupta[3], Darshan Vinod Shah[4], Rohan Kumar Karan[5], Anju Anant[6], Tanmay Ghosh[7], Satyabrat Sarma[8], Keshamma E[9]*

[1]Associate professor, Department of Zoology, Govt. First Grade College, Vijayanagar, Bengaluru, Karnataka, India
[2]Head & Assistant Professor, Department of Chemistry (PG), Sahibganj College Sahibganj, Jharkhand, India
[3]Assistant Professor, Department of Pharmacology, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India.
[4]Associate Professor, Department of Pharmacology, SVPM'S College of Pharmacy Malegaon, Bk Baramati Pune, Maharashtra, India
[5]M. Pharm scholar, Department of Pharmaceutical chemistry, Guru Nanak Institute of pharmaceutical science and technology, Kolkata, West Bengal, India
[6]Assistant Professor, Department of Pharmacy, Shri Rawatpura Sarkar University, Near Shadanidarbar, New Dhamtari Raipur, Chhattisgarh, India
[7]Assistant Professor, Departmennt of Microbiology, Dinabandhu Andrews College, Baishnabghata, South 24 Parganas, Kolkata, West Bengal, India
[8]Assistant Professor, Faculty of Pharmaceutical Science, Assam downtown University, Guwahati, Assam, India
*[9]*Associate Professor, Department of Biochemistry, Maharani Science College for women, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India*

***Corresponding Author:** Dr. Keshamma E*
Department of Biochemistry, Maharani Science College for women, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India.

Abstract

The relevance of probiotic, especially lactic acid bacteria cannot be over emphasized. In this present study three honey sources were serially diluted and cultured on De Man, Rogosa and Sharpe (MRS) agar among which only one of this sources grew on MRS agar. The pure Lactobacilli isolate were subjected to gram staining, biochemical tests, physiological test, molecular analysis using Polymerase Chain Reaction (PCR) techniques and Deoxyribonucleotide (DNA) sequencing. Only one isolate was obtained known as *Enterococcus fecalis*. The isolate was subjected to probiotic selection and was found fit for consumption, however their effect when consumed by type-2 diabetic are alarming and based on the outcome of this study, diabetic patient are advised not consume honey.

Key-words: Diabetes, Probiotic, Enterococcus fecalis,

Introduction

In the bodies of human and animal, lactic acid bacteria (LAB) are part of normal microbiota of gastrointestinal tract and genitourinary tracts. They are gram positive bacteria, non-spore former, carbohydrate fermenter, aerotolerant anaerobes, catalase and oxidase negative. They have been given the Qualified Presumption of Safety (QPS) status. They belong mostly to family of Lactobacillaceae. Majority of probiotics are lactic acid bacteria. The sources of Lactic acid bacteria (LAB) are mostly fermented and dairy food. The isolation of Lactic Acid Bacteria involve culturing of the potential source in a selective medium such as de Man, Rogosa, and Sharpe medium commonly known as MRS agar in an anaerobic condition (Razmposh *et al*., 2016).

Statement of Problems

The relevance of honey to human cannot be overemphasized. Honey is presently being used for wound healing. Although, the rationale behind it use for wound healing could be due to the fact that it harbors some probiotic lactic acid bacteria which can be used as an alternative to antibiotic treatment. However, their effect on metabolic disease such diabetes when consumed orally has not been scientifically claimed. Hence there is need to isolate and characterize probiotic lactic acid bacteria that could be present in honey and the effect of honey consumption by type-2 diabetic patient by assessing some clinical parameters.

Material and Methods

Sample Source and Inclusion and Exclusion Criteria

Honey of three different producers which are original and not expired was selected for the research study.

Sample Collection

The Honey were collected into a universal sterile container observing universal sterile technique using sterile syringes, methylated spirit and then analysis was commenced immediately.

