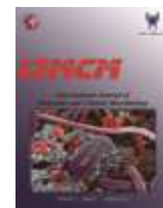
IJMCM 14(1) (2024) 2027-2037 Doi: 10.22034/IJMCM.2024.710056

International Journal of
Molecular and Clinical Microbiology



Research Article

Effect of extracted bacteriocin *L. plantarum* ATCC8014 on the expression of FUM1 secreted by *Fusarium verticillioides*

Mahtab Afrashi[1], Mansour Bayat[2]*

1. MSc degree in Microbiology, Department of Microbiology, Faculty of Veterinary Specialized Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Professor of Microbiology, Department of Pathobiology, Faculty of Veterinary Specialized Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran

ARTICLE INFO

Article history:
Received 25 March 2024
Accepted 13 June 2024
Available online 1 July 2024

Keywords:
FUM1;
RT-PCR;
MIC;
MFC;
Fusarium verticillioides

ABSTRACT

The Fusarium fungus is a pest that harms food plants and affects agricultural production. One way to control this fungus is by using biological methods, such as employing bacteria from the lactic acid family. In this study, we researched the impact of bacteriocin produced by Lactobacillus plantarum (L. plantarum) on the growth of Fusarium verticillioides (F. verticillioides) and the expression of FUM1, a protein secreted by this fungus. We acquired ten confirmed strains of F. verticillioides (f1-f10) and one strain of L. plantarum ATCC8014 from the Atiyeh Salamat Alborz company laboratory. The impact of bacteriocin extracted from L. plantarum on inhibiting the growth of the fungus and on FUM1 gene expression in F. verticillioides was examined using specific primers and the RT-PCR method. The research data revealed that the fungus growth was halted in the concentration range of 0.014 to 0.113 µg/ml (with an average µg/ml MIC of 0.42). Further, the lethal concentration of the bacteriocin (MFC) ranged between 0.028 and 0.225 µg/ml. After treatment with this bacteriocin, electrophoresis of the RT-PCR product showed that two sensitive strains, f4 and f8 (20% of the total 10 isolates), exhibited a complete lack of gene expressio*n. L. Plantarum* is highly effective in preventing the growth of *F.Verticillioides*. It can also prevent Fumonisin toxin production completely in two strains, making it a promising candidate to inhibit the fungus.

1. Introduction

Fungal diseases can severely limit the growth and performance of plants, causing significant economic losses. The excessive use of chemical fertilizers and pesticides, which are used to control plant diseases, can also lead to environmental pollution and the spread of resistant pathogens. Studies have shown that Fusarium species, in addition to causing diseases and pests in plants, can also cause infection in animals and humans. Among Fusarium species, *F. verticillioides* is one of the important fungi related to pathogenicity (Darvishnia *et al.,* 2023; Do Carmo Silver*et al*., 2023). The fungus produces toxins that can contaminate human and animal food, leading to significant damage in this area (Daie *et al*., 2011). The severity of poisoning and the resulting clinical diagnosis in humans and animals depend on various factors such as the type of mushroom, the concentration of poison, age, gender, health status, and the duration of exposure (Xu *et al.* 2023).

*Corresponding author: prof. Mansour Bayat
E-mail address: bayat1349m@gmail.com

The prevention of fungal growth and reduction of toxin production can be an expensive process using physical and chemical methods. Fumonisin is a toxin produced by the *F. verticillioides* fungi and it consists of four types: B1, B2, B3, and B4. Fumonisin B1 is the most significant type and it is soluble in water (Yan *et al.,* 2023). Most FUM genes are crucial for fumonisin production in *F. verticillioides*. FUM1 and FUM8 play a role in the initial stage, while FUM6 is responsible for the third step (Mao *et al*., 2023).

To control toxin production, biological detoxifiers like lactic acid bacteria (LAB) can be helpful. LAB are gram-positive bacilli found in sourdough, sour milk, and vegetables (Gagiu *et al.,* 2013). They can grow and survive in the human digestive system and have numerous health benefits. The inhibitory effects of LAB can prevent fungal toxin production by binding to the carbohydrates and protein components of the fungus' cell wall, thereby preventing the biosynthesis of mycotoxins (Li *et al., 2023*; Samaoui *et al.,* 2023).

