**Benha Journal of Applied Sciences (BJAS)** **print: ISSN 2356–9751**
**Vol. (10) Issue (2) (2025), (51-59)** **online: ISSN 2356–976x**
**http://bjas.bu.edu.eg**
**Applied and Basic Science**

# Screening and characterization of potential probiotic lactic acid bacteria isolated from traditional dairy products

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**Abstract**

Lactic acid bacteria (LAB) have been used as probiotic agents due to their ability to produce a variety of beneficial compounds for cultivars and their status as safe microorganisms. Discovering and evaluating new probiotics in dairy products and other normal habitats poses a global economic and health importance. Therefore, in our study, a total of 43 traditional dairy product samples were collected from Menoufia and Qalyubia governorates for isolation of lactic acid bacteria with probiotic characteristics. Seventy-nine bacterial isolates were obtained and identified morphologically and biochemically. Among these isolates, only 23 were found to have LAB characteristics as catalase-negative, gram-positive and non-motile. The most potent five isolates (P18, P19, P28, P41, and P42) were tested for their probiotic characteristics, including low pH tolerance and bile salt stability. They showed high tolerance against pH 3.0 and 0.3 % bile salt concentration. Thus, this research provides a foundation for developing dairy products that can positively affect human health by incorporating probiotic microorganisms.

**Key words:** Lactic acid bacteria, Isolation, Screening, Probiotics, Dairy products.

## 1. Introduction

Dairy products manufactured from raw milk that is produced locally continue to play a significant role in everyday diets; The characteristics of these products vary by region, influenced by the local indigenous microbiota, This subsequently mirrors the climatic conditions of the area. These products share a similar characteristic: the incorporation of fermentation by lactic acid bacteria (LAB) is essential to their production.

LAB are a diverse group of Gram-positive, non-spore-forming and anaerobic or microaerophilic rods or cocci in shape, known for their ability to ferment carbohydrates into lactic acid, serving a vital function in the creation of diverse fermented foods [1]. LAB are commonly found in nutrient-rich environments such as dairy products (milk, cheese), fermented foods (meat, vegetables, beverages), and baked goods. They also naturally occur in various ecosystems, including soil, water, decaying plant matter, and the mucosal surfaces of humans and animals, such as the gastrointestinal tract, oral cavity, and skin [2].

LAB are pivotal in the fermentation of a wide array of foods, contributing to their flavor, texture, preservation, and nutritional value. They are integral to the production of fermented dairy products like yogurt and cheese, as well as fermented vegetables, meats, and beverages. LAB function by converting carbohydrates into lactic acid, which not only imparts a characteristic tangy flavor but also acts as a natural preservative by lowering the pH and inhibiting the growth of spoilage organisms. This process is fundamental in creating products such as sourdough bread, pickles, and fermented sausages. Moreover, LAB can produce other compounds like bacteriocins and exopolysaccharides, enhancing the safety and texture of fermented foods [3].

LAB are renowned for creating a variety of antibacterial compounds that prevent pathogenic microorganisms from growing. These include bacteriocins, hydrogen peroxide, and organic acids [4]. [5,6] Because of the presence of various bioactive compounds that confer antimicrobial activity, *L. acidophilus* and *Lb. plantarum* have significant probiotic activity, exhibiting both antibacterial and antibiofilm effects against MDR Uropathogenic *E. coli* (UPEC) at both phenotypic and genotypic levels.

A new and alternative form of preservation, food bio-preservation, is gaining popularity among consumers as a safe and environmentally friendly post-harvest procedure. LAB's strong antibacterial qualities make them a natural inhibitor of harmful microbes and food deterioration, as well as a bio-preservative. [7]. Furthermore, it is known that certain LAB strains produce the alleged health-promoting benefits of mannitol [8].