Sample Processing, Phenotypic Diagnosis and characterization of Lactic acid bacteria

Sample processing

One millilitre (1ml) of the honey from three different producers was collected from the containers using sterile needle and syringe under universal aseptic technique. A serial dilution of 10^{-3} of the three different brands of honey using sterile water solution (Microxpress**R**, a division of Tulip Diagnostics Ltd.) and incubated at 25^{o}C for 30 minutes. Streak plating method was used as described by Sun-young and his colleagues with some slight modification (Sun-young *et al*., 2019). Then 100µL from each dilution of the samples was inoculated and cultured into de Man, Rogosa, and Sharpe agar using micropipette and incubated at 37^{o}C for 24 hours to 72 hours anaerobically to obtain different colonies and then purified by streak plate techniques on MRS agar for subculture and incubated at 37^{o}C for 24 hours to 48 hours anaerobically again to obtain pure culture. The pure colony from each sample was further inoculated into MRS broth containing 15% glycerol and stored at – 20^{o}C to preserve the isolates for DNA extraction, sequencing and in-vivo evaluation (Nabi *et al*., 2020).

Phenotypic Diagnosis of Presumptive LAB

Pure culture of Presumptive LAB plate immediately after isolation was assessed for colony morphology and was further characterized microscopically via gram stain reaction and biochemically via oxidase, catalase and carbohydrate fermentation test using Mohan and Murugalatha method. Microscopically, they are gram positive bacteria with rod-shaped or cocci-shaped. Biochemically, presumptive Lactic Acid Bacteria colonies are catalase negative and oxidase negative or positive; and carbohydrate fermentation test is positive (Forhad *et al*., 2015). For additional confirmation of LAB, temperature and sodium chloride tolerance test were done. A loop full of suspected LAB was inoculated into 10mls of MRS broth and examined for their ability to grow at different temperatures (15, 37, 45^{o}C) for three (3) days and saline concentration (2, 3, 4, and 6.5% (w/v) NaCl) for 24 to 48hours and then incubated at 37^{o}C anaerobically. Both procedures was described by Estifanos (Estifanos, 2014), observing their turbidity rate. Lactic acid bacteria that show tolerance to the

subjected temperatures and different saline concentration was confirmed as LAB and then stored in MRS broth containing 15% of glycerol at -10^{o}C prior further analysis (Davodabadi *et al*., 2015).

Molecular Identification of the Isolated Presumptive Probiotic LAB

The DNA extraction was done using an extraction kit on ZR fungal/bacterial DNA mini prep (Inqaba in South Africa).The DNA extracted was quantified using a spectrophotometer called Nanodrop 1000 spectrophotometer. The amplification of the DNA was done using the polymerase chain reaction (PCR) machines operating at 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers, followed by checking for purification using agarose gel electrophoresis technique (Pei *et al*., 2012). The Sequencing of LAB was done using a machine known as a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The PCR template used is per 100 base pair (Anna *et al*., 2017).

Characterization of Functional and Safety Properties of Confirmed Probiotic LAB Isolates
Acid Tolerance Assay

Each confirmed LAB isolates obtained from overnight culture in MRS agar was cultured overnight in MRS broth at 37^{o}C and settled by centrifugation for 10min at 5000rpm. The supernatant was discarded while it precipitate containing suspension of grown confirmed LAB isolates was left in the test tube. One normality of hydrochloric acid was used to modify the precipitate to obtain a final pH of 2.5 as measured by pH meter and then incubated anaerobically at 37^{o}C for 2 hours and assessed for growth by assessing their turbidity in each tube. To further evaluate the resistant of confirmed LAB to acid by being turbid, the methodology used by Rajesh and his colleagues was applied with slight modification where hundred microliters (100µL) of the bacteria culture from those test tubes that are turbid was inoculated in MRS agar in duplicates and examined after 24 to 48 hours of incubation anaerobically at 37^{o}C. Those inoculated plate which showed growth of more 10^{7} CFU/ml (number of colonies of bacteria in each plate was between 5 and 20) were considered as resistant to acid (Rajesh *et al*., 2016).

Bile Tolerance Assay

Bile tolerance was evaluated through growth studies by the method of Succi and his colleagues with some modifications in 2005 where hundred microliters (100µL) of bacterial culture of each isolates of confirmed LAB isolates was transferred to 9mL of modified MRS broth containing 0.15 and 0.3% bile salt concentration and then incubated at 37^{o}C anaerobically and viable growth on MRS broth was assessed for turbidity after 0, 4, 24, and 48hours and their ability to reduce the pH (pH meter, Eutech instrument, Malaysia) of the modified MRS broth solution at 0, 4, 24 and 48hours of incubation Those test tube showing turbidity and reduce the pH of the modified MRS broth was considered to be resistance to bile salt (Succi *et al*., 2005).

