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Detection of virulence genes in *Pseudomonas fluorescens* isolates from local cheese in Nineveh province

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Abstract

Cheese is a product highly consumed in Nineveh province, it is easily spoiled with *pseudomonas fluorescens* through preparation and processing, and the growth of these bacterium causes alteration in cheese quality, consequently reducing their shelf-life periods. Fifty samples of local cheeses sold in Nineveh province were screened to detect the existence of *P. fluorescens* as a food spoiler from October 2023 till March 2024.The *P. fluorescens* isolates from positive samples were tested to evaluate their virulence in producing exoenzymes causing cheese spoilage including, protease and lipase, by genetic approach of target genes using polymerase chain reaction assay. Out of 50 samples, 10(%20) were positive for the presence of *P. fluorescens* according to the *16SPflu* gene. The activity of protease and lipase producing enzymes to positive isolates was detected depending on the *AprX* gene and *LipM* genes; the results revealed that 3(%30) of strains positive for *AprX* gene presence and 1(%10) of *P. fluorescens* strains possess the *LipM* gene indicated low lipase activity. Results of DNA partial sequencing of the *16SPflu* gene revealed four strains recorded in the GenBank nucleotide sequence database with accession numbers PP727372, PP727373, PP727374, and PP727375. Our results shed light on the risk of *P. fluorescens* existence as a spoilage indicator in local cheese and confirm following the hygienic and sanitation conditions during cheese processing from milk and ensure the safety of raw milk during milk collecting, processing and preservation of cheese under chilling environments to prolong the shelf life of the products and ensure consumer health.

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Introduction

Many people in Nineveh province consumed local cheeses using raw milk. During this process, cheese was exposed to contamination from the milk, handler, and surrounding environment during preservation under chilling temperature 4°C. *P. fluorescens* as a psychotropic bacterium, may grow and multiply in many foods such as milk and cheese and become the dominant microflora displaying spoilage defects, including changes in color, odor-flavor, and texture, which reduce the shelf life of cheese and affect the quality (1,2). These bacteria have been reported in many types of cheeses (3) *P. fluorescens* was the most specific spoilage microorganisms of milk and dairy products during storage under refrigeration temperatures (4) it is highly spread and can enter the dairy products plants through post-pasteurization contamination, it is already present in soil, dust, and water in small fraction within dairy animal environments (5). The high genomic diversity of *P. fluorescens* strains needs a genetic approach to confirm the strains, such as *16srRNA* sequencing (6-8). Many strains of *P. fluorescens* produce extracellular heat-stable protease, lipase, and lecithinase, which contribute to the spoilage of milk and milk products, including cheese (9). Their activities degrade milk constituents such as casein, when protease digest casein, milk gelatin will occur and milk fat will be

hydrolyzed (10,11). An alkaline zinc metalloprotease has a molecular mass of about 42 Kilo Dalton (12,13). Also, *P. fluorescens* can produce pigments such as pyoverdin, fluorescein, and pyomelanin, which cause food discoloration (14). Several studies highlighted the effect of thermo-resistant protease on the sensorial features of cheese (15,16). Some studies screen the bacterial contamination of milk, and cheese and the antimicrobial susceptibility of pseudomonas (17-19).

There was no study on the protease and lipase activity of *P. fluorescens* from milk and cheese in Nineveh province, therefore, the current study was designed to monitor the existence of *P. fluorescens* in local cheese and their abilities to cause cheese spoilage according to protease and lipase activity in using conventional polymerase chain reactions assay.

Materials and methods

Ethical approval

All samples were obtained after the owner's approval, and the research was carried out according to the ethical guidelines of the Institutional Animal Care and Use Committee at the College of Veterinary Medicine, University of Mosul, which included an authorized ID of UM.VET. 2023.083.

Samples

The study included 50 samples of local fresh cheese collected randomly from different regions in Nineveh province between Oct. 2023 and Mar. 2024.All samples were preserved in an ice box and then transported to the Laboratory of Veterinary Public Health, College of Veterinary Medicine, University of Mosul.

