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Isolation And Diagnosis Of Lactic Acid Bacteria From Iraqi Labneh And Study Of Its Manufacturing Traits

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Abstract:

This study included isolating and diagnosing lactic acid bacteria from Iraqi labneh, as 36 isolates were obtained according to phenotypic and microscopic characteristics, and after conducting biochemical tests, 8 bacterial isolates belonging to the genus Lactobacillus were obtained out of 36 bacterial isolates. After that, the eight isolates were diagnosed using the VITEK 2 device at the species level using a special cut for the genus Lactobacillus, and it was found that 6 of them belong to the type Lactobacillusparacasei with a probability ranging between 85-91%, and one isolate belonged to the type Lactobacillusplantarum with a probability of 88%.and one isolate was diagnosed as Lactobacillusparabuchneri, with a probability of 85%. According to the synthetic characteristics, which included acid production, curd Hardness and whey Syneresis, the two isolates Lactobacillusparacasei3 and Lactobacillusplantarum 10 were selected from among the eight isolates for the purpose of manufacturing yogurt. Physical and rheological tests were conducted for yogurt made from these two isolates.As the tests showed no difference between the yogurt made with the commercial starter and the yogurt made from the single isolated in this study,The sensory evaluation of yogurt was also conducted, and the results of the statistical analysis showed that there were no clear significant differences between yogurt made from single isolates compared with the commercial starter treatment.

Key words:-labneh, lactic acid bacteria, Lactobacillusparacasei

Introduction

Lactic acid bacteria include a variety of bacteria and have been widely used in the dairy and medicinal food industry (Rather etal., 2014), as well as their health importance to increase the numbers of microflora in the intestinal tract (Patel et al., 2010). And its ability to produce peptides that have activity against pathogenic microorganisms(Algboory&Muhialdin 2021)Nowadays, there are different types of fermented dairy products all over the world and they mainly contain lactic acid bacteria (LAB), the nature of which depends on the type

of milk used, the pre-treatment of the milk, and the conditions of fermentation and post-processing historically. Fermented dairy products were produced to extend the shelf life of milk. These traditional foods persisted over centuries and often evolved from traditional home processing to large-scale industrial production using specific starter farms and modern equipment. The use of these initiators has improved the technological quality of dairy products, but at the same time has limited their biodiversity as well as the sensory variability of the final products, so there is a growing demand for new strains that show desirable effects on the properties of the product. Handcrafted dairy products are a candidate source of such microorganisms (Zamfir et al., 2006).Moreover, the growing interest in products with functional properties has encouraged scientists to search for new lactic acid bacteria found in natural sources (Margalho et al., 2021)and the traditional phenotypic diagnosis may not give an accurate and reliable diagnosis of lactic acid bacteria. Recently, with the rapid development of molecular biology and the increasing knowledge of the genetic structures of LAB, many molecular classification techniques have been developed (Bao et al., 2012).This allowed for more consistent and accurate identification of individual strains, so it is important to take advantage of the new techniques available for characterization and identification of new LAB strains (Mokoena, 2017) and the diagnosis of bacterial strains of traditional dairy products helps to identify and select the strains that may complement or replace the current industrial initiators (Jensen et al., 2009).So far, only limited information is available about the microorganisms that are included in traditional dairy products that are locally manufactured in Iraq, and the scarcity of taxonomic studies for these bacteria, especially those that study local products, As there are few published reports on microorganisms and their technological traits of fermented dairy products in Iraq and that most of the dairy factories in Iraq import the starters from outside the country, and thus it costs this country a currency in dollars,Given the importance of lactic acid bacteria as starter used in the production of fermented milk and therefore, it is necessary to select LAB strains with beneficial function and stable fermentation properties from conventional dairy products, Then it was used to design starter farms for commercial production, so this study was conducted with the aim of isolating and diagnosing lactic acid bacteria from local dairy products and studying their manufacturing traits.

Materials and Methods

First: Collection of samples

Samples of locally manufactured labnehwere collected from the local markets of the holy Karbala and Babylon province. The samples were placed in airtight, refrigerated, and sterilized plastic containers. The samples were transferred to the laboratory under sterile and refrigerated conditions.

Second: Bacterial isolation and identification

The selective medium MRS Agr supplied by Oxiod Company was used to grow the isolates. After sterilization, the medium was distributed on Petri dishes containing 1 ml of the appropriate decimal dilutions for the Iraqi labnehsamples and incubated under selective anaerobic conditions at a temperature of 45 ° C for 48 hours according to Guess and Kihal, (2004)The medium was also used to purify the isolates by the planning process on the medium, and the process was repeated three times in order to obtain pure single colonies.

