NTU Journal of Pure Sciences (2025) 4 (3) : 1 - 6
DOI: https://doi.org/10.56286/v0cc5p67

IRAQI Academic Scientific Journals

ISSN: 2789-1089 EISSN: 2789-1097
NTU Journal of Pure Sciences
Available online at: https://journals.ntu.edu.iq/index.php/NTU-JPS/index

Characterization of a Lactobacillus strain found in obese women in Kirkuk, Iraq

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Article Information

Received: 13-07- 2023
Accepted: 09-09-2023
Published online: 12-10-2025

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Key Words:
Lactobacillus,
Obesity,
Kirkuk,
BMI,
Diabetes.

A B S T R A C T

Background: Since the origin of obesity is multifactorial and complex, resulting from the interaction between genetic/epigenetic, environmental, and socio-psychological determinants, in addition to the bacterial composition of the intestinal flora could be a third element involved in the development of excess weight. therefor intestinal flora (especially *Lactobacillus*) plays an intermediary role in the metabolism and inflammation and involved in the pathogenesis of diabetes type 2 and obesity.Objective: This study aimed to determine the relationship between identified *Lactobacillus spp.* and obesity in women.Material and method: This study included the collection of 90 samples from participants (20-50 Years) during the period from December 2022 to March 2023. Body mass index was calculated for study participants and *Lactobacillus* strain was microbiologically and biochemically identified.Results: Results showed that *Lactobacillus* of obese participants are significantly ($p<0.001$) different from that of thin and normal participants.Conclusion: This study showed a microbiological analysis revealed that obese diabetic and non-diabetic patients had higher percentages of many distinct types of *lactobacillus* than thin and healthy diabetic and non-diabetic patients. Female participants with type 2 diabetes had FBG values that were greater than those of non-diabetic participants, and it also rose with age.

Introduction

The bacterial flora of the gastrointestinal tract (GI) of humans, there are differences in the composition of the flora which are influenced by age, diet, cultural conditions, and the use of antibiotics (1). In addition, the flora of the large intestine (colon) is qualitatively similar to that found in feces (2, 25, 26). Lactobacillus is a genus of gram-positive, aerotolerant anaerobes or microaerophilic, rod-shaped, non-motile, non-spore-forming bacteria and Lactobacillus species constitute a significant component of the human and animal microbiota at a number of body sites, such as the digestive system, and the female genital system (3). Lactobacillus bacteria are distinguished by their inability to manufacture the enzyme catalase in the presence of oxygen, due to the creation of hydrogen peroxide, and it produces lactic acid as the main product of fermentation, which is negative for catalase and oxidase tests (4, 26). These bacteria have antagonistic activity against pathogenic microorganisms and secrete metabolites (hydrogen peroxide, lactic acid, organic acids, and bacteriocins), which are generally considered to be aerobic or facultatively anaerobic and acid-loving. They can exist in temperatures between (2 and 53°C) and the ideal temperature for their growth is between (30 and 40°C) (3, 4). Obesity also refers to the buildup of fat in the body (5, 24). It results in a gain in weight due to a flaw or disturbance in the mechanism that regulates lipid exchange, as well as from an imbalance between the energy intake from food and the energy used by the body (6). In comparison to men, women are more likely to be obese (30–50%) than men (20–30%) (7). On the other hand, an important finding in humans was that switching from a fat-rich, low-fiber diet to a low-fat, fiber-rich diet induced significant changes in gut bacteria intestinal in only 24 h. In addition, multiple evidence suggests that, in humans, an increase in dietary fat causes an increase in Gram-negative bacteria while decreasing the number of Lactobacillus organisms (2). In particular, obesity (2) was linked to higher levels of Lactobacillus reuteri and lower levels of Lactobacillus casei/paracasei and Lactobacillus plantarum. Therefor, this study implicated to determine the effect of Lactobacillus spp. as a potential risk factor increasing BMI.

