*Egypt. J. Vet. Sci.* **Vol. 52,** No. 2, pp. 195-202 (2021)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

# The First Report of Bacteriocin Production by the *Bacillus coagulans* IS2 and its Antibacterial Effects

**Mohadese Sharifi[1], Hatef Ajoudani Far[1], Abolfazl Pahlevanlo[2], Marzieh Hosseininezhad[2]**

[1]*Department of Microbiology, Damghan Branch, Islamic Azad University, Damghan, Iran.*

[2]*Research Institute of Food Science and Technology, Mashhad, Iran.*

*BACILLUS coagulans* is a probiotic bacterium with high beneficial effects on human health. The present research was performed to assess the antimicrobial effects of bacteriocin extracted from *B. coagulans* IS2 against different food-borne bacteria. *B. coagulans* IS2 was cultured on tryptic soy broth and incubated for 24 h at 37°C. Then, bacteria were sub-cultured on Man, Rogosa, Sharpe (MRS) broth. Produced bacteriocin was extracted from the MRS culture of *B. coagulans* IS2 by 70% ammonium sulphate. *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* were cultured on Mueller Hinton Agar. Then, two separate 6 mm wells were created and filled with 200 µL of extracted bacteriocin. Presence of growth inhibition zones around wells weas considered as antimicrobial effect. The molecular weight of the bacteriocin was assessed by the Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE analysis showed presence of low molecular weight protein (<10 KD) in the gel which confirm the presence of bacteriocin-type protein. Clear growth inhibition zones were occurred around the wells contained bacteriocin for all *E. coli*, *S. enterica*, and *L. monocytogenes* bacteria which can directly confirmed the antimicrobial effects of bacteriocin produced by *B. coagulans* IS2 toward *E. coli*, *S. enterica*, and *L. monocytogenes*. However, it is an introductory work but our findings show an extracted bacteriocin had antimicrobial effects toward both Gram-negative (*E. coli* and *S. enterica*) and Gram-positive (*L. monocytogenes*) bacteria. Nevertheless, additional investigates should perform to found more information about the antimicrobial effects of bacteriocin produced by the *B. coagulans* IS2.

**Keywords:** *Bacillus coagulans* IS2, Bacteriocin, Production, Antimicrobial effect.

## Introduction

Recently, probiotic application, including lactic acid producing bacteria, has established abundant consideration to avert and treat diverse kinds of diseases with particular attentions to gastrointestinal disorders [1]. Probiotics are live useful microorganisms with high benefits on human health when administered in suitable amounts [2]. These bacteria can colonize into the epithelial cells and layers and produce diverse metabolites with antimicrobial effects [3].

*Bacillus coagulans* (*B. coagulans*) is a lactic acid producing bacterium responsible for diverse good effects in the probiotic manufacturing. *B. coagulans* is considered as an effective agent to improve diverse kinds of gastrointestinal disorders such as colitis-induced by bacteria, particularly *Clostridium difficile*, irritable bowel syndrome (IBS), diarrhea, abdominal cramps, rheumatoid arthritis and nausea [4-6]. Furthermore, *B. coagulans* spores can germinate in the gut and act as a probiotic organism. Of late, *B. coagulans*, has considered as an important probiotic

Corresponding auther: Hatef Ajodani Far, E.mail: Ajoudanifar@gmail.com.
(*Received* 23/10/2020, *accepted* 07/02/2021)
DOI. 10.21608/ejvs.2021.47454.1199

bacterium in clinical, laboratory and food related experiments [6]. Furthermore, *B. coagulans* has a higher survival rate and resistance toward the environmental condition than other probiotic bacteria and as a result, it is a perfect agent to progress more resistant probiotics for functional food industry [7, 8].

Several investigations revealed that the bacteriocin, ribosomally synthesized antimicrobial peptide, is the main factor synthesis by different probiotic bacteria such as *B. coagulans* to cause diverse biological effects [4, 9, 10]. Bacteriocins are low molecular weight peptides with antimicrobial activities synthesized by diverse kinds of bacteria and archaea which institute a varied bundle of peptides respecting structure, size, antimicrobial potency, mode of action, target cell receptors and immunity mechanisms [10-12].

