Research Article

BIOTECHINTELLECT, 2026, 3(1) e1 (1-9)
https://jbiotechintel.com/index.php/biotechintel

eISSN: 3115-7920

OPEN ACCESS

PCR Identification of Subtilosin and Coagulin synthetase gene in *Bacillus coagulans*, Cultured in an Artificial Medium

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Article History:
Received 02 Nov 2025
Revised 22 Dec 2025
Accepted 29 Dec 2025
Published online 02 Jan 2026

Keywords: *Bacillus coagulans*, Coagulin, Subtilosin, PCR identification

How to cite this article: Mousavi E, Jalili Tabaii M, Ghoreishi F, Abkar M, Emtiazi G. PCR identification of subtilosin and coagulin synthetase gene in *Bacillus coagulans*, cultured in an artificial medium, 2026: 3(1) e1 (1-9)
https://doi.org/10.66224/BiotechIntellect.3.1.38

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Abstract

Purpose and aim: Bacillus spp. is particularly important among probiotic microorganisms due to its resistance to extreme conditions. *Bacillus coagulans*, producing coagulin is a probiotic bacteria used in food products. This study aimed to genetically identify bacteriocins in *B. coagulans* IBRC-M 10807.

Method: The agar-well diffusion method was used to examine the antimicrobial property of the 24-hour crude supernatant. The heat and storage resistance of the crude supernatant was studied, and the HPLC technique was utilized to analyze its percentage of amino acids. Finally, the PCR method detected the presence of coagulin, subtilin, and subtilosin genes.

Results and conclusion: The results showed the antimicrobial properties of the CS against gram-positive/gram-negative bacteria. The best storage condition for the CS was -20 °C for 4 weeks, and it was resistant to <50 °C for 30 minutes. Furthermore, the artificial medium in this study with similar efficiency and a lower price, was valuable compared to the other two commercial media. PCR results showed the presence of a 3000-3500 bp band related to coagulin (observed only after plasmid extraction) and a 250-300 bp band related to subtilosin after sequencing. The sequence of the subtilosin band had 100% identity with the *B. subtilis* strain L4 subtilosin (albA) gene. These broad-spectrum antimicrobial properties, and their relative resistance to temperature and storage, make this strain a valuable candidate for use in probiotic products.

What is "already known":	• *Bacillus* spp. is an important probiotic due to its resistance to extreme conditions. • probiotic *B. coagulans* producing coagulin is potent for application in food sector. • spore-forming Bacillus can overcome probiotic loss during food processing
What this article adds:	• Antimicrobial properties of bacteriocins have been proven against Gram+ & Gram-, and yeasts by Abada • Application of spore-forming strains like Bacillus can overcome probiotic loss during food processing

1. INTRODUCTION

In recent years, the application of probiotics in promoting human health has received attention due to their role in preventing and treating diseases. The use of conventional probiotics such as Bifidobacterium spp., *Lactobacillus* spp., *Streptococcus* spp., *Propionibacterium* spp., as well as some Saccharomyces species is limited because they are sensitive to extreme conditions like high temperature and acidic pH. Therefore, the use of spore-forming strains like *Bacillus* spp. has been considered. Among the probiotic *Bacillus* spp., *B. coagulans* has been studied more than others. This bacterium was first isolated from spoiled canned milk in 1915. At first, some of its strains were called Lactobacillus sporogenes [1], and today it is classified as Weizmannia coagulans. In addition to its role in probiotic food products, this strain is used in the medical industry to treat gastrointestinal disorders, depressive symptoms, immunomodulation, and non-alcoholic fatty liver. *B. coagulans* is a gram-positive, facultative anaerobic spore-forming probiotic bacterium that utilizes a wide range of sugars and produces lactic acid [2-4]. This bacterium and other Bacillus strains produce a many bacteriocins or bacteriocin-like inhibitory substances [5].