Materials and Methods

Study group

The total of (90) participated females in this study at the age range of (20-50 year) for the period from December 2022 to March 2023 after signing a consent to participate.They were divided according to their BMI as in table (2). The BMI was calculated depending on the law cited in (23, 24) following weight equation:

$$BMI = Weight\ (kg) / Height2\ (m2)$$

The criteria of classification is supported in table (1)

Table 1. Classification of body mass index

Classification	BMI
Severe thinness	< 16 kg/ m2
Moderate thinness	16- 17 kg/ m2
Mild thinness	17- 18.5 kg/ m2
Normal	18.5- 25 kg/ m2
Overweight	25- 30 kg/m2
Obese class I	30- 35 kg/ m2
Obese class II	35- 40 kg/ m2
Obese class III	> 40 kg/ m2

Table 2. Classification of study group according to body mass index (BMI).

Classification	Diabetic patient (N=30)			Non-diabetic patient (N=30)		
BMI	Obese (BMI >30kg/m²)	Thin (BMI 16-18.5kg/m²)	Normal (BMI 18.5-25kg/m²)	Obese (BMI >30kg/m²)	Thin (BMI 16-18.5kg/m²)	Normal (BMI 18.5-25kg/m²)
Total	22	5	3	3	7	20

Collection of Sample and processing

a) Collection of Stool samples and analyses

The stool sample was collected in a clean container according to the method in (8). Once the sample is collected, it was transferred to the laboratory directly for culture.

b) Collection of Blood samples and analyses

Whilst, then, 5ml of blood was drawn by venipuncture from study participants and placed in gel tube for the purpose of separating the serum and process biochemical analyzes and left it for 15 minutes for clotting. The serum was separated by centrifugation at 3000 rpm for 15 minutes, and used to measurement FBG by device Cobas Integra Plus 400 (Roche/Switzerland) as in (9).

Identification of Intestinal bacteria in stool samples

Total of (30) stool samples were directly inoculated onto (MRS agar/Himedia/India) and incubated at 37°C for 48 hrs for Macroscopic and biochemical tests according to the procedure mentioned in (10, 25). Complete diagnosis of isolated bacteria were performed by using API 50CHL for Lactobacillus (BioMerieux, France). Stock cultures were inoculated into (heart and brain infusion broth medium/Himedia/India) and kept in the temperature at -20 ºC for about 6-8 months as mentioned in Ref. (11, 25).

Statistical analysis

The Chi-square test was used with conventional equations to compare the results,and reported with P.value <0.001 as the accepted level of significance accordingly.

Results and Discussion

The likelihood and severity of diabetes type 2 are closely linked with body mass index (BMI) (12, 24). There is a seven times greater risk of diabetes in obese people compared to those of healthy weight, with a threefold increase in risk for overweight people (13). Two studies in 2021 found that severely obese people (BMI ≥40) were at an even greater risk of diabetes type 2, when compared to obese people with a lower BMI (BMI 30-39.9) (12, 14). Also, these findings matched those of Palmer & Toth (15) who found that BMI was one of the factors that increase the chance of developing diabetes type 2.