Diagnostic tests for bacterial isolates

Lactobacillus isolates were diagnosed according to Holt etal. (1994).The phenotypic traits of colonies, microscopic and biochemical tests included growth with different saline consentpercentages and growth at different temperatures as well as the catalase tests were relied on.

Diagnosis of isolates using the VITEK2 system

The VITEK2 system was used according to the instructions of the company that supplied it, Bimerieux and according to Fritsche et al. (2011) in the diagnosis of isolates. This test was conducted in the main health laboratory of Hilla Teaching Hospital in Babylon Province.

Yogurt manufacture

The yogurt was manufactured according to the method mentioned by Tamime and Robinson (1999), as the isolates identified in this study were used in the manufacture of yogurt , and the commercial mixed starter prepared from the French company Danisco was used.

Physical and rheological tests of yogurt

Total acidity

The total acidity of yogurt manufactured using personalized isolates and a commercial starter was determined using the A.O.A.C (2008) method.

Viscosity Estimation

The apparent viscosity of yogurt samples was estimated at 10°C by using a Brookfield DVII+ viscometer according to the method mentioned by Donkor et al. (2007).

Texture determination

The texture of the fortified yogurt samples was estimated by using a texture analyzer equipped with a 5 kg load cell as reported by Joon et al. (2017).

Sensory evaluation for yogurt

Sensory tests for yogurt samples were conducted in the Department of Dairy Science and Technology - College of Food Sciences - Al-Qasim Green University by a number of specialized professors according to the sensory evaluation form, which included six characteristics of taste, flavour, texture, external appearance and acidity developed by Nelson and Trout (1964) .The statistical analysis was also conducted using the statistical program (Genstat Release 10. 3DE17) (2011) in analyzing the data to study the effect of different treatments on the studied traits according to a complete random design (CRD), and the significant differences between the means were compared with the least significant difference (LSD) test.

Results and discussion

Isolation and identification of lactic acid bacteria

The 36 isolates were obtained according to the phenotypic and microscopic traits of the colonies, where the colonies isolated on MRS solid medium by the method of pouring dishes were of different shapes (spindle, star, oval) and yellowish-white color (cream) and their edges are smooth, and most of these colonies are glossy, smooth to the touch and sticky. Some of them are slightly convex, while others are relatively flat. The indicated characteristics agreed with the characteristics of lactic acid bacteria of the genus Lactobacillus.As mentioned by Wang et al. (2014) and as shown in Table (1-1), the results of the microscopic tests of the isolates showed that they were rod-shaped, Gram-positive, and these characteristics often belong to the genus Lactobacillus,This applies with what Ahirwar et al. (2017) mentioned for the specifications of lactobacilli and as shown in Figure (1-1), then the colonies were purified using the planning method on MRS agr medium, as shown in Figure (2-1),Also, its ability to produce acid was tested by growing it on MRS-CaCo3 agr medium. The isolates growth test was based on this medium as one of the confirmatory tests for lactic acid bacteria, and through the tests conducted on the isolates, 8 out of 36 bacterial isolates were selected, which showed clear agreement With the characteristics of the genus Lactobacillus.

Figure(1-1) shows the process of colony planning on MRS medium agr

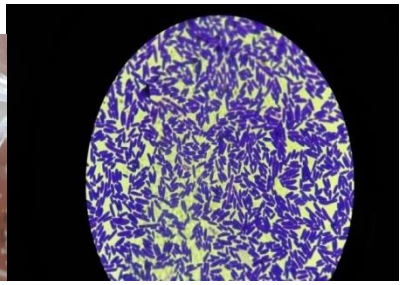

Figure (2-1) shows isolates of Lactobacillus under the microscope

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Biochemical Tests

The ability of the eight isolates understudy was tested for growth at different temperatures, as well as for growth in different Growth at saline concentration of Nacl, as well as for catalase assay.The results of the biochemical tests for the isolates under study showed that the eight isolates had the ability to grow at a temperature of 37°C and 45°C.The results also showed that all isolates were able to grow at Growth at saline concentration of 4% and 6.5% of NaCl, as shown in Table (1-1).These results agreed with what Fguiri et al. (2017) stated that isolates of the genus Lactobacillus were able to grow at different temperatures, including (10, 39 and 45 °C), as well as growth in Growth at saline concentration of 4% and 6.5%, Through Table (1-1), the results of the biochemical tests showed that the eight isolates gave a negative result for catalase, and this is consistent with the characteristics of species belonging to the genus Lactobacillus (Thakur et al, 2017)

