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PRESERVING TRADITION AND PROMOTING INNOVATION: "QUESILLO," ARTISANAL CHEESE FROM OAXACA, MEXICO

PRESERVAR LA TRADICIÓN Y PROMOVER LA INNOVACIÓN: "QUESILLO", QUESO ARTESANAL DE OAXACA, MÉXICO

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Preserving Tradition and Promoting Innovation: "Quesillo," Artisanal Cheese from Oaxaca, Mexico

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ABSTRACT

The quesillo is a traditional food originally produced in the state of Oaxaca, Mexico, is fresh, spun paste or strands, made with raw milk and has unique sensory attributes due to its extensive native microbiota, mainly lactic acid bacteria, which are fermenting microorganisms, safe, are used in the fermentation and preservation of food helping to improve flavor, aroma, and texture. They are part of the natural microbiota of raw milk and its derivatives, mainly traditional cheeses, where the greatest diversity and heterogeneity is found. The objective of this study was to microbiologically identify lactic acid bacteria (LABs) present in the traditional quesillo of Oaxaca, Mexico. Quesillo samples were collected in three municipalities in Oaxaca, Mexico. Serial dilutions were prepared from 10 g of sample, then seeded in a Man, Rogosa, and Sharpe (MRS) selective medium. Subsequently, they were incubated at 30 °C for 24 to 48 h. The strains were identified macroscopically and microscopically, catalase and oxidase tests were performed as confirmation tests for LAB. From the three traditional quesillo samples, 86 colonies with BAL macroscopic morphology were identified, strains with yeast morphology, coccobacillary, catalase and oxidase positive were discarded, and finally 56 strains were identified with macroscopic and microscopic characteristics of LAB type, gram positive, bacillary and coccobacillary morphology, catalase and oxidase negative. In conclusion, quesillo is a traditional product that presents lactic acid bacteria as important microorganisms, which, in addition to improving sensory attributes and food preservation, can present probiotic properties that when administered in adequate amounts confer a health benefit to the host.

***Keywords*:** quesillo, lactic acid bacteria, traditional food, probiotic

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Preservar la Tradición y Promover la Innovación: “Quesillo”, Queso Artesanal de Oaxaca, México

RESUMEN

El quesillo es un alimento tradicional producido originalmente en el estado de Oaxaca, México, es fresco, de pasta hilada o en hebras, elaborado con leche cruda y posee atributos sensoriales únicos debido a su extensa microbiota nativa, principalmente bacterias lácticas, que son microorganismos fermentadores, inocuos, se utilizan en la fermentación y conservación de los alimentos ayudando a mejorar su sabor, aroma y textura. Forman parte de la microbiota natural de la leche cruda y sus derivados, principalmente quesos tradicionales, donde se encuentra la mayor diversidad y heterogeneidad. El objetivo de este estudio fue identificar microbiológicamente las bacterias lácticas (BAL) presentes en el quesillo tradicional de Oaxaca, México. Se recolectaron muestras de quesillo en tres municipios de Oaxaca, México. Se prepararon diluciones seriadas a partir de 10 g de muestra y se sembraron en un medio selectivo de Man, Rogosa y Sharpe (MRS). Posteriormente, se incubaron a 30 °C durante 24 a 48 h. Las cepas se identificaron macroscópica y microscópicamente, se realizaron pruebas de catalasa y oxidasa como pruebas de confirmación de BAL. De las tres muestras de quesillo tradicional se identificaron 86 colonias con morfología macroscópica BAL, se descartaron cepas con morfología levaduriforme, coccobacilares, catalasa así como oxidasa positivas, y finalmente se identificaron 56 cepas con características macroscópicas y microscópicas de tipo BAL, grampositivas, morfología bacilar y coccobacilares, catalasa y oxidasa negativas. En conclusión, el quesillo es un producto tradicional que presenta bacterias lácticas como microorganismos importantes, que además de mejorar los atributos sensoriales y la conservación de los alimentos, pueden presentar propiedades probióticas que administradas en cantidades adecuadas confieren un beneficio a la salud del hospedero.

Palabras clave: quesillo, bacterias ácido lácticas, alimento tradicional, probióticos.

