Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

The cytolytic capacity of *Enterococcus faecalis* isolates from cheese, yogurt, and curd

W.R. Atiyah[1], O.M. Faja[1], Q.Z. Bneed[2] and Z.M. Al-khozai[2]

[1]Department of Public Health, College of Veterinary Medicine, [2]Department of Medical Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Al-Qadisiyah, Iraq

Article information

Article history:
Received 27 March, 2024
Accepted 22 June, 2024
Published online, 18 September, 2024

Keywords:
Enterococcus faecalis
Food-borne diseases
Food contamination
Food safety

Correspondence:
W.R. Atiyah
wisam.atyiah@qu.ed.iq

Abstract

Food safety is an important issue that concerns scientific and commercial communities worldwide for its direct impact on people's health. The current study used conventional methods to isolate Enterococcus faecalis from dairy products. The study was also evaluated its pathogenicity (cytolysin) and detected the genes responsible for the production of cytolysin (cylM, cylB, asa, cylA, and cylLs) using polymerase chain reaction (PCR). Here, the study was first started with collecting of 72 dairy product samples (21 cheese, 31 yogurts, and 20 curd samples). The samples were subjected to conventional cultivation, cytotoxicity, and PCR methods. The results of the cultivation revealed the presence of the E. faecalis in 43/72 (59.7%) samples, which is distributed over 20/21 (95.2%) cheese, 14/31 (45.2%) yogurt, and 9/20 (45%) curd samples. The findings of the hemolysis analysis demonstrated that the hemolytic capability of the isolated bacteria of types (γ, α, and β) differed, depending on the type of blood agar (BA) employed (human (Hn), sheep (S), and horse (Hs)). On HnBA plates, 17 isolates (39.5%) exhibited γ hemolytic action; however, 21 isolates (48.8%) exhibited β hemolytic activity. The cytotoxic activity on Vero cells showed various rates of dead cells and ranged from 0.2% (for the control) to 50.1% for specific isolates. The result of the PCR revealed the amplification of the examined genes in the bacterial isolates of dairy products. The current data demonstrate the presence of Enterococcus faecalis in dairy products with the indications of its pathogenicity due to the presence of cytolysin-coding genes and the cytotoxic activity of this protein on Vero cells.

DOI: 10.33899/ijvs.2024.148181.3556,

Introduction

Enterococci are Gram-positive bacteria naturally inhabited in the GIT of most terrestrial mammals, including humans. As a group, they are responsible for many infections, including difficult-to-treat infections in people whose immune systems are compromised. Two enterococcal species, *E. faecium* and *E. faecalis*, account for at least 75 per cent of all enterococcal infections (1). *E. faecalis* is a common cause of UTIs as well as deeper abscesses. Resistant strains of *E. faecalis* are the main culprits behind infections that people acquire while receiving care in hospitals, including bloodstream infections, infections of the heart valves, UTIs, infections within the abdomen, surgical site infections, and infections from medical devices that are implanted in the body, such as catheters and intraocular lenses (2). What brought enterococci to the modern hospital? On one hand, they're naturally resistant to many antibiotics that doctors use. Secondly, they're tough to survive in extreme places. Thirdly, many can acquire mobile genetic elements that bring along drug resistance and other waste genes that help them cause disease. Finally, they biofilm on medical devices implanted in the body, such as catheters and intraocular lenses (3-6). Bacterial toxins are virulence determinants involved in pathogenesis – a host-pathogen interaction where toxins play a major role in transforming

commensals into pathogens (i.e., the infection process). More specifically, these factors have unique functions to inhibit protein synthesis, assist the pathogen with evading host immune responses, and disrupt the membrane integrity of the target cell (7-10). Bacterial toxins can be grouped into numerous functional classes based on their biophysical properties, structures, and known modes of action. The most diverse protein toxins class is pore-forming toxins (PFTs). PFTs are proxies of the process known as pore-forming as they assemble on the membrane of a selected cell and create pores within it, thus increasing anion or cation permeability of the target membrane. In so doing, PFTs induce catastrophic alterations inside the cell, leading ultimately to cell lysis and cell death (11-14). Based on the secondary structures of the domains that construct the transmembrane pore, PFTs have been classified into two structural families: alpha-PFTs and beta-PFTs which, respectively, form helical and sheet-like pores. Well-studied examples of alpha-PFTs are the colicins produced by *Escherichia coli*, whereas *Staphylococcus aureus* produces a beta-PFT known as alpha-hemolysin (15-17). Cytolysin A (ClyA) (also called HlyE or SheA) is a 34-kilodalton α-PFT produced by some members of the Enterobacteriaceae family such as *E. coli* and *Salmonella enterica*. This toxin has been exhaustively studied in terms of its structure, its activity, and its importance as a virulence factor (18).