Safety Assay
Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was carried out with Muller Hinton agar diffusion by Kirby Buer method (phenotypic methods) using commercially available antibiotic discs (MAST, Berkshire, UK) in Nigeria as described by Vijayakumar and his colleagues in 2015. The antibiotic disc includes amoxicillin (AML, 20µg), norfloxacin (NB, 10µg), streptomycin (S, 30µg), levofloxacin (LEV, 20µg), chloramphenicol (CHL, 30µg), ciprofloxacin (CIP, 10µg), rifampicin (RIF, 20µg) erythromycin (ERY, 30µg), ampiclox (APX, 20µg) and gentamicin (CN, 10 µg). After streaking, antibiotic discs of gram positive disc was placed on the inoculated MHA agar surface using sterile forceps ensuring that each disc are gently pressed down to ensure complete contact with the agar and the plate was left over for 20min at 4^{o}C for diffusion of antibiotics and then incubated at 37^{o}C for 24 to 48 hours under anaerobic condition, after which the inhibition zone diameters was measured with vernier caliper and results was (Vijayakumar *et al*., 2015). *Lactobacillius bulgaricus* were used for positive quality control.

Hemolytic Test

To assess their safety via hemolytic testing, Yasmin and his colleague's method in 2020 was used. Fresh overnight confirmed LAB isolates was streaked on Human blood agar and incubated at 37°C for 24 to 48 hours in an anaerobic condition. Hemolytic activity of the confirmed LAB isolates was examined for alpha, beta and gamma hemolysis. Probiotic LAB undergoes gamma-hemolysis (Yasmin *et al*., 2020).

In-vivo Evaluation of Honey for its Anti-diabetic Properties
Ethical consideration and study design

The rules guiding the use of animal for experimental study were strictly adhered to after ethical consent was granted. This research utilized randomized subject control experimental design and observational study. Eighteen (18) adult male wistar rats (*Rattus norvegicus*) were randomly divided into two (2) main groups. The two main groups are normal group and diabetes induced group. The diabetes-induced group was further subdivided into two (2) subgroups namely diabetic group on treatment and those not on treatment. Prior experimental design, a pilot study was done on the standardization of streptozocin injection required to induce type-2 diabetes on the adult wistar rat and the LD50 of streptozotocin injection.

Management of Experimental Animals

The rat was maintained under standard laboratory conditions in the animal house. The cages used for keeping the rat was measuring 38×23×15cm with not more than seven rats per cage. They were fed with pelletized growers feed. Prior treatment, the wistar rats were fed with goya oil as a source of high fat diet to induce obesity via gastric gavage. Obesity in wistar rat was assessed using two anthropometrical parameters such as Body mass index (BMI) and Lee index. The formula used for calculating the basal mass index and lee index are given below.

$$\text{Body Mass Index} = \frac{\text{Body weight (g)}}{Length^2(cm^2)}$$

$$\text{Lee's Index} = \frac{\text{Cube root of body weight (g) x } 1000}{Nose - to - anus\ length\ (cm)}$$

Obesity is estimated in rat using BMI and lee's index. The normal BMI for rat is between 0.45g/cm^2 and 0.68g/cm^2 while the normal Lee's index for rat is less than 300g/cm (Novelli *et al*., 2006).

Parameters evaluation and Treatment of Animals

Prior administration of Streptozotocin intraperitoneally at lower left or right quadrant of the abdomen, the anthropometric parameter (basal mass index and Lee's index), biochemical parameters (blood glucose, total cholesterol) and hematological parameter (packed cell volume) was estimated before and seven (7) days after Streptozotocin administration. The male adult wistar rats with blood glucose of greater or equal to 180mg/dL and total serum cholesterol level of greater than or equal to 100mg/dL; and change in body weight via assessment of their BMI/ Lee index (5% decrease of initial weight) were confirmed for the presence of obesity and metabolic syndrome with type-2-diabetes mellitus. Blood samples were collected from the tail vein at 0, 4, 6 and 8weeks for estimation of biochemical parameters. Treatment was carried out for four (4) weeks (Rajesh *et al*., 2016). The treatment where divided into two groups namely:

Group A: Normal Study. The rats in this group was fed with HFD, normal rat feed and water orally daily via gastric gavage but where not induced with Type-2 diabetes

Group B: Diabetes-Induced Study

Group B subgroup I: Control. The rats in this group are diabetic-induced and were given commercial feeds and treated with 0.9% physiological saline (3mL/Kg/BW) orally daily via gastric gavage. They serve as positive control (Rajesh *et al*., 2016).