L. plantarum is a significant and widely distributed member of the Lactobacillus genus. It has the largest genome among Lactobacilli and possesses the ability to liquefy gelatin (Noktehsanj *et* al. 2023).This fungus shows a high level of variability in its shape and size and possesses antimicrobial properties (Kavkova *et* al., 2023; Petrucci *et al.,* 2023). Consequently, researchers have investigated alternative approaches, such as biological control, to combat these harmful fungi (Al-Zaban *et al.,* 2023; Krishnan *et al*., 2024). Therefore, in the upcoming research, the main goal was to investigate the effect of bacteriocin probiotics on the growth of *F. verticillioides* and FUM1 gene expression in the production of fumonisin toxin by reverse transcription RT-PCR method. The RT-PCR test is a highly sensitive and effective method to identify and determine the toxins of this fungus and its effective genes.

2. Materials and Methods

2.1. Samples

This study examined ten different species of *F. Verticillioides* (f1-f10), including one non-toxin-producing species as a control. Additionally, a strain of *L. plantarum* ATCC 8014 was prepared to investigate the impact of probiotic bacteriocin on FUM1 gene expression. All fungi and bacteria used in the study were the approved strains obtained from the Alborz Science Foundation Laboratory Center. The study employed an analytical design and a simple non-random sampling method.

2.2. Extraction of bacteriocin

In this study, bacteriocin that was previously extracted by ethyl acetate was used. Precipitation of bacteriocin in this way is a suitable method for extracting bacteriocin. The purification involved ammonium sulphate precipitation, centrifugal micro-concentration with a 10 kDa membrane cutoff, gel filtration using Sephadex G-25, and C18 reverse-phase HPLC (Lu *et al*., 2010; Wang *et al*., 2023). The next step involved preparing a solution of 100 mM PMSF. PMSF powder and isopropyl alcohol solvent were used to create this solution, and the solution was kept in the freezer next to ice and under the hood. A 1 mM solution was prepared using the C1V1-C2V2 formula from the stock solution, and it was used in the next steps. Then, 50 1 mM PMSF solution microliters were added to the centrifuged Falcon suspension. Alkenes were transferred to Falcon containing a 3KD Amicon filter and centrifuged at 4000 rpm and 4°C for 10-30-60 minutes. The final step was to transfer the concentrated bacteriocin into a sterile microtube and store it in a refrigerator at 0-4°C.

2.3. Method of preparation of Bradford solution

The reagent of this method was obtained by dissolving 10 mg of Coomassie Brilliant Blue in 5 ml of 95% ethanol. In the next step, 10 ml of 85% orthophosphoric acid was added to it, and after the color was completely dissolved, its volume was increased to 100 ml with the help of distilled water, and the solution was obtained using Whatman No. 1 filter paper. It was filtered and stored in a dark container in the refrigerator. Sometimes it is necessary to repeat the filtering process several times to remove the reagent blue particles (Misbah *et al.,* 2020).

2.4. Preparation of standard curve

To determine the protein of a solution by the Bradford method, we must draw a standard

curve. For protein measurement, the protein we choose as a standard is the purified form of the desired protein for measurement. To draw a curve, using bovine serum albumin, different concentrations of the protein standard were prepared (at least three standards in the concentration range of 20-200 μg/ml) and the optical absorbance of each tube was measured against the control at a wavelength of 595 nm by the spectrophotometer was read. Coomassie colour strongly binds to quartz, and glass or polystyrene cuvettes are used to do this (Leslie and Summerel, 2006).

2.5. MIC determination

Preparation of fungal suspension and 0.5 McFarland.

To prepare the fungal suspension, we incubated tested fungal isolates on a PDA medium for one week at 30°C. We then added a PST solution containing normal physiological serum and Tween 80 onto the slanted agar medium. After washing the spores' surface, we vortexed the suspension for 15 seconds, and placed it in the laboratory door for 5 to 10 minutes until the mycelium settled, and the spores remained in the solution. Finally, we read the suspension concentration using a spectrophotometer at a wavelength of 530 nm and compared the prepared suspension with the McFarland half.