Food safety is an important issue that concerns scientific and commercial communities worldwide for its direct impact on people's health. The current study used conventional methods to isolate *Enterococcus faecalis* from dairy products. The study also evaluated its pathogenicity (cytolysin) and detected the genes responsible for the production of cytolysin (*cylM*, *cylB*, *asa*, *cylA*, and *cylL$_s$*) using PCR.

Materials and methods

Ethical approve

The current study was approved by the Ethical Committee at the College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq, under the No.4414 in 23/11/2023.

Samples

The study first started with collecting of 72 dairy product samples (21 cheese, 31 yogurts, and 20 curd samples). These samples were purchased from local stores in Al-Diwaniyah City, Iraq.

Bacterial cultivation and identification

Conventional methods were used to cultivate and identify bacterial species (9). The identification process included using selective media, such as Bile Esculin Agar, Enterococcsel Agar, and Slanetz and Bartley medium.

Hemolysis activity

The isolated bacteria of *E. faecalis* were grown on three different types of blood agar, horse blood agar (HnBA), HsBA and SBA. The HnBA is provided by the Health Clinic Centre situated in UKM, Bangi while HsBA and SBA are provided by Era Bumi Sains Sdn Bhd, Malaysia. After the overnight incubation (at 37ºC), the data were collected regarding the examination of hemolysis zone surrounding the colonies (19).

Cytotoxic activity

The isolated bacteria were grown in 5 mL of brain heart infusion (BHI) medium with 1% glucose and 0.03% L-arginine at a temperature of 37ºC for 18 hrs, as previously described by Booth *et al.* (20). The colonies were centrifuged in 6000 xg for 15 mins at 4ºC. The liquid above the sediment was taken and filtered through the 0.22 µm Millipore membrane, then later used to test Vero cells, originating from the African green monkey kidney, for identifying the ability of strains of *E. faecalis* to produce cytolysin. The survivability of the Vero cell to detects the toxicity caused by the strains was done in an MTT assay. Method for MTT assay as described by Raheel *et al.* (21). Vero cell lines gained from a stock of Virology Laboratory Faculty of Science and Technology UKM. Negative control uses sterilized broth without culture. The samples DMEM was taken, and 20 µL per well of the MTT was added (5 mg/ml in PBS) then the plates were incubated for another 4 hrs. The OD was measured in an ELISA reader (BioRad, Chapan) at 540 nm. Percentages of cell death were calculated using the formula as follows:

Cells Die % = [Mean OD control / Mean OD treated cell] × 100.

Extraction of DNA

Ten bacterial contents of genomic DNA were extracted using the Wizard® Genomic DNA purification kit (Promega, USA). According to the manufacturer's protocol, the bacteria grown overnight in BHI broth was harvested by centrifuging the solution at 13,000 RPM for 1 minute. Then the pellet formed was suspended in 480 µL of 50 mM EDTA solution. The solution was then centrifuged at 13,000 rpm for 2 minutes, and the liquid portion above the sediment was separated and eluded.

PCR

The 50 µL of PCR reaction mixture contained 10 µL of 5X Go Taq Flexi buffer, 1 µL of 0.2 mM dNTPs, 8 µL of 4 mM MgCl2, 1 µL of 1 µM of each primer, 1 µL of 0.5 µg DNA, 0.25 µL of 1.25 U DNA polymerase, and 27.75 µL water. The PCR was performed in a Bio-Rad MyCycler thermal cycler (Bio-Rad, USA). The program was set for 2 minutes at 95ºC for an initial denaturation, followed by 35 cycles of 95ºC for 1 minute (denaturation), annealing at 40ºC to 60ºC for 1 minute and extension at 72ºC for 1 minute, and

a final extension product of 5 minutes at 72ºC. The amplified DNA product was separated on 1.5% agarose gel with an applied voltage of 80 V for 45 minutes. Before loading the gel, a DNA stain (FloroSafe stain) was added. Finally, all gels were observed and recorded using a UV trans-illuminator (syngene, UK).