Group B subgroup II: Honey (3mL/Kg/BW). The rats in this group are diabetic-induced and were given commercial feeds and treated with honey (3mL/Kg/BW) orally daily via gastric gavage. This was done to also assess the glycemic index of honey. The treatment was preserved in a refrigerator after each use daily.

Each parameter were estimated using appropriate device and instruments such as glucometer (Accu-check), urinalysis combi-9 strips and automated four in one machine (Accu-answer) that assess total cholesterol level, fasting blood sugar, uric acid and packed cell volume with separate strips.

Statistical Analysis

All the measurements were performed in triplicates and the results (data) were expressed as mean ± standard deviation (SD). Data were analyzed by the use of two-way analysis of variance (ANOVA, SPSS 17.0) test.

Results and discussion

Table 1 showed that out of the entire three (3) different honey source used, only one (vickag's honey) of them grown on MRS agar after incubation anaerobically at 37ºC for 24 to 48hours of incubation having a colony size of 0.5mm and designated as J_1.

Table 1: Isolation of presumptive Lactic acid producers from samples collected

Market (s)	Fermented food (s)	No of colonies	No. of isolates
Market square	Vickag's honey	1	J_1
	Bema honey, Omphalos honey	0	
Total	3	1	1

The isolate obtained was subjected to gram stain, catalase test, oxidase test and carbohydrate fermentation test. This is illustrated in plate 1 and figure 1. The isolate was confirmed gram positive bacteria with cocci shape, catalase-negative, oxidase-negative and sugar fermenters (lactose and dextrose). The isolate was further subjected to physiological characterization using temperature and saline (sodium chloride) tolerance test as showed in table 2. The bacterium did not grow at a temperature of 10ºC, but rather grew optimally at a temperature of 37ºC and 45ºC. The isolate grew optimally at a saline concentration of 2%, 4% and 6.5% that's has been incorporated into MRS agar. After DNA sequencing and agarose gel electrophoresis was done, the isolate obtained was confirmed probiotics known as *Enterococcus faecalis*.

Table 2: Microscopic, biochemical and physiological characterization of Lactic acid Bacterium isolated

Tests/Isolates	*Enterococcus faecalis*
Morphology	Cocci
Biochemical testing	
Catalase	Negative
Oxidase	Negative
Carbohydrate fermentation	
Glucose	Positive
Lactose	Positive
Physiological growth at different temperature	
10ºC	Negative
37ºC	Positive
45ºC	Positive
Growth at different NaCl concentration	
2%	Positive
4%	Positive
6.5%	Positive

The sequence homologies detected through phylogenetic analysis showed *Enterococcus feacalis* has 100% similarity with an already previously identified OR271976 *Enterococcus feacalis* from the gene bank. **Table 3, 4, 5, and 6** showed the tolerance of the confirmed LAB to acid and bile salt. Table 3 shows the susceptibility of *Enterococcus fecalis* cells after being inoculated in MRS broth modified with hydrochloric acid of pH 2.5 and bile salt at a concentration of 0.15 and 0.3%. The two modified media will be incubated at

37ºC anaerobically and examined for turbidity at 0hr and 24hour. The isolate was showed to be acid and bile tolerant.

Table 3: Probiotic characteristics (acid and bile tolerance test) of *Enterococcus fecalis*

Inhibitory condition/ LAB isolates	***Enterococcus fecalis***
Acid tolerance test	+
pH 2.5 at 0hour	+
pH 2.5 at 24hours	+
Bile tolerance test	+
0.15% bile at 0hour	+
0.15% bile at 6hours	+
0.15% bile at 24ouhrs	+
0.15% bile at 48hours	+
0.3% bile at 0hour	+
0.3% bile at 6hours	+
0.3% bile at 12hours	+
0.3% bile at 24hours	+
0.3% bile at 48hours	+

T**able 4** showed that *Enterococcus fecalis* isolated from Vickags honey had bacterial count (no bacterial growth) after its inoculum from the modified MRS broth with a pH of 2.5 after 24hours was inoculated into MRS agar and incubated anaerobically at 37ºC for 24 to 48hours.