Isolation

Cheese samples were examined to isolate psychotropic *P. fluorescens* on Pseudomonas cetrimide agar (Neogen, USA). Plates were incubated at 25°C for two days, and colonies were identified according to phenotypic characteristics (20).

Identifications of bacterial isolates

The identification of *P. fluorescens* isolates was related to some biochemical tests, including the Oxidase test, Catalase test, Starch hydrolysis, pyoverdine production, and Gelatin liquefaction abilities (21). Molecular identification of *P. fluorescens* was used to confirm the diagnosis using a polymerase chain reaction assay (PCR).

DNA Extraction

According to the manufacturer's profile, suspected colonies were subjected to DNA extraction using a Bacterial DNA kit (Add a bio, Korea).

Polymerase chain reaction (PCR)

The *P. fluorescens* strains isolated from cheeses were confirmed depending on PCR assay using the *16SPflu* gene, a universal primer provided by (Macrogen/Korea). The primer consists of forward and reverse primer sets following with a molecular weight of 850 bp. The products exposed to a thermal profile included denaturation of 2 min. at 95ºC then 35 cycles of 94ºC for 45s, followed by annealing 56ºC for 60 s. and extension at 72ºC for 1 min. and final extension at 72ºC for 2 min. with cooling at 4ºC. The products were illustrated by electrophoresis (1.5% agarose gel) manufactured by (AddBio, Korea) with three µl GelRed dye (AddBio, Korea). The PCR products were analyzed in 300mA 75 volts for 1 hour. 5 µl of DNA ladder with 100 base pairs (GeNet Direx, Korea) was standard. The specific band of DNA was identified using the gel documentation system (Bio-Rad, USA). The positively identified strains of *P. fluorescens* were screened to detect their protease and lipase activity depending on *AprX* and *LipM* genes with product size of 1434 and 1422 base pairs, respectively (Table 1), the PCR reactions done according to manufacturer instructions.

Sequencing of the *16SrRNA* gene

After the PCR products were purified, the sequencing of the *16SPflu* gene was assessed according to Sanger dideoxy sequencing and the Blast algorithm at the NCBI server. Then phylogenic analysis was done using ClustalX (NCBI) software programs [available at]. The phylogenetic tree structure was done using the Maximum Likelihood approach depending on the Tamura-Nei model in MEGA11 software.

Table 1: Oligonucleotide Primers sequence for *P. flouresence* used in the current study

Primers	Primers sequence 5"-3"	Tmemperature (ºC)	Size (bp)	Reference
16SPflu-F	5'-TGCATTCAAAACTGACTG-3'	56	850	(22)
16SPflu-R	5'-AATCACACCGTGGTAACCG-3'			
APrX-F	5'-TTATGTCAAAAGTAAAAGAC-3'	58	1434	(23)
AprX-R	5'-TCAGGCTACGATGTCACTG-3'			
LipM-F	5'-ATGGGTRTSTTYGACTATAAAAACC-3'	55	1422	(23)
LipM-R	5'-TTAACCGATCACAATCCCCTCC-3'			

Results

The results revealed a successful recovery of *P. fluorescens* strains in local fresh cheese (14/50) %28 by conventional culture methods and (10/50) %20 was positive for *P. fluorescens* strains using PCR techniques (Table 2). The PCR results confirmed the detection of *P. fluorescens* isolates according to the *16SrRNA* gene producing bands with 850 base pairs (Figure 1). Additionally, screening of *P. fluorescens* protease activity was detected only (3/10) strains at (%30) according to the presence of *AprX* gene producing bands at 1434 bp and (1/10) 10% for the existence of *LipM* gene with amplicon 1422 bp (Figure 2 and 3). Sequencing of the *16SPflu* gene exhibits that strains of *P. fluorescens* isolated from cheese have been submitted to the Genebank database with accession numbers PP727372, PP727373, PP727374, and PP727375 were registered in the National Center for Gene Bank. The alignment of local *P. fluorescens* with NCBI GenBank is shown in (Figure 4). According to Blast, the local isolates accession number matches the China isolates of *P. fluorescens* OP341878, MW582677 gene with a percentage of 100% (Table 3). The relationship between Iraqi local isolates and global isolates was obtained according to the phylogenic tree using the Maximum Likelihood approach depending on the Tamura-Nei model in MEGA11 software. The *P. azotoformans* (MT998034-Austria) were rooted as outgroup (Figure 5).

