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Exploring Lactic Acid Bacteria in Nasiriyah's Locally Manufactured Cheese

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Abstract

This investigation aimed to identify and diagnose lactic acid bacteria in locally produced cheese. Forty-eight isolates were obtained by categorizing them according to their phenotypic and microscopic features. After conducting biochemical tests, we acquired ten bacterial isolates from the Lactobacillus species. The VITEK 2 instrument was utilized to distinguish the samples to the species level with a threshold for the Lactobacillus genus. In the city of Nasiriyah, visiting the various local markets could obtain samples of locally produced cheese. The samples were placed in plastic containers, sterilized, sealed, and refrigerated. Sterile and refrigerated conditions were maintained throughout transporting samples to the laboratory.The analysis revealed that seven samples were identified as Lb. Paracasei species, with a probability range of 85 91%. Additionally, two samples could potentially be categorized as Lb. Plantarum species, with a probability of 88%. Lastly, one sample showed a likelihood exceeding 85% of being Lb. Parabuchneri. The assessment examined properties like acid production, curd firmness, and whey interactions.

Keywords: Lactic acid bacteria, dairy product, Nasiriyah ,VITEK 2 system. Identification.

INTRODUCTION

Acid curd cheese is an affordable choice for people. However, its short shelf life of 7 8 days is due to labor processing infections caused by low salt and high moisture levels. This cheese is prone to deterioration from yeast and mold, leading to food waste and challenges for producers and consumers [1].

Certain strains of acid curd cheese have shown promise in combating pathogens, highlighting their importance in promoting health and food safety. Recent research in the Middle East on dairy product compositions has sparked interest, with a study focusing on isolating LAB from cheeses and revealing Lactobacillus species with abilities that could offer health benefits. Exploring the role of Lactobacillus in cheese varieties can deepen our understanding of these bacteria found in microbiota [2].

A recent study emphasized the significance of microbiota in cheese production by showcasing the variety of LAB in dairy products. Researchers stressed the importance of studying and identifying these microorganisms to enhance dairy products' quality and safety standards.

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Ongoing research has been focusing on studying the microbiota present in cheeses from various regions. Some studies have identified Lactobacillus species that contribute to the flavors of these cheeses. Another investigation concentrated on cheeses from Southern Italy, isolating Lactobacillus strains essential for the fermentation process. These studies highlight the significance of diversity and environmental factors in cheese making [3].

Furthermore, a separate study explored a range of dairy products in Asturias, Spain, revealing a community by employing techniques that classify Lactobacillus species. These findings reinforce the idea that traditional production methods and local conditions play a role in shaping the characteristics present in cheeses [4].

The importance of isolating and recognizing Lactobacillus species goes beyond understanding cheese fermentation. These strains are recognized for their effects on gut health, immune system fortification, and prevention of bacterial growth. In a research endeavor, scientists investigated the advantages of Lactobacillus isolates derived from cheeses. By employing phenotypic and genotypic techniques, researchers could identify the lactic flora strains present in traditional Urfa cheese. Crafted from sheep's milk [5].

Lactic acid bacteria (LAB) play a role in enhancing food safety by preserving fermented foods. The development of flavors in dairy fermentations is attributed to a series of reactions that bring out the qualities of classic dairy varieties – emphasizing the importance of assessing newly discovered wild LAB strains sourced from premium raw milk sources [6].

The natural world offers a dynamic environment that provides a setting for discovering new types of LAB species or varieties. The different strains of LAB used in cheese production shape the taste of dairy products. These strains are also used as starters or supplements for acid, autolysis, proteolysis, and diacetyl production [7].

This study aims to explore the diversity in dairy products from Iraq by identifying the types of Lactobacillus bacteria present in locally-made cheese from Nasiriyah. It also aims to pinpoint strains with fermentation capabilities and potential health benefits to enhance our knowledge of bacteria (LAB) found in cheeses. The findings from this research could have implications for the dairy industry regarding developing products and starter cultures using beneficial Lactobacillus strains sourced locally [8].