Results

The results of the cultivation revealed the presence of the bacterium *E. faecalis* in 43/72 (59.7%) samples, which is distributed over 20/21 (95.2%) cheese, 14/31 (45.2%) yogurt, and 9/20 (45%) curd samples. The findings of the hemolysis analysis demonstrated that the hemolytic capability of the isolated bacteria of types (γ, α, and β) differed, depending on the type of blood agar employed HnBA, SBA, and HsBA. On HBA plates, 17 isolates (39.5%) exhibited γ hemolytic action; however, 21 isolates (48.8%) exhibited β hemolytic activity. The results revealed that only 5 isolates showed α blood hemolysis. Overall, 39.5% of the isolates (n=17) showed γ counts, 11.6% (n=5) were α and 48.8% (n=21) were β hemolysis colonies on human blood agar. For SBA, the report revealed differences in some of the ratios. Notably, none showed β in SBA. γ blood hemolysis was recorded in 39.5% (n=17) of the isolated pluses. While α blood, in this case, was 60.5% (n=26). Interestingly, some isolates were unable to show β blood hemolysis on SBA even though they had already demonstrated hemolysis on HnB and HsB agars. The second was the delay in the process, as some of the isolates were able to show it after 72 hours (Table 1 and Figure 1). The cytotoxic activity on Vero cells showed various rates of dead cells and ranged from 0.2% (for the control) to 50.1% for certain isolates (Figure 2). The result of the PCR revealed the amplification of the examined genes in the bacterial isolates of dairy products (Figure 3).

Table 1: Pattern of hemolysis of *E. Faecalis* from cheese, yogurt, and curd

Isolates	Horse	Sheep	Human	Pattern
S1, S2, S5, S16, S22, S40, S100, S124, S133	β	α	β	A
S21, S31, S37, S52, S56, S88, S98, S131	γ	γ	γ	B
S29, S46, S106	α	α	α	C
H5, H11, H29, H53, H75, H87	β	α	β	A
H14, H21, H54, H72, H79, H85	γ	γ	γ	B
H16, H27	α	α	α	C
M1, M2, M20, M34, M40, M42	β	α	β	A
M10, M29, M39	γ	γ	γ	B

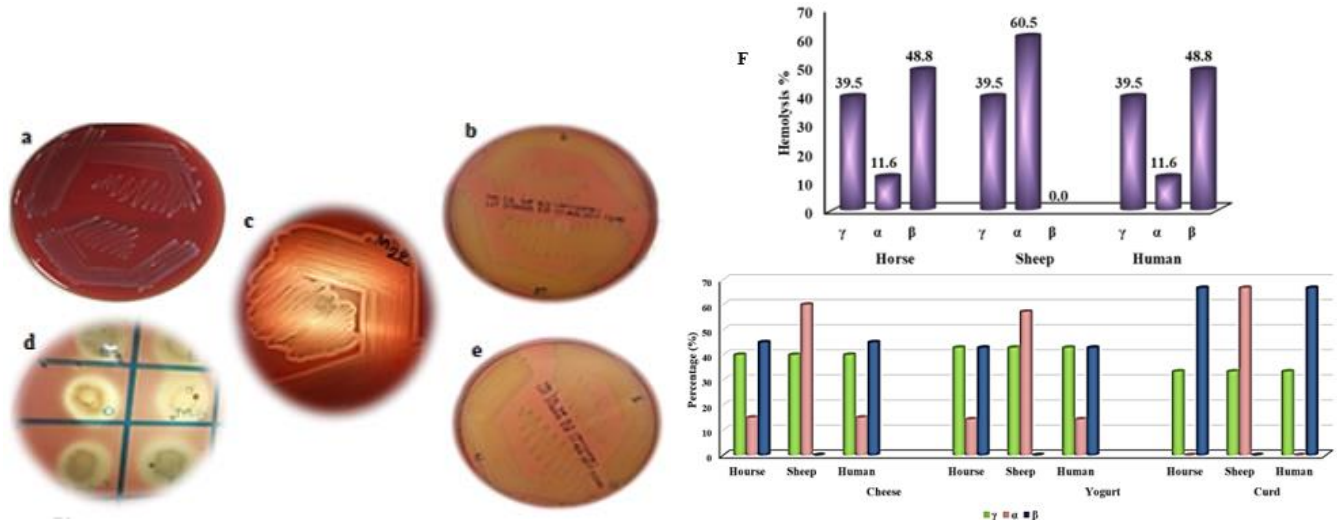

Figure 1: Hemolysis activity on different blood agars of *Enterococcus faecalis* bacteria isolated from cheese, yogurt, and curd samples. (a) Gamma hemolysis on sheep blood agar (*E. faecalis* S21), (b) alpha hemolysis on sheep blood agar (*E. faecalis* H57), (c) beta hemolysis on human blood agar (*E. faecalis* S2), (d) beta hemolysis on horse blood agar (E. faecalis S133, H85, H87 and M1), (e) alpha hemolysis on horse blood agar (*E. faecalis* S29).