A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4).

We used a 96-well plate to determine the minimum inhibitory concentration (MIC) of Lactobacillus plantarum bacteriocin solution. We added the solution from high to low concentrations in wells 1 to 10, and used wells 11 and 12 as negative and positive controls respectively. We then added standardized suspension to each well, incubated the plates at 35°C for 48 hours and recorded the pit that prevented fungus growth as the MIC (Khosravi *et al.* 2015).

2.6. MFC determination

The minimum fungicidal concentration (MFC) is the lowest concentration that can destroy 99.9% of microorganisms. To determine the MFC, 50 microliters are taken from the MIC well and cultured before the MIC with a higher amount of bacteriocin. These are cultured on potato dextrose agar medium for a week. The concentration that prevents complete growth of the fungus or shows less than 3 colonies is the MFC. The difference between the MFC and MIC values indicates the resistance or sensitivity of the isolate to the bacteriocin (Nouri *et al.,* 2018).

2.7. Molecular test

Fumonisin-inducing liquid medium

To enhance the expression of RNA and the FUM1 gene, a specific culture medium was utilized to stimulate the production of fumonisin toxin. This medium was prepared as broth in the laboratory with carefully sourced ingredients. The components of this prepared medium (PH 2) are shown in Table 1.

Table 1. The ingredients required for making fumonisin toxin induction culture medium

Material	Amount
malt extract	**0.5 g/L**
yeast extra	**1 g/L**
peptone	**1 g/L**
KH_2PO_4	**1 g/L**
$MgSO_4$-$7H_2O$	**0.3 g/L**
KCl	**0.3 g/L**
$ZnSO_4$- $7H_2O$	**0.05 g/L**
$CuSO_4$-$5H_2O$	**0.01 g/L**
Fructose	**20 g/L**

10^6 spores of *F. verticillioides* were sterilized and inoculated into a falcon-containing toxin production medium. The fungus was then placed on potato dextrose agar once it showed growth. After that, 106 fungal spores were inoculated into a fumonisin toxin induction medium and kept in a greenhouse for a week at 20°C and 200 rpm. After a week, mushroom samples can be extracted under sterile conditions with a Buchner funnel and sterile filter paper Whatman No. 1. The fungal mass should be collected in sterile tubes and frozen in liquid nitrogen. The culture medium liquid can be filtered to measure fumonisin toxin levels and stored at -20 ℃ .

2.8. RNA extraction

The RNA extraction of *F. verticillioides* was done using the commercial kit of MBST Company (Tehran, Iran). To remove DNA from RNA, 1µg RNA, 1µ 10 X DNase I reaction buffer with MgCl2 9 µl, DEPC- Treated Water, 1µl DNase I, RNase-free was added to nuclease-free streel microtube and incubated at 37 ℃ for 30 seconds. Then add 1 microliter of 25mM EDTA solution to the microtube and incubate for 10 minutes at 65℃, which can be used to convert to cDNA.

To determine the absence of DNA, a PCR test was conducted using the desired primers. For this, 5 microliters of the DNA from the samples affected by DNase and 5 microliters of the extracted DNA from the samples with the 5 microliter kit were used.

2.9. Convert RNA to cDNA

This step was performed by Synthesis Kit Revert Aid First Strand cDNA of Sina Clone Company

First step- The compounds listed in Table 2 were added to a sterile nuclease-free microtube and simultaneously on ice.

The second step - mix the ingredients inside the microtube slowly and centrifuge, then incubate at 65°C for 5 minutes and quickly cool on ice. In the next step, The compounds listed below were added to the above microtube:

4 µl 5x Reaction Buffer, 1 µl Ribolock RNase Inhibitor (20 u/µl), 2 µl 10mM dNTP Mix, 1 µl RevertAid M-MulV Reverse Transcriptase (200 u/µl)

Mix the ingredients in the microtube again and then centrifuge. Finally, the oligo (dt) primer was incubated for 60 minutes at 42 degrees Celsius and at the end of this part, we heated the microtubes to 70 degrees for 5 minutes. This reverse transcribed version can be used as a product in the PCR test, or stored at -20°C for less than a week.

