CENTRAL ASIAN JOURNAL OF THEORETICAL AND APPLIED SCIENCE

https://cajotas.centralasianstudies.org/index.php/CAJOTAS

Volume: 07 Issue: 03 | July 2026 ISSN: 2660-5317

Article

Biotyping of Gram positive and negative bacteria from milk sample

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Abstract: Food contains high-risk pathogens and is the primary cause of human illnesses. To verify the presence of dangerous bacteria, food safety must be assessed. These studies have revealed a promising role in identifying various milk and dairy samples. The information gathered from this study may serve as a foundation for the prompt detection of foodborne bacteria in the food industry. Using culture-dependent microbial techniques, the variation of lactic acid bacteria (LAB) species in milk was investigated in order to determine the prevalence and antibiotic sensitivity of Campylobacter species present in raw milk. Agar media, which is commonly used to count LAB, was used to obtain dominant strains. Arcobacter butzleri, a novel zoonotic foodborne pathogen associated with enteritis and occasionally causing bacteremia in humans, was added to the milk samples to increase the amount of acidifying LAB present. This bacterium's biotyping is essential for determination. The enzymatic patterns of Lactobacillus delbrueckii and Streptococcus thermophilus were identified using the quick APIZYM method. Alkaline and acid phosphatase, naphthol-AS-BI-phosphohydrolase, esterases, glycosidases, peptidases, and proteases (trypsin and chymotrypsin) were among them. Eating at restaurants can cause foodborne illnesses, but there are many ways that cross-contamination can occur. These include grocery stores, farmers' markets, secondary food production involving food processing and manufacturing, transportation, storage, and distribution, and primary food production from farm plants and animals during the harvesting or slaughter of food animals. In addition, homes, restaurants, and other foodservice establishments prepare and serve food Considering the various phases

Keywords: Bacteria, milk sample, biotyping approch

Citation: Mahmood W. S. Biotyping of Gram positive and negative bacteria from milk sample. Central Asian Journal of Theoretical and Applied Science 2026, 7(3), 384-393.

Received: 10th Apr 2026
Revised: 11th May 2026
Accepted: 20th Jun 2026
Published: 20th Jul 2026

1. Introduction

Safety and quality are important issues for food producers, regulators, and consumers. Toxins, viruses, fungi, bacteria, and chemical residues can pose major health and financial risks. Conventional detection techniques may not have the sensitivity, specificity, or capacity to consistently identify novel pathogens, and they are frequently expensive, time-consuming, and labor-intensive. Furthermore, these techniques may not enable prompt identification and detection, which could cause delays that increase the risks to public health. Given these problems, biotyping methods have transformed food safety by offering quick, precise, and sensitive ways to find and identify pollutants.due to their frequent occurrence in food-related infections, a variety of contaminants and foodborne pathogens, including Salmonella, Escherichia coli, Listeria, Lactobacillus, Bacillus cereus, and Staphylococcus, are included in colony analysis in food safety.. When compared to other methods, the results are encouraging in terms of sensitivity and specificity, and this technique allows for quick pathogen [1]

Compilations of dairy microbes from unprocessed milk environments contain strains with the capacity to produce novel dairy products Therefor, new variations with desired characteristics may have originated from native type lactococci obtained from unpasteurized milk samples. Furthermore, compared to cheeses made with pasteurized or microfiltered dairy, handmade, yeast-free Classic cheeses made from raw milk mature faster and have a stronger flavor). In another study, fecal lactococci strains isolated from fermented milk, unpasteurized milk, and nondairy sources generally produced a unique flavor that was different from those produced by industrial varieties). A common food additive, ribosomally produced bacteriocin antimicrobial peptides are produced by various bacterial strains and have antimicrobial properties against closely related bacterial varieties [2].

The two most important naturally occurring inhibitors that are present in higher concentrations in milk and help to preserve its cleanliness and quality are lactoferrin and lysozyme. Recent research has also demonstrated the antiviral properties of protein fractions from milk . However, the clear link between the higher levels of antimicrobial compounds and the lower levels of bacteria in milk is unclear and requires more investigation .Several researchers have previously observed that a plentiful supply of lactose appears to promote the development and persistence of adapted probiotic lactobacilli despite the elevated levels of lysozyme [3].