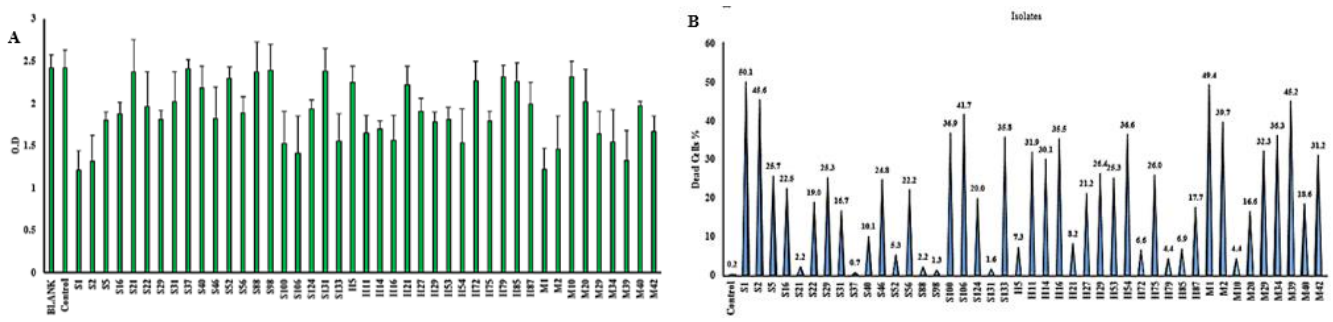

Figure 2: Cytotoxicity activity of *E. faecalis* isolated from cheese, yogurt, and curd tested on Vero cell line. A. Optical density. B. Dead cell percentage.

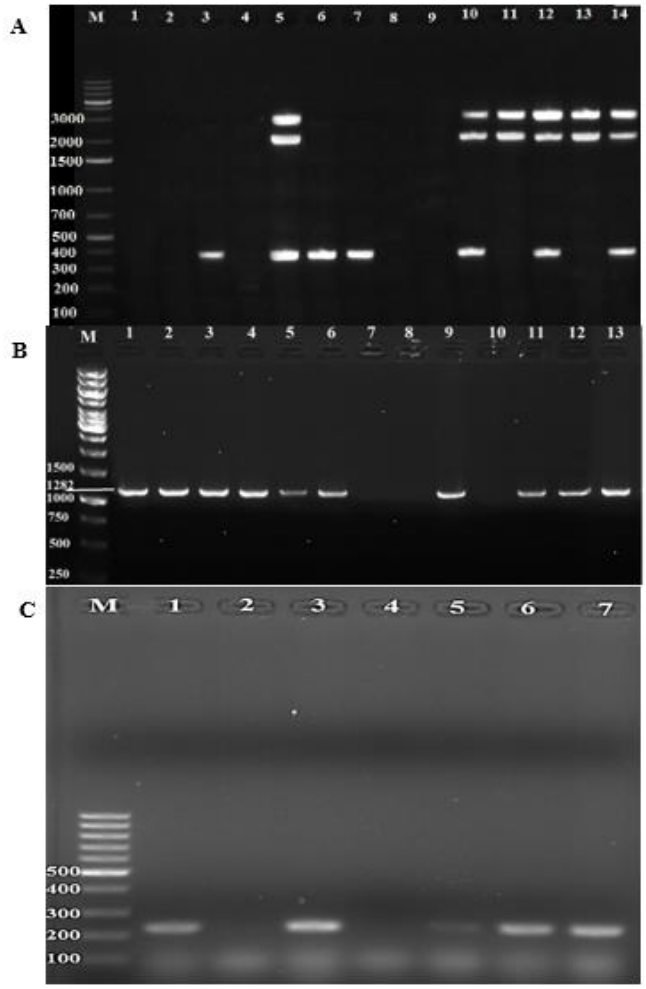

Figure 3: Agarose gel electrophoresis of A. *cylM*, *cylB*, *asa*, B. *cylA*, C. and *cylL*$_s$ genes responsible for cytolysin coding in *E. faecalis* isolated from cheese, yogurt, and curd.