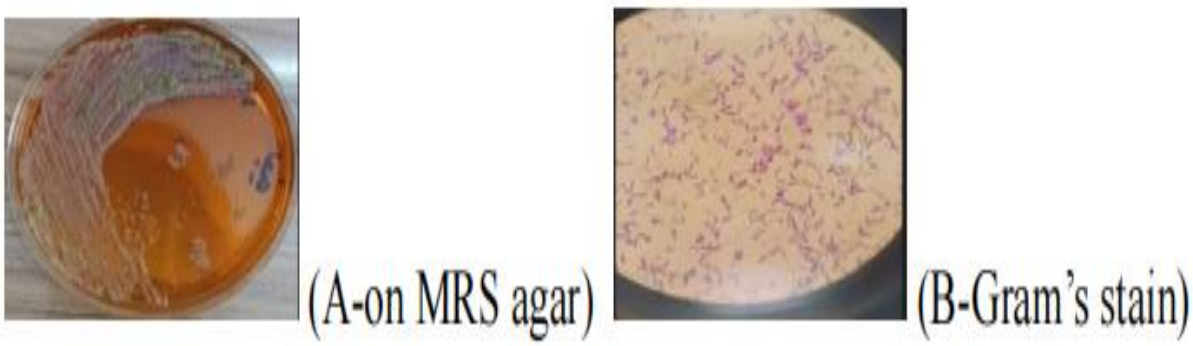

Figure 1. Photo of *Lactobacillus* bacteria cultivated on MRS agar medium for 48 hrs at 37 °C isolated from the stool samples of the study groups.

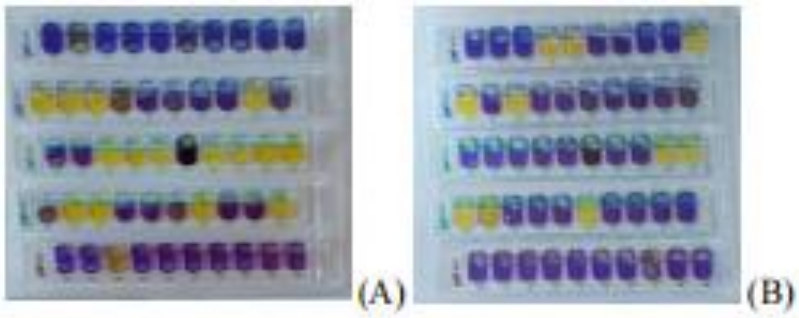

Figure 2. API 50CHL test results for *Lactobacillus* bacteria isolated from stool samples. (A) *L. gasseri* (B) *L. reuteri.*

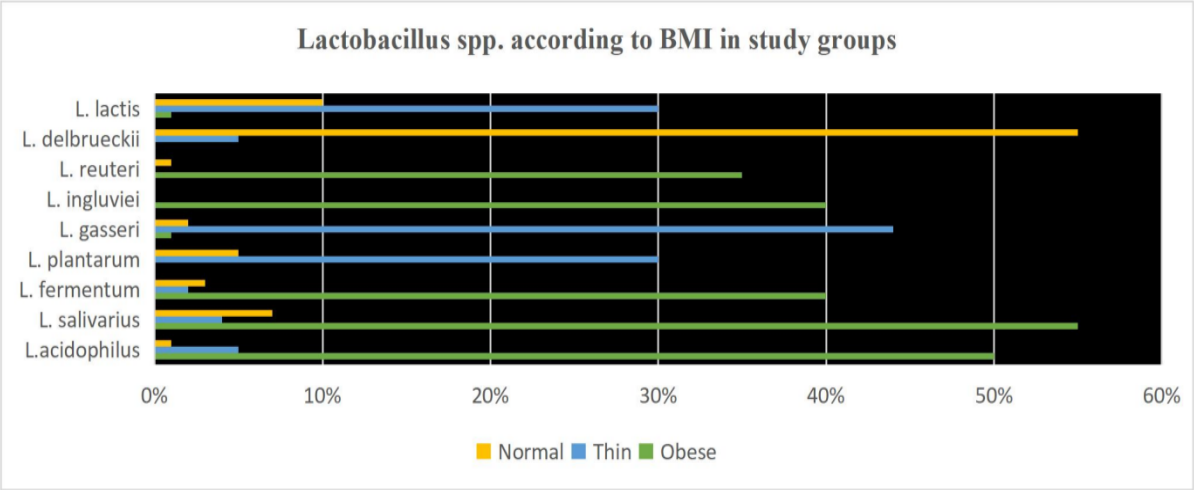

Figure 3. Distribution of identified *Lactobacillus* bacteria isolated from stool samples according to the body mass indexed in study participants.