Table 2: The prevalence of *P. fluorescens* in local fresh cheese by conventional and PCR assay

No. examined	Target microbe	Conventional methods		PCR	
		No.	%	No.	%
50	Positive	14	28	10	20
	Negative	36	72	40	80

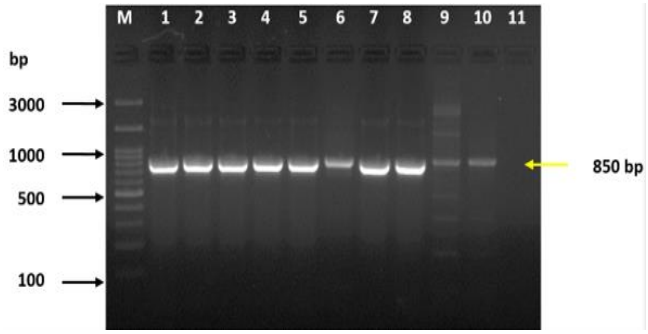

Figure 1: Genomic characterization of PCR products for the *16SPflu* gene of *Pseudomonas fluorescens*, M lane represents a 100 base pair DNA ladder. Lanes 1-10 are positive cheese samples at 850 base pairs, and lane 11 is a negative control.

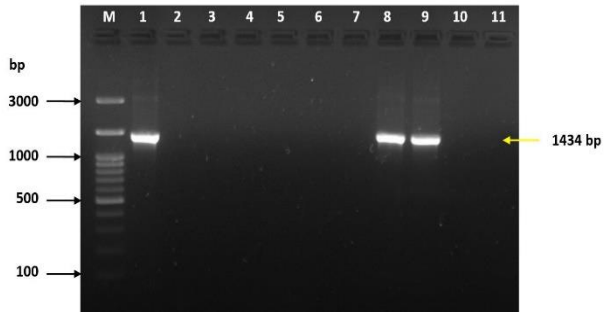

Figure 2: Genomic characterization of PCR products for the *AprX* gene of *Pseudomonas fluorescens.* M lane represents a 100 base pair DNA ladder. Lanes 1,8, and 9 are positive samples at 1434 base pairs, lanes 2-7 and 10 are negative samples, and lane 11 is a negative control.

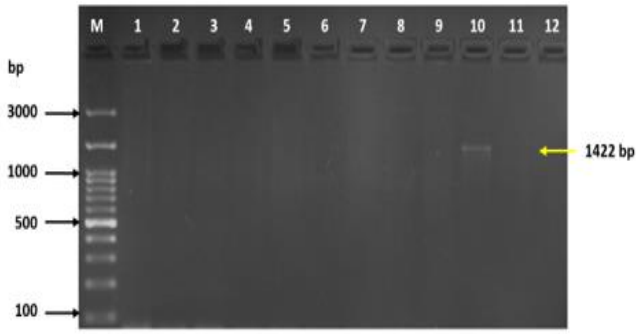

Figure 3: Genomic characterization of PCR products for the *Lip* gene of *Pseudomonas fluorescens*. M lane represents a 100 base pair DNA ladder. Lane 10 has positive samples at 1422 base pairs, lanes 1-9 and 11 are negative samples, andlane 12 inegative control.