Table 4: Acid tolerance test of *Enterococcus fecalis* on MRS agar after incubation in MRS broth modified with acid

Isolates	Log_{10}CFU/mL	Log_{10}CFU/mL
	Bacterial count at 0hour ($\times 10^7$)	Bacterial count at 24hours ($\times 10^7$)
Enterococcus fecalis	5.50 ±0.10	5.20 ±0.23

A mean ± standard deviation of 0.00 ± 0.00 signifies that those isolates had no growth (no bacterial count).

Table 5 and 6 showed the results of the analysis of pH values of the inoculated MRS supplemented with 0.15 and 0.3% (w/v) bile salt interpreted via the use of the mean or standard deviation. In MRS without bile, the inoculum (Enterococcus fecalis cell) grew faster than MRS supplemented with 0.15% and 0.3% bile at every sampling time (0, 4, 6, 24 and 48 hours) with growth appearing after 24hours of inoculation. *Enterococcus feacalis* showed attribute in lowering the pH of the MRS agar that has been supplemented with 0.15% and 0.3% bile salt medium when compared with at 0 hour and 48hours of inoculation.

Table 5: Tolerance to 0.15% bile salt of lactic acid bacteria isolated

Culture media/	**MRS agar + 0.15% Bile salt**			
Sampling time (hours)	**0**	**6**	**24**	**48**
Enterococcus fecalis	5.70±0.00	5.68±0.01	5.64±0.20	5.60±0.00

Table 6 showed the antibiotic susceptibility testing (which was carried out with Muller Hinton agar diffusion with Kirby Buer method) of *Enterococcus fecalis* that was assessed through determination with minimum inhibitory concentration (MIC) of ten common (10) antibiotics. The ten antibiotics include amoxicillin (AML, 20µg), norfloxacin (NB, 10µg), streptomycin (S, 30µg), levofloxacin (LEV, 20µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 10µg), rifampicin (RIF, 20µg) erythromycin (ERY, 30µg), ampiclox (APX, 20µg) and gentamicin (CN, 10 µg). Enteroccocus fecalis cells were all susceptible to all the ten antibiotics.

Table 6: Antimicrobial susceptibility test of the confirmed *Enterococcus fecalis*

Antibiotics	Disc potency (µg)	Isolates *Enterococcus fecalis*
Amoxicillin	20	S
Norfloxacin	10	S
Streptomycin	30	S
Levofloxacin	20	S
Chloramphenicol	30	S
Ciprofloxacin	10	S
Rifampicin	20	S
Erythromycin	30	S
Ampiclox	20	S
Gentamycin	10	S

Where inhibition zone diameter
≤ 8mm= Resistant (R)
8−10mm= Moderate susceptibility (M)
≥10mm = susceptibility (S)

Finally hemolytic assay test confirmed that this strain of *Enteroccus fecalis* isolated from vickag's honey was gamma hemolytic. These means they are safe for consumption by human.
Table 6 shows the effect of Honey on some anthropometric (weight, body mass index and Lee's index), biochemical (total cholesterol, fasting blood glucose), hematological (Packed cell volume) and microbiological parameter (glucosuria and proteinuria) at four (4) weeks. However, the standardized value and LD_{50} value obtained for this present study is 65mg/kg body weight and 80mg/kg body weight respectively.
The FBG of T2D rat fed with honey had significant increase in their FBG (from 241 to 300mg/dL) compared to normal (non-diabetic) (82.8 to 85.4mg/dL) rat and T2D induced rat (220 to 300.3mg/dL). The weight and Total cholesterol level of T2D rat fed with honey decreased significantly. The PCV of those T2D rat fed with honey increased when compared toT2D induced rat (60 to 63.5%).