In a 2025 comprehensive review, Nicolas [6] highlights the potential of secondary metabolites from *Bacillus* spp. probiotics as novel treatments against multidrug-resistant pathogens, emphasizing their diverse chemical structures and mechanisms of action that offer promising alternatives to conventional antibiotics. Complementing this, Meskhi and colleagues [7] reported bacteriocins-ribosomally synthesized antimicrobial peptides from bacteria including Bacillus as sustainable substitutes for antibacterial agents in primary food production systems, under-lining their role in reducing chemical preservatives and antibiotic use in livestock. Similarly, Geda and co-authors [8]. provide an overview of bacteriocins as a promising antimicrobial strategy against multidrug-resistant pathogens, detailing their mechanisms of action and broad applications across human and animal health as well as the food industry. Together, these reviews converge on the growing recognition of Bacillus-derived metabolites and bacteriocins as effective, eco-friendly tools to combat antimicrobial resistance while enhancing food safety and animal production.

Bacteriocins are antimicrobial peptides of bacterial origin that act against closely related species [9]. The most well-known studied bacteriocin is nisin A, which was approved by World Health Organization as a food additive. Coagulin is one of the bacteriocin-like inhibitory substances produced by *B. coagulans* I4, coded by the plasmid pI4, approximately 14 kb in size, and is only effective against gram-positive bacteria [5]. Subtilin and Subtilosin are two other bacteriocins initially discovered in *B. subtilis*, but they are also produced by other *Bacillus* spp [10,11]. Considering the importance of *B. coagulans* in probiotic products and the medical industry, further study and identification of its bacteriocins and antimicrobial substances are valuable. This research studied the bacteriocins and their genes in *B. coagulans* IBRC-M 10807.

2. Materials and Methods

2.1. Bacterial Strain, Media, and Growth Curve

The bacterial lyophilized powder of *B. coagulans* with accession no. IBRC-M 10807 (obtained from the Iranian Biological Resource Centre) was sterilized by heat shock method (80°C for 10 min). Then, the powder was added to the Nutrient Broth (NB) medium and incubated at 37°C for 24 h. The Gram and spore staining and morphology of isolated colonies were investigated to confirm the purity and correctness of the strain. Then, the bacterial colony was dotted on the lawn culture of Staphylococcus aureus for antibacterial assay [12]. The bacterial colonies were kept on the slant medium and cultured using three different media, including NB, MRS broth, and an artificial medium with cow bone extract.

The bacterial growth curve was analyzed in NB medium at 37°C for 48 h with shaking at 160 rpm. The bacterial growth rate was detected by measuring OD600 nm absorbance in three replicates with a spectrophotometer. Finally, the growth curve was made using Excel software [6].

2.2. Antibacterial Effect of the Crude Supernatant (CS) in Three Different Media

Bacterial suspensions with a concentration of half McFarland from three different media were inoculated separately into the flasks containing the respective

media at the rate of 5% and incubated at 37°C for 24 h with shaking at 160 rpm. Finally, the bacterial supernatants were separated by centrifugation at 3000 rpm (centrifugal radius=7 cm) for 15 min, sterilized by a 0.45 µm filter membrane, and their protein content was compared by the Bradford method. The antibacterial effect of all CSs (15 µl) against S. aureus was analyzed by agar-well diffusion methods on Mueller Hinton Agar (MHA) and compared in three different media including cow bone extract. [9].

2.3 Antibacterial Effects of Concentrated CSs

Bacterial CSs were concentrated 20 times by a freeze-dryer and sterilized by passing through a 0.45 µm filter membrane. Then, the antibacterial effects were determined by the agar-well diffusion method on MHA against S. aureus and some other bacteria [9].