Probiotics are live microorganisms that provide health benefits to the host when given in sufficient quantities [9]. LAB, encompassing a diverse range of genera, are recognized as potent probiotics that contribute significantly to gut health. Their beneficial effects extend beyond the digestive system, including the potential to bind and neutralize carcinogens, thereby reducing their absorption and promoting excretion. LAB also play a role in lowering plasma cholesterol levels, which can support cardiovascular health. Furthermore, they stimulate the immune system, enhancing the body's defense against pathogens. Some strains have been associated with alleviating allergic responses, such as those related to allergic rhinitis and atopic dermatitis

[10].

Many probiotic species and strains are members of the *Bifidobacterium* and *Lactobacillus* genera. Probiotic bacteria include *Bacillus, Propionibacterium,* the yeast *Saccharomyces*, and other LAB families (*Streptococcus* and *Enterococcus*) [11].

Bacteria predominate among the various microorganisms that reside in the gastrointestinal tract (GIT). The GIT also contains yeasts, molds, and other archaeal domains [12]. Numerous health advantages are believed to be associated with probiotic bacteria. They increase nutritional status, have anti-inflammatory, anti-cholesterol, and anti-colon cancer properties, activate the host's mucosal and systemic immune responses, and have antibacterial activity against enteric pathogenic bacteria. Additionally, they lessen the signs of allergic responses, lactose intolerance, and diarrhea [12]. Multistrains or multispecies probiotic mixtures are therefore gaining popularity due to their ability to provide a wider range of health benefits and additive or even synergistic effects [13]. There is growing scientific interest in the advantages of probiotic intervention in the gut microbiota for preserving and regaining health [14]. In addition to adhering to and colonizing the gastrointestinal epithelium layer, probiotic organisms must be able to survive in the presence of bile and gastric acid. Additionally, they ought to be harmless and non-pathogenic to the host [15].

Isolating and screening LAB that naturally occur in traditional dairy products from Egypt and other typical ecosystems was the aim of this study. In order to look into these isolates' probiotic qualities, they were characterized and identified using both morphological and biochemical techniques.

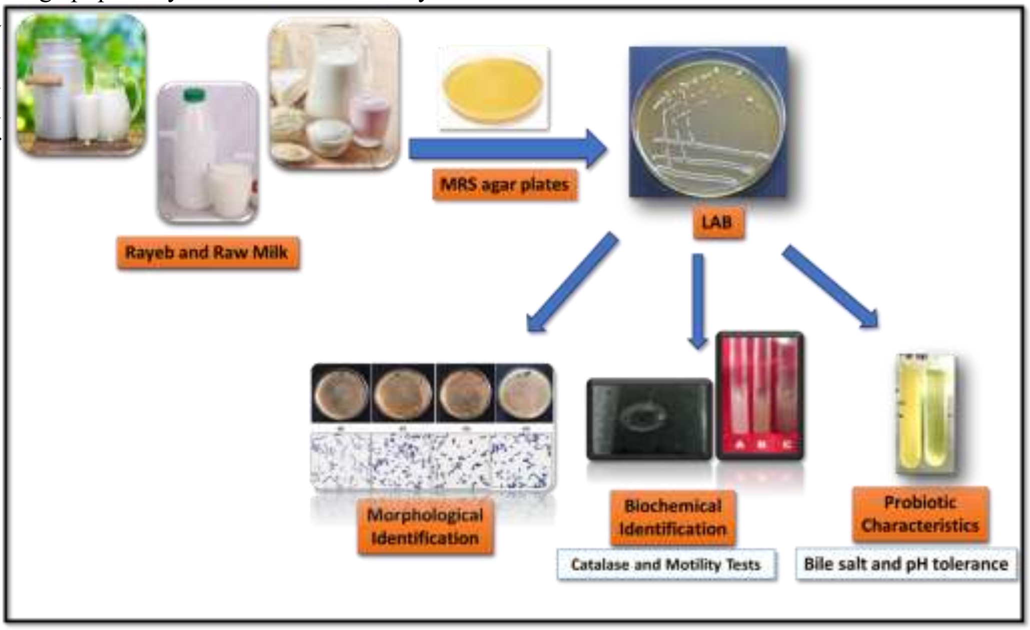


**Fig. (1)** Graphical abstract for isolation and characterization of LAB.

## 2. Materials and Methods

Data in Figure 1. Showed the different steps for isolation, morphological and biochemical characterization, and probiotic characteristics of LAB.