Discussion

Blood agar is used to identify and test the antibiotic susceptibility of many different bacterial pathogens. The high cost and harsh atmosphere required to raise wool sheep or horses to collect the blood lead microbiologists in developing countries to go for the cheaper and more suitable human blood agar. Several pathogens do not grow or exhibit morphologies and hemolytic profiles so it is difficult to differentiate their colonies (22). Hence, this study intended to use different types of erythrocytes other than those obtained from humans such as horse blood and sheep blood. This study used three different types (human, sheep and horse) of blood agar to evaluate *E. faecalis* isolates (n=43) for the activity of hemolysis (22). As shown in Figure 1, the results from the hemolysis test done on the erythrocytes showed that the three isolates (γ, α and β) have varied degrees of hemolysis depending on the type of blood agar used among the isolates sampled, whether human, sheep, or horses. The overall, a total of 17 isolates (39.5%) showed γ hemolysis on horse blood agar while a total of 21 isolates (48.8%) were β hemolysis. Only 5 isolates showed α blood hemolysis (22).

Cell proliferation (cell growth is an augmentation in cell viability. Cell death (cell loss) is deterioration in cell viability as characterized by cell death caused by the toxic effects of the test material (23). The predominance of *E. faecalis* in infections can be partially attributed to its ability to inhibit the growth of other bacteria in the same environment. This inhibition is likely due to the Enterococcal cytolysin (encoding genes of *E. faecalis*, as they all act against both Gram-positive and Gram- negative species (24). Furthermore, the presence of cytolysin also increases the likelihood of *E. faecalis* dominating a mixed infection and providing an ecological advantage that potentially can lead to diseases in humans and animals.

No cytolysin genes were found in the phenotypic non-γ hemolysin isolates. Moreover, the data show differences in toxicity between the bacterial isolates, with the cells infected with *E. faecalis* mediated γ hemolysin and lacking cytolysin genes showing a decreased toxicity level compared with the other isolates. The isolates exhibiting γ hemolysin capability and lacking cytolysin genes such as S21, S37, S46, S52, S88, S98, S131, H79 and M10 had, respectively, dead cell percentages of 2.2, 0.7, 0.8, 5.3, 2.2, 1.3, 1.6, 4.4, and 4.4%.

Since cytolysin may cause hemolysis and cytotoxicity, the absence of the cytolysin genes could also account for these results (25-34).

Enterococci are Gram-positive bacteria naturally inhabited in the GIT of most terrestrial mammals, including humans. Food safety is an important issue that concerns scientific and commercial communities worldwide for its direct impact on people's health (35-48).

Conclusion

The current data demonstrate the presence of *Enterococcus faecalis* in dairy products with the indications of its pathogenicity due to the presence of cytolysin-coding genes and the cytotoxic activity of this protein on Vero cells.

Acknowledgement

Not applicable

Conflict of interest

The study has no conflict of interest as it is declared by the authors.