2.4. Heat and Storage Stability of the Concentrated CS

The concentrated CS obtained from the cow bone medium was re-suspended in 1 ml of sterile 0.9% normal saline and divided into three microtubules. The extracts were kept in three conditions, -20, 4, and 30°C, for one to eight weeks. In addition, the heat shock stability was analyzed at different temperatures (30, 37, 50, 60, 80, and 100°C for 30 min and 50°C for 10-60 min). Finally, the antibacterial effects of treated extracts were analyzed using the agar-well diffusion method against S. aureus. All examinations were performed in three replicates [9].

2.5. Optimization of Antibacterial Metabolite Production

The rate of antibacterial substance production in different media was detected at three different incubation times: 24, 48, and 72 h, by protein content measurement (Bradford method) and antibacterial clear zone diameter analysis. Then, the optimized conditions were investigated.

2.6. HPLC Analysis

The amino acid content of the concentrated extract , obtained from the best solid medium, was investigated using the HPLC method in the Nobel laboratory [13]. The amino acid in washed solide medium was not included the enriched food in medium.

2.7. Identification of Coagulin, Subtilin, and Subtilosin Genes by PCR

The presence of coagulin, subtilin, and subtilisin genes in this bacterium was investigated using PCR. The specific primers for coagulin and subtilin were obtained from previous research [9], and the subtilisin primer was designed by the National Centre for Biotechnology Information (NCBI) website. The sequence of each primer is given in Table 1.

Table 1 Characteristics of primers used for PCR.

Gene	Accession No. In NCBI	Primer sequence
Coagulin	AF300457.1	Forward: GGTGGTAAATACTACGGTAATGGGGT Reverse: ACGAATCAACCAGTAATTTAGACAC
Subtilin	J03767.1	Forward: CAAAGTTCGATGATTTCGATTTGGATGT Reverse: TTCCTTCAAACACTAACTTGTAACTGC
Subtilosin	AJ430547.1	Forward: TTGCCGGTGCTACAGGTCTA Reverse: ACTTGCGCGATAATGTCTCCA

The primers were made by Cinaclone Company as a lyophilized powder, then diluted to 10 mM concentration. The total bacterial DNA was extracted by boiling for 20 min, from a 24 h bacterial culture4. Gene amplification was performed at a 25 µl mixture, containing 9.5 µl of distilled water, 12.5 µl of Master-mix, 1 µl of each primer, and 1 µl of DNA sample. The PCR program for subtilin and subtilosin genes included a denaturation stage at 94 °C for 5 min, 35 cycles of three stages (35°C for 30 sec, 52°C for 30 sec, and 72° C for 30 sec), and a final extension by 5 min incubation at 72°C. The Bacterial direct solution from Zeanbiotech Company was used for the coagulin gene extraction, located on a plasmid. The PCR mixture of this gene was first incubated at 94 °C for 5 min, then 35 cycles were performed as follows: 95°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min. A final extension was performed at 72 °C for 10 min. The final amplicons were evaluated on 2% (for subtilin and subtilosin) and 1% (for coagulin) agarose gel electrophoresis with TBE

buffer. Finally, Bio Magic Gene Company sequenced the resulting PCR products in both directions.

3. Results and discussion

3.1. Morphological & Antibacterial Properties

The Gram, spore staining, and its antibacterial effect against S. aureus were analyzed to confirm the purity and correctness of the strain. The antibacterial analysis showed a clear zone on the MHA medium. The Gram and spore staining confirmed this strain is a Gram-positive bacilli and spore production so that its antimicrobial effect against Gram-positive bacteria was greater than against Gram-negative bacteria. Besides, the most antimicrobial effect among gram-negative bacteria was observed against Escherichia coli. In other literature, the antimicrobial effect of this bacterium has been determined against S. aureus, E. coli, L. monocytogenes, and K. pneumonia [5,14,15]. However, but to the best of our knowledge, no similar study has been conducted against Acinetobacter strains.

3.2. Bacterial growth curve

The bacterial growth curve was analyzed at 37°C in NB medium. The resulting graph showed that this strain completes the logarithmic phase within 15-20 h (Figure 1).