MATERIALS AND METHODS

Samples Collection

In the city of Nasiriyah, visiting the various local markets could obtain samples of locally produced cheese. The samples were placed in plastic containers, sterilized, sealed, and refrigerated. Sterile and refrigerated conditions were maintained throughout transporting samples to the laboratory.

Counting and Isolation of Strains

A twenty-gram piece of cheese was mixed with 180 mL of clean 2% sodium citrate solution. In the blender, cheese specimens were mixed. Following the rules set by the International Dairy Federation (IDF), decimal dilutions were created by mixing 10 mL with 90 mL of sterile peptone water (0.1% w/v) from Merck (Darmstadt, Germany).

To find out how many lactic acid bacteria were present, two types of media were employed: MRS agar (Merck), which can be used to count and separate lactobacilli after being incubated at 37 °C for 48 hours, and M17 agar (Merck), which can be used to count and separate lactococci after being incubated at 30 °C for 48–72 hours.

Three randomly selected colonies from M17, MRS, and plates were obtained for every batch of the 48 cheese samples. As a result, 432 colonies from each medium were collected in total. Following purification in the same media, all isolates were kept as frozen stocks in either MRS or M17 broth with 40% glycerol at 80 °C.

Each isolate was subjected to Gramme stain, morphology, and catalase output tests [9]. Gram-positive and catalase-negative isolates were tested for generating gas from glucose using inverted

Durham tubes containing MRS and M17 broth [10]. Various temperatures (4, 15, and 45 °C) and pH levels were employed to evaluate the growth of bacillus-shaped strains in 4% and 6.5% NaCl broth.

Acidifying Properties of the Isolates

Titrimetric and potentiometric methods were implemented to evaluate acid production capabilities [11]. In order to accomplish this, lactobacilli strains were initially activated from frozen stocks in MRS and M17 broth at 37 °C for 24 hours. Then, 100 mL of incubation cultures were placed into 10 mL sterile UHT skim milk broths. The pH changes and titratable acidity of the aseptically produced 2 mL aliquots were assessed using a pH meter (Orion model 250 A, Orion Research Inc., Boston, MA) at 3, 6, 9, and 24 hours.

Growth at 4°C, 15°C, and 45°C

Bacilli are categorized according to their growth at 4°C, 15°C, and 45°C [12]. The modified MRS and M17 media determined the growth at particular temperatures. In essence, every component was identical, except Bromocresol purple. The color change in acidity from purple to yellow, which indicates the production of lactic acid and the growth of cells, was determined using bromocresol purple. 5 mL of test media was inoculated with 50 mL of overnight activating cultures to determine the growth and color change. Incubation time was tracked at the designated temperatures for seven days. The fermentation process of sugars was a component of the biochemical tests for all strains. After inoculation, two drops of sterile liquid paraffin have been added to each tube to ensure anaerobic conditions.

RESULTS

Identification

Depending on the phenotypic and microscopic features of the colonies, 48 isolates were produced. Using the pouring dish procedure, the colonies were isolated on MRS solid media and displayed a variety of shapes (spindle, star, oval), a creamy yellowish-white colour, and smooth edges. The majority of these colonies had an adhesive, glossy, and smooth aspect. They exhibit different shapes, with some slightly convex while others flatter. All the traits mentioned are characteristic of lactic acid bacteria in the Lactobacillus genus. The microscopical examinations confirmed that the isolates were classified under the Lactobacillus genus. They exhibited a rod-shaped morphology and were Gram-positive, as reported by [13]. The colonies were cultivated on MRS and M17 agar medium for purification, following the lactobacilli specifications mentioned by [14], as depicted in Figure (1). In addition, their acid production capacity was assessed by cultivating them on an MRS-CaCo3 agar medium. To confirm the presence of lactic acid bacteria, the growth of the isolates was assessed on this specific medium. Ten of the 48 bacterial isolates were selected, and their characteristics were unequivocally aligned with those of the Lactobacillus genus.