2. Materials and Methods

Around 7% of this bacteria show alkaline phosphatase activity, 7% show phosphohydrolase activity, and 77% show acid phosphatase activity. A notable number of strains generate acid phosphatase, essential for the normal ripening process of cheese by degrading phosphopeptides containing phosphoseryne groups. No strain can generate the trypsin enzyme, and 13% of strains show chymotrypsin activity. It is important to note that the esterases do not demonstrate complete enzymatic activity.(3) The extent of lipolysis required for fat product preparation is considered when selecting strains. For example, low-activity strains ought to be utilized for fresh cheeses and yogurts. All strains show activity for P-D-galactosidase, a-L-arabinosidase, and P-D-fucosidase. 3-D-galactosidase is the specific enzyme responsible for lactose fermentation and is essential to the dairy process. None of the S. thermophilus strains were observed to generate P-D-glucosidase, P-galacturonohydrolase, P-D-glucuronidase (a procarcinogenic enzyme), p-maltosidase, IV-acetyl-cu-D-glucosaminidase, N-acetyl-P-D-glucosaminidase, /3-L-fucosidase, /3-D-lactosidase, or P-D-xylosidase. (4)Ninety-three percent of strains possess cy-D-xylosidase and cw-maltosidase. Additional glycosidase activities include cY-D-glucosidase (over half of strains), a-D-galactosidase and /3-D-mannosidase (around half of strains), phospho$-D-galactosidase (more than a quarter of strains), a-L-fucosidase (one-fourth of strains), and cY-D-mannosidase, which are less prominentCu-D-galactosidase hydrolyzes di- and tri-saccharides that contain a galactose residue, including stachyose and raffinose (Tochikura), confirming its physiological importance.Streptococcus macedonicus, recently reclassified under Streptococcus gallolyticus subsp. Schlegel was found to be the most frequently recovered species, with coming in second. E. faecalis and Enterococcus faecium/hirae/durans species could not be differentiated through sequence alignment of the initial 500 bps of the 16S rRNA, while other enterococci such as Streptococcus, Enterococcus gilvus, and Enterococcus casseliflavus were obtained.While strains obtained from Italian cheeses and natural milk cultures are also efficient acidifiers, macedonicus is recognized as a moderate acidifier in milk, exhibiting many properties related to Streptococcus acidification. The strains of macedonicus that were obtained in this study were evaluated. It was established that merely a few strains could coagulate cow's milk in 65 hours, while most did so in 8–9 hours.

To promote the growth of thermophilic and heat-resistant bacteria, they are located in natural milk cultures often generated by incubating raw milk that has been subjected to heat treatment beforehand. Opportunistic pathogens affecting humans and animals, such as Streptococcus equi subsp, Streptococcus equinus/bovis, and zooepidemicus have been

identified. Reports have indicated the presence of S.equi, Streptococcus equisimilis, and other possibly harmful bacteria in donkey milk.

3. Results and Discussion

Phytoremediation Campylobacter species prevalence. identification. Campylobacter is a zoonotic . germ, a small, curved or S-shaped gram-negative bacterium. It is widely recognized as the main cause of bacterial milk-related illnesses. The genus Campylobacter has expanded to include several significant human and animal pathogens since its initial taxonomic confirmation [4]. Over the past few decades, it has been the most common pathogen found during outbreaks in both developed and developing nations a common component of the intestinal microbiota from a variety of hosts, including birds and both domestic and wild mammals, are several species of Campylobacter. The main cause of Campylobacter infections is ingesting contaminated food or liquids. The frequent presence of Campylobacter in partially cooked foods and raw milk or dairy products indicates that they may be hazardous. of zoonotic transmission to humans. According to epidemiological research, Campylobacter causes diarrhea that affects 400–500 million people annually and results in 37,600 deaths worldwide [5]. However, many of its epidemiological characteristics in Middle Eastern nations are still unknown. Individual-to-individual contact, contaminated food and liquids, and contact with contaminated animals are the main ways that Campylobacter spreads from its natural habitats .Human infections have been linked to several species of Campylobacter, with accounting for 95% of cases C.. jejuni as well as C. coli . as well as C. fetus .However, in both developed and developing countries, various species including are also referred to as gastrointestinal pathogens. C.lari, C. helveticus, C. upsaliensis, C. hominis, C. gracilis, C. peloridis, C lanienae. C.concisus, C. mucosalis, C. hyointestinalis, C. sputorum, C. insulaenigrae. C.curvus, C. rectus, C. showae in addition to C. ureolyticus. Human campylobacteriosis typically manifests as gastroenteritis and other extraintestinal manifestations, mostly as aftereffects, after a three-day incubation period. Your message appears to be lacking something. Gastrointestinal symptoms include nausea, sudden watery or bloody diarrhea, and stomach discomfort. Please send me the entire text you want me to paraphrase. Diarrhea [6, 7].

