Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Investigation of the Antifungal Activity of *Bacillus megaterium* Against *Fusarium* Species

Esra Nur KELEŞ[1], Ayşe Feyza TUFAN DÜLGER*[2], Emre YÖRÜK[3]

[1]Haliç University, Graduate Institute of Education, Program of Molecular Biology and Genetics, 34445, İstanbul, Türkiye
[2]Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, 55139, Samsun, Türkiye
[3]İstanbul Yeni Yüzyıl University University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, 34010, İstanbul, Türkiye

[1]https://orcid.org/0000-0002-6665-4723, [2]https://orcid.org/0000-0003-4779-6811, [3]https://orcid.org/0000-0003-2770-0157

*Corresponding author e-mail: feyzadulger@omu.edu.tr

Article Info

Received: 17.01.2013
Accepted: 31.03.2023
Online published: 15.06.2023
DOI: 10.29133/yyutbd.1237451

Keywords

Bacillus megaterium,
Biocontrol,
Fusarium culmorum,
Fusarium graminearum,
Gene expression

Abstract: Several *Fusarium* species are emerging as serious pathogens on small grain cereals worldwide. The use of fungicides is a short-term strategy in the fight against *Fusarium* diseases. The use of biocontrol agents is an attractive alternative strategy by reducing the chemical input to the environment as well as being economical. *Bacillus* species have received attention as biocontrol agents. In this study, the antagonistic activities of *Bacillus megaterium* CTBmeg1 and HMA5 strains on *Fusarium culmorum* UK99 and *F. graminearum* PH-1 isolates were investigated *in vitro* and at molecular level. On the 7th day of the dual culture assay, both of *B. megaterium* strains significantly reduced the mycelial growth of *Fusarium* isolates, with very high antifungal activity with the inhibition rate between 72.7% and 77.7%, respectively. Similarly, both strains caused high antifungal activity in the volatile organic compound (VOC) analysis between 52.1% and 62.4%, respectively. At the molecular level, in all tested groups, transcript levels of the *tri5* gene, which is associated with trichothecene production, decreased, while the transcript levels of *cat*, an antioxidant gene, and *mst20*, a gene related to apoptosis, increased. Findings from this study showed that *B. megaterium* CTBmeg1 and HMA5 strains could be accepted as highly effective biocontrol agents against worldwide phytopathogens *F. culmorum* and *F. graminearum*.

To Cite: Keleş, E N, Tufan Dülger, A F, Yörük, E, 20xx. Investigation of the Antifungal Activity of *Bacillus megaterium* Against *Fusarium* Species. *Yuzuncu Yil University Journal of Agricultural Sciences*, 33(2): 183-191. DOI: https://doi.org/10.29133/yyutbd.1237451

1. Introduction

F. culmorum and *F. graminearum* are the major causal agents of the *Fusarium* head blight and crown rot diseases of small grain cereals worldwide. Epidemics of these diseases result in yield losses reaching up to billions of dollars in economic damages and mycotoxin contamination e.g., deoxynivalenol, nivalenol, and zearalenone (Pasquali and Migheli, 2014; Matny, 2015). Several strategies including fungicide usage, biocontrol agents and the development of disease resistant cultivars have been used in order to combat *Fusarium* diseases (Moya-Elizondo and Jacobsen, 2016; Tufan et al., 2017; Uluhan et al., 2019). The use of fungicides is a short-term strategy in the fight against diseases

and is not effective because pathogenic fungi can easily become resistant to these fungicides and cause environmental pollution. The use of biocontrol agents is the more friendly strategy by reducing the chemical input to the environment as well as being economical. Among various bacterial genera, *Bacillus*, *Pseudomonas,* and *Streptomyces* species are widely used as biocontrol agents (Legrand et al., 2017). However, the *Bacillus* genus has an advantage over other microorganisms by endospore production that is tolerant to stress conditions such as heat and high concentrations of chemicals. They produce a broad spectrum of antibiotics, a wide range of antifungal lipopeptides (iturine, fengycin, surfactins, etc.), and hydrolytic enzymes and act as bacterial and fungal antagonists against phytopathogens. They promote plant growth through mechanisms such as siderophore production, potassium solubility, and phytohormone synthesis (Khan et al., 2017; Nayak et al., 2017; Bolivar-Anillo et al., 2021).

The aim of this study was to examine the antagonistic effects of *B. megaterium* CTBmeg1 and HMA5 on *F. culmorum* and *F. graminearum*. In this context, first, the antagonistic effects of *B. megaterium* on *Fusarium* species were tested *in vitro* by dual culture assay and VOC analysis. Then, the effects of *B. megaterium* strains on oxidative stress, trichothecene biosynthesis, and apoptosis in Fusarium spp. were analyzed by qPCR at the transcript level. Finally, the toxigenic effects of *B. megaterium* strains on *Fusarium* spp. were determined by WST-1- assay.

2. Material and Methods

2.1. Culture conditions of *Fusarium* isolates and *Bacillus megaterium* strains

The reference strains of *F. graminearum* (PH-1) and *F. culmorum* (UK99) isolates were used for experiments. Carboxymethylcellulose (CMC) liquid medium (1.0 % CMC, 0.3 % NaCl, 0.1 % KH_2PO_4) was used for *Fusarium* spore production. *Fusarium* isolates were grown in ½ potato dextrose agar (PDA) medium for 5 days at 25 °C. Then, fungal discs were added to a CMC medium and incubated at 28°C, 100 rpm for 5 days. The harvested fungal spores were standardized to 1 x 10^5 macroconidia/mL (Nalam et al., 2016). Then, 20 µL of *Fusarium* spores were placed in the center of the PDA medium and grown for 7 days at 25°C, 50 % humidity. Fungal discs with a diameter of ~3mm were used for the following experiments.

Bacillus megaterium CTBmeg1 (Akcay and Kaya, 2019) and HMA5 (Aksoy et al., 2018) strains were obtained from Ondokuz Mayıs University, Agricultural Biotechnology, and Plant Protection Departments, respectively. The strains were grown in Luria Bertani (LB) medium at 30°C, 200 rpm overnight, and diluted to an OD_{600} of 0.4. Strains were stored as 50% glycerol stock at -80°C.

2.2. Dual culture assay

Fusarium discs with a diameter of ~3mm were placed in the centers of new PDA media and bacterial strains were swab inoculated ~2.5 cm away from the *Fusarium* disc and ~1 cm away from the petri dish. Radial growth diameter from *Fusarium* disc center to bacteria was measured after 7 days and the percentage of inhibition of radial growth (PIRG) was determined according to the following formula: PIRG (%) = $[(R1-R2) / R1] \times 100$ % where R1 is the radial growth of *Fusarium* in the control plate, R2 is the radial growth of *Fusarium* towards antagonist bacteria. This study used a scale described by Bivi et al. (2010) as follows; PIRG <30 % indicates low antifungal activity, 30 - <50 % indicates moderate antifungal activity, 50 - <70 % indicates high antifungal activity, ≥70 % indicates very high antifungal activity. A Petri dish containing only *Fusarium* isolate was used as a negative control. Experiments were performed with 5 technical and 3 biological replicates.

2.3. Effects of VOCS on radial mycelial growth and hyphae morphology

A ~3mm diameter fungal disc was inoculated into the center of a new PDA medium, and the bacterial strain was inoculated into another PDA medium with