The following are the steps for controlling the production of single-stranded cDNA. To ensure accurate results, positive controls (RNA and specific primers provided in the kit) and negative controls were used. The control RNA used was human GAPDH (1.3 kb).

To construct a positive control, follow the steps below:

1. Take 2 µl of Control GAPDH RNA (50 ng/µl), 1 µl of Oligo (dt) Primer, 4 µl of 5x Reaction Buffer, 1 µl of Ribolock RNase Inhibitor (20 u/µl), and 2 µl of mixed dNTP Mix (10 mM).
2. Mix the above reagents well and centrifuge.
3. Incubate the mixture with oligo(dt) primer at 42 degrees Celsius for 60 minutes.
4. Then, heat the microtube to 70 degrees Celsius for 5 minutes.
5. Finally, add 9 µl of DEPC-treated water to the mixture and mix well. The final volume of the mixture should be 20 µl.

Table 2. Material used to convert RNA to cDNA

Template RNA Oligo(dt)primer	1 µl
Total RNA	0.1-5 µg
DEPC-treated water	to 12 µl
Total volume	12 µl

2.10. Positive control PCR amplification steps

The positive control cDNA was prepared and diluted with DEPC-treated water at a ratio of 1 to 1000. All the reagents required for the PCR test were mixed and heated. Then, we added the necessary compounds to the microtube (see Table 3) while keeping it on ice. The PCR steps were performed in the thermocycler. Next, 5 to 10 microliters of the product were transferred to a 1% agarose gel and stained with ethidium bromide. Finally, the specific bands of the RT-PCR product can be seen at a distance of 496 bp.

Table 3. Required materials for amplification of positive control PCR

cDNA from control RT reaction (1/1000 dilution)	2 µl
10X PCR buffer	5 µl
10 mM dNTP Mix	1 µl (0.2 mM)
25 mM $MgCl_2$	3 µl
Forward GAPDH Primer	1.5 µl
Reverse GAPDH Primer	1.5 µl
Taq DNA Polymerase (5 u/µl)	0.5 µl
Water, nuclease-free	35.5 µl
Total volume	50 µl

2.11. The steps of performing the PCR test on the samples

To study the effect of bacteriocin, the RNA of *F. verticillioides* isolates was extracted before and after exposure. The isolates were cultured in a toxin-induction environment for 7 days at a temperature of 20 degrees Celsius. The resulting fungal mass was collected using a vacuum pump and then frozen with liquid nitrogen. After following the instructions for pounding the frozen mass in a sterile mortar, RNA was extracted and converted into cDNA to ensure its stability (Petrocci et al., 2023).

2.12. RT-PCR test with FUM1 gene

To perform this step, the primer related to the FUM1 gene, which is involved in the production of fumonisin toxin, was used (Table 4).

The following were added to a microtube of 0.1 ml for one sample with FUM1 gene, as well as positive and negative controls: 11.5 µl of Distilled Water, 2.5 µl of PCR buffer, 1 µl of MgCl2, 0.5 µl of dNTP, 1 µl of Forward Primer, 1 µl of Reverse Primer, 0.5 µl of Taq DNA Polymerase (5 u/µl), and 5 µl of cDNA. The final volume was 25 µl. The contents were transferred to the thermocycler and the procedure was carried out according to the schedule in Table 5. The negative control was the non-toxin-producing *F. Verticillioides* fungus, while the positive control was the toxin-producing *F. Verticillioides* fungus. Table 5.RT-PCR test program with FUM1 gene.

After the thermocycler was finished, the RT-PCR product was transferred to 1.2% agarose gel.