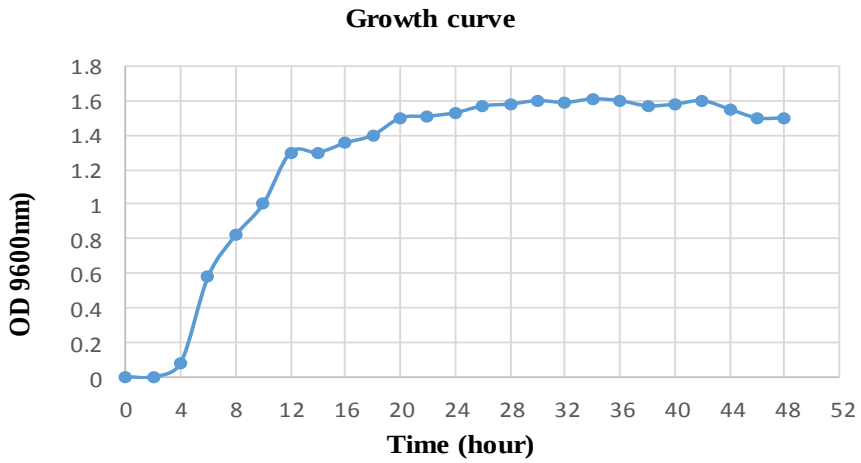

Fig. 1 Growth curve of *B. coagulans* at 37°C, with shaking at 160 rpm, in NB medium.The results are mean of 3 experiments

The Best Medium for Antibacterial Substances Production

In the first stage, the antibacterial effect of filtrated CS of all three media, NB, MRS broth, and the artificial medium, was investigated against S. aureus by the agar-well diffusion method without concentrating. The clear zone diameters showed that all three media had almost the same results (clear zone diameters were 10, 11, and 10 mm for MRS broth, artificial medium, and NB, respectively). The artificial medium was selected for further experiments because it is more economical than other media. Afterward, the CS obtained from the artificial medium was concentrated 20 times by a Lyophilizer. Then, the antibacterial effect against S. aureus and some other bacteria was analyzed using the agar-well diffusion method. The clear zone diameter against S. aureus was 14 mm (Table 2).

Table 2 Antibacterial effect of the concentrated CS, against some bacteria by agar-well diffusion method.

Bacterial strain	Clear zone diameter (mm)
S. aureus	14
Escherichia coli	12
Listeria monocytogenes	15
Acinetobacter spp.	7
Klebsiella pneumoniae	6.5

Thirty µl of CS, was obtained from the artificial medium.

Table 2 demonstrates the clear zone diameter of the concentrated extract (15 µl) obtained from the artificial medium against some other bacteria by the agar-well diffusion method. The results suggest that this extract can be effective against Gram-negative bacteria, even the antibiotic-resistant Acinetobacter strain. However the observed inhibition of Gram-negative bacteria suggests that compounds other than coagulin or subtilosin may be involved.

3.3.Heat & Storage Stability of Concentrated CS

The heat and storage stability of the concentrated CS, which was identified as the best extract and obtained from the artificial medium, were analyzed. The results (Table 3) showed that -20°C is the best temperature; the antibacterial effect is maintained for 4 weeks, after which it starts to decrease. The heat shock effect on this extract showed that its antimicrobial properties begin to decrease at 50°C. The stability of the extract at this temperature is constant for 30 min and then starts to decline (Tables 4A & 4B). The heat stability results indicated the relative heat resistance of antimicrobial substances to 50 °C for 30 min, similar to Hyronimus et al [5]. The best temperature and time for CS storage were -20 °C and 4 weeks, respectively. Therefore, the relative resistance of antimicrobial substances obtained from the concentrated CS to heat and storage has made it a suitable candidate for use in food products.

Table 3 Storage stability of *B. coagulans* concentrated CS in three different temperatures.