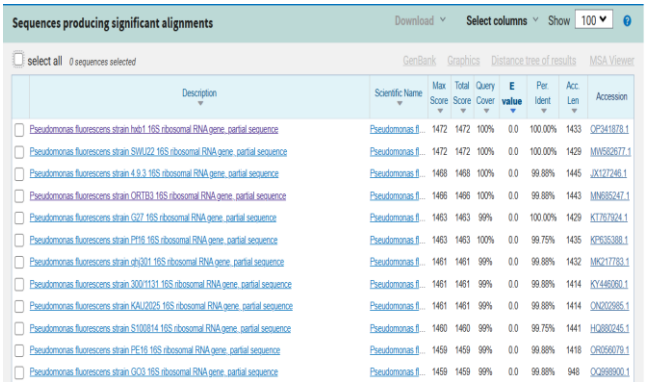

Figure 4: Identifying the query sample, *Pseudomonas fluorescens*, by alignment in NCBI gene bank.

Table 3: Percentage distribution of *P. fluorescens* based on *16SrRNA* gene according to BLAST in GenBank of NCBI

Local sample	Query Cover (%)	Identity (%)	GenBank Accession Number	Country
	100	100	OP341878	China
	100	100	MW582677	China
	100	99.88	JX127246	Turkey
	100	99.88	MN685247	Taiwan
PP727372	100	99.75	KP635388	Iran
PP727373	99	100	KT767924	China
PP727374	99	99.88	MK217783	China
PP727375	99	99.88	KY446060	New Zealand
	99	99.88	ON202985	Egypt
	99	99.88	OQ998900	Nigeria
	99	99.88	OR056079	Chile
	99	99.75	HQ880245	China

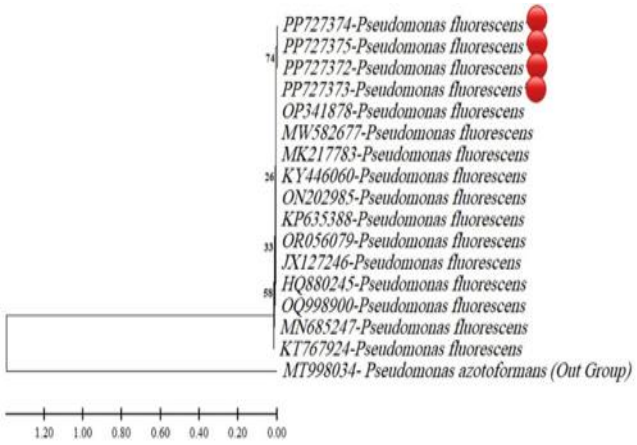

Figure 5: The phylogenic tree of *P. fluorescens* from cheese in Nineveh province is pointed in a red circle. The *P.azotoformans* (MT998034-Austria) represent the outgroup.

Discussion

The source of the milk used for cheese manufacture is essential factor affecting final products. psychotropics bacteria are involved in the defects in dairy products due to prolonged chilling storage (24,25). *Pseudomonas* spp. is one of the most common microbiotas in raw milk under cold storage. Physicochemical traits of fresh cheeses are suitable for the growth of *P. fluorescens*, especially the a^w and pH (26,27). Our results confirmed the findings of other studies on the prevalence of *P. fluorescens* in cheese in Australia (28,29) and from Damietta cheese in Egypt at 35.14% (30). which may attribute to the Hydrolysis of casein liberate plasmin and plasminogen which altering the cheese yield and affecting the sensory traits of the final product of cheeses (2,31,32) or may be attributed to the microbiota of milk supplied for cheese production and affect cheese quality. The composition of cheese and pH may affect proteolysis patterns with cheese hardness, which affects cheese texture and flavor (33-35). Similarly, a thermoresistant protease produced by a *P. fluorescens* strain hydrolyzed β-casein in milk increasing protease activity over storage time before cheese processing (16,36,37). Higher ripening pH and temperature affect the protease activity of *P. fluorescens* in cheese Both *AprX* and *LipM* genes are depended on as an indicator to detect the virulence of *P. fluorescens* to induce spoilage (38,39) and by using PCR assay as a more flexible method for early detection of *P. fluorescens* to predict the shelf life of the products (40).The study revealed the prevalence of *P. fluorescens* in dairy chains in Nineveh province as mentioned in the Genebank database using partial specific region genetic sequencing of *16srRNA* of *P.fluorescens* isolated from local cheese for the first time in my city, the comparative. study of our local strains with global strains recorded in Genebank database referred to highly aggregation cluster of local isolates indicating transmission due to contamination as well as the essential role of environmental effects on this genetic diversity where the flexibility of the Pseudomonas genome, permitting the accession of nutrient-scavenging pathways through variant environments (41). The 99% identity may be attributed to mini nucleotide differences from world strains due to mutation (42). Therefore, adequate hygienic conditions should be provided to reduce bacterium growth and reduce dairy product spoilage.