Table 4. Nucleotide pairs used to examine FUM1 gene expression

Primer	Target gene	Length	Sequence (5´- 3´)	Amplification Product (bp)
FUM_1-F	*FUM_1*	20	5´- CCA TCA CAG TGG GAC ACA GT-3'	183
FUM_1-R	*FUM_1*	22	5´- CGT ATC GTC AGC ATG ATG TAG C-3'	183

Table 5. PCR timetable reaction

Initial denaturation	94˚ C , 4 min
Amplification (35 cycles)	Denaturation : 94˚ C , 1 min Annealing 58˚ C , 1 min Extension : 72˚ C , 1 min
Final extension	72˚ C , 10 min

3. Results

3.1. Results of protein determination by Bradford method

We mixed the contents of 11 numbered tubes with values from a standard solution and distilled water. Then, we added Bradford's reagent to each tube and left them at room temperature for five minutes. After measuring the optical absorption at a wavelength of 595 nm and creating a standard curve, we connected the read values to indicate protein concentration.

3.2. Sensitivity test results

The results of investigating the effect of Lactobacillus plantarum bacteriocin on 10 studied strains of Fusarium verticilloides (f1-f10) showed that this bacteriocin prevented the growth of the fungus at MIC=0.014 g/ml(Fig 2).

The minimum bacteriocin lethal concentration (MFC) against Fusarium strains was determined after finding the minimum bacteriocin growth inhibition concentration (MIC).

The killing power of *L. plantarum* bacteriocin ranged from 0.028 to 0.225 µg/ml. The MFC of f1-f4 strains was equivalent to 0.056 µg/ml, while f4 strains had an MFC equivalent to 0.028 µg/ml. The MFC of the f5-f8 strains as well as the f10 strain was equivalent to 0.113 µg/ml, and the MFC of the f9 strain was 0.225 µg/ml.

3.3. The results of RT-PCR tests

RT-PCR before and after the bacteriocin effect to check FUM1 gene expression.

After preparing biomass, extracting RNA, and converting it to cDNA, RT-PCR was conducted with the FUM1 gene. The results showed the FUM1 gene in RNA samples, with a 183 bp product visible in the image. Positive control in the cDNA kit was used to confirm the conversion test, with the RT-PCR product appearing at 496 bp.

The obtained results indicate that this gene was not identified in some samples after the effect of bacteriocin. No product indicating the presence of this gene was observed. In this test, the positive control of the cDNA kit was used.

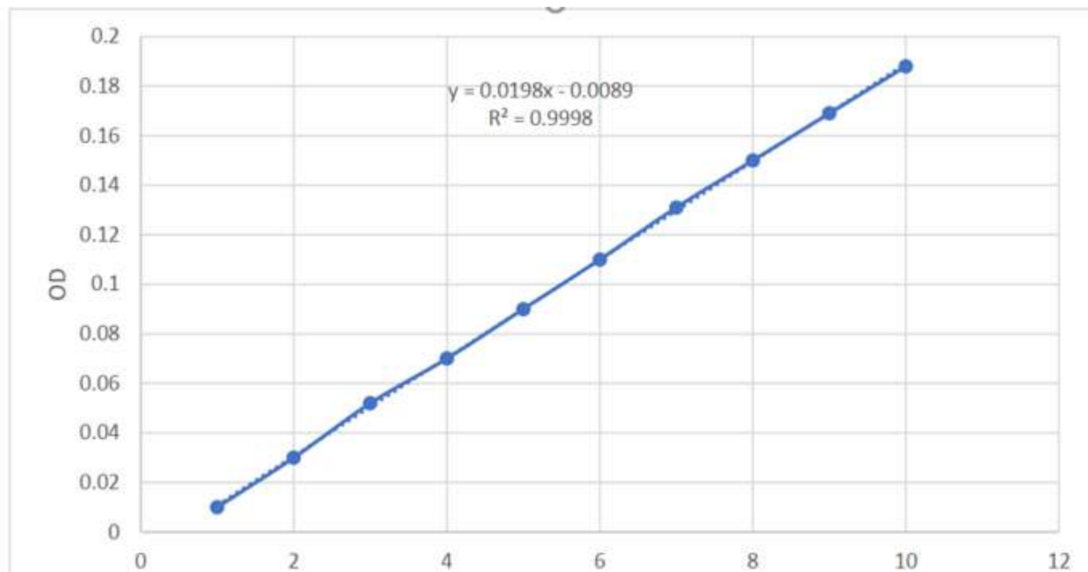


Figure 1. The standard curve

Figure 1. Microplate image after MIC test. The 1st to 11th wells of the tested samples, the 12th wells are the positive control (containing bacteriocin), the G3 well is the negative control (containing the culture medium).