References

1. Van Tyne D, Gilmore MS. Friend Turned Foe: Evolution of enterococcal virulence and antibiotic resistance. Annu Rev Microbiol. 2014;68:337-356. DOI: 10.1146/annurev-micro-091213-113003
2. García-Solache M, Rice LB. The Enterococcus: A model of adaptability to its environment. Clin Microbiol Rev. 2019;32:e00058-18. DOI: 10.1128/CMR.00058-18
3. Fiore E, Van Tyne D, Gilmore MS. Pathogenicity of Enterococci. Microbiol Spectr. 2019;7. DOI: 10.1128/microbiolspec.GPP3-0053-2018
4. Kristich CJ, Rice LB, Arias CA. Enterococcal infection-treatment and antibiotic resistance. In: Kaiser PK, Friedman NJ, editors. The Massachusetts Eye and Ear Infirmary Illustrated Manual of Ophthalmology. USA: Saunders; 2014. 1-63 pp. DOI: 10.1016/B978-1-4557-7644-3.09988-1
5. Price VJ, Huo W, Sharifi A, Palmer KL. CRISPR-Cas and restriction-modification act additively against conjugative antibiotic resistance plasmid transfer in *Enterococcus faecalis*. mSphere. 2016;1:e00064-16. DOI: 10.1128/mSphere.00064-16
6. Noskin GA, Stosor V, Cooper I, Peterson LR. Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. Infect Control Hosp Epidemiol. 1995;16:577-581. DOI: 10.2307/30141097
7. Henkel JS, Baldwin MR, Barbieri JT. Toxins from Bacteria. In: Luch A, editor. Molecular, Clinical and Environmental Toxicology. Germany: Springer; 2010. 1-29 pp. 10.1007/978-3-7643-8338-1_1
8. Rudkin JK, McLoughlin RM, Preston A, Massey RC. Bacterial toxins: Offensive, defensive, or something else altogether?. PLoS Pathog. 2017;13:e1006452. DOI: 10.1371/journal.ppat.1006452
9. Alouf JE. The Comprehensive sourcebook of bacterial protein toxins. Netherlands: Elsevier Ltd.; 2015. DOI: 10.1016/B978-0-12-800188-2.00038-0
10. Lemichez E, Barbieri JT. General aspects and recent advances on bacterial protein toxins. Cold Spring Harb Perspect Med. 2013;3:a013573. DOI: 10.1101/cshperspect.a013573
11. Bischofberger M, Gonzalez MR, van der Goot FG. Membrane injury by pore-forming proteins. Curr Opin Cell Biol. 2009;21:589–595. DOI: 10.1016/j.ceb.2009.04.003
12. Dal Peraro M, van der Goot FG. Pore-forming toxins: Ancient, but never really out of fashion. Nat Rev Microbiol. 2016;14:77–92. DOI: 10.1038/nrmicro.2015.3
13. Los FC, Randis TM, Aroian RV, Ratner AJ. Role of pore-forming toxins in bacterial infectious diseases. Microbiol Mol Biol Rev. 2013;77:173–207. DOI: 10.1128/MMBR.00052-12
14. Gouaux E. Channel-forming toxins: Tales of transformation. Curr Opin Struct Biol. 1997;7:566–573. DOI: 10.1016/S0959-440X(97)80123-6
15. Parker MW, Pattus F, Tucker AD, Tsernoglou D. Structure of the membrane-pore-forming fragment of colicin A. Nature. 1989;337:93–96. DOI: 10.1038/337093a0
16. Parker MW, Feil SC. Pore-forming protein toxins: From structure to function. Prog Biophys Mol Biol. 2005;88:91–142. DOI: 10.1016/j.pbiomolbio.2004.01.009
17. Song L, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE. Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science. 1996;274:1859–1866. DOI: 10.1126/science.274.5294.1859
18. Murase K. Cytolysin A (ClyA): A Bacterial virulence factor with potential applications in nanopore technology, vaccine development, and tumor therapy. Toxins. 2022;14(2):78. DOI: 10.3390/toxins14020078
19. Furumura MT, Figueiredo PS, Carbonell GV, Darini C, Yano T. Virulence-associated characteristics of *Enterococcus faecalis* strains isolated from clinical sources. Braz J Microbiol. 2006;37(3):230–6. DOI: 10.1590/S1517-83822006000300007
20. Booth NJ, Elkamel A, Thune RL. Intracellular replication of *Edwardsiella ictaluri* in channel catfish macrophages. J Aquat Anim Health. 2006;18:101-108. DOI: 10.1577/H05-025.1
21. Raheel R, Ashraf M, Ejaz S, Javeed A, Altaf I. Assessment of the cytotoxic and anti-viral potential of aqueous extracts from different parts of *Acacia nilotica* (Linn) Delile against Peste des petits ruminants virus. Environ Toxicol Pharmacol. 2013; 35(1):72-81. DOI: 10.1016/j.etap.2012.11.005
22. Yeh E, Pinsky BA, Banaei N, Baron EJ. Hair sheep blood, citrated or defibrinated, fulfills all requirements of blood agar for diagnostic microbiology laboratory tests. PLoS One. 2009;4(7):e6141. DOI: 10.1371/journal.pone.0006141
23. Rampersad SN. Multiple applications of Alamar Blue as an indicator of metabolic function and cellular health in cell viability bioassays. Sensors. 2012;12(9):12347-60. DOI: 10.3390/s120912347
24. Kayaoglu G, Ørstavik D. Virulence factors of *Enterococcus faecalis*: Relationship to endodontic disease. Crit Rev Oral Biol Med. 2004;15(5):308-20. DOI: 10.1177/154411130401500506
25. Rahman IR, Sanchez A, Tang W, van der Donk WA. Structure-activity relationships of the Enterococcal Cytolysin. ACS Infect Dis. 2021;7(8):2445-2454. DOI: 10.1021/acsinfecdis.1c00197
26. Arias CA, Murray BE. The rise of the enterococcus: Beyond vancomycin resistance. Nat Rev Microbiol. 2012;10(4):266–16. DOI: 10.1038/nrmicro2761
27. Janka T, Tornai T, Borbely B, Tornai D, Altorjay I, Papp M, Vitalis Z. Deleterious effect of proton pump inhibitors on the disease course of cirrhosis. Eur J Gastroenterol Hepatol. 2020;32(2):257–264. DOI: 10.1097/MEG.0000000000001499
28. Lin HF, Liao KF, Chang CM, Lin CL, Lai SW. Correlation between proton pump inhibitors and risk of pyogenic liver abscess. Eur J Clin Pharmacol. 2017;73(8):1019–1025. DOI: 10.1007/s00228-017-2256-9
29. Imhann F, Bonder MJ, Vila AV, Fu J, Mujagic Z, Vork L, Tigchelaar EF, Jankipersadsing SA, Cenit MC, Harmsen HJ, Dijkstra G. Proton pump inhibitors affect the gut microbiome. Gut. 2016;65(5):740–748. DOI: 10.1136/gutjnl-2015-310376
30. Zhang J, Wang C, Wang J, Zhang F. Relationship between intestinal flora and inflammatory factors in patients with nonalcoholic steatohepatitis. Exp Ther Med. 2018;15(1):723–726. DOI: 10.3892/etm.2017.5490