Despite the high importance of the bacteriocin producing probiotic bacteria, scarce data are available about the exact antimicrobial effects of the bacteriocin produced by the *B. coagulans* IS2 strain. Thus, the present research was performed to assess the bacteriocin production of *B. coagulans* IS2 and confirmation of its antimicrobial effects against some food-borne bacteria.

## Materials and Methods

*B. coagulans unique IS2 growth condition*

*B. coagulans* unique IS2 pure culture was purchased (Unique Biotech Limited, Hyderabad, India). The bacterium was cultured on to nutrient broth, tryptic soy broth (TSB) and Man, Rogosa, Sharpe (MRS) media (Merck, Germany) separately to found the best medium suitable for bacteriocin production by the bacterium. All media were incubated at for h. The optical density (OD) of all cultures were measured (Spectrophotometer, CT5700, Taiwan) to found the best one for bacteriocin production. Finally, the TSB medium by the mean OD value of 1.1 to 1.2 after 12 to 24 h incubation was selected as the best one for growth and bacteriocin production by the *B. coagulans* IS2.

*Bacteriocin extraction*

Bacteriocin was extracted from the *B. coagulans* unique IS2 culture using the ammonium sulphate method [13]. For this purpose, 250 mL of *B. coagulans* IS2 which was activated one day before on TSB media was inoculated on 50 mL MRS broth medium and incubated at 30 °C for 72 h. Then, obtained culture was added into 450 mL MRS broth and incubated at 30 °C for 18 h. Then, cultured were transferred to 50 mL falcons and incubated on ice for about 1 h. Cultures were centrifuged at rpm for about 10 min (Sigma, Germany). The supernatant was filtered through the 0.22 µm syringe filter and its pH was adjusted to 7-7.5. Then, 22.86 g of 70% ammonium sulphate powder were added to 50 mL of obtained supernatant and incubated at 4 °C for 24 h. Then, obtained solution were centrifuged at rpm for about 10 min. Sediment was mixed with the lowest volume of distilled water (Merck, Germany) for further analysis [13].

*Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis*

The molecular weight of an extracted bacteriocin was analyzed by the SDS-PAGE using the 14% separating gel, a 4% stacking gel and a 10% spacer gel. Ultra-Low-Molecular-Weight Protein Marker I (3.3–22 kDa, Realtimes, China) was applied to protein size estimation. Coomassie brilliant blue G-250 stain was used to gel staining [14, 15].

*Antimicrobial effects of extracted bacteriocin*

Presence of growth inhibition zone around the extracted bacteriocin for *Escherichia coli* (*E. coli* ATCC 8739), *Salmonella enterica* (*S. enterica* ATCC 35664), and *Listeria monocytogenes* (*L. monocytogenes* ATCC 19111) (Obtained from the Department of Microbiology, Damghan Branch, Islamic Azad University, Damghan, Iran) was considered as its antimicrobial effect. For this purpose, *E. coli*, *S. enterica*, and *L. monocytogenes* were cultured on the Mueller Hinton Broth (Merck, Germany) media and incubated at 37 °C for 24 h. Then, activated bacteria were sub-cultured on Mueller Hinton Agar (Merck, Germany) media and in order to well-dried, they were incubated at 30 °C for 1 h. Then, two separate 6 mm wells were created in media contained 200 µL of extracted bacteriocin. Plated were incubated at 4 °C for 4 h and then, all plates were incubated at 30 °C for 24 h [16].

## Results

The present survey was performed to synthesis of bacteriocin from the *B. coagulans* unique IS2 and confirmation of its antimicrobial effects against *E. coli*, *S. enterica*, and *L. monocytogenes* bacteria.