Table (1-1) shows the phenotypic, microscopic and biochemical tests of the isolates under study

biochemical properties					Phenotypic and microscopic properties			
Catalog test	**Growth in 6.5% concentration of Nac**	**Growth in 4% concentration of Nacl**	**growth at 45°C**	**growth at 37°C**	**solid medium MRS**	**cell shape under the microscope**	**Gram stain**	**Isolation Number**
–	+	+	+	+	fusiform, small, with smooth, regular, convex edges, soft to the touch, and sticky	bacilli	+	**1**
–	+	+	+	+	Round, small, with smooth, regular, convex edges that are smooth and sticky	bacilli	+	**3**
–	+	+	+	+	Round, creamy in color, small, regular, convex, smooth and sticky	bacilli	+	**9**
–	+	+	+	+	Round, small and creamy in color, small to convex, smooth to texture, and sticky to convex	bacilli	+	**10**
–	+	+	+	+	Round, small and creamy in color, small to convex, smooth in texture and sticky to convex	bacilli	+	**12**
–	+	+	±	+	Oval, small and cream in colour, small to convex, smooth in texture and sticky to convex	bacilli	+	**13**

_	±	+	±	+	Starfish, small and cream in color, small, regular, convex, smooth to the touch, sticky, convex	bacilli	+	**14**
_	±	+	±	+	Round, small and creamy in color, small to convex, smooth in texture and sticky to convex	bacilli	+	**17**

(+)positive result, (-) negative result, (+_) weak growth

Diagnosis of Lactobacillus isolates by using VITEK2

Table (2- 1) shows the results of the diagnosis of the eight local isolates using the VITEK2 system, which was conducted in the Central Health Laboratory at Hilla Teaching Hospital.The results showed that six of the eight isolates were diagnosed as Lacto bacillus paracasei with a probability ranged between 85-91% and one isolate was diagnosed as Lacto bacillus plantarum with a probability of 88%and one isolate was diagnosed as Lactobacillusparabuchneri, with a probability of 85%.

Table (2 - 1) results of the diagnosis of the eight local isolates using VITEK2 system

% probability	Diagnosis	Isolates numbers
85%	Lacto bacillus para casei	1
87%	Lacto bacillus para casei	3
85%	Lacto bacillus para buchneri	9
88%	Lacto bacillus plantarum	10
85%	Lacto bacillus para casei	12
87%	Lacto bacillus para casei	13
91%	Lacto bacillus para casei	14
87%	Lacto bacillus para casei	17

The results of our current study agree with what was found by Demir (2020), where it diagnosed isolates of lactic acid bacteria that were isolated from samples of traditional and commercial fermented kefir using the VITEK2 system.Where 6 isolates belonged to the genus Lactobacillus, and two of them were affiliated to the type Lactobacillusplantarum,The results of this study are also consistent with what was indicated by Coton et al. (2008) who isolated and diagnosedLb.parabuchneri and Lb. plantarum andLb. Para casei of dairy products.

Study of manufacturing characteristics

The isolates Lb Para casei3 and Lb.plantarum10 were selected for the purpose of studying the manufacturing characteristics of isolates through the manufacture of yogurt compared to using the commercial starter, since these two isolates have given a good, cohesive, smooth and soft curd .This is consistent with what was mentioned (Sharpe, 1981). As well as to achieve an appropriate acidity in milk, which amounted to 1.68% and 1.18%, respectively, which means that the highest growth rate of lactic acid bacteria. This result is in

agreement with what was reached by Ebrahimi et al. (2011) in his study where the acidity of Lb.plantarum, Lb. casei 1,20% and 1.40%, respectively.

Physical and rheological properties of processed yogurt

Titration Acidity percentage

Through the results shown in Table (3 -1), the values of the acidity (calculated on the basis of lactic acid) for different yogurt treatments,As the acidity percentage of the different treatments immediately after manufacturing for the control treatment (T1) was 0.84%, and as for the yogurt treated (T2) made from the initiator Lb.paracasei3, it amounted to 0.83%, while the yogurt treated (T3) made from the initiator Lb.plantarum10 The acidity was 0.81%.These results are close to what Al-Sheikh (2018) found for yogurt , which is 0.81%. Through the obtained results, we note that the use of single starter in the manufacture of yogurt did not affect the acidity of yogurt treatments compared to the control treatment

Table(3 - 1) shows the results of the physical and rheological properties of the control treatment yogurt and the yogurt treatments added to the local isolates under study.