The study involved subjecting the eight isolates to a series of tests (tables 1,2 and 3), which included a catalase assay, assessing their ability to grow at different temperatures, and evaluating their growth in different concentrations of sodium chloride. According to the biochemical analyses, all eight tested isolates exhibited growth within the 4°C to 45°C range. All isolates demonstrated successful growth at saline concentrations of 4% and 6.5% NaCl, as shown in Table (1). The results confirmed the findings of [15], which demonstrated that various strains of the bacterium Lactobacillus can flourish in diverse environmental conditions, such as temperatures of 10, 39, and 45 °C and saline concentrations of 4% and 6.5%. Based on the characteristics of species in the Lactobacillus genus, the biochemical tests showed that all ten isolates tested negative for catalase [16].

VITEK2 System

The VITEK2 technique was applied to diagnose isolates according to the guidelines provided by the supplier [17, 18]. The outcomes of the VITEK2 system's assessment of the ten local isolates are presented in Table (4), which can be found attached. We can tell that seven out of the ten specimens are from the genus Lb. paracasei, with a probability that ranged from 85-91%. Additionally, two isolates were identified as belonging to the genus Lactobacillus plantarum, with a probability of 88%. There was an 85% chance that one of the samples belonged to the genus Lb. parabuchneri.

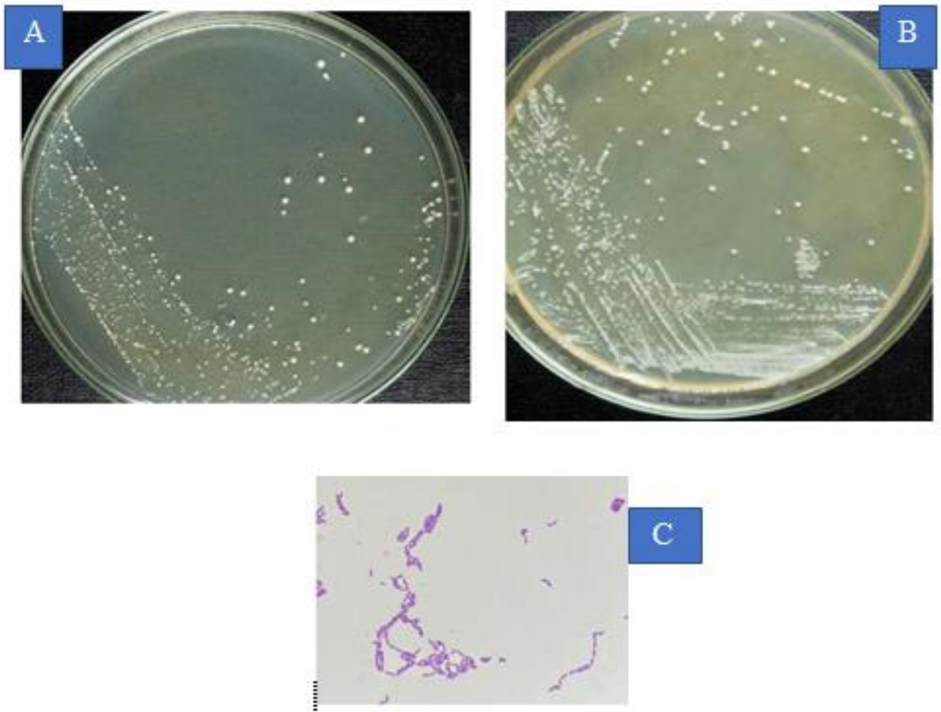

Figure 1. Illustrates a typical isolated Lactobacillus spp. colonies on MRS media (A) , M17 media (B), and slide of gram (+ve) bacillus colons (C).

Table 1. Biochemical properties for Lb. strains at NaCl.

Strains	**No. of Samples**	**Biochemical Properties**		
		Catalase test	***Growth in 6.5% of NaCl***	***Growth in 4% of NaCl***
Lb. paracasei	6 (60%)	_	+	+
Lb. plantarum	1 (10%)	_	+	+
Lb. parabuchneri	1 (10%)	_	+	+
Lb. plantarum	1 (10%)	_	+	+
Lb. paracasei	1 (10%)	_	_	+

(+) positive result , (-) negative result,

Table 2. Biochemical properties for Lb. strains at various temperature.