### 2.1. Sample collection

The study was carried out between January and November 2024 to isolate and identify the naturally occurring potential probiotic LAB from normal habitats and traditional dairy products as fresh raw animal milk, mish liquid, and traditional rayeb milk.

A total of forty-three samples were collected aseptically from local producers from Menoufia (39 samples) and Qalyubia governorate (4 samples). Samples were gathered in sterile sample vials and transported to the lab with an icebox. The gathered samples were stored at 4°C in a refrigerator until the analysis started.

### 2.2. Isolation of lactic acid bacteria

LAB isolation was accomplished using the streak plate method as determined by **El Kahlout et al.** [16]. Ten milliliters of each dairy product sample were homogenized with sterilized peptone physiological saline solution (1% peptone (Oxoid), 0.9% NaCl) for about 1-3 minutes aseptically. Then, using 1 mL of homogenate, the proper serial dilution ($10^{-1}$ to $10^{-6}$) was made for every sample. On MRS (OXOID) agar media, 100 microliters of the proper dilutions were spread out. The plates were then incubated at 37 °C for 48 hours in an anaerobic jar. Common LAB traits include the following: colonies were selected at random and purified by streaking them twice or three times on fresh MRS agar plates, then examined under a microscope and under a microscope. From each plate, the colonies exhibiting the typical traits of LAB were selected for biochemical and physiological analysis.

### 2.3. Morphological and biochemical identification of the isolated bacteria

Multiple colonies underwent comprehensive biochemical and morphological analysis, encompassing examination of colony morphology, Gram staining, catalase enzyme activity, and motility assessments.

**2.3.1. Gram staining**

For each isolated strain, a conventionaatly gram staining applied to each isolated strain in accordance with standardised procedures. as shown by [17,18]. A microbial smear was deppsited onto a glass slide, subsequently dried using air currents and heat fixation. Following fixation, the smear was exposed to a crystal violet stain for a period of one minute prior to water rinsing. Thereafter, Gram's iodine solution was applied and left in contact with the smear. 95 percent ethyl alcohol was used to achieve decolorization, followed by water rinsing. Then counterstaining by exposing the smear to safranin for one to two minutes, which was then followed by water rinse prior to microscopic examination by 100x magnification.

**2.3.2. Catalase test**

A drop of 3% hydrogen peroxide was added to a fresh culture that had been prepared on a sanitized glass slide and properly mixed. The formation of bubbles or froth indicated catalase positivity, whereas the lack of these signs indicated catalase negativity [17,18].

**2.3.3. Motility test**

Using the hanging drop slide method, the motility of the isolated bacteria was examined. MRS broth was used to cultivate pure cultures of the separated materials. To create a hanging drop, one drop of broth culture was poured over the coverslip and inverted over the hanging drop slide's concave depression. To improve the cover slip's attachment and stop air currents and fluid evaporation, Vaseline was applied around the hanging drop slide's concave depression. By employing an immersion oil lens to view the slide under a compound microscope, the motile and nonmotile organisms were distinguished [19].

**2.4. Probiotic characteristics of the isolated LAB**

**2.4.1. pH tolerance test**

3 N HCl and 1 N NaOH were used to adjust the MRS broth's pH to 3.0. In test tubes, fresh bacterial cultures were added to the proper MRS broth, and the mixture was incubated for 4 hours at 37 °C. A spectrophotometer was used to detect the optical density at 620 nm. The optical density of the culture media was measured in order to obtain the results [20].

**2.4.2. Bile salt tolerance test**

A sterilized MRS broth medium containing 0.3% bile salt was inoculated with 100 micro-liters of overnight-grown cultures. A spectrophotometer was used to detect the optical density at 620 nm after 24 and 48 hours of incubation, which started at 37◦C. The results were obtained by examining the optical density of the culture media [20].