	Clear zone diameter (mm) in:		
Time (week)	30°C	4°C	-20°C
0	14	14	14
1	10	11	14
2	10	11	13
3	10	11	13
4	10	10	13
7	8	9.5	12
8	8.5	8.5	12

The comparison was based on antibacterial clear zone diameter analyses.

Table 4 The effect of heat shock on the antibacterial clear zone.

A: The effect of 30 min heat shock in different temperatures.

Temperature (°C)	Clear zone diameter (mm)
30	13
37	13
50	11.5
60	11
80	9.5
100	8.5

B: The effect of different time heat shock in 50 °C.

Time (min)	Clear zone diameter (mm)
10	11.83
20	11.66
30	11.5
40	11.33
50	10.33
60	9.66

3.4. Optimized Conditions for Antimicrobial Metabolite Production

The protein content and antibacterial clear zone diameter were investigated in three incubation times and three different media. The results indicated that the best time in NB and artificial media for antibacterial metabolite production is 24 h incubation, and the clear zone of the extract obtained from the artificial media is greater than that of NB medium. Therefore, the production of antimicrobial substances is in the stationary phase. However, in the case of the MRS medium, the clear zone diameter in three incubation times of 24, 48, and 72 hours was almost the same (Table 5).

Table 5 The best time and media for the most antibacterial and protein production extract from solid medium

Culture media	Incubation time (h)	Bradford (OD)	Clear zone diameter (mm)
NB	24	0.099	10
	48	0.124	9.66
	72	0.020	9
Artificial	24	0.217	11
	48	0.194	11
	72	0.085	9.5
MRS	24	0.288	10
	48	0.330	10
	72	0.337	12.5

Table 6 Amino Acid HPLC Analysis (µmol/L) of the extract obtained from different solid media.

A: Results obtained from the artificial medium cow bone extract solid medium.

Amino Acid	µmol/L	Amino Acid	µmol/L
Aspartic acid	551.36	Tyrosine	1315.92
Glutamic acid	1089.65	Valine	3202.33
Serine	1044.65	Methionine	1869.84
Glutamine	275.28	Tryptophane	178.24
Histidine	598.94	Phenylalanine	5095.42
Glycine	8791.46	Isoleucine	1175.13
Threonine	1161.69	Ornithine	834.79
Citrulline	157.16	Leucine	3118.46
Arginine	1775.12	Lysine	5452.25
Alanine	4689.27	Aspargine	862.77
Allo Isoleucine	0	Taurine	242.64

B: Results obtained from the NB medium.

Amino Acid	µmol/L	Amino Acid	µmol/L
Aspartic acid	11.35	Tyrosine	95.79
Glutamic acid	23.83	Valine	156.48
Serine	9.43	Methionine	320.41
Glutamine	7.76	Tryptophane	84.97
Histidine	122.65	Phenylalanine	400.41
Glycine	11.28	Isoleucine	30.88
Threonine	175.36	Ornithine	52.36
Citrulline	3.84	Leucine	283.38
Arginine	3.21	Lysine	837.05
Alanine	16.57	Aspargine	9.47
Allo Isoleucine		Taurine	1.13

3.5. HPLC Analyses

Amino acid profiles of crude solid growth extracts do not directly reflect the identity of specific anti-microbial peptides. The amino acid components of two extracts, including concentrated CS from the artificial and NB media, were detected by the HPLC method. The higher glycine concentration observed in the extract cannot be considered evidence of subtilosin production. Although the Amino acids profiles of crude solid growth extract do not directly reflect the identity of specific antimicrobial peptides but the amino acid analysis in the cell-free suspension showed more glycine in soluble protein at artificial medium, which increases the possibility of producing subtilosin in this medium.This 35 amino acids peptide has a high amount of glycine in its sequence (Asn-Lys-Gly-Sys-Ala-Thr-Cys-Ser-Ile-Gly-Ala-Ala-Cys-Leu-Val-Asp-Gly-Pro-Ile-Pro-Asp-Phe-Glu-Ile-Ala-Gly-Ala-Thr-Gly-Leu-Phe-Gly-Leu-Trp-Gly). However, the bacterial suspension grown in nutrient agar contains more lysine, which gives the possibility of producing other peptides. Other amino acid content in each extract is listed in the following Tables 6A & 6B.