31. Liu J, Wu D, Ahmed A, Li X, Ma Y, Tang L, Mo D, Ma Y, Xin Y. Comparison of the gut microbe profiles and numbers between patients with liver cirrhosis and healthy individuals. Curr Microbiol. 2012;65(1):7–13. DOI: 10.1007/s00284-012-0105-8
32. Freedberg DE, Zhou MJ, Cohen ME, Annavajhala MK, Khan S, Moscoso DI, Brooks C, Whittier S, Chong DH, Uhlemann AC, Abrams JA. Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection. Intensive Care Med. 2018;44(8):1203–1211. DOI: 10.1007/s00134-018-5268-8
33. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, Lee YJ, Dubin KA, Socci ND, Viale A, Perales MA. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2012;55(7):905–914. DOI: 10.1093/cid/cis580
34. Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, Viale A, Socci ND, Van Den Brink MR, Kamboj M, Pamer EG. Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. J Clin Invest. 2010;120(12):4332–4341. DOI: 10.1172/JCI43918
35. Alsanjary LH, Sheet OH. Molecular detection of uidA gene in *Escherichia coli* Isolated from the dairy farms in Nineveh governorate/Iraq. Iraqi J Vet Sci. 2022;36(3):599-603. DOI: 10.33899/ijvs.2021.131046.1913
36. Alkhafaje WK, Olama ZA, Ali SM. Molecular characterization and microbial resistance of different bacterial isolates in some dairy products. Iraqi J Vet Sci. 2022;36(2):333-339. DOI: 10.33899/ijvs.2021.130206.1764
37. Taher DD, Yassin SA, Abdulkareem MH. Isolation and molecular detection of enterotoxigenic *Staphylococcus aureus* from raw milk of cows. Iraqi J Vet Sci. 2021;35:137-141. DOI: 10.33899/ijvs.2021.131957.2030
38. Mahmood FR, Ahmed IM. Molecular detection of ESBL/AmpC ß-lactamase *Escherichia coli* isolated from sheep in Mosul city. Iraqi J Vet Sci. 2022;36(2):387-392. DOI: 10.33899/ijvs.2021.130380.1810
39. Atiyah AS, Alkhafaji MH. Isolation of Enterococcus Species from food sources and its antibacterial activity against *Staphylococcus aureus*. Iraqi J Sci. 2020;61(12):3164-3171. DOI: 10.24996/ijs.2020.61.12.3
40. Eman AM, Suhad MH, Enas YS. Molecular detection and identification of *Enterococcus faecium* isolated from dental root canals. Iraqi J Sci. 2021;62(5):1447-1451. DOI: 10.24996/ijs.2021.62.5.7
41. Al-Alhially AA. First analysis of beak and feather disease of psittacine in Nineveh province, Iraq. Iraqi J Vet Sci. 2024;38(1):173-81. DOI: 10.33899/ijvs.2023.141118.3104
42. Abbas LA, Rady AM. Isolation and molecular detection of *Staphylococcus aureus* from feline otitis in Baghdad. Iraqi J Vet Sci. 2023;37(I-IV):141-6. DOI: 10.33899/ijvs.2023.1375560.2696
43. Jabori EA, Ismail HK, Khaleel LW, Al-Hadidy AA. Hematological, biochemical, and histological alteration induced by nano silver material on male rats. Iraqi J Vet Sci. 2023;37(3):707-17. DOI: 10.33899/ijvs.2023.137239.2660
44. Saadh MJ. Green synthesis of silver nanoparticles using Piper betle exhibit high antimicrobial activity against *Salmonella enteritidis* and *E. coli*. Iraqi J Vet Sci. 2023;37(3):759-63. DOI: 10.33899/ijvs.2023.136475.2585
45. Khayoon T, Abbas R, Abdullah F. Effects of feeding various levels of postbiotics produced by lactic acid bacteria on growth performance, gastrointestinal microbiota count, and digestibility of some nutrients in broiler chickens. Mesopotamia J of Agric. 2024;52(2):68-81. DOI: 10.33899/mja.2024.145531.132019
46. Hanoush N. Honey as antibacterial agent used against *Bacillus spp.* isolated from locally produced juice. Mesopotamia J of Agric. 2023;51(4):72-85. DOI: 10.33899/mja.2023.143201.1273
47. Alkhashb A, Alhaji T, Thalij K. Effectiveness of chitosan and Ag-nanoparticle films on the quality of chicken meat. Mesopotamia J of Agric. 2024;52(2):14-26. DOI: 10.33899/mja.2024.145729.011337
48. Alzubaidi E. The reality of sustainable development in developing countries and the prospects for achieving it in 2030. Mesopotamia J of Agric. 2024;52(2):27-45. DOI: 10.33899/mja.2024.145075.1311