Conclusion

The detection of *P. fluorescens* in local fresh cheese indicates the possibility of spoilage by microorganisms arising from contaminated milk supplies for manufacturing. The risk of these bacteria comes from the liberation of protease and lipase which accelerate spoilage and reduce cheese shelf life. Therefore, due to their high diversity we need to restrict the growth of pseudomonas spp. in dairy products to minimize spoilage and maintain cheese quality.

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Conflict of interest

The authors confirm there was no conflict of interest.

References

1. Hantsis-Zacharov E, Halpern M. Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits. Appl Environ Microbiol. 2007;73(22):7162-8. DOI: 10.1128/AEM.00866-07
2. Dogan B, Boor KJ. Genetic diversity and spoilage potentials among *Pseudomonas spp*. isolated from fluid milk products and dairy processing plants. Appl Environ Microbiol. 2003;69:130-138. DOI: 10.1128/AEM.69.1.130-138
3. del Olmo A, Calzada J, Nuñez M. The blue discoloration of fresh cheeses: A worldwide defect associated to specific contamination by *Pseudomonas fluorescens*. Food Control. 2018;86:359-366. DOI: 10.1016/j.foodcont.2017.12.001
4. Simões M, Simões LC,Vieira MJ. A review of current and emergent biofilm control strategies. Food Sci Technol. 2010;43:573-583. DOI: 10.1016/j.lwt.2009.12.008
5. Martin NH, Murphy SC, Ralyea RD, Wiedmann M, Boor KJ. When cheese gets the blues: *Pseudomonas fluorescens* as the causative agent of cheese spoilage. J Dairy Sci. 2011;94:3176-3183. DOI: 10.3168/jds.2011-4312
6. Garrido-Sanz D, Meier-Kolthoff JP, Göker M, Martín M, Rivilla R, Redondo-Nieto M. Genomic and genetic diversity within the *Pseudomonas fluorescens* complex. PLoS One. 2016;25;11(2):e0150183. DOI: 10.1371/journal.pone.0150183
7. Caputo L, Quintieri L, Bianchi DM, Decastelli L, Monaci L, Visconti A, Baruzzi F. Pepsin-digested bovine lactoferrin prevents Mozzarella cheese blue discoloration caused by Pseudomonas fluorescens. Food Microbiol. 2015;46:15-24. DOI: 10.1016/j.fm.2014.06.021
8. Cenci-Goga BT, Karama M, Sechi P, Iulietto MF, Novelli S, Mattei S. Evolution under different storage conditions of anomalous blue coloration of Mozzarella cheese intentionally contaminated with a pigment-producing strain of *Pseudomonas fluorescens*. J Dairy Sci. 2014;97:6708-18. DOI: 10.3168/jds.2014-8611
9. Morales P, Fernandez-Garcia E, Nunez M. Production of volatile compounds in cheese by *Pseudomonas fragi* strains of dairy origin. J Food Prot. 2005;68:1399–1407. DOI: 10.4315/0362-028x-68.7.1399
10. Zhang C, Bijl E ,Svensson B, Hettinga K. The extracellular protease AprX from Pseudomonas and its spoilage potential for UHT milk: A review. Compr Rev Food Sci Food Saf. 2019;4:834-852. DOI: 10.1111/1541-4337.12452
11. Chen L, Daniel RM, Coolbear T. Detection and impact of protease and lipase activities in milk and milk powders. Int Dairy J. 2003;13(4):255-275. DOI: 10.1016/S0958-6946(02)00171-1
12. Marchand S, Vandriesche G, Coorevits A, Coudijzer K, De Jonghe V, Dewettinck K, De Vos P, Devreese B, Heyndrickx M, De Block J. Heterogeneity of heat-resistant proteases from milk Pseudomonas species. Int J Food Microbiol. 2009;133(1-2):68-77. DOI: 10.1016/j.ijfoodmicro.2009.04.027
13. Dufour D, Nicodème M, Perrin C, Driou A, Brusseaux E, Humbert G, Gaillard JL, Dary A. Molecular typing of industrial strains of *Pseudomonas spp*. isolated from milk and genetic and biochemical characterization of an extracellular protease produced by one of them. Int J Food Microbiol. 2008;125:188-196. DOI: 10.1016/j.ijfoodmicro.2008.04.004
14. Andreani NA, Martino ME, Fasolato L, Carraro L, Montemurro F, Mioni R, Bordin P, Cardazzo B. Tracking the blue: A MLST approach to characterise the *Pseudomonas fluorescens* group. Food Microbiol. 2014;39:116-26. DOI: 10.1016/j.fm.2013.11.012