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INTRODUCTION

Quesillo or string cheese is an artisanal product originally produced in Oaxaca, Mexico (Villegas, 2016) is an integral part of the culture, traditions, and gastronomy of the region, so its denomination acquires a heritage dimension (Cervantes et al., 2019), is very popular, and in other states of the country it is called Oaxaca cheese (Villanueva-Carvajal et al., 2012). Traditional cheeses in Mexico are characterized mainly by being made with raw bovine milk, the process is done by hand by members of the family (Patiño-Delgado et al., 2022), on a small scale in workshops or roof houses set up inside the home (Camacho-Vera et al., 2019), also incorporate traditional ingredients that give the product its own identity (Villegas de Gante et al., 2013); quesillo is a fresh type of cheese, spun paste or strands, with great melting capacity; its production requires skill and knowledge; during its manufacture the paste or curd undergoes a natural fermentation that causes its acidification, so that when it is kneaded with hot water it plasticizes and can be stretched to form strips or fibrous threads (Caro et al., 2013), thus acquiring the unique sensory attributes (flavor, aroma and texture) conferred by its diverse native microbiota, among which lactic acid bacteria are the most important. Lactic acid bacteria (LAB) are safe, nonpathogenic, fermenting microorganisms (FAO, 2006), produce lactic acid as the main product when fermenting carbohydrates (Mokoena, 2017); are unicellular, nonmotile, nonspore-forming, Gram-positive, anaerobic, catalase and oxidase negative organisms of bacillary or coccoid morphology (Miranda et al., 2021). These microorganisms are used mainly in the food industry in fermented food preservation processes due to the production of bacteriocins, hydrogen peroxide, and diacilli, which prevent the proliferation of pathogenic microorganisms (Arena et al., 2018); are used as starter cultures to promote the development of flavor, aroma, improve texture, and increase the shelf life of fermented products (Wang et al., 2021).

LAB can be found in fermented products of vegetable and meat origin (Ashaolu and Reale, 2020), however, these microorganisms appear to be predominant because they play a fundamental role in lactic fermentation during the traditional cheesemaking process and exhibit a complex microbiota with greater diversity and heterogeneity (Mathur et al., 2020), these bacteria play an essential role in the processes of fermentation, maturation and flavor development (Coelho et al., 2022), also make it possible to maintain or even improve the nutritional value of the raw material (Caro et al., 2013), there are several

strains of LAB such as *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Enterococcus*, *Weissella* and *Pediococcus* (Terzić-Vidojević et al., 2020). However, there are also several strains of LAB that are used in cheese production, such as *Lactococcus*, *Streptococcus*, *Lactobacillus* and *Leuconostoc*, as they produce lactic acid, which acidifies the medium and contributes to the characteristic flavor and texture of the cheese. These bacteria may be added to the cheese directly or may be naturally present in the environment or in the milk used to make the cheese. LAB have been proposed as a novel approach due to their ability to confer health benefits as they are recognized as probiotics, mainly the genus *Lactobacillus* (Reuben et al., 2020), due to the transformation of substrates and the formation of bioactive or bioavailable end products, with the capacity to aid in digestive health (Kim et al., 2019). Generally used as dietary supplements, they are also considered an important group of functional foods (Soemarie et al., 2021). Therefore, the objective of this study was to isolate and microbiologically identify the LAB present in quesillo traditionally made in Oaxaca, Mexico.

METHODOLOGY

Sample collection

Samples of quesillo made from raw bovine milk were collected from municipalities belonging to the Valle Eteco region, Oaxaca, located northwest of the Central Valleys, at coordinates 17° 12'27'N 96°48'00'W (see Figure 1). Samples were collected under hygienic conditions and transported at refrigerated temperature (4 to 7 ºC).

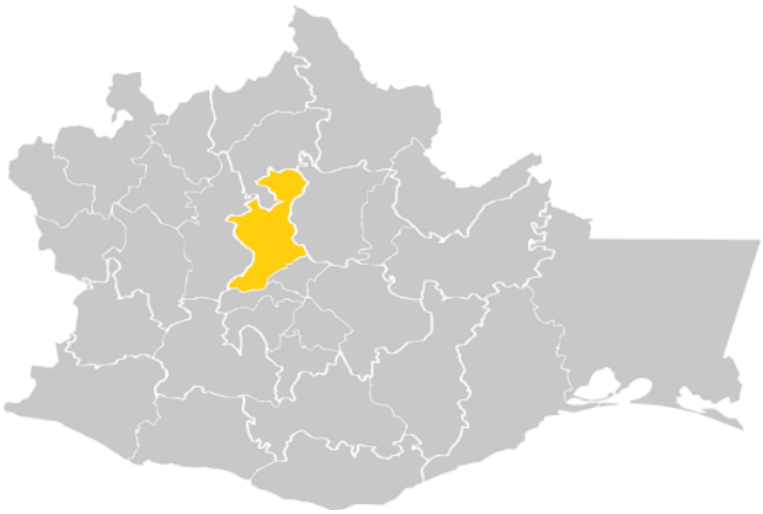

Figure 1. Location of the district of Etla, in the state of Oaxaca.

Isolation and purification of strains

Samples were coded with Q1, Q2, Q3 then homogenized to prepare serial dilutions from 0.1 to 0.0000001, then 100 µl of each dilution was placed in solid Man, Rogosa and Sharpe (MRS) medium, using the surface spreading technique, then incubated at 30 °C under anaerobic conditions for 24 to 48 h. Differentiated colonies were selected, then successive reseeding was performed by the cross streaking method, subsequently incubated at 30 °C for 24 to 48 h.

Macroscopic and microscopic identification

Colonies were identified with characteristics of white or cream color, round or dotted shape, creamy or moist appearance, diameter of 1 to 2 mm, convex surface and entire edges. For the identification of microscopic morphology, Gram staining was performed and later, they were observed under a 100x optical microscope. Colonies identified as gram positive and bacillary, coccobacillary and coccoaceous morphology were selected. Catalase and oxidase tests were performed as confirmatory tests.