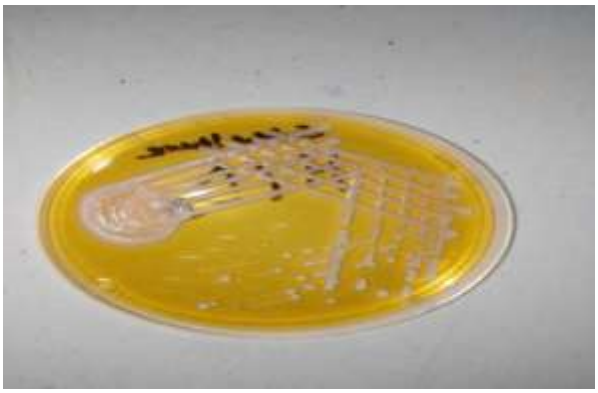

Plate 1.: Showing the growth of *Enterococcus fecalis* on MRS agar

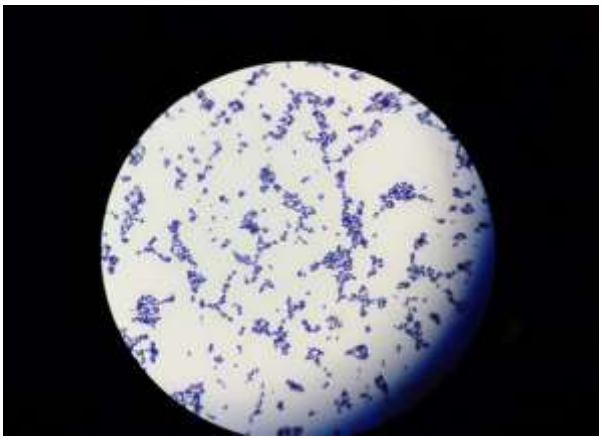

Figure 1: Showing *Enterococcus fecalis* on gram stain

Discussion

Only 1 (33.3%) of the honey out of three different source of honey (100%) used had growth on MRS agar. The reasons why the entire different honey source used in this study did not grow on MRS agar, could be due to some physical factors that can affect probiotic survival such as temperature, temperature of fermentation and pH according to Karin and Barbel (2000).

The bacterium isolated fit the classification of lactic acid bacteria (LAB) as gram positive, catalase-negative, oxidase-negative and carbohydrate fermentation-positive. This finding is in accordance with Akalu *et al*.(2015) who isolated *Lactobacillius* species from Ergo (pickled vegetable). The LAB isolated in this study grew well at 37^{o}C and 45^{o}C after incubation period of 24 to 48 hours which is the optimum temperature range for their growth. However, there was no growth of these isolates at 10^{o}C. This is similar to the study of Ayodeji *et al*. (2020). The isolate obtained in this study grew well at 4.0 % and 6.5% salt concentration. Amplified DNA of the Lactobacilli bacteria isolates were sequenced and the results were compared with the ones in Genebank database using local alignment search tool (BLAST) for complete identification which came out to be *Enterococcus feacalis*, This is support with the work of Abdulmumini *et al*.(2021) which isolated Lactic bacteria from cabbage and Soumitra, (2020) from traditional fermented rice. The bacterium isolated and subjected to a pH of 2.5 at 0 and 24hours showed tolerance to pH of 2.5 for 0 and 24hours. Chan *et al*.(2011) reported that acids such as the hydrochloric acid (HCl) found also in human stomach, disrupt the biomolecules of cells, such as fatty acids , proteins and DNA. Our findings are consistent with previous studies where *Weissella confusa* demonstrated its ability to survive in high acidic environments. The bacterium isolated in this study exhibited high resistance to bile concentrations of 0.15% and 0.3% at 0, 6, 24 and 48hours with reduction in pH of MRS broth. Based on Chateau *et al*. (2011) classification of lactobacilli based on their delay in their growth in the presence of bile salt, the bacterium isolated in this study are sensitive to bile salt.

The LAB isolated was found to be susceptible to all the antibiotics. The outcome of the antibiotic susceptibility testing in this study is not similar to the work of Casado *et al*. (2014). The LAB isolated in this study was non-hemolytic and as such they were not selected for other tests since their safety were confirmed. This is not in accordance with the work done by Olufemi *et al*. (2018) where most LAB strains they isolated were non-hemolytic.

Table 7 shows Comparism of some Anthropometric, biochemical, and hematological parameter of normal, control and treatment group. However, the fasting blood glucose of Type-2 diabetes rats treated with Honey (from 241mg/dL as at 0 week from 3000.3mg/dL as at fourth week) when compared to the fasting blood glucose (FBG) of control group was high. In this present, *Enterococcus fecalis* was isolated from honey. Honey has been found to harbor *Enterococcus feacalis* in previous researches. This probiotic in honey in previous study has been found to regulate in Honey bee developmental genes (Yating *et al*., 2021). The result of this present study obtained from the effect of honey consumption on T2D rat is in concordance with the some previous research such as those obtained by Marzieh and his colleagues (2021), they stated that high intake of honey might increase glucose levels and worsen other metabolic parameters in patients with Type-2 diabetic mellitus (T2DM).