15. Caldera L, Franzetti L, Van Coillie E, De Vos P, Stragier P, De Block J, Heyndrickx M. Identification, enzymatic spoilage characterization and proteolytic activity quantification of *Pseudomonas spp*. isolated from different foods. Food Microbiol. 2015;54:142-153. DOI: 10.1016/j.fm.2015.10.004
16. Matéos A, Guyard M, Baglinière F, Jardin J, Gaucheron F, Mourot A, Humbert G, Gaillard J. Proteolysis of milk proteins by AprX, an extracellular protease identified in Pseudomonas isolated from bulk raw milk, and implications for the stability of UHT milk. Int Dairy J. 2015;49:78-88. DOI: 10.1016/j.idairyj.2015.04.008.hal-01160599
17. Aziz TA, Lafta IJ. Isolation and antimicrobial resistance of *Staphylococcus spp.*, enteric bacteria and *Pseudomonas spp.* associated with respiratory tract infections of sheep. Iraqi J Vet Sci. 2021;35(I-III):53-58. DOI: 10.33899/ijvs.2021.131098.1917
18. Al-Rudhan AM, Khalil NK, Altaai NA. Evaluation of bacterial contaminants and heavy metals in cow and buffalo raw milk sold in Baghdad governorate. Iraqi J Vet Sci. 2021;35:101-105. DOI: 10.33899/ijvs.2021.131744.1999
19. AL-Hamdany M, Hassan A. Microbiological quality of white local sheep cheese in Mosul city markets. Iraqi J Vet Sci. 2017;31(1):1-6. DOI: 10.33899/ijvs.2017.126712
20. Brooks G, Carroll K, Butel J, Morse S, Mietzner T. Jawetz Melnick and Adelberg Medical Microbiology, USA: McGraw-Hill Education; 2013. [available at]
21. Roberts D, Greenwood M. Practical food microbiology. 3rd ed. UK: Blackwell Publishing Ltd; 2008. 273-274 pp.
22. Scarpellini M, Franzetti L, Galli A. Development of PCR assay to identify *Pseudomonas fluorescens* and its biotype. FEMS Microbiol Lett. 2004;15;236(2):257-60. DOI: 10.1016/j.femsle.2004.05.043
23. Martins ML, Pinto UM, Riedel K, Vanetti MC. Milk-deteriorating exoenzymes from *Pseudomonas fluorescens* 041 isolated from refrigerated raw milk. Braz J Microbiol. 2015;46(1):207–217. DOI: 10.1590/S1517-838246120130859
24. Bellassi P, Rocchetti G, Morelli L, Senizza B, Lucini L, Cappa F. A milk foodomics investigation into the effect of *Pseudomonas fluorescens* growth under cold chain conditions. Foods. 2021;10(6):1173. DOI: 10.3390/foods10061173
25. Ibrahim shaker A, Mohammed Saleem R. Effect of fortifying by whey protein and proiotic bacteria on properties of soft cheese during cold storage 1- effect of powdered and curd whey on coagulation time and curd tension. Mesopotamia J Agric. 2013;41(3):157-161. DOI: 10.33899/magrj.2013.80312
26. Ribeiro Júnior JC, de Oliveira AM, Silva FG, Tamanini R, de Oliveira ALM, Beloti V. The main spoilage-related psychrotrophic bacteria in refrigerated raw milk. J Dairy Sci. 2018;101(1):75-83. DOI: 10.3168/jds.2017-13069
27. Ercolini D, Russo F, Nasi A, Ferranti P, Villani F. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. Appl Environmental Microbiol. 2009;75(7):1990–2001. DOI: 10.1128/aem.02762-08
28. Carrascosa C, Millán R, Jaber JR, Lupiola P, Rosario-Quintana CD, Mauricio C, Sanjuán E. Blue pigment in fresh cheese produced by *Pseudomonas fluorescens*. Food Control. 2015;54:95-102. DOI: 10.1016/j.foodcont.2014.12.039
29. Leriche F, Bordessoules A, Fayolle K, Karoui R, Laval K, Leblanc L, Dufour E. Alteration of raw-milk cheese by *Pseudomonas spp*. monitoring the sources of contamination using fluorescence spectroscopy and metabolic profiling. J Microbiol Methods. 2004;59:33-41. DOI: 10.1016/j.mimet.2004.05.009
30. Al-Leboudy AA, Nasief AM, Shaimaa M, Eltony SM. Occurrence and behavior of Pseudomonas organisms in white soft cheese. Alex J Vet Sci. 2015;44:74-79. DOI: 10.5455/ajvs.166387
31. Samaržija D, Zamberlin Š, Pogačić T. Psychrotrophic bacteria and milk and dairy products quality. Mljekarstvo. 2012;62(2):77–95. [available at]