Strains	**No. of Samples**	**Growth at Various Temperature**		
		Growth in 4°C	***Growth in 15°C***	***Growth in 45°C***
Lb. paracasei	5 (50%)	+	+	+
Lb. plantarum	1 (10%)	+	+	+
Lb. parabuchneri	1 (10%)	+	+	+
Lb. paracasei	2 (20%)	_	+	+
Lb. plantarum	1 (10%)	_	+	+

Our study employed the VITEK2 system to identify lactic acid bacteria in commercially available and traditionally fermented kefir, which aligns with previous research [19, 20]. The study's findings align with the research conducted by [21], which successfully identified Lb. Parabuchneri, Lb.

Paracasei, in dairy products. Two of the six isolates classified under the Lactobacillus genus were explicitly recognized as Lb. Plantarum.

Table 3. Biochemical properties for Lb. strains at various PH.

Strains	No. of Samples	Growth at Vairous PH		
		PH 5.2	*PH 7.8*	*PH 9.2*
Lb. paracasei	4 (40%)	+	+	_
Lb. plantarum	1 (10%)	+	+	_
Lb. parabuchneri	1 (10%)	+	+	_
Lb. paracasei	3 (30%)	+	_	_
Lb. plantarum	1 (10%)	+	_	+

Table 4. Shows the outcomes of the ten local strains' VITEK2 diagnostics.

% Probability	Diagnosis	Isolates Numbers
0.85	Lb. paracasei	1 (10%)
0.87	Lb. paracasei	1 (10%)
0.88	Lb. plantarum	1 (10%)
0.87	Lb. paracasei	2 (20%)
0.91	Lb. paracasei	3 (30%)
0.87	Lb. parabuchneri	1 (10%)
0.86	Lb. paracasei	1 (10%)

DISCUSSION

Identifying Lactobacillus bacteria from cheese made in Nasiriyah provides insights into the diversity and adaptability of microbes that benefit human health. Our research confirmed that 48 strains were identified as part of the Lactobacillus family using analysis. These colonies displayed shapes like ovals, stars, and spindles with edges and a yellowish-white appearance commonly found in lactic acid bacteria from the Lactobacillus family. Similar physical characteristics were noted by [22] when they studied Lactobacillus samples from dairy goods.

The biochemical tests showed that these strains could thrive in salt concentrations (4% to 6.5% NaCl) shown in figure 2 and over a temperature range of 4°C to 45°C shown in figure 3. These results align with research by [23] showing that similar environmental conditions support the growth of Lactobacillus strains found in dairy products. Additionally, none of the tested strains exhibited catalase activity, a trait of Lactobacillus species consistent with the findings presented in the study [24]. This consistency highlights the adaptability and flexibility of Lactobacillus strains in different settings.

During our research, we encountered strains of Lactobacillus bacteria with Lb. Paracasei is prevalent, followed by Lb. Parabuchneri. These bacteria could thrive under pH 7.8 and pH 5.2 (shown in figure 4) conditions, although their survival rates in alkaline conditions (pH 9.2) differed. Specifically, 40% of the Lb. Paracasei bacteria were able to flourish at pH levels of 5.2 and 7.8 but not at pH 9.2, indicating a preference for environments. This discovery supports a study [25] that noted how Lb. Paracasei from fermented dairy products can adapt to pH levels for survival in the digestive system.

On the hand Lb. Plantarum showed a different behavior, with only one isolate (10%) able to grow at all tested pH levels, including pH 9.2). Research [26] has also shown that strains of Lb. Plantarum from dairy products can withstand various pH levels, supporting their role as versatile probiotics in various food products—consistent with their research findings.