Table 7: Comparing some Anthropometric, biochemical, and hematological parameter of normal, control and treatment group

Parameter (s)	Experimental group					
	Normal group		Control group		treatment group	
	0week	4weeks	0week	4weeks	0week	4weeks
APM						
Wt(g)	209.8±35.3	229.6±13.77	227.8±1.70	220.8±13.83	180±9.2	169.8±1.83
BMI (g/cm^3)	0.54±0.04	0.59±0.04	0.53±0.03	0.5±0.06	0.53±0.03	0.5±0.06
LI (g/cm)	307.3±5.5	351.6±6.51	298.6±7.0	288.6±12.4	270.6±7.0	261.6±12.4
BCM						
FBG (mg/dL)	82.8.3±8.2	85.4±5.1	220±96.6	262.3.±27.9	241.4±96.6	300.3±12.7
TC (mg/dL)	95±7.0	97.2±2.1	123.8±14.5	220.8±13.83	102.3±4.4	93.7±2.5
HEM.						
PCV (%)	62.8±4.1	63.7±23.08	60±2.6	53.5±5.05	61±2.6	63.5±2.05

Key:
APM= Anthropometric parameter; Wt = Weight ;BMI= Body mass index ;LI= Lees index
BCM= Biochemical parameter ;FBG= Fasting blood glucose ;TC= Total cholesterol
HEM= Hematological parameter ; PCV= Packed cell volume; %= percentage
mg/dL=milligram per decilitre ; g/cm= gram per centimeter; g/cm^3

Conclusion and recommendation

In conclusion, honey harbor *Enterococcus fecalis*, a probiotic lactic acid bacterium, however it was found to increase the glycemic index of type-2 diabetic wistar rat. Based on this present study,it is recommended that diabetic patient avoid the consumption of honey. Honey should be preserved in the refrigerator to ensure survival of the probiotic they harbor which might aid in wound healing and weight loss among obsessed patient.

References

1. Abdulmumini, B., Mohammed, S. and Yabaya, A. (2021). Isolation and molecular identification of *Weissella cibaria* from cabbage. *Science World Journal*, 16, 3.
2. Akalu, N., Assefa, F. and Dessalegn, A. (2017). In vitro evaluation of lactic acid bacteria isolated from traditional fermented Shamita and Kocho for their desirable characteristics as probiotics. *African Journal of Biotechnology*, 16 (12), 594–606.
3. Anna, V., Kristi, B., Michael, W., Kim, G. and Richard, G. (2017). Evaluating the impact of DNA extraction method on the representation of Human Oral Bacterial and Fungi contamination, *PLOS ONE*, 12, 1
4. Ayodeji, A., Bimpe, O., Michael, A., Cecilia, K., Osarobo, B. and Olayemi, O. (2020). Screening of probiotic characteristics of LAB isolated from some fermented Nigeria food products. *Tropical Journal of Natural Product Research*, 4 (12), 1166-1169.
5. Casado, C., Benomar, N., Lerma, L., Gálvez, A. and Abriouel, H. (2014). Antibiotic resistance of *Lactobacillus pentosus* and *Leuconostoc pseudomesenteroides* isolated from naturally-fermented Aloreña table olives throughout fermentation process. *International Journal of Food Microbiology*, 172, 110–118.
6. Cavallini U., Roselino, M. and Pauly-Silveira, D. (2012). A potential synbiotic product improves the lipid profile of diabetic rats. *Lipids in Health and Disease*, 11(1), 1–9.
7. Charteau, N., Deschamps, A. Hadj, A. (2011). Heterogeneity of bile salts resistance in the lactobacillus isolates of a probiotic concosortium. *Letters in Applied Microbiology*, 18, 42.
8. Chan, H., Sahadeva, R. and Leong, S. (2011). Survival of commercial probiotic strains to pH and bile. *International Food Resources Journal*, 18(4), 1515-1522.
9. Davodabadi, A., Dallal, M., Foroushan, A., Douraghii, M., and Harati, F. (2015). Antibacterial activity of *Lactobacillius* spp, isolated from feces of healthy infant against enteropathogenic bacteria. *Anaerobe*, 3 (4), 53-58.
10. Estifanos, H. (2014). Isolation and identification of probiotic lactic acid bacteria from curd and in- vitro evaluation of its growth inhibition activities against pathogenic bacteria. *African journal of microbiology Research*, 8(13), 1419-1425.
11. Forhad, M., Khaledur, R., Shahedur, R., Forhad, K. and Krishno, C. (2015). Probiotics properties analysis of isolated lactic acid bacteria from buffalo milk. *Archives of Clinical microbiology*, 7:1-35.
12. Karin, H. and Barbel, H. (2000). Factor affecting the fermentative lactic acid production from renewable resources. *Enzymes and microbial technology*, 26, 87- 107.
13. Marzieh, A., Masoumeh, J., Mohammed, H. and Nazli, N. (2021). The effects of oral consumption of Honey on key metabolic profiles in adult patients with type-2 diabetes mellitus and non-diabetic individuals: a systematic review of Clinical trials. *Evidenced based complement Alternate Medicine*, 3.
14. Mohan, K. and Murugalatha, N. (2011). Isolation of Lactobacillius plantarum from cow meat and screening for the presence of sugar alcohol producing gene. *Journal of Microbiology and Antimicrobials*, 4(1), 16-22.
15. Nabi, J., Hazhir, J., Mansour, A., Morteza, S., Mushtak, T., Hajar, H., Maryam, S. and Zahra, G. (2020). Isolation and identification of potential probiotic Lactobacillius species from feces of infants in southwest Iran. *International Journal of Infectious diseases*, 96, 524-530.