species occurrence. identification. Campylobacter is a zoonotic pathogen, a tiny, curved or S-shaped gram-negative organism. It is generally acknowledged as the primary cause of bacterial illnesses associated with milk. The Campylobacter genus has grown to encompass several important pathogens affecting both humans and animals since its original taxonomic identification [8]. In recent years, it has become the most prevalent pathogen identified during outbreaks in both developed and developing economies. Various Campylobacter species, typically present in the intestinal microbiota of various hosts such as birds and both domestic and wild mammals, are involved. The primary source of Campylobacter infections is consuming contaminated food or beverages [9]. The common detection of Campylobacter in uncooked dairy items and insufficiently cooked foods suggests a potential risk of zoonotic spread to humans Twelve percent of the 375 raw milk samples showed a positive result for Campylobacter spp. Cow's milk accounted for as much as 15.8% of the positive samples. The proportion of raw milk available in markets that is infected with Campylobacter species varies between 9.12 percent and 16.08 percent. The prevalence of Campylobacter does not notably vary among milk types. A summary of the Campylobacter species found in raw milk indicates that C. jejuni represented 65.9 percent (29/44 isolates) of the total isolates, making it the predominant species. Three biotypes of Campylobacter. C. jejuni detected: biotype II (24.2%), biotype III (17.2%), and the most common biotype IV (58.6%). Only the biotype II of Campylobacter [10].

A notable connection exists between the occurrence of Campylobacter in milk and the advancement of the warm spring and summer months. Twelve frequently used

antibiotics were utilized to evaluate Campylobacter isolates. Antimicrobial susceptibility testing revealed total resistance to ampicillin and trimethoprim. Conversely, complete susceptibility to tetracycline, rifampicin, and neomycin was observed. Nonetheless, the elevated sensitivity to gentamicin and streptomycin found in this study is not as high as reported from India. Additionally, the resistance levels to ampicillin, erythromycin, and gentamicin correspond with a recent study from Tanzania involving raw milk samples and beef carcasses [11].

Varieties that thrive inVarious ecological habitats, geographic regions, antibiotic usage, and the horizontal transfer of resistance traits all affect these variations in resistance phenotypes. Globally, Campylobacter is a significant cause of bacterial gastroenteritis outbreaks. The Global Enteric Multicenter Study (GEMS) and the European Centers for Disease Control and Prevention (ECDC) both show that Campylobacter spp. is regarded as the primary cause of bacterial gastroenteritis globally, particularly with higher incidence rates in children under five [12]. The total prevalence of Campylobacter species found in this research is 12.6%. These findings correspond with a previous study conducted in Pakistan that identified the highest prevalence.A recent investigation in Iraq discovered two different biotypes (I and II) of two Campylobacter species, with C. jejuni and C. coli being the most common biotypes. Moreover, biotype II of C. coli was the predominant biotype of the species, accounting for 25% of occurrences. Still, the current study recognized biotypes I of each species. The finding that C. jejuni and C. coli biotype I is more common in humans, whereas biotype II is primarily found in animals, indicates that domesticated animals, including those tested, are probably the source of milk contamination. Concerning the seasonal occurrence of Campylobacter, late spring and early summer displayed the peak rates, while winter had the lowest frequency. These findings correspond with a study conducted in Nigeria, which found that campylobacteriosis instances increase during the summer and then decrease in the winter [13]. Numerous studies have linked higher temperatures to warm periods