Figure 1 shows the dimeter of the growth inhibition zone of *S. enterica* toward the extracted bacteriocin. Figure 2 shows the dimeter of the growth inhibition zone of *E. coli* toward the

extracted bacteriocin. Figure 3 shows the dimeter of the growth inhibition zone of *L. monocytogenes* toward the extracted bacteriocin. Presence of the growth inhibition zone around bacteriocin wells confirmed the antimicrobial effects of extracted bacteriocin against three examined bacteria.

The molecular weight of an extracted bacteriocin was analyzed by the SDS-PAGE. Figure 4 shows the SDS-PAGE pattern of the extracted bacteriocin. Findings revealed the presence of a sharp band with the molecular weight lower than 10 KD in the SDS-PAGE gel.

**Fig. 1. Dimeter of the growth inhibition zone of *S. enterica* toward the extracted bacteriocin.**

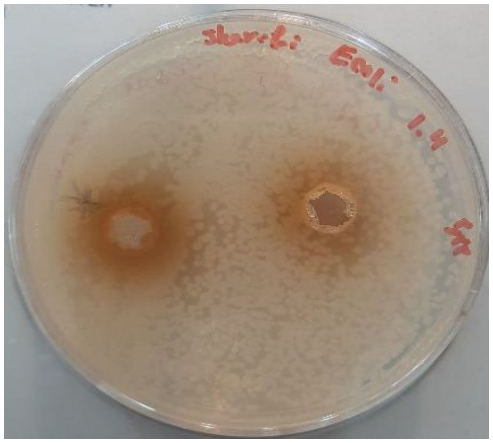

**Fig. 2. Dimeter of the growth inhibition zone of E. coli toward the extracted bacteriocin.**

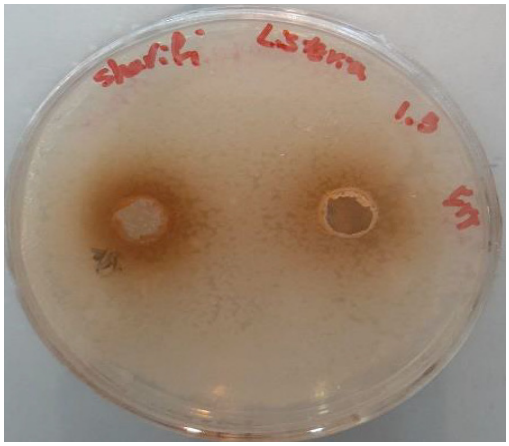

**Fig. 3. Dimeter of the growth inhibition zone of L. monocytogenes toward the extracted bacteriocin.**

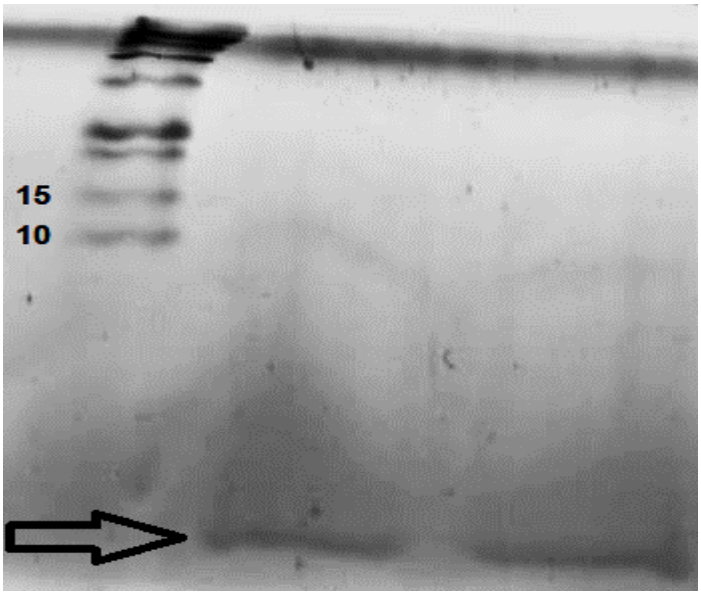


**Fig. 4. The molecular weight of an extracted bacteriocin in the SDS-PAGE.**

## Discussion

*B. coagulans*, as a sporogenous probiotic bacterium, has develop a emphasis of investigate owing to its high resistance toward hard conditions and probiotic features. Numerous valuable effects of *B. coagulans* including improvement of intestinal digestion, regulation of symbiotic microbiota of host, contamination the growth and survival of pathogens, and improvement of host regional immunity, have been stated [6]. Consequently, *B. coagulans* administration may be beneficial to avoid and treat diverse human diseases.