Table 6. MIC and MFC abundance percentage in micrograms per milliliter (g/mlμ)

	Dilution of the wells (μg/ml)	Frequency	Percent
MIC	0.028	4	40
	0.056	4	40
	0.014	1	10
	0.113	1	10
MFC	0.056	4	40
	0.113	4	40
	0.028	1	10
	0.225	1	10

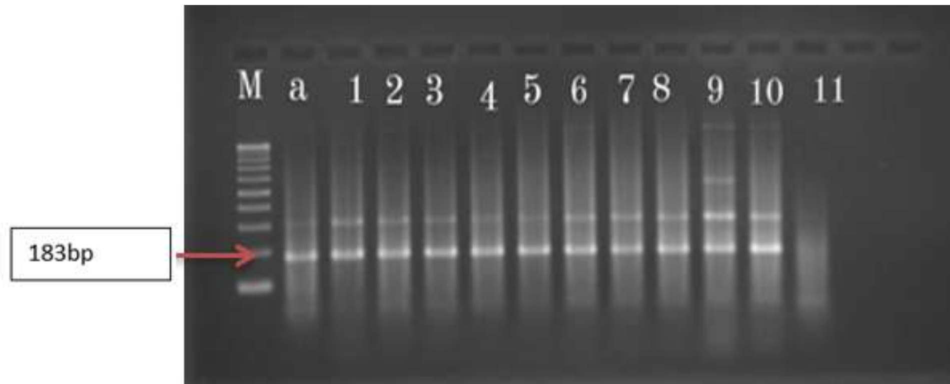


Figure 2. Agarose gel of RT-PCR test on RNA samples before the effect of bacteriocin with FUM1 gene, from left to right: M : marker 100bp, row a: positive control; rows 1 to 10 visible band formation on 183bp for Fusarium verticilloides fungus samples investigated (f1-f10); 11: negative control

Figure 3. Agarose gel of RT-PCR test on RNA samples after the effect of bacteriocin with FUM1 gene, from left to right: row M : marker 100bp, a: positive control (confirmed strain with fumonisin gene), row 1 to 10 strains Fusarium verticillioides examined, the absence of a visible band at 183bp in samples 4 and 8 of F. verticilioides (f4 and f8). Other strains had a visible band at 183bp, 11: negative control. Row C-RT positive control cDNA kit with a length of 496bp

4. Discussion

Fungal diseases hinder plant growth and cause economic losses. Fungal plant pathogens such as F. plantarum affect plants, animals, and humans. Using lactic acid bacteria and yeasts is effective in reducing pesticide use in agriculture (Deepthi *et al.,* 2016; Guan *et al*., 2023). Lactic acid bacteria can produce various antimicrobial compounds such as lactic acid, propionic acid, bacteriocins, and hydrogen peroxide. Although the exact mechanism by which these compounds act is not yet fully understood, it is widely recognized that they can effectively inhibit pathogenic fungi and reduce the expression of genes that play a role in pathogenesis (Chandra Nayaka *et al*., 2009).

In this research, the presence of the FUM1 gene was checked before and after *L. plantarum* bacteriocin treatment using the RT-PCR that showed 2 strains (20%) did not show any band formation, while 4 strains showed very weak bands. The extracted bacteriocin in MIC50=0.014 $\mu g/$ml prevented the growth of the fungus and the MFC results exhibited that this toxin inhibited the growth of nine strains in the culture medium, with only one colony able to grow (MFC=0.225-0.28 µg/ml).

Guan and his colleagues (2023) have also found similar results by using probiotic bacteria

such as *L. plantarum* and *Bacillus lactiplanti*, which reduced the growth of Fusarium and Aspergillus fungi and their toxin production. Smaoui *et al.*(2010), also reported good antimicrobial activity of *L. plantarum* sp on Gram-negative bacteria and *Candida tropicalis* at PH=3 and PH=11.