القدرة التحللية للمكورات المعوية البرازية المعزولة من الجبن والزبادي واللبن الرائب

وسام رحيم عطية[١]، عروبة متعب فجة[١]، قاسم زامل بنيد[٢] و زياد متعب الخزاعي[٢]

[١]فرع الصحة العامة، كلية الطب البيطري، [٢]قسم التقانات الطبية، كلية التقانات الإحيائية، جامعة القادسية، القادسية، العراق

الخلاصة

تعتبر سلامة الغذاء من القضايا المهمة التي تشغل المجتمعات العلمية والتجارية حول العالم لتأثيرها المباشر على صحة الإنسان. أجريت الدراسة الحالية لعزل المكورات المعوية البرازية من منتجات الألبان باستخدام الطرق التقليدية. أجريت الدراسة أيضًا لتقييم قدرتها المرضية (السيتوليزين) والكشف عن الجينات المسؤولة عن إنتاج السيتوليزين (*cylM*، *cylB*، *asa*، *cylA*، و*cylLs*) باستخدام تفاعل أنزيم البلمرة. بدأت الدراسة بجمع ٧٢ عينة من منتجات الألبان (٢١ عينة من الجبن، و٣١ عينة من الزبادي، و٢٠ عينة من اللبن الرائب). تم إخضاع العينات للزراعة التقليدية، والسمية الخلوية، وطرق تفاعل أنزيم البلمرة. أظهرت نتائج الزراعة وجود البكتيريا في ٧٢/٤٣ (٥٩,٧%) عينة، موزعة على ٢١/٢٠ (٩٥,٢%) جبن، ٣١/١٤ (٤٥,٢%) زبادي، و٢٠/٩ (٤٥%) عينات اللبن الرائب. أظهرت نتائج تحليل انحلال الدم أن القدرة الانحلالية للبكتيريا المعزولة بأنواعها (كاما والفا وبيتا) تختلف باختلاف نوع أكار الدم المستخدم (إنسان وأغنام وحصان). بشكل عام، أظهرت اكار الدم من الإنسان ١٧ عزلة (٣٩,٥٪) عمل انحلالي من نوع كاما؛ ومع ذلك، أظهرت ٢١ عزلة (٤٨,٨%) نشاط بيتا الانحلالي. أظهر النشاط السام للخلايا على خلايا فيرو معدلات مختلفة للخلايا الميتة تراوحت بين ٠,٢% (للسيطرة) إلى ٥٠,١% لبعض العزلات. أظهرت نتيجة تفاعل أنزيم البلمرة تضخيم الجينات المفحوصة في العزلات البكتيرية لمنتجات الألبان. توضح البيانات الحالية وجود المكورات المعوية البرازية في منتجات الألبان مع مؤشرات على قدرتها المرضية بسبب وجود الجينات المشفرة للسيتوليزين والنشاط السام للخلايا لهذا البروتين على خلايا فيرو.