The present survey was conducted to assess the antimicrobial effects of bacteriocin produced by the *B. coagulans* IS2 toward *E. coli*, *S. enterica*, and *L. monocytogenes*. Findings revealed that the extracted bacteriocin from the *B. coagulans* IS2 had the molecular weight lower than 10 KD which can directly confirm the presence of bacteriocin as they are classified as low molecular weight proteins [17, 18]. Additionally, extracted bacteriocin from the *B. coagulans* IS2 exhibited high antimicrobial effects against *E. coli*, *S. enterica*, and *L. monocytogenes* bacteria. Findings showed the presence of growth inhibition zones for *E. coli*, *S. enterica*, and *L. monocytogenes* around the wells contained extracted bacteriocin which directly can confirm its antimicrobial effect. It seems that the extracted bacteriocin from the *B. coagulans* IS2 had the highest antimicrobial effects against *S. enterica* and *E. coli*. However, its exact confirmation need more accurate experiments. Put together, this is the first report of the confirmation of the antimicrobial effects of the bacteriocin extracted from the *B. coagulans* IS2 bacterium toward *E. coli*, *S. enterica*, and *L. monocytogenes* bacteria.

Diverse researches confirmed the therapeutic effects of *B. coagulans* in different gastrointestinal disorders including inflammatory bowel disease (IBD) [19, 20], antibiotic-associated diarrhea [21, 22], rheumatoid arthritis [23, 24], and colorectal cancer [25, 26]. Moreover, United States Food and Drug Administration (FDA) introduced the *B. coagulans* as Generally Recognized As Safe (GRAS) bacterium [27]. Garrison (2019) [28] conducted a survey on the characterization and evaluation of the probiotic properties of the sporeforming bacteria, *B. coagulans* unique IS2. The author of this survey showed that the *B. coagulans* spores were stable at 4˚C in broth culture contained pH 3-8 up to 4 months. The author recommended the inclusion of *B. coagulans* IS2 to foodstuffs as a probiotic culture and also preservative. Diverse researches showed that the Coagulin is a definitive bacteriocin produced by the Bacillus species [12, 14]. Antimicrobial effects of different types of bacteriocin against *E. coli* [29], *S. enterica* [30] and *L. monocytogenes* [31] have been reported previously.

The present survey is an initial work about the antimicrobial effects of the bacteriocin produced by the *B. coagulans* IS2. This study was limited

by the absence of quantitative data especially on the dimeter of the growth inhibition zone of bacteriocin produced by the *B. coagulans* IS2 toward *E. coli*, *S. enterica* and *L. monocytogenes* bacteria. Additionally, absence of antibiotic disks to compare their antimicrobial effects with an extracted bacteriocin is another limitation of the present survey. However, assessment of antimicrobial effect of extracted bacteriocin against both Gram-negative (*E. coli* and *S. enterica*) and Gram-positive (*L. monocytogenes*) bacteria is the most important strength of the present research. Totally, it seems that application of *B. coagulans* IS2 as a potential source of bacteriocin can improve the microbial quality of diverse foodstuffs.

Food-borne diseases are a constant threat to human beings. Several reports showed high prevalence of food-borne diseases amongst Iranian foodstuffs [32-53]. Thus, extraction and synthesis of bacteriocin-based antimicrobial agent with edible nature can help humans to decrease the rates of food-borne diseases globally. Bacteriocin application as antimicrobial in food system does not change the taste, taste or smell of the food as it has a natural source.