3-Lactic acid bacteria

Isolation and identification of lactic acid bacteria from unprocessed cow milk, these bacteria are naturally present in the microbiota of raw milk and are mainly used as a starter culture for manufacturing fermented dairy products [14]. Consequently, raw milk is considered an excellent source for isolating LAB with technological potential. To isolate lactic acid bacteria, samples of raw cow milk were collected from eleven different dairy farms in Turkey. Lactic acid bacteria were identified as gram-positive, catalase-negative, oxidase-negative, and non-spore-producing isolates. According to physiological and biochemical analysis, 90 LAB were extracted from raw cow samples and classified as Lactococcus (36.67 percent), Enterococcus (20.00 percent), Streptococcus (4.44 percent), Leuconostoc (1.11 percent), and Lactobacillus (37.78 percent). Members of the Lactococcus genus usually thrive in a medium with 4% NaCl concentration [15].

Three LAB isolates included E. faecalis strains (AKS419, AKS424, and AKS313.1), along with E. faecium AKS350 and E. avium AKS365. The Lactobacillus strains that were recognized included L. acidophilus, L. fermentum, L. paracasei ssp, L. plantarum, and L. delbrueckii ssp. L. lactis. The current study identified only three isolates from the heterofermentative group of Lactobacillus spp., with one designated as L. fermentum AKS222. This aligns with findings indicating that LAB were present in raw cow's milk in Italy. A limited quantity of lactobacilli was obtained from milk samples and recognized as Lactobacillus in another research. L. plantarum, L. casei, L. brevis. These characteristics spark the pursuit of novel strains with technological potential [16]. The majority of Lactobacillus isolates showed better acid production than Lactococcus and Enterococcus isolates in terms of their capacity to lower the pH of 10% reconstituted skim milk after 24 hours. The majority of Lactobacillus isolates decreased the milk pH to under 4. Strain of L. delbrueckii subspecies. The growth of specific isolates in 10% reconstituted skim milk

showed that lactis AKS369 demonstrated the highest acidifying activity in milk, reaching a pH of 3.67. The capacity of the cocci to acidify skim milk ranged from pH 4.33 to pH 5.31. Additional reports indicate that Lactobacillus isolates may be regarded as important acidifiers in this scenario [17]. The proteolytic system of LAB, responsible for generating proteolytic enzymes, is essential for optimal development in milk and starch breakdown. LAB can take advantage of milk protein casein because of their sophisticated system of proteases and peptidases