**3. Results and Discussion**

**3.1. Isolation of probiotic bacteria**

Data in **Table (1)** and **Fig. (2)** showed that a total of 43 samples were collected from local producers in Menoufia Governorate (39 samples) and Qalyubia Governorate (4 samples) for the isolation of LAB with probiotic characteristics from traditional dairy products and other natural habitats.

Among these forty-three samples, 29 samples were from Buffalo, 13 samples from cow, and only one sample from Goat. The samples collected were: Rayeb milk (17 samples), raw milk (16 samples), Mish liquid (7 samples), calves' infant feces (2 samples) and traditional butter (one sample).

**Hernández-Castellano et al.** [21] showed that in Egypt, South American, African, Asian, and Mediterranean nations, water buffaloes (*Bubalus bubalis*) are regarded as the primary dairy animal and the most significant dairy species. About 70% of Egypt's yearly milk production comes from water buffalo [22], It is the second-most popular milk in the world [23]. Egyptian consumers love this milk because it is white, has a good flavor, and has a higher fat content than cow's milk [24].

**Quigley et al** [25] showed that because LAB species constitute the predominant bacterial population, buffalo milk is widely accepted. The most prevalent LAB in buffalo milk are Lactobacillus, Lactococcus, Streptococcus, Leuconostoc, and Enterococcus.

Additionally, Reque & Brandelli [26] discovered that the most common and well-known probiotic microorganisms are lactobacilli and bifidobacteria, which are typically found as commensals in the human gastrointestinal tract, while LAB (e.g., *Lactococcus, Streptococcus, Pediococcus,* and *Enterococcus*) are the primary probiotic group in buffalo milk.

**Table (1)** Different sources for isolation of probiotic lactic acid bacteria.

| Sample Code | Sample type | No. of isolates | Sample animal source | Sampling region | Sample Code | Sample type | No. of isolates | Sample animal source | Sampling region |
|---|---|---|---|---|---|---|---|---|---|
| MB01 | Raw milk | 2 | Buffalo | Menoufiya gov. | SH23 | Mish liquid | 2 | Buffalo | Menoufiya gov. |
| MB02 | Raw milk | 1 | Buffalo | Menoufiya gov. | SB24 | Traditional butter | 2 | Buffalo | Menoufiya gov. |
| MC03 | Raw milk | 2 | Cow | Menoufiya gov. | SH25 | Mish liquid | 1 | Buffalo | Menoufiya gov. |
| FB04 | Feaces | 3 | Buffalo | Menoufiya gov. | SH26 | Mish liquid | 2 | Buffalo | Menoufiya gov. |
| MB05 | Raw milk | 2 | Buffalo | Menoufiya gov. | SH27 | Mish liquid | 2 | Buffalo | Menoufiya gov. |
| MB06 | Raw milk | 1 | Buffalo | Qalyubia gov. | SH28 | Mish liquid | 2 | Buffalo | Menoufiya gov. |
| MC07 | Raw milk | 2 | Cow | Menoufiya gov. | RB29 | Rayeb milk | 2 | Buffalo | Menoufiya gov. |
| MC08 | Raw milk | 2 | Cow | Menoufiya gov. | RB30 | Rayeb milk | 2 | Buffalo | Menoufiya gov. |
| FC09 | Feaces | 2 | Cow | Menoufiya gov. | RB31 | Rayeb milk | 2 | Buffalo | Menoufiya gov. |
| RC10 | Rayeb milk | 1 | Cow | Menoufiya gov. | RB32 | Rayeb milk | 2 | Buffalo | Menoufiya gov. |
| RC11 | Rayeb milk | 2 | Cow | Menoufiya gov. | MC33 | Raw milk | 1 | Cow | Menoufiya gov. |
| RC12 | Rayeb milk | 2 | Cow | Menoufiya gov. | MB34 | Raw milk | 2 | Buffalo | Menoufiya gov. |
| RC13 | Rayeb milk | 2 | Cow | Menoufiya gov. | MB35 | Raw milk | 2 | Buffalo | Menoufiya gov. |
| RC14 | Rayeb milk | 1 | Cow | Menoufiya gov. | MB36 | Raw milk | 2 | Buffalo | Menoufiya gov. |
| RC15 | Rayeb milk | 2 | Cow | Qalyubia gov. | SH37 | Mish liquid | 2 | Buffalo | Menoufiya gov. |
| RC16 | Rayeb milk | 2 | Cow | Qalyubia gov. | RB38 | Rayeb milk | 1 | Buffalo | Menoufiya gov. |
| RC17 | Rayeb milk | 3 | Cow | Qalyubia gov. | RB39 | Rayeb milk | 2 | Buffalo | Menoufiya gov. |
| SH18 | Mish liquid | 2 | Buffalo | Menoufiya gov. | RB40 | Rayeb milk | 1 | Buffalo | Menoufiya gov. |
| RB19 | Rayeb milk | 2 | Buffalo | Menoufiya gov. | MB41 | Raw milk | 2 | Buffalo | Menoufiya gov. |
| MB20 | Raw milk | 2 | Buffalo | Menoufiya gov. | MG42 | Raw milk | 2 | Goat | Menoufiya gov. |
| MB21 | Raw milk | 1 | Buffalo | Menoufiya gov. | MB43 | Raw milk | 2 | Buffalo | Menoufiya gov. |
| RB22 | Rayeb | 2 | Buffalo | Menoufiya gov. | | | | | |