The present study was limited to the lack of quantitative data on the diameter of the growth inhibition zone of the *B. coagulans* IS2 against bacteria and also lack of the comparison of diameters with specific antibiotic agents.

## Conclusion

In conclusion, this is the first report of the antimicrobial effects of bacteriocin produced by *B. coagulans* IS2 in the world. However, it is a preliminary work in its field but our findings show that an extracted bacteriocin had antimicrobial effects toward both Gram-negative (*E. coli* and *S. enterica*) and Gram-positive (*L. monocytogenes*) bacteria *in vitro* condition. However, supplementary researches should address to found the exact role of bacteriocin produced by the *B. coagulans* IS2 against numerous kinds of bacteria and its further uses in food systems.

*Acknowledgements*

Many thanks from staffs of the Researches Center of Microbiology of Damghan Branch and also Research Institute of Food Science and Technology, Iran for their laboratory supports.

*Conflict of interest:*The authors declared that no conflict of interest.

*Funds statement:* Self-funding.

## References

1. Pandey, K.R., Naik, S.R. and Vakil, B.V., Probiotics, prebiotics and synbiotics-a review. *J. Food. Sci. Techno.,* **52**(12),7577-7587 (2015).
2. Soccol, C.R., Vandenberghe, L.P.d.S., Spier, M.R., Medeiros, A.B.P., Yamaguishi, C.T., Lindner, J.D.D., Pandey, A. and Thomaz-Soccol, V., The potential of probiotics: a review. *Food. Techno. Biotech.,* **48**(4),413-434 (2010).
3. Aureli, P., Capurso, L., Castellazzi, A.M., Clerici, M., Giovannini, M., Morelli, L., Poli, A., Pregliasco, F., Salvini, F. and Zuccotti, G.V., Probiotics and health: an evidence-based review. *Pharma. Res.,* **63**(5),366-376 (2011).
4. Jurenka, J.S., Bacillus coagulans. *Alternative. Med. Rev.*, **17**(1),76-82 (2012).
5. Cutting, S.M., Bacillus probiotics. *Food. Microbiol.,* **28**(2),214-220 (2011).
6. Cao, J., Yu, Z., Liu, W., Zhao, J., Zhang, H., Zhai, Q. and Chen, W., Probiotic characteristics of Bacillus coagulans and associated implications for human health and diseases. *J. Function. Foods.,* **64**(103643)pages 1-11 (2020).
7. Drago, L. and De Vecchi, E., Should Lactobacillus sporogenes and Bacillus coagulans have a future? *J. Chemotherap.,* **21**(4),371-377 (2009).
8. Konuray, G. and Erginkaya, Z., Potential use of Bacillus coagulans in the food industry. *Foods.,* **7**(6),92, pages 1-10 (2018). doi: 10.3390/ foods7060092
9. Yusuf, M.A. and Hamid, T., Lactic acid bacteria: bacteriocin producer: a mini review. *IOSR. J. Pharm.,* **3**(4),44-50 (2013).
10. Malik, D.K., Bhatia, D., Nimbriya, A. and Kumar, S., Lactic acid bacteria and bacteriocin: a review. *J. Pharma. Res.,* **5**(5),2510-2513 (2012).
11. Marrec, L., Coagulin, a bacteriocin-like inhibitory substance produced by Bacillus coagulans I4. *J. App. Microbiol.,* **85**(1),42-50 (1998).
12. Le Marrec, C., Hyronimus, B., Bressollier, P., Verneuil, B. and Urdaci, M.C., Biochemical and genetic characterization of coagulin, a new antilisterial bacteriocin in the pediocin family of bacteriocins, produced by Bacillus coagulans I4. *App.Env. Microbiol.,* **66**(12), 5213-5220 (2000).

13. Meyer, T.E., Isolation and characterization of soluble cytochromes, ferredoxins and other chromophoric proteins from the halophilic phototrophic bacterium Ectothiorhodospira halophila. *Biochim. Biophys. Acta. B.,* **806**(1),175-183 (1985).