الكشف عن جينات الضراوة في الزوائف المتألقة المعزولة من الجبن المحلي في محافظة نينوى

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الخلاصة

يستهلك الجبن كمنتج غذائي بدرجة كبيرة في محافظة نينوى وهو مادة غذائية سريعة الفساد بفعل الزوائف المتألقة خلال عمليات تصنيع الجبن حيث أن نمو الزوائف يغير نوعية الجبن ويختزل مدة حفظه، تم فحص خمسون عينة من الجبن المحلي المستهلك في محافظة نينوى للكشف عن تواجد جراثيم الزوائف المتألقة كأحد أدلة الفساد الغذائي وتم اختبار عترات الزوائف المتألقة المعزولة من عينات الجبن المحلي لتقييم ضراوتها من حيث قابليتها على إنتاج الإنزيمات الخارجية التي تسبب فساد الأجبان وبضمنها أنزيم البروتيز وأنزيم اللاييز باعتماد الطرق الجينية للكشف عن الجينات الهدف بتقنية تفاعل البلمرة المتسلسل. من مجموع خمسون عينة مفحوصة من الجبن أعطت عشرة عينات نتيجة موجبة لعزل جراثيم الزوائف المتألقة وبنسبة ٢٠% تبعا للجين *16SPflu* ،وشخصت قابليتها على إنتاج إنزيم البروتيز واللاييز اعتمادا على كل من جين *AprX* و جين *LipM* بالتتابع، وأظهرت النتائج أن ثلاث عزلات من جراثيم الزوائف المتالقة موجبة لتواجد الجين *AprX* وبنسبة ٣٠% في حين أعطت عزلة واحدة نتيجة موجبة لتواجد جين *LipM* وبنسبة ١٠% مما يؤكد انخفاض قدرتها على إنتاج إنزيم اللاييز وأشارت نتائج التسلسل الجيني لدنا عزلات الزوائف المتألقة وتبعا للجين *16SPflu* تسجيل أربعة عزلات في بنك الجينات بالأرقام المعرفة PP727372 PP727373,PP727374,PP727375, وتوكّد هذه النتائج خطورة تواجد وانتشار الزوائف المتألقة في الجبن المحلي ومن الممكن اعتمادها كدليل على التنبؤ بحدوث فساد الأجبان مع ضرورة التأكيد على متابعة الشروط الصحية أثناء عمليات تصنيع الجبن وضمان سلامة الحليب الخام أثناء عمليات جمع الحليب ومعاملته وحفظه تحت ظروف التبريد لإطالة مدة حفظ منتجات الحليب وصحة المستهلك.