Similarly, studies have indicated that Lb. Parabuchneri strains found in cheese have limited tolerance to alkaline conditions—a trend noted by researchers [27]. Furthermore Lb. Parabuchneri showed development at pH 5.2 and 7.8. It did not thrive at pH 9.2.Our samples' predominance of Lb. paracasei, which accounts for 40% of the isolates, is consistent with the findings of [28], who identified Lb. paracasei as a dominant species in artisanal cheeses. The study underscored the probiotic properties of Lb. paracasei, which includes its capacity to improve gut health and enhance the sensory qualities of cheese.

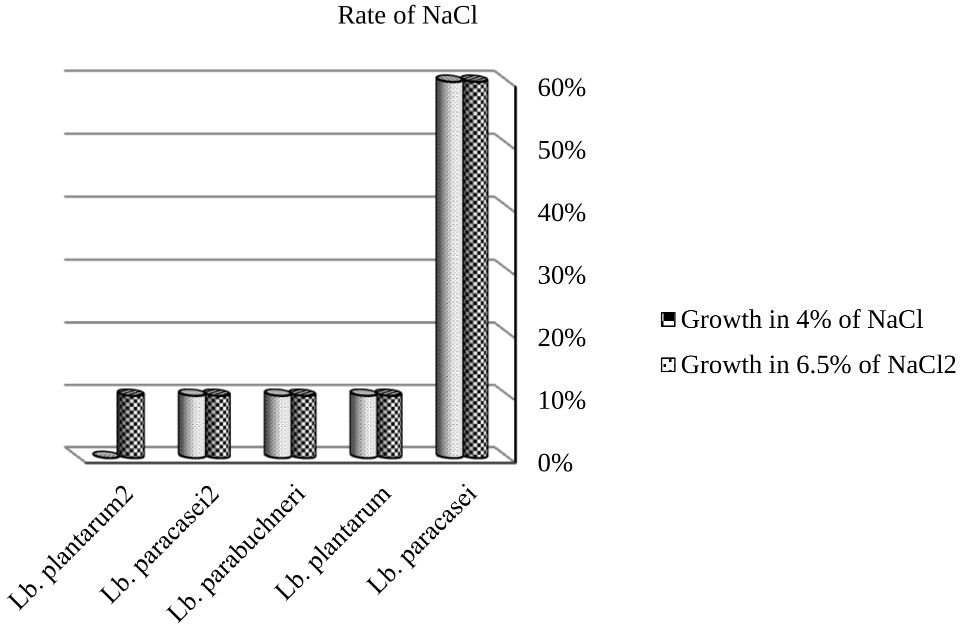

Figure 2. Illustrate of Lb. strains at NaCl.