14. Schägger, H., Tricine-sds-page. *Nature. Protocols.,* **1**(1),16-22 (2006). doi: 10.1038/nprot.2006.4.

15. Haider, S.R., Reid, H.J. and Sharp, B.L.,Tricine-sds-page. Protein electrophoresis, *Springer.,* 2012,p.151-160 (2012).

16. Fu, L., Wang, C., Ruan, X., Li, G., Zhao, Y. and Wang, Y., Preservation of large yellow croaker (Pseudosciaena crocea) by Coagulin L1208, a novel bacteriocin produced by Bacillus coagulans L1208. *Int J. Food. Microbiol.,* **266**, 60-68 (2018).

17. Zacharof, M. and Lovitt, R., Bacteriocins produced by lactic acid bacteria a review article. *Apcbee. Procedia.,* **2**, 50-56 (2012).

18. Balciunas, E.M., Martinez, F.A.C., Todorov, S.D., de Melo Franco, B.D.G., Converti, A. and de Souza Oliveira, R.P., Novel biotechnological applications of bacteriocins: a review. *Food. Control.,* **32**(1),134-142 (2013).

19. Majeed, M., Nagabhushanam, K., Arumugam, S., Majeed, S. and Ali, F., Bacillus coagulans MTCC 5856 for the management of major depression with irritable bowel syndrome: a randomised, double-blind, placebo controlled, multi-centre, pilot clinical study. *Food. Nutr. Res.,* **62**,pages 1-15(2018). doi: 10.29219/fnr.v62.1218. eCollection 2018.

20. Shinde, T., Perera, A.P., Vemuri, R., Gondalia, S.V., Beale, D.J., Karpe, A.V., Shastri, S., Basheer, W., Southam, B. and Eri, R., Synbiotic supplementation with prebiotic green banana resistant starch and probiotic Bacillus coagulans spores ameliorates gut inflammation in mouse model of inflammatory bowel diseases. *European. J. Nutrition.,volume?* 1-21 (2020).

21. Horosheva, T.V., Vodyanoy, V. and Sorokulova, I., Efficacy of Bacillus probiotics in prevention of antibiotic associated diarrhoea: a randomized, double blind, placebo controlled clinical trial. *JMM. Case. Reports.,* **1**(3),e004036 (2014).

22. Maity, C. and Gupta, A.K., A prospective, interventional, randomized, double-blind, placebo-controlled clinical study to evaluate the efficacy and safety of Bacillus coagulans LBSC in the treatment of acute diarrhea with abdominal discomfort. *European. J. Clin. Pharma.,* **75**(1),21-31 (2019).

23. Mandel, D.R., Eichas, K. and Holmes, J., Bacillus coagulans: a viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial. *BMC. Complement. Alter. Med.,* **10**(1),1-7 (2010).

24. Abhari, K., Shekarforoush, S.S., Hosseinzadeh, S., Nazifi, S., Sajedianfard, J. and Eskandari, M.H., The effects of orally administered Bacillus coagulans and inulin on prevention and progression of rheumatoid arthritis in rats. *Food. Nutr. Res.,* **60**(1),30876 (2016).

25. Madempudi, R.S. and Kalle, A.M., Antiproliferative effects of Bacillus coagulans Unique IS2 in colon cancer cells. *Nutr.Cancer.,* **69**(7),1062-1068 (2017).

26. Yin, L., Meng, Z., Zhang, Y., Hu, K., Chen, W., Han, K., Wu, B.-Y., You, R., Li, C.-H. and Jin, Y., Bacillus spore-based oral carriers loading curcumin for the therapy of colon cancer. *J. Control. Release.,* **271**,31-44 (2018).

27. GRAS, GRAS Notification for Bacillus coagulans SNZ1969 spore preparation. GRAS notice (GRN) No. 000597. Retrieved from https://wayback.archive-it.org/7993/20180124093141/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM475920.pdf. 2018.