milk

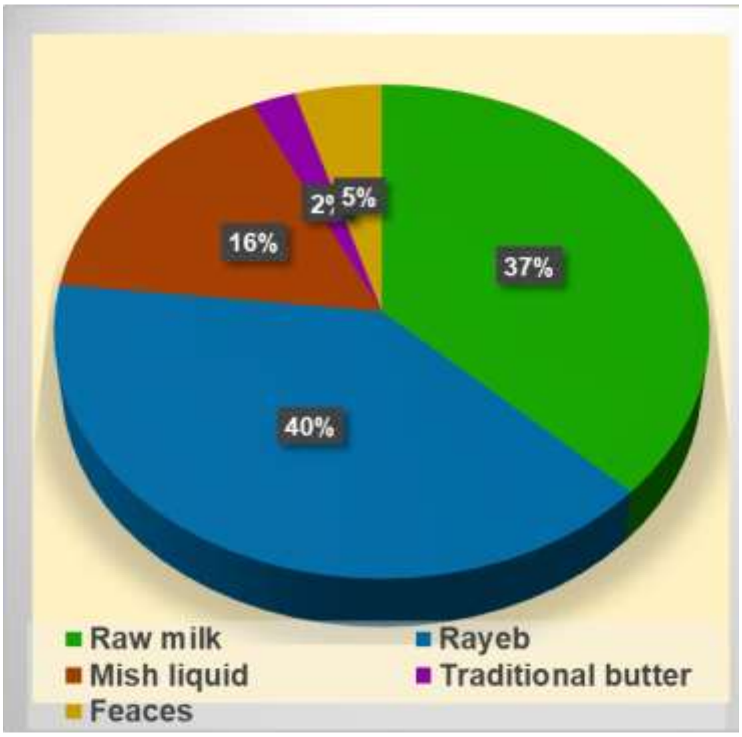


**Fig. (2)** Number of isolated bacteria from different source

**3.2. Morphological and Biochemical identification of bacterial isolates**

A total of 79 isolates were obtained and identified morphologically and biochemically as shown in **Table 2** and **Fig. 3**.

Microscopic observation was performed to observe the shape and Gram reaction of the isolates. Among the 79 isolates, 42 isolates were found to be gram-negative, whereas 37 isolates were found to be gram-positive.