Furthermore, Deepthi *et al*., (2016), (had similar findings to our research, showing that the use of the extracellular metabolite of *L. plantarum* reduced the growth of fungi and the level of fumonisin toxin production by 61.7%. Our research has shown that using bacteriocin from a certain bacterium can reduce the growth and production of toxins by decreasing the expression of the gene that encodes fumonisin. However, out of the strains we investigated, only two showed a complete lack of gene expression.

In another study by Nayaka and his colleagues (2023), it was discovered that the bacterium *Pseudomonas fluorescens* effectively controls the pathogenicity of *F. verticillioides* and fumonisin production. In addition, the bacteriocin Bacillus subtilis and various species of Streptomyces were found to have an inhibitory effect on the fungus *Fusarium graminarum*. This suggests a strong inhibitory effect of biocontrol on this fungus, confirming the inhibitory effect of these probiotic bacteria, in line with our research. In agreement with our work, Kavkova et al. (2023) revealed in their study that *L. plantarum* and *L. pentosus* had an inhibitory effect on the mycelial growth and conidial germination of Fusarium spp (Kavakova *et al*.2023). The use of molecular methods to examine changes in gene expression after treatment with a substance is precise, accurate, and efficient. Among the molecular methods, RT-PCR is an extremely sensitive method for detecting and quantifying mRNA. Gene expression analysis provides a better understanding of gene and protein action, making it a suitable choice to determine mRNA levels of the gene under investigation. Studies have shown that genes such as FUM1 play a direct role in the production of Fusarium fumonisine (Abd-Elsalam *et al*., 2003).

In this research, the bacteriocin inhibited the expression of the FUM1 gene, which produces fumonisin toxin, suggesting it is a good candidate for controlling the growth of the fungus and preventing contamination in food sources. More research is needed to confirm these findings.

Ethics considerations

The authors have completely observed ethical issues.

Acknowledgments

We would like to express our special appreciation to the personnel of Atiyeh Salamat Alborz

Author Contributions

M.B. and M.A. conceived and designed the experiments. Both authors wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

There is no conflict of interest based on the writers

Refereces

Abd-Elsalam, KA., Aly, IN., Abdel-Satar, MA., Khalil MS, Verreet JA. (2003). PCR identification of Fusarium genus based on nuclear ribosomal-DNA sequence data. African Journal of Biotechnology 2003;2(4):82-5.

Al-Zaban, MI., Alrokban, AH., Mahmoud, MA. (2023). Development of a real-time PCR and multiplex PCR assay for the detection and identification of mycotoxigenic fungi in stored maize grains. Mycology. 14(3):227-238.

Chandra Nayaka, S., Udaya Shankar, AC., Reddy, MS., et al. (2009). Mortensen CN. Control of Fusarium verticillioides, cause of ear rot of maize, by Pseudomonas fluorescens. Pest Management Science: formerly Pesticide Science 2009. 65(7):769-75.

Daie, GR., Mirhendi, H., Ghiasian, SA., et al. (2011). Genotyping of Fusarium verticillioides strains producing fumonisin B1 in feed associated with animal health problems. Iranian Journal of Veterinary Research. 12(4): 309-316.

Darvishnia, M., Senshenas, S., Bazgir, E., Darvishnia F. (2023). Identification of Fusarium verticillioides fumonisin B1 mycotoxin and the effects of pH, relative humidity and light on its production on maize substrate. Journal of Applied Research in Plant Protection. 11(4):15-27.

Deepthi, BV., Poornachandra Rao, K., Chennapa, G., et al. (2016). Antifungal attributes of Lactobacillus plantarum MYS6 against fumonisin-producing Fusarium proliferatum associated with poultry feeds. PLoS One. 11(6):e0155122.

do Carmo Silvestre, K., da Silva, IF., Rodrigues, N. (2023). Influence of the application of biofertilizers on the control of Fusarium root rot and Fusarium wilt and on the growth of common bean plants. Semina. 44(3): 1225-1244.