28. Garrison, A., Characterization and Evaluation of the Probiotic Properties of the Sporeforming Bacteria, Bacillus coagulans Unique IS-2. College at the University of Nebraska In Partial Fulfillment of Requirements. Master of Science. 1-160 (2019).

29. Belfiore, C., Castellano, P. and Vignolo, G., Reduction of *Escherichia coli* population following treatment with bacteriocins from lactic acid bacteria and chelators. *Food. Microbiol.,* **24**(3),223-229 (2007).

30. Tatsadjieu, N., Njintang, Y., Sonfack, T.K., Daoudou, B. and Mbofung, C., Characterization of lactic acid bacteria producing bacteriocins against chicken *Salmonella enterica* and *Escherichia coli*. *African J. Microbiol. Res.,* **3**(5),220-227 (2009).

31. Rodrıguez, E., Calzada, J., Arqués, J., Rodrıguez, J., Nunez, M. and Medina, M., Antimicrobial activity of pediocin-producing Lactococcus lactis on Listeria monocytogenes, Staphylococcus aureus and Escherichia coli O157: H7 in cheese. Int. Dairy. Journal., 15(1),51-57 (2005).

32. Nejat, S., Momtaz, H., Yadegari, M., Nejat, S., Safarpour Dehkordi, F. and Khamesipour, F., Seasonal, geographical, age and breed distributions of equine viral arteritis in Iran. Kafkas. Univ. Vet. Fak. Derg., 21(1),111-116 (2015).

33. Nayebpoor, F., Momeni, M., and Dehkordi, F.S., Incidence of ochratoxin A in raw and salted dried fruits using high performance liquid chromatography. *Am. Eur. J. Toxicol. Sci*., **5**,1-6 (2013).

34. Abdolmaleki, Z., Mashak, Z., and Dehkordi, F.S., Phenotypic and genotypic characterization of antibiotic resistance in the methicillin-resistant Staphylococcus aureus strains isolated from hospital cockroaches. *Antimicrob. Res. Infecti. Control,* **8**(1),1-14 (2019).

35. Dehkordi, F.S., Prevalence study of Bovine viral diarrhea virus by evaluation of antigen capture ELISA and RT-PCR assay in Bovine, Ovine, Caprine, Buffalo and Camel aborted fetuses in Iran. *AMB. Express,* **1**(1),1-6 (2011).

36. Dehkordi, F.S., Saberian, S. and Momtaz, H., Detection and segregation of Brucella abortus and Brucella melitensis in Aborted Bovine, Ovine, Caprine, Buffaloes and Camelid Fetuses by application of conventional and real-time polymerase chain reaction. *Thai. J. Vet. Med.,* **42**(1),13-20 (2012).

37. Dehkordi, F.S., Valizadeh, Y., Birgani, T.A., and Dehkordi, K.G. Prevalence study of Brucella melitensis and Brucella abortus in cow's milk using dot enzyme linked immuno sorbent assay and duplex polymerase chain reaction. *J. Pure. Appl. Microbiol.,* **8**(2),1065-1069 (2014).

38. Dehkordi, F.S., Prevalence study of Coxiella burnetii in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. *Am. J. Anim. Vet. Sci*., **6**(4),180-186,(2011).

39. Ranjbar, R., Yadollahi Farsani, F., and Safarpoor Dehkordi, F., Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. *J. Food. Safety*., **39**(2):e12594 (2019).

40. Ranjbar, R., Seyf, A., and Dehkordi, F.S., Prevalence of Antibiotic Resistance and Distribution of Virulence Factors in the Shiga Toxigenic Escherichia coli Recovered from Hospital Food. *Jundishapur. J. Microbiol*., **12**(5), e82659, (2019).

41. Ranjbar, R., Farsani, F.Y. and Dehkordi, F.S., Phenotypic analysis of antibiotic resistance and genotypic study of the vacA, cagA, iceA, oipA and babA genotypes of the Helicobacter pylori strains isolated from raw milk. Antimicrobial. Resist. *Infecti. Control.,* **7**(1),115, pages 1-14 (2018).