Results obtained from figures (3) showed that the main Lactobacillus spp. isolated from the obese diabetes type 2 females and non diabetes was L. acidophilus, L. salivarius, L. fermentum, L. ingluviei, L. reuteri,this is because the bacteria in the obese participants create certain enzymatic activity and as a result of their fermentation of the sugars. whilst, Lactobacillus spp. isolated from the thin diabetes and non- diabetes females was L. plantarum, L. fermentum, L. gasseri, L. lactis ,due to the fact that it creates digestive enzymes that break down food and convert it to energy. and in normal L. delbrueckii, L. plantarum. The results of the current study agreed with those results of Falah Salem Daoud and colleague in 2016, who found that the types of lactic acid bacteria (L.acidophilus, L. fermentum, L. reuteri) may cause weight gain, and that the reason for weight gain is that lactic acid bacteria stimulate appetite, produce digestive enzymes, improve the microbial balance in the intestine, and their ability to reduce the amount of the lipids available in the intestine are triglycerides or cholesterol, which have the largest role in the occurrence of weight gain, through their association with other compounds of steroids that are able to fully exploit and metabolize the lipids present in food, turning them into fat layers in the body, which causes an increase in body weight (16).

Similarly, Million and Choi, concluded that Lactobacillus reuteri was associated with obesity (17&18). Other paradoxical results have been demonstrated in these work by Youn, in overweight adolescents, that weight loss due to a low-calorie diet and physical activity increases Lactobacillus (19). On the other hand, the level of fasting blood glucose was also evaluated and figure (4) showed the results.

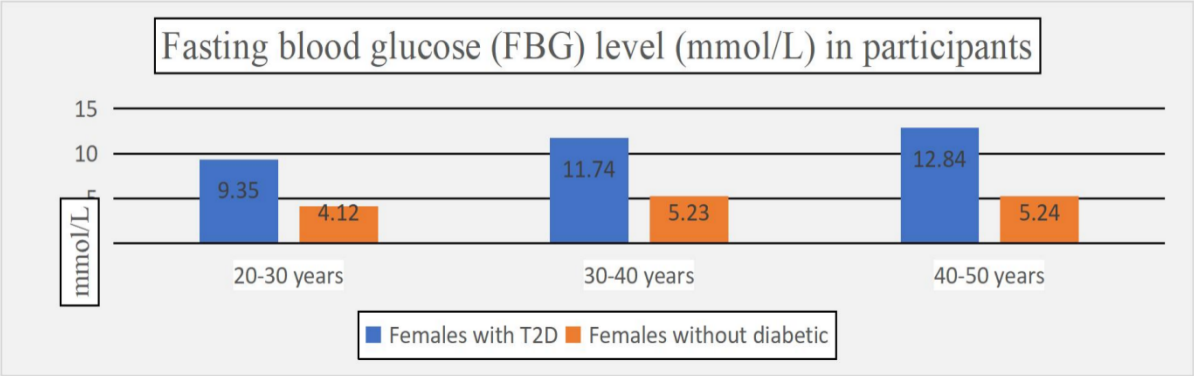

Figure 4. Fasting blood glucose level in participants according to age group.

Moreover, the current results showed that the highest mean of fasting glucose was in the age group (40-50) years about (12.84 mmol/l) in diabetic females. Whereas, the percentages about (5.24 mmol/l) in non-diabetic females as shown in figure (4) at all age groups.

These result, however, agreed with the results of (20, 21) which showed a high level of fasting blood glugose increase with age. This increase in the blood sugar level changes with age as a result of poor glucose tolerance, which decreases with age, with poor control of blood sugar level and deterioration in glucose tolerance result from aging (22). Therefor, microbiological examination showed the isolation of many different types of Lactobacillus with increased Lactobacillus acidophilus, L. salivarius, L. fermentum, L. reuteri, L. ingluviei percentage in obese patients than thin and normal ones.

Conclusion

This study showed a significant relationship between Lactobacillus spp. and diabetic patient with increased body mass index leading to probable useful tools for developing strategies to prevent obesity.

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