3.6. Identification of Coagulin, Subtilin, and Subtilosin Genes

Amplification of the bacterial DNA with a subtilisin primer by PCR showed a 250-300 bp band (Figure 2a), which was 100% identical to the *B. subtilis* strain L4 subtilisin (albA) gene. PCR results with coagulin primer showed a band of 3000-3500 bp (Figure 2b), similar to the coagulin gene weight but could not be sequenced due to insufficient sharpness.

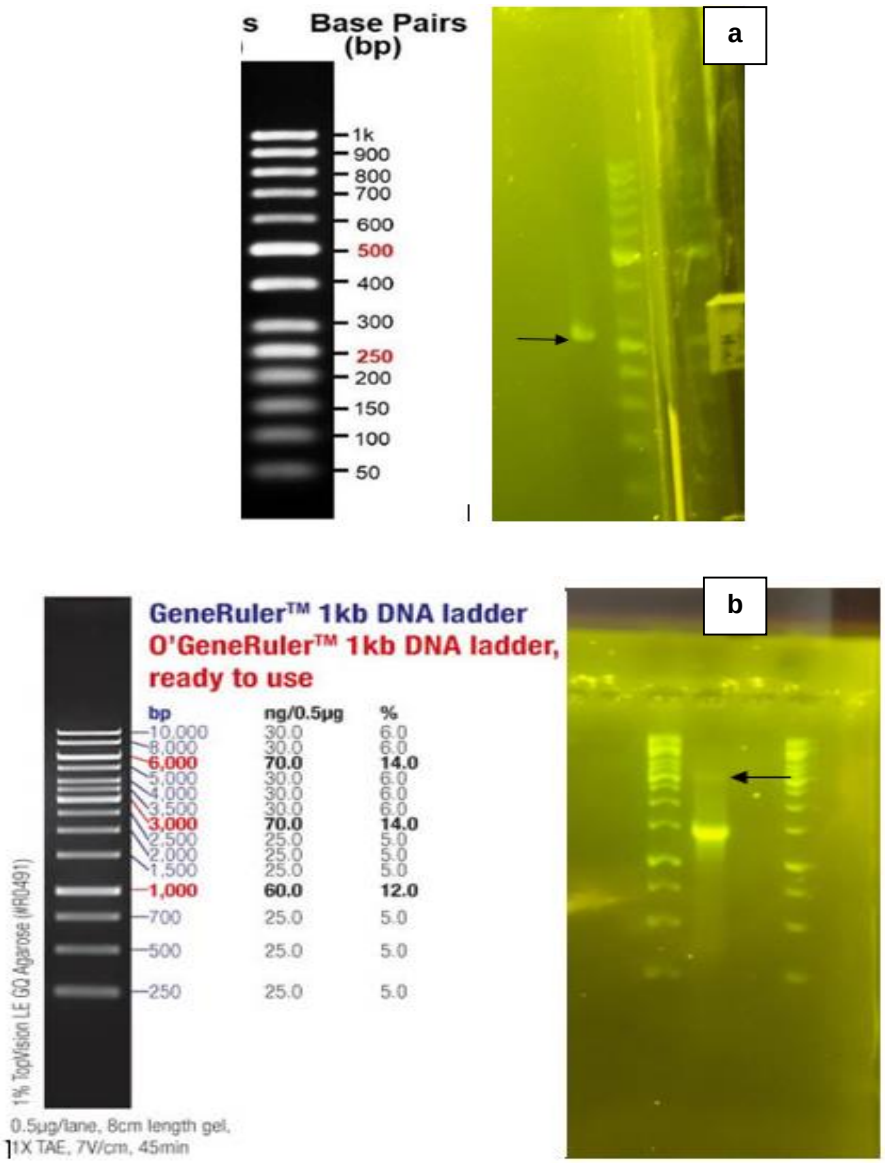

Fig. 2 Electrophoresis results of PCR amplicon with subtilosin (a) and coagulin (b) primers