42. Dehkordi, F.S., Momtaz, H., and Doosti, A., Application of Real-Time PCR for detection of Aspergillus species in aborted ruminant foetuses. *Bulg. J. Vet. Med*., **15**, 30-36 (2012).

43. Dehkordi, F.S., Tirgir, F., and Valizadeh, Y., Effects of Guajol® ointment synthesized from medicinal smoke condensate of jennet feces on burn wound healing on Wistar rat. *Vet. Res. Forum*., **8**(3), 215-221 (2017).

44. 44. Rahi, A., Kazemeini, H., Jafariaskari, S., Seif, A., Hosseini, S., and Dehkordi, F.S., Genotypic and Phenotypic-Based Assessment of Antibiotic Resistance and Profile of Staphylococcal Cassette Chromosome mec in the Methicillin-Resistant Staphylococcus aureus Recovered from Raw Milk. Infect. *Drug. Res.,* **13**,273-283 (2020).

45. Abdolmaleki, Z., Mashak, Z., and Safarpoor Dehkordi, F. Molecular and Virulence Characteristics of Methicillin-Resistant Staphylococcus aureus Bacteria Recovered From Hospital Cockroaches. *Jundishapur. J. Microbiol*., **12**(12), e98564.(2019).

46. Mashak, Z., Banisharif, F., Banisharif, G., Reza Pourian, M., Eskandari, S., Seif, A., Safarpoor Dehkordi, F., and Alavi, I., Prevalence of Listeria Species and Serotyping of Listeria monocytogenes Bacteria Isolated from Seafood Samples. *Egypt. J. Vet. Sci*., **52**(1),1-9 (2020).

47. Mashak, Z., Jafariaskari, S., Alavi, I., Shahreza, M.S., and Dehkordi, F.S., Phenotypic and Genotypic Assessment of Antibiotic Resistance and Genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 Alleles of Helicobacter pylori Bacteria Isolated from Raw Meat. *Infect. Drug. Res*., **13**, 257-272 (2020). doi: 10.2147/IDR.S233612

48. Dehkordi, F.S., and Rafsanjani, M.S., Prevalence study of Coxiella burnetii in aborted fetuses of small ruminants in various partum and seasons in Iran. *Afr. J. Microbiol. Res*., **6**(27),594-600 (2012).

49. Ranjbar, R., Dehkordi F.S., and Heiat, M. The Frequency of Resistance Genes in *Salmonella enteritidis* Strains Isolated from Cattle. *Iran. J. Publ. Health*., **49**(5), 968-974 (2020).

50. Afshar, D., Moghadam, S.O., Dehkordi, F.S., Ranjbar, R., and Hasanzadeh, A., Psp140: an immunodominant antigen in the supernatant of *Streptococcus pneumoniae* culture. *Iran. J. Microbiol*., **12**(4),338-342 (2020). doi: 10.18502/ijm.v12i4.3938

51. Sheikhshahrokh, A., Ranjbar, R., Saeidi, E., DehkordI, F.S., Heiat, M., Ghasemi-Dehkordi, P., and Goodarzi, H., Frontier therapeutics and vaccine strategies for sars-cov-2 (COVID-19): A review. *Iran. J. Publ. Health*., **49**,18-29 (2020).

52. Mirzaie, A., Halaji, M., Dehkordi, F.S., Ranjbar, R., and Noorbazargan, H., A narrative literature review on traditional medicine options for treatment of corona virus disease 2019 (COVID-19). Compl. Ther. Clin. Prac., 40,101214, pages 1-8 (2020). doi: 10.1016/j.ctcp.2020.101214.

53. Halaji, M., Farahani, A., Ranjbar, R., Heiat, M., and Dehkordi, F.S., Emerging coronaviruses: first SARS, second MERS and third SARS-CoV-2: epidemiological updates of COVID-19. Le Infezioni. Medicina., 28(1),6-17 (2020).