Strain conservation

Strains of lactic acid bacteria with gram-positive, catalase-negative and oxidase-negative bacillary, coccobacillary and coccal morphology were preserved in MRS broth with 30% glycerol and stored at -20 °C in cryopreservation vials.

RESULTS AND DISCUSSION

From three samples of quesillo made with raw bovine milk, 86 colonies were identified with macroscopic morphology of lactic acid bacteria, which presented characteristics of white or cream color, diameter of 1 to 2 mm, round shape, punctiform, entire edges, convex or flat surface, butyrous and moist consistency. All isolates were identified as gram-positive, presenting blue-purple coloration in microscopic identification at 100x, and were also characterized by catalase and oxidase test. According to the results obtained, 65 isolates were identified as negative catalase, since they did not react when 3% H_2O_2 was added, while 21 isolates were positive for gas production; in the oxidase test, 65 isolates were identified as negative oxidase since they did not react when the Kovacs reagent was added (Table 1). In the microscopic morphology identification, of the 65 LAB isolates, 12 were rods, while 44 were coccobacilli and 9 were cocci. Finally, 56 Gram positive strains were selected, morphologically bacilli and coccobacilli, with negative catalase and oxidase (Table 2).

Table 1. Strains with macroscopic and microscopic characteristics presumptive for lactic acid bacteria, growth at 30 °C for 24 h in MRS culture medium**.**

		Microscopic morphology					
Isolated strains	**Macroscopic characteristics**	**Gram (+)**	**Bacillary**	**Cocácea**	**Cocobacillary**	**Catalase (-)**	**Catalase (+)**
86	White or cream color, diameter 1-2 mm, round shape, pointed, entire edges, convex or flat surface, buttery and moist consistency.	**86**	13	29	44	65	21

Table 2. Microscopic morphology of catalase-negative lactic acid bacteria.

Sample	**Q1**	**Q2**	**Q3**	**Total**
Catalase (-)	18	26	21	**65**
Bacillus	0	6	6	**12**
Cocobacillus	16	16	12	**44**
Cocos	2	4	3	**9**

The quesillo or Oaxaca cheese is a dairy food that during its elaboration process the paste or curd goes through a fermentation process under the action of lactic acid bacteria present in raw milk, traditionally this process lasts from 18 to 24 hours, which confers unique and distinctive sensory attributes of this product, mainly the characteristic of texture in long strands or threads, with great melting capacity. In addition to its nutritional contribution, mainly protein and fat, this traditional cheese may have some functional properties due to the lactic acid bacteria it contains, such as probiotic properties. Roldán-Pérez et al. (2023) points out that artisanal cheeses could provide new native strains with probiotic properties to develop innovative dairy products with positive effects on human health. Rolim et al. (2020) indicates that cheese is considered a better carrier of probiotics compared to many dairy products, due to its lower water activity (aw > 0.90), low storage temperature (4-8 °C), relatively higher pH compared to traditional dairy products, and textural characteristics such as high fat content, which

protects probiotics during gastrointestinal transit.

Lactic acid bacteria (LAB) isolated from traditional quesillo from Valle Eteco, Oaxaca, made with raw milk, show that these microorganisms are part of the natural microbiota of cheeses; Miranda et al. (2021) points out that LAB can be found in various types of traditional cheeses, which favors the development of sensory attributes characteristic of these cheeses. Coelho et al. (2022) indicates that the presence of lactic acid bacteria predominates in raw milk cheeses produced in an artisanal way, mainly the genera *Lactococcus spp*, *Leuconostoc spp*, *Lactobacillus spp* and *Streptococcus spp.* The macroscopic and microscopic characteristics presented by the lactic acid bacteria strains isolated from the three quesillo samples, such as size 1-2 mm, white or creamy color, round shape, punctiform, entire edges, convex or flat surface, buttery, dry and moist consistency, correspond to typical characteristics of lactic acid bacteria previously reported by Santander-Cortés & Castro-Rosas (2024); as well as the microscopic characteristics of Gram-positive bacteria, morphology of cocci, bacilli and coccobacilli, catalase and oxidase negative, coincides with that reported by Goa et al. (2022).

CONCLUSIONS

In conclusion, we can say that quesillo or Oaxaca cheese belongs to the fresh type classification, with spun paste or strands, with great melting capacity, made with raw bovine milk, it has unique sensory attributes due to its wide native microbiota, mainly lactic acid bacteria. Lactic acid bacteria (LAB) are safe microorganisms, widely used in the food industry, mainly in the production of fermented dairy foods; the various by-products derived from the fermentation of these microorganisms, confer antimicrobial properties, conservation and improvement of sensory attributes (flavor, aroma, texture, color), which allows improving the quality and safety of food. From the tests carried out on the quesillo, 56 strains of lactic acid bacteria were isolated that showed macroscopic and microscopic characteristics typical of these bacteria, which indicates that this traditional cheese from Valle Eteco, Oaxaca, could be a promising source for the isolation of lactic acid bacteria that can present probiotic properties and later its application in the elaboration of functional foods.

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