Rheological tests				physical tests	Treatments
whey Syneresis ml/g	water % holding capacity	viscosity (centipedes)	hardness (g)	acidity%	
5	28.94	2788	103.2	0.84	**T1**
5.4	25.2	2700	104.34	0.83	**T2**
5.2	26.44	2654	**99.8**	0.81	**T3**

Rheological properties of yogurt

Viscosity

The results in Table (3- 1) show that the viscosity values for the yogurt treatments immediately after processing (T1,T2,T3) where T1 was 2788 centipoise, while the viscosity values for the T3 and T2 treatments

were 2700 and 2,654 centipoise, respectively.These results are close to what was found by Shaghaghi et al,(2013), who mentioned that the viscosity of the yogurt treatment was 2123 centipoise immediately after processing.The reason for this may be due to the activity of the starter bacteria, which leads to a decrease in the pH of the yogurt , which leads to an increase in its hardness and then an increase in its viscosity (Walstra et al., 2006).Figure (3 -1) shows the yogurt made from the isolates under study. Through the obtained results, we note that the use of a single starter in the manufacture of yogurt has increased the viscosity of yogurt treatments.Compared with the control treatment, since these single starters have the ability to produce exogenous polysaccharides, and this agrees with what was mentioned (Li et al., 2013; Bajpai et al. 2016)These sugars are among the contributing factors in improving the texture of yogurt , where they interfere with milk proteins and increase its viscosity and improve its nutritional quality characteristics (Ruas - Madiedo et al., 2002).

Figure (3- 1) shows the yogurt made from the isolates under study.

Automatic whey Syneresis

The results in Table (3 - 1) show the whey **Syneresis**quantities for the aforementioned different yogurt treatments, as it was 5 ml/50 gm immediately after manufacturing for treatment T1 and for treatments (T2 and T3) 5.4 and 5.2 ml/50 cloudy straight . This result is close to what was found by Khattab and Dosh (2017), when the amount of whey Syneresis for T1 immediately after manufacturing was 5.65 ml / 50 g of yogurt .

water-holding capacity

It is noted from the results shown in Table (3- 1) the percentage of water retention for the aforementioned different yogurt treatments, where it is clear that the water retention capacity for T1 treatment immediately after manufacturing is 28.94%As for the water retention capacity of (T2, T3) treatments, it amounted to 25.2 and 26.44%, respectively. These results are similar to Matter et al (2016), where the water retention capacity of the yogurt sample reached 26.80% immediately after manufacturing. Through the obtained results, we note that the use of a single starter in the manufacture of yogurt did not affect the water retention capacity of yogurt treatments compared to the control treatment.

Hardness

The results in Table (3 -1) show the hardness values of yogurt for the control treatment T1 and T2 and T3 treatments, where the hardness values for the treatments were 103.2, 104.34 and 99.8 g, respectively.This result is consistent with what was mentioned by Jassim (2020), where the hardness values of yogurt samples immediately after processing ranged between 96.0-106.2 g. Through the obtained results, we note that the use of single starter in the manufacture of yogurt did not affect the hardness of yogurt treatments compared to the control treatment.

Sensory evaluation

Through sensory evaluation of yogurt made using isolates compared to yogurt made using a commercial starter. The awarded scores showed no significant differences in terms of taste, flavour, texture, acidity and external appearance.

Conclusions

We conclude from this study that there are important types of lactic acid bacteria in the traditional Iraqi dairy products, which can be used as initiators in the manufacture of these products, such as Lacto bacillus Plantarum, Lacto bacillus Para casei, and Lacto bacillus para buchneri. Also, the diagnosis of bacterial species by VITEK2 technology is an easy and quick method, and the results showed the possibility of using isolated strains as a single starter in the manufacture of yogurt without a change in the composition compared to the yogurt for the control treatment,The results of the sensory evaluation also showed the consumer's acceptance of yogurt added to the locally isolated and diagnosed single starters, and no negative effect was observed on the texture, texture, taste, flavor and external appearance of the yogurt compared to the yogurt manufactured using the commercial starter.

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