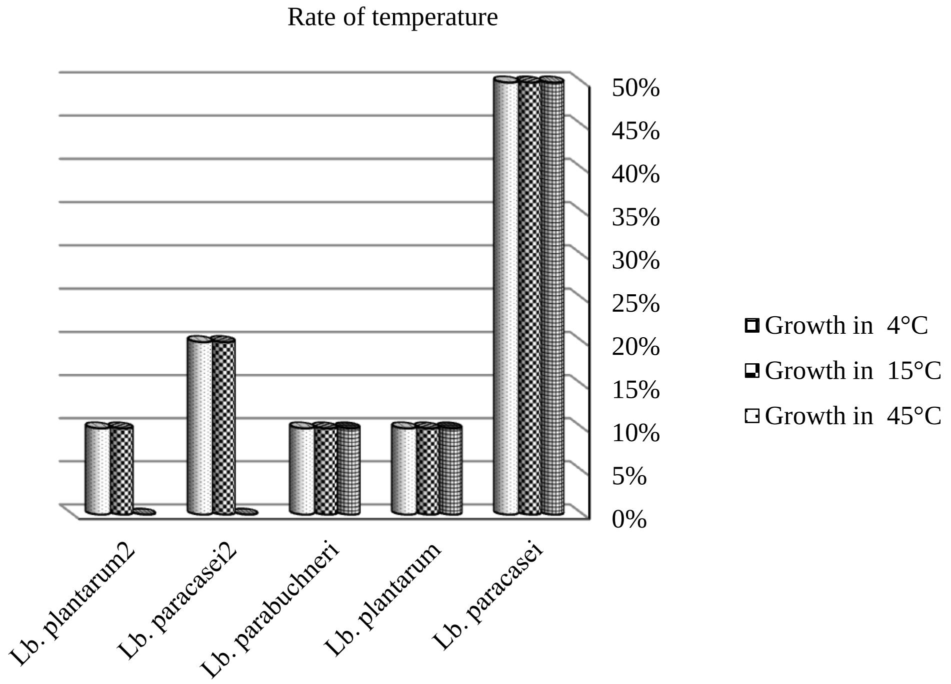

Figure 3. Illustrate of Lb. strains at various temperature.

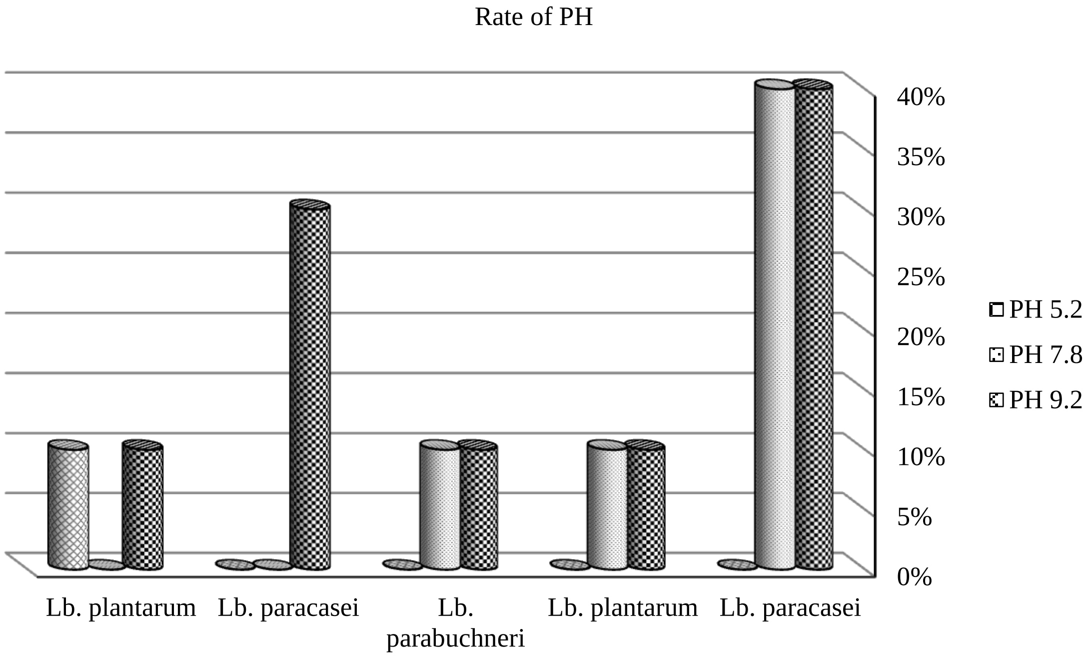

Figure 4. Illustrate of Lb. strains at various PH.

CONCLUSION

Lb. paracasei, Lb. plantarum, and Lb. parabuchneri were successfully identified in locally produced cheese in Nasiriyah by this study. Their remarkable adaptability to various environmental conditions underscored the isolates' potential as probiotics. The common type of bacteria found was Lb. Paracasei grows well in environments with acidic to neutral pH levels and is vital for surviving in the digestive system. It is suggested that future studies focus on exploring the properties of these Lactobacillus strains, their impact on gut health, and how they can enhance the taste of dairy products. Additionally, delving into their makeup through analysis could provide deeper insights into their functionalities.

Recommendations

1. Work with cheese makers to establish a fermentation process that ensures these beneficial Lactobacillus strains are always present in their products.
2. Investigate the potential of these strains to boost both the quality and probiotic content of dairy products and cheeses on a scale.

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