LAB isolates generate lactic acid, diacetyl, bacteriocins, and a variety of antimicrobial substances that combat pathogenic and spoilage microorganisms (21,22). Based on the diameter of halos (mm) observed in agar well assays, our results showed that the majority of LAB isolates exhibited antimicrobial effects against at least one food item. Three LAB isolates (Lc. AKS320.1, AKS320.2, and E lactis ssp. lactis. faecalis AKS424) showed notable antimicrobial activity and were examined in more depth. To assess the characteristics of the inhibitory effects, α-amylase, catalase, lipase, and several proteases including proteinase K, pepsin, α-chymotrypsin, trypsin, and papain were employed. The antimicrobial properties of E. Faecalis AKS424 were partially inactivated by lipase and completely inactivated by α-chymotrypsin, pepsin, and proteinase K. Heat treatment (5 m at 100ºC) somewhat diminished the activity, influencing the size of the inhibition zone with and without heat treatment. Following that, the antimicrobial activity toward Lactobacillus was examined. The protein nature of the antimicrobial compounds generated by S. aureus ATCC 25923 and L. monocytogenes ATCC 7644 was shown by their vulnerability to the proteolytic enzyme protease K, with respective values of 26.67 percent and 23.33 percent. Bacteriocins generated by E. coli have demonstrated comparable results. E. faecalis. L. lactis [18]. Among the evaluated LAB, our results indicated that L. Lactis ssp. lactis AKS320.1, AKS320.2, and E. Faecalis AKS424 demonstrated potential for application as a probiotic culture or starter in both human and veterinary nutrition. The author states that E. faecium . To assess the biochemical traits of the isolated strains, the enzyme activities of these selected 17 strains (8 cocci, 9 Lactobacillus spp.) may represent an ecological enzyme activity concerning the ability of bacteriocin production. were analyzed through the API ZYM system, and the findings showed that the tested strains were positive for 18 of 19 enzyme activities. Nonetheless, no activity was detected for the enzyme α-mannosidase. Our results indicated that the enzyme activities varied both among and within strains. All tested strains exhibited activities of leucinearylamidase, acid phosphatase, and naphthol-AS-BIphosphohydrolase. Among the 17 LAB strains studied, Lactobacillus spp. Léucine arylamidase activité était élevée dans les souches. These findings align with Lactobacillus spp. results reported by other researchers, isolated from unprocessed milk [19]. L-leucine arylamidase is an enzyme (EC 3.4). 11.2) facilitates the removal of an N-terminal leucine from p- or arylamides. Consequently, the low activity of the β-glucuronidase enzyme enhances the prospective application of these strains as starter cultures in dairy. The strains of Lactobacillus spp. showed the greatest activity levels of β-galactosidase. However, it was missing in the other strains that were analyzed. Our findings showed that the Lactobacillus strains were part of the LAB that were analyzed. L. plantarum AKS382 and L. plantarum AKS389 showed activity of the N-Acetyl-β-glucosaminidase enzyme. These findings correspond with those documented for various L. plantarum strains obtained from caper berry fermentations. L-fucosidases (EC 3.2). 1.51), which are exoglycosidases that can sever fucosyl oligosaccharides from bound L-fucose residues, are crucial for bacterial adaptation to specific environments. Lfucose (6-deoxy-L-galactose) is a monosaccharide found at the nonreducing terminus of intestinal mucin, blood group antigens, human milk oligosaccharides, and several glycans on the surfaces of mammalian cells [20].

Lactobacillus delbrueckii subspecies. Half of the L.bulgaricus strains show acid phosphatase activity, whereas 61% display phosphohydrolase activity. None of the strains can produce trypsin and chymotrypsin. Strains demonstrate esterase activity. All strains

show P-D-galactosidase and P-D-fucosidase activity, yet they do not produce P-maltosidase, N-acetyl-cu, P-D-glucosaminidase, P-D-xylosidase, P-D-glucosidase, or P-D-galacturonohydrolase. Ninety percent of strains contain a-L-arabinosidase and cY-D-xylosidase, eighty-three percent possess ymaltosidase, and sixty percent have a-D-galactosidase and a-D-glucosidase. It seems that other glycosidase functions are ancillary. Nine dipeptidases, two tripeptidases, one tetrapeptidase, and eleven mono- peptidases. The presence of pept, overall. In the dairy industry, lactic acid bacteria (LAB) are an important group of bacteria with industrial significance, acting as starter cultures for the production of fermented milk products like yogurt and different types of cheese. The genera Aerococcus, Alloicoccus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Symbiobacterium, Tetragenococcus, Vagococcus, and Weissella are categorized as gram-positive, catalase-negative, non-spore-forming LAB. These groups of bacteria have been sourced from unpasteurized milk and a variety of raw food ingredients [21], in addition to fruits. The plant organisms found in raw milk could be an important resource for new LAB species.

In another study, lactococci strains were isolated from raw milk in the cheese member region and identified through genotypic (RAPD) methods and phenotypic criteria (physiological and biochemical evaluations). The microbial flora present in raw milk is essential for cheese aging. The presence of nonstarter lactic acid bacteria (NSLAB) in Cheddar cheese made by industrial makers in the United Kingdom was identified using a commercially available identification system (API)50channel system. Subspecies of Lactobacillus paracasei. Paracasei and Lactobacillus plantarum were the most frequently identified species. The main sources of bacteria in raw milk originate from within the udder, along with the outer surfaces of the teats and udder, the milking equipment, storage containers, the environment, bedding, feed, air, and water [22]. The creation of fermented dairy products mainly entails the generation of lactic acid from dairy sucrose, lactose, and several other sugars, aroma compounds, and extracellular polysaccharides