Catalase and motility tests were conducted to identify LAB. Among the 79 isolates, 56 isolates were found to be catalase-positive and 23 isolates were found to be catalase-negative. In addition, 56 isolates were found to be motile and 23 isolates were found to be non-motile.

Similar results were obtained by Leroy and De Vuyst [27] who found that the main LAB groups are Gram-positive, catalase-negative and non-motile organisms.

Also, **El–Zawahry et al.** [28] succeeded in isolating LAB that were Gram-positive, catalase-negative, and non-motile from traditional dairy products (Qarish Cheese). Furthermore, **Vanniyasingam et al.** [4] isolated 8 LAB isolates from dairy products, and those that were gram-positive, catalase negative, and non-motile.

**Table (2)** Biochemical characteristics of Lactic acid bacteria

| Source sample | iso-late code | Catalase test | Gram stain | Motili-ty | Source sample | isolate code | Catalase test | Gram stain | Motility |
|---|---|---|---|---|---|---|---|---|---|
| MB01 | P1 | Positive | Negative / Rods | Motile | RC15 | P27 | Positive | Positive / cocci | Motile |
| MB01 | P2 | Negative | Positive / Rods | Non-motile | RC16 | P28 | Positive | Negative / Rods | Motile |
| MB02 | P3 | Positive | Positive / cocci | Motile | RC16 | P29 | Positive | Negative / Rods | Motile |
| MC03 | P4 | Negative | Positive / Rods | Non-motile | RC17 | P30 | Positive | Negative / Rods | Motile |
| MC03 | P5 | Positive | Negative / Rods | Motile | RC17 | P31 | Positive | Negative / Rods | Motile |
| FB04 | P6 | Positive | Negative / Rods | Motile | RC17 | P32 | Positive | Negative / Rods | Motile |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| FB04 | P7 | Positive | Negative / Rods | Motile | SH18 | P33 | Negative | Positive / Cocci | Non-motile |
| FB04 | P8 | Positive | Negative / Rods | Motile | SH18 | P34 | Positive | Negative / Rods | Motile |
| MB05 | P9 | Negative | Positive / Rods | Non-motile | RB19 | P35 | Negative | Positive / Rods | Non-motile |
| MB05 | P10 | Positive | Negative / Rods | Motile | RB19 | P36 | Positive | Negative / Rods | Motile |
| MB06 | P11 | Positive | Negative / Rods | Motile | MB20 | P37 | Negative | Positive / Rods | Non-motile |
| MC07 | P12 | Negative | Positive / Rods | Non-motile | MB20 | P38 | Positive | Positive / cocci | Motile |
| MC07 | P13 | Positive | Negative / Rods | Motile | MB21 | P39 | Positive | Negative / Rods | Motile |
| MC08 | P14 | Positive | Negative / Rods | Motile | RB22 | P40 | Negative | Positive / Rods | Non-motile |
| MC08 | P15 | Positive | Negative / Rods | Motile | RB22 | P41 | Positive | Positive / cocci | Motile |
| FC09 | P16 | Positive | Positive / cocci | Motile | SH23 | P42 | Negative | Positive / Cocci | Non-motile |
| FC09 | P17 | Positive | Positive / cocci | Motile | SH23 | P43 | Positive | Negative / Rods | Motile |
| RC10 | P18 | Negative | Positive / Rods | Non-motile | SB24 | P44 | Positive | Negative / Rods | Motile |
| RC11 | P19 | Positive | Negative / Rods | Motile | SB24 | P45 | Positive | Negative / Rods | Motile |
| RC11 | P20 | Positive | Negative / Rods | Motile | SH25 | P46 | Negative | Positive / Rods | Non-motile |
| RC12 | P21 | Positive | Negative / Rods | Motile | SH26 | P47 | Negative | Positive / Rods | Non-motile |
| RC12 | P22 | Positive | Negative / Rods | Motile | SH26 | P48 | Positive | Negative / Rods | Motile |
| RC13 | P23 | Positive | Negative / Rods | Motile | SH27 | P49 | Positive | Negative / Rods | Motile |
| RC13 | P24 | Positive | Negative / Rods | Motile | SH27 | P50 | Positive | Negative / Rods | Motile |
| RC14 | P25 | Positive | Negative / Rods | Motile | SH28 | P51 | Negative | Positive / Rods | Non-motile |
| RC15 | P26 | Positive | Positive / cocci | Motile | SH28 | P52 | Positive | Positive / cocci | Motile |