32. Mahmood AW. Effect of microbial transglutaminase treatment on soft cheese properties. Mesopotamia J Agric. 2009;37(4):19-27. DOI: 10.33899/magrj.2009.27525
33. Bintsis T, Litopoulou-Tzanetaki E, Robinson RK. Existing and potential applications of ultraviolet light in the food industry: A critical review. J Sci Food Agric. 2000;80(6):637–645. DOI: 10.1002/(sici)1097-0010(20000501)80:6<637::aid-jsfa603>3.0.co;2-1
34. Paludetti LF, O'Callaghan TF, Sheehan JJ, Gleeson D, Kelly AL. Effect of *Pseudomonas fluorescens* proteases on the quality of Cheddar cheese. J Dairy Sci. 2020;103(9):7865-7878. DOI: 10.3168/jds.2019-18043
35. Paludetti LF, Kelly AL, Gleeson D. Effect of thermoresistant protease of pseudomonas fluorescens on rennet coagulation properties and proteolysis of milk. J. Dairy Sci. 2020;103:4043–4055. DOI: 10.3168/jds.2019-1777
36. Stuknytė M, Decimo M, Colzani M, Silvetti T, Brasca M, Cattaneo S, Aldini G, De Noni I. Extracellular thermostable proteolytic activity of the milk spoilage bacterium pseudomonas fluorescens PS19 on bovine caseins. J Dairy Sci. 2016;99:4188–4195. DOI: 10.3168/jds.2016-10894
37. Baglinièrea F, Matéos A, Tanguya G, Jardina J, Briard-Bion V, Rousseau F, Robert B, Beaucher E, Gaillard J, Amiel C, Humbert G, Daryc A, Gaucheron F. Proteolysis of ultra high temperature-treated casein micelles by AprX enzyme from *Pseudomonas fluorescens* F induces their destabilization. Int Dairy J. 2013;31:55–61. DOI: 10.1016/j.idairyj.2013.02.011
38. Machado SG, Bazzolli DS, Vanetti MD. Development of a PCR method for detecting proteolytic psychrotrophic bacteria in raw milk. Int Dairy J. 2013;29(1):8-14. DOI: 10.1016/j.idairyj.2012.09.007
39. Martins ML, de Araújo EF, Mantovani HC, Moraes CA, Vanetti MC. Detection of the apr gene in proteolytic psychrotrophic bacteria isolated from refrigerated raw milk. Int J Food Microbiol. 2005;102(2):203-11. DOI: 10.1016/j.ijfoodmicro.2004.12.016
40. Rosenau F, Jaeger K. Bacterial lipases from pseudomonas regulation of gene expression and mechanisms of secretion. Biochim. 2000;82(11):1023-32. DOI: 10.1016/s0300-9084(00)01182-2
41. Barton MD, Petronio M, Giarrizzo JG, Bowling BV, Barton HA. The genome of *Pseudomonas fluorescens* strain R124 demonstrates phenotypic adaptation to the mineral environment. J Bacteriol. 2013;195(21):4793-803. DOI: 10.1128/JB.00825-13
42. Kiewitz C, Tümmler B. Sequence diversity of *Pseudomonas aeruginosa*: Impact on population structure and genome evolution. J Bacteriol. 2000;182:3125–3135. DOI: 10.1128/jb.182.11.3125-3135.2000