Dairy microbe collections from raw milk sources contain strains that can create unique dairy products. As a result, new strains with desirable characteristics may have emerged from native lactococci obtained from unpasteurized milk samples. Additionally, unlike cheeses made from pasteurized or microfiltered milk, artisanal, yeast-free Classic cheeses made from raw milk mature faster and have a stronger flavor. The nisin Z variety of Lactococcus lactis that produces bacteriocin was isolated from a traditional source, the isolation of raw E. faecium strains, causing donkeys to yield enterocin milk. To use these isolates as starter cultures for creating fermented foods, it is crucial to obtain wild LAB isolates from both unprocessed food items and fermented products. A comparative analysis of LAB genomic data obtained via next-generation sequencing techniques showed no notable variations within the LAB group at various taxonomic categories, including order, family, group, genus, and species (24). A strain of Lactococcus lactis that produces the bacteriocin nisin Z was obtained from a traditional ,the extraction of unprocessed E. faecium strains that stimulate donkeys to produce enterocin milk [23].

To select both acid-producing and heat-resistant LAB isolates and to address the difficulties associated with LAB isolation due to its low abundance in the microbiota, enrichment strategies utilizing (i) milk culturing and (ii) pasteurization were applied with milk. The selection of lactic acid bacteria strains in the dairy industry depends on their cultural, biochemical, and technological characteristics; acidity and volatile aroma compounds (such as diacetyl, acetoin, and acetaldehyde, among others) are the main factors, alongside proteolysis (International Dairy Federation, 1991a). Different strains of the same species may show differing levels of activity and result in markedly distinct outcomes [24].

4-Arcobacter

Arcobacter butzleri

It has been demonstrated that there is a new foodborne pathogen that may pass from animals to individuals, inflammation of the intestines and possibly causing more serious bloodstream infections. Poor hygiene, close contact with diseased animals, and tainted food or water can all spread the infection. In-depth studies of its transmission patterns are essential to understanding how it's spreading. Simpler techniques continue to be employed to detect and describe these bacteria, giving valuable information, even though not all labs have access to state-of-the-art technology.For example, researchers analyzed strains of A. butzleri found in poultry products like meat, liver, and gizzard in southern Chile, and were able to pinpoint the most common types in the area. To make sure test results are accurate, it's a good idea to give certain tests more time to run their course. For instance, letting urea hydrolysis and sodium acetate tests run for a bit longer - 48 and 72 hours, respectively - can help avoid false negatives that might pop up due to inconsistent timing. Interestingly, when one test was allowed to run longer, no new cases were uncovered, suggesting that tweaking the testing process mainly helps confirm existing results rather than identify new ones. By fine-tuning our testing methods, we can develop a better grasp of how this pathogen behaves and come up with more effective ways to detect and manage it [25].

5-Pseudomonas fluorescens

Pseudomonas spp. may act as potential bacterial reservoirs and endure the effects of chemicals and sanitizers when the milking system remains dirty for a prolonged period. Different Pseudomonas species have been isolated from raw milk, with P. fluorescens being the most frequently observed. Notably, P. fluorescens made up more than 55.6% of all bacteria isolated from raw milk and was present in 84% of the unpasteurized milk samples. P. fluorescens produces proteinases and lipases that break down milk fats and proteins. The harmful effects of these enzymes can be detected via sensory evaluation, especially when P. fluorescens levels exceed 10 cfu/ml [26]. P. fluorescens obtained from bulk tank milk was analyzed using phenotypic and genotypic markers while conducting phylogenetic analysis on P. fluorescens to understand the species' relatedness. The results of this research may provide valuable information about Pseudomonas.