**Table (2)** continued

| **Source sample** | **isolate code** | **Catalase test** | **Gram stain** | **Motility** |
|---|---|---|---|---|
| RB29 | P53 | Negative | Positive / Rods | Non-motile |
| RB29 | P54 | Positive | Positive / cocci | Motile |
| RB30 | P55 | Negative | Positive / Rods | Non-motile |
| RB30 | P56 | Positive | Negative / Rods | Motile |
| RB31 | P57 | Negative | Positive / Rods | Non-motile |
| RB31 | P58 | Positive | Positive / cocci | Motile |
| RB32 | P59 | Positive | Negative / Rods | Motile |
| RB32 | P60 | Positive | Negative / Rods | Motile |
| MC33 | P61 | Negative | Positive / Rods | Non-motile |
| MB34 | P62 | Positive | Positive / cocci | Motile |
| MB34 | P63 | Positive | Positive / cocci | Motile |
| MB35 | P64 | Positive | Negative / Rods | Motile |

| | | | | |
|---|---|---|---|---|
| MB35 | P65 | Positive | Negative / Rods | Motile |
| MB36 | P66 | Negative | Positive / Rods | Non-motile |
| MB36 | P67 | Positive | Positive / cocci | Motile |
| SH37 | P68 | Negative | Positive / Cocci | Non-motile |
| SH37 | P69 | Positive | Negative / Rods | Motile |
| RB38 | P70 | Positive | Negative / Rods | Motile |
| RB39 | P71 | Negative | Positive / Rods | Non-motile |
| RB39 | P72 | Positive | Negative / Rods | Motile |
| RB40 | P73 | Positive | Negative / Rods | Motile |
| MB41 | P74 | Negative | Positive / Rods | Non-motile |
| MB41 | P75 | Positive | Negative / Rods | Motile |
| MG42 | P76 | Negative | Positive / Rods | Non-motile |
| MG42 | P77 | Positive | Positive / cocci | Motile |
| MB43 | P78 | Negative | Positive / Rods | Non-motile |
| MB43 | P79 | Positive | Negative / Rods | Motile |