Gagiu, V., Israel-Roming, F., Belc, N., Dima, R. (2013). Inhibitory activity of Lactobacillus plantarum strains and calcium propionate on spoilage fungi. Romanian Biotechnological Letters. 18(2):8215.

Guan, Y., Lv, H., Wu, G., et al. (2023). Effects of lactic acid bacteria reducing the content of harmful fungi and mycotoxins on the quality of mixed fermented feed. Toxins 15(3):226.

Kavková, M., Bazalová, O., Cihlář, J., et al. (2023). Characterisation of Wild Strains of Lactic Acid Bacteria Isolated from Legumes and Their Biocontrol Potential against Fusarium spp. Agronomy. 13(12):2911.

Khosravi, AR., Shokri, H., Mokhtari, AR. (2015). Efficacy of Cuminum cyminum essential oil on FUM1 gene expression of fumonisin-producing Fusarium verticillioides strains. Avicenna Journal of Phytomedicine. 5(1):34.

Krishnan, SV., Nampoothiri, KM., Suresh, A., et al. (2024). Fusarium biocontrol: antagonism and mycotoxin elimination by lactic acid bacteria. Frontiers in Microbiology. 14:1260166.

Leslie, JF., Summerell, BA. (2006). The Fusarium Laboratory Manual. 1st ed, Blackwell Publishing Ltd., Hoboken, NJ, USA. 388 pp.

Li, Q., Zeng, X., Fu, H., et al. (2023). Lactiplantibacillus plantarum: A comprehensive review of its antifungal and anti-mycotoxic effects. Trends in Food Science & Technology. 136: 224-238.

Lu, TS., Yiao, SY., Lim, K., et al. (2010). Interpretation of biological and mechanical variations between the Lowry versus Bradford method for protein quantification. North American Journal of Medical Sciences. 2(7):325

Mao, Z., Wang, Y., Li, M., et al. (2024). Vacuolar proteomic analysis reveals tonoplast transporters for accumulation of citric acid and sugar in citrus fruit. Horticulture Research. 11(1):uhad249.

Misbah, MM., Ferdous, J., Bulbul, MZ., et al. (2020). Evaluation of MIC, MBC, MFC and anticancer activities of acylated methyl β-D-galactopyranoside esters. Int. J. Biosci 16(4):299-309.

Noktehsanj Avval, M., Hosseininezhad, M., Pahlavanlo, A., Ghoddusi, HB. (2023). Creating optimal conditions for bacteriocin production from Lactiplantibacillus plantarum isolated from traditionally fermented fruits and vegetables. Research and Innovation in Food Science and Technology. 11(4):351-66.

Nouri, S., Nazeri, S., Hosseyni, P. (2018). Isolation and Biochemical and Molecular Identification of Lactobacillus Plantarum Bacteria from Rhizosphere of Lenjan Rice. Biological Journal of Microorganism. 7(27):61-71.

Petrucci, A., Khairullina, A., Sarrocco, S., et al. (2023). Understanding the mechanisms underlying biological control of Fusarium diseases in cereals. European Journal of Plant Pathology. 167(4):453-76.

Smaoui, S., Agriopoulou, S., D'Amore, T., et al. (2023). The control of Fusarium growth and decontamination of produced mycotoxins by lactic acid bacteria. Critical Reviews in Food Science and Nutrition. 63(32):11125-52.

Wang, Z., Zhang, Y., Chen, C., et al. (2023). A novel bacteriocin isolated from Lactobacillus plantarum W3-2 and its biological characteristics. Frontiers in Nutrition. 9:1111880.

Xu, Y., Zhang, Z., Lu, P., et al. (2023). Increasing Fusarium verticillioides resistance in maize by genomics-assisted breeding: Methods, progress, and prospects. The Crop Journal .11(6): 1626-1641.

Yan, H., Zhou, Z., Zhang, H., Shim, WB. (2023). Vacuole proteins with optimized microtubule assembly are required for Fum1 protein localization and fumonisin biosynthesis in mycotoxigenic fungus Fusarium verticillioides. Journal of Fungi. 16;9(2):268.