Atempts to observe the band related to the subtilin gene were unsuccessful and this gene was not observed in the PCR products. Therefore, the presence of the coagulin gene cannot be conclusively demonstrated. Additional sequencing or alternative molecular confirmation is recommended. Identification of subtilosin requires stronger evidence.The PCR product identified as albA represents only one component of the subtilosin biosynthetic pathway. Detection of a single gene does not confirm production of subtilosin. Analysis of additional biosynthetic genes and direct peptide characterization would strengthen this conclusion. The PCR results with coagulin primers showed a 3000-3500 bp band corresponding to the coagulin gene. This amplicon was produced only after plasmid extraction from the bacteria, which, like other studies, confirms the presence of the coagulin gene in the plasmid. This

bacteriocin has already been proven in *B. coagulans* I4 in previous research [5].

The PCR product of the subtilosin gene with a weight of 250-300 bp was sequenced, which had 100% identity with the *B. subtilis* strain L4 subtilosin (albA) gene. The HPLC results revealed more glycin content in the extract from the artificial media than in the NB. Since subtilosin has a large amount of glycin, the amount of subtilosin produced in the artificial medium is higher than that of NB, which confirms the presence of subtilosin in the extract.

4. Conclusion

Natural antimicrobial substances derived from microorganisms, especially probiotic bacteria such as *B. coagulans*, have attracted special attention due to their application in food products and the increasing resistance of pathogens to conventional antibiotics. Bacteriocins are one of these antimicrobial substances, which have been identified in *B. coagulans*. Coagulin is one of the bacteriocins produced by *B. coagulans* I4, whose gene is located on a plasmid8. In the present study, the coagulin gene band was observed only in the PCR products obtained from the plasmid amplification, confirming the presence of this gene on the plasmid. Although coagulin and subtilosin are only effective against Gram-positive bacteria, the antimicrobial property of *B. coagulans* CS in this study, as well as in some other studies, against Gram-negative bacteria indicated the presence of a wide range of other antimicrobial substances such as antimicrobial lipopeptides, in this bacterium1. Since *B. coagulans* has no side effects and has been approved as a probiotic additive in food products, it is essential to investigate its antimicrobial properties and find new products for future applications. Finally, among the three studied culture media, the artificial medium was valuable due to its low price and efficiency, similar to commercial ones.

5. DECLARATIONS

5.1. Acknowledgements

The authors acknowledge the Shahid Ashrafi Esfahani University for providing support for this research.

5.2. Conflict of Interest

The authors declare no conflict of interest.

5.3. Authors Contributions

Elham Mousavi: data curation, investigation, Writing-original draft preparation, methodology, Maryam Jalili Tabaii: formal analysis , visualization, investigation, Fatemeh Sadat Ghoreishi: writing-review and editing, investigation, resources, Morteza Abkar: supervision, Conceptualization, Giti Emtiazi: project administration, Conceptualization, resources

5.4. Using Artificial Intelligent chatbots

No AI chatbots or tools were used in this research

REFERENCES

1. 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, *Journal of Functional Foods,* 2020; 64, 103643. https://doi.org/10.1016/j.jff.2019.103643

2. Abdhul K., Ganesh M., Shanmughapriya S., Vanithamani S., Kanagavel M., Anbarasu K., and Natarajaseenivasan K., Bacteriocinogenic potential of a probiotic strain *Bacillus coagulans* [BDU3] from Ngari, *International journal of biological macromolecules,* 2015; 79, 800-6.