16. Novelli, B., Diniz, Y., Galhardi, M., Ebaid, X., Rodrigues, G., Mani, F., Fernandes, H., Cicogna, C. and Novelli, B. (2007). Anthropometrical parameters and markers of Obesity in rats. *Laboratory animals*, 41, 111-119.
17. Olufemi, F., Chijioke, O., Popoola, S., Oluwafemi, O. (2018). In vitro study of potential probiotic lactic acid Bacteria isolated from the gut of chickens in Abeokuta, Nigeria. Alex. *Journal of Veterinary Science*, 58(1), 73-84.
18. Rajesh, K., Ipseeta, R., Manjusha, K., Ujurale, M., and Deshmukh, A. (2016). Development of an experimental model of diabetes co-esiting with metabolic syndrome in rats. *Advance in pharmacological and pharmaceutical Science*, 6.
19. Razmpoosh, E., Javadi, M., Ejtahed, H. and Mirmiran, P. (2016). Probiotics as beneficial agents in the management of diabetes mellitus: a systematic review. *Diabetes/Metabolism Research and Reviews*, 32 (2), 143–168.
20. Succi, M., Tremonte, P., Reale, A., Sorrentino, E., Grazia, L., Pacifico, S. and Coppola, R. (2005). Bile salts and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. *FEMS Microbiology Letter*, 244, 129-137.
21. Sun-Young, K., Eui-Chun, C., Yaelim, L., Dong, L. and Seokjin, K. (2019). Isolation and characterization of five nove probiotic strains from Koreans infants and children feces. *Plos ONE Journal*, 14 (10), 1-17.
22. Vijayakumar, M., Ilavenil, S., Kim, D., Arasu, M., Priya, K. and Choi, K. (2015). In vitro assessment of the probiotic potential of *Lactobacillus plantarum* KCC-24 isolated from Italian rye-grass (Lolium multiflorum) forage. *Anaerobe*, 2 (32), 90–97.
23. Yasmin, I, Saeed, M., Ali, W., Adnan, K., Farhan, M., Iqbal, A., Tehseen, S., Naz, S., Liaquat, A., Mehmood, T., Ahsan, S. and Tanweer, S. (2020). In-vitro probiotic potential and safety evaluation (Hemolytic and cytotoxic activity) of *Bifidobacterium* strains isolated from raw camel milk. *Microorganism*, 8, 354.
24. Yating, D., Shiqi, L. and XIN, Z. (2021). Enterococcus feaccium regulates honey bee developmental genes. *International Journal of Molecular Science*, 22(22), 1-20.