The identification kit API 20 NE (BioMérieux, Hazelwood, MO) was utilized to identify P. fluorescens biotypes. The test was performed at least twice for each isolate to confirm the precision of the API 20 NE profiles generated. The API 20 NE profile number, consisting of seven digits, is based on 21 biochemical and enzymatic assessments. Each of the seven digits represents the sum of three enzymatic or biochemical evaluations. The production assay for proteinases by P. fluorescens was performed utilizing skim milk agar as The development of a noticeable zone surrounding the colonies was regarded as a beneficial proteolytic response. The generation of lipases by P. fluorescens was evaluated utilizing Spirit Blue agar (Difco Laboratories, Detroit, MI) supplemented with lipase reagent (Difco) as described by A culture of P. fluorescens cultivated overnight was streaked onto three Spirit Blue agar plates, which were subsequently incubated at 7, 22, and 32°C for 168, 72, and 48 hours, respectively. The presence of deep blue around the colonies was seen as a positive reaction. The evaluation of proteolytic and lipolytic (PL) profiles was similar to the API 20 NE scoring system. The profile for each isolate was established based on these criteria: A score of 1, 2, or 4 was given for the proteolytic reaction at 7, 22, and 32°C, respectively. Non-proteolytic isolates scored 0. Scores for proteolysis at 7, 22, and 32°C were merged to form a proteolytic profile. For example, a P. fluorescens isolate with a score of 3 indicated that the isolate showed proteolytic activity only at 7 and 22°C [27].

However, a major drawback of biotyping pertains to its reproducibility. The standardization of biotyping is crucial for identifying the precise phenotype. When carried out effectively, biotyping can possess sufficient distinguishing power to differentiate

isolates that belong to the same species. Our research findings indicate that API 20 NE profiles can be used to differentiate strains based on their enzymatic and biochemical traits.Isolates of P. fluorescens were examined for their PL activity at temperatures of 7, 22, and 32 degrees Celsius. Based on PL activity assessed at three different temperatures, the 55 isolates were grouped into 14 distinct PL profiles (29). The results showed that 92.7% of isolates (PL-04 to PL-77) demonstrated proteolytic and/or lipolytic activity at 7, 22, and/or 32°C. Out of the 55 isolates, 80%, 91%, and 58% displayed proteolytic activity at 7, 22, and 32°C, respectively. Only 7, 44, and 7% of the isolates showed lipolytic activity at 7, 22, and 32°C, respectively (30). These results suggest that P. fluorescens's PL activity can be detected across different temperatures; nonetheless, PL activity was more prominently observed at 22°C than at 7 or 32°C. The PL profiles PL-30, PL-70, and PL-72 accounted for 64% of the isolates, all of which displayed proteolytic activity at 7 and 22°C (In this study, the proteolytic activity of P. fluorescens was significantly higher at 7 and 22°C in comparison to 32°C). Significantly, only 45.5% of P. fluorescens isolates displayed lipolytic activity at 22°C, which is markedly higher than the activities observed at 7 (7.3%) and 32°C (7.3%) [28].

The optimal temperature for the PL activity of P. fluorescens ranges from 20 to 25°C. Our results showed that PL activity at 32°C was significantly lower than at 22°C. The incubation temperature of 32°C may have exceeded the optimal level, potentially causing some degree of inhibition in PL activity. While 80% of P. fluorescens isolates showed proteolytic activity at 7°C, only 7.3% displayed lipolytic activity at that temperature, indicating that the proteinases of P. fluorescens are more likely to create problems in raw milk during refrigeration. This was evidenced by the fact that after incubating raw milk inoculated with Pseudomonas spp. at 7°C, the PL activity after 3 days was 1000- and 280-fold greater than prior to incubation [29, 30].

4. . Conclusion

The instability and poor reproducibility of phenotypic features limit the utility of biotyping procedures. A more cost-effective option for an assessment tool is the biotyping approach. Our findings indicate that this phenotypic biotyping approach is straightforward, feasible, and produces consistent results; it may be employed as a phenotypic tool. Microbes are the best source of natural compounds, hence microbial biotyping is the finest substitute for synthetic techniques. The need for environmentally safe and human-friendly materials is now growing rapidly across a wide range of industries. suitable for application in a number of industries, including the food, textile, and pharmaceutical sectors as well as the effective large-scale manufacture of biomaterial derived from bacteria.The main species found was Lactobacillus pentosus. L. aunquespecific in an industrial scale , mesa-olaaceitunas. Additionally, L.plantarum were found.

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