3. Wang L., Wang J., Du L., Fang X., and Liao Z., Application of *Weizmannia coagulans* in the medical and livestock industry, *Annals of Microbiology*, 2022; 72(1), 1-9. https://doi.org/10.1186/s13213-022-01687-3

4. Ghoreishi F.S., Roghanian R., and Emtiazi G. Simultaneous Production of Antibacterial Protein and Lipopeptides in *Bacillus tequilensis*, Detected by MALDI-TOF and GC Mass Analyses, *Probiotics and antimicrobial proteins,* 2022; 2022 1-12

5. Marrec L., (1998) Coagulin, a bacteriocin-like inhibitory substance produced by *Bacillus coagulans* I4, *Journal of applied microbiology,* 1998; 85(1), 42-50

6. Nicolas GM. Secondary Metabolites from *Bacillus* spp. probiotics as potential treatments for multidrug-resistant pathogens: A comprehensive review. Curr Res Microb Sci. 2025 Apr 16;8:100392. https://org.doi/10.1016/j.crmicr.2025.100392. PMID: 40337641; PMCID: PMC12056956

7. Meskhi B, Todorov SD, Rudoy D, Olshevskaya A, Shevchenko V, Maltseva T, Mirzoyan A, Kozyrev D, Odabashyan M, Teplyakova S, Mazanko M. Bacteriocins, a New Generation of Sustainable Alternatives to Antibacterial Agents in Primary Food Production Systems. Molecules. 2026 Jan 19;31(2):356.

https://org.doi/10.3390/molecules31020356. PMCID: PMC12844023

8. Geda AM, Tumebo AW, Abey SL, Kasse GE, Temesgen AB, Wassie ZG. Bacteriocins as a Promising Antimicrobial Strategy Against Multidrug-Resistant Pathogens: Mechanisms of Action, Applications in Human and Animal Health, and the Food Industry. Biomed Res Int. 2026;2026(1):e1527677. https://org.doi/10.1155/bmri/1527677. PMCID: PMC13148635

9. Riazi S., Wirawan R., Badmaev V., and Chikindas M., Characterization of lactosporin, a novel antimicrobial protein produced by *Bacillus coagulans* ATCC 7050, *Journal of applied microbiology,* 2009; 106(4), 1370-7

10. Halami P.M., Sublichenin, a new subtilin-like lantibiotics of probiotic bacterium *Bacillus licheniformis* MCC 2512T with antibacterial activity, *Microbial Pathogenesis,* 2019; 128, 139-46

11. Manikandan P., Moopantakath J., Imchen M., Kumavath R., and SenthilKumar P., Identification of multi-potent protein subtilosin A from halophilic bacterium *Bacillus firmus* VE2, *Microbial Pathogenesis*, 2021; 157, 105007. https://doi.org/10.1016/j.micpath.2021.105007

12. Park S., Classen A., Gohou H.M., Maldonado R., Kretschmann E., Duvernay C., Kim G.J., and Ronholm J., A new, reliable, and high-throughput strategy to screen bacteria for antagonistic activity against *Staphylococcus aureus*, *BMC. Microbiology,* 2021; 21(1), 1-10. https://doi.org/10.1186/s12866-021-02265-4

13. Tumbarski Y., Deseva I., Mihaylova D., Stoyanova M., Krastev L., Nikolova R., Yanakieva V., and Ivanov I., Isolation, characterization and amino acid composition of a bacteriocin produced by *Bacillus methylotrophicus* strain BM47, *Food Technology and Biotechnology,* 2018; 56(4), 546-552. https://doi.org/10.17113/ftb.56.04.18.5905

14. Abada E.A.E.M., Isolation and characterization of an antimicrobial compound from *Bacillus coagulans*, *Animal cells and systems,* 2008; 12(1), 41-6

15. Kim Y.S., Lee J., Heo S., Lee J.H., and Jeong D.W. Technology and safety evaluation of *Bacillus coagulans* exhibiting antimicrobial activity for starter deveopment. *LWT*, 2021; 137, 110464