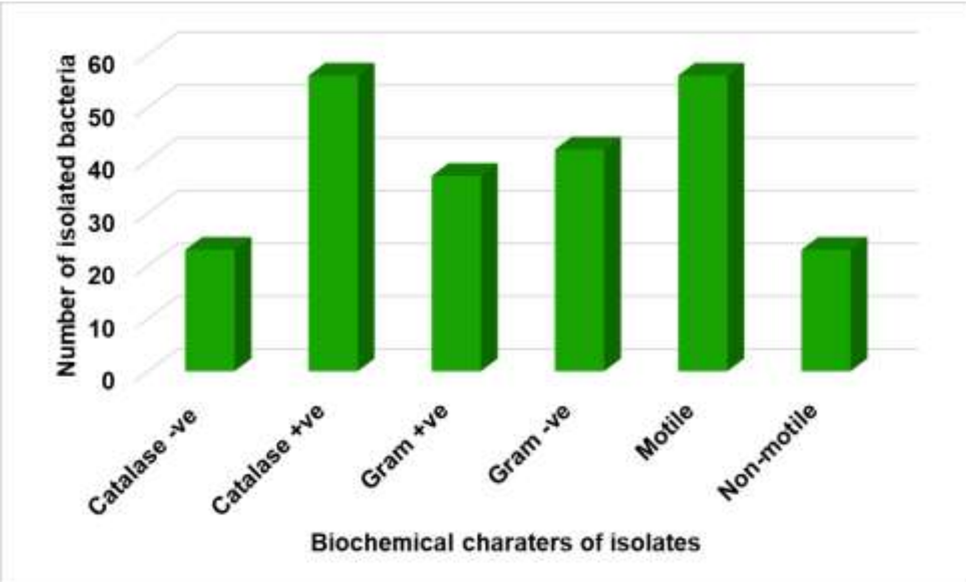


**Fig. (3)** Biochemical characteristics of isolated bacteria.

### 3.3. Characteristics of LAB

#### 3.3.1. pH tolerance

**Table (3)** Low pH tolerance exhibited by the selected bacterial isolates.

| Isolate name | Wavelength of normal MRS broth (control) | Wavelength of MRS broth with 3.0 PH | Survival rate |
|---|---|---|---|
| P18 | 1.792 | 1.628 | 91% |
| P19 | 1.895 | 1.810 | 96% |
| P28 | 1.738 | 1.540 | 98% |
| P41 | 1.832 | 1.802 | 98% |
| P42 | 1.728 | 1.571 | 91% |

Among the 79 isolates, only 23 isolates were found to have LAB characteristics as catalase-negative, gram-positive, and non-motile. We have chosen five isolates to test their probiotic characteristics including low pH tolerance and bile salt stability.

**Table (3)** shows the results of Low pH tolerance exhibited by the selected bacterial isolates. In present study, the selected isolates P18, P19, P28, P41, and P42 showed high tolerance against pH 3.0, with survival rates more than 90% compared to the control. The main feature of probiotics throughout the selection process is their resistance to low pH levels. As they leave the stomach, probiotics must be able survive at low pH levels, roughly 1.5 to 3.5. Before being eliminated from the stomach, the probiotic must continue to be viable for three hours following its initial ingestion. [29].

**Azat et al.** [30] showed that, For certain probiotic strains, the survival rate at pH 3.0 is regarded as the ideal acid tolerance

Our results are in agreement with those obtained by **Vanniyasingam et al.** [4] who reported that LAB strains obtained from dairy products could thrive at pH 3.0, indicating that they are acid-tolerant LAB bacteria. Furthermore, several LAB that were isolated from traditional dairy products by **El Zawahry et al.** [28] performed well, exhibiting low pH tolerance and an 80% survival rate when compared to the control.

#### 3.3.2. Bile salt tolerance

Bile tolerance, which enables the bacteria to survive, grow, and produce their therapeutic effects in the gastrointestinal tract, is another crucial characteristic of probiotic LAB employed as adjuncts. In this study, the selected isolates P18, P19, P28, P41, and P42 showed high tolerance to 0.3 % bile salt concentration with survival rates more than 72% compared to the control as shown in Table 4.

These results are in accordance with El–Zawahry et al. [28] who found that isolated LAB treated with 0.3% bile salt showed high resistant compared to bacteria treated without bile salt. Moreover, Rahman et al. [29] evaluated 8 isolates from local yogurt for resistance against 0.3 % bile salt, and all of them showed resistance.

**Table (4)** Bile salt stability exhibited by the selected bacterial isolates.

| Isolate name | Wavelength of normal MRS broth (control) | Wavelength of MRS broth with 0.3% Bile salt | Survival rate |
|---|---|---|---|
| P18 | 1.792 | 1.405 | 78% |
| P19 | 1.895 | 1.600 | 84% |
| P28 | 1.738 | 1.271 | 73% |
| P41 | 1.832 | 1.552 | 85% |
| P42 | 1.728 | 1.258 | 73% |

## 4. Conclusion

From this study, we can conclude that traditional dairy products are a rich source of probiotic LAB as we could isolate and identify a number of these bacteria. In order to enhance community health, additional research should focus on the discovered LAB's economic advantages.

## 5. Acknowledgments

The Benha University Faculty of Science is acknowledged by the authors for their cooperation and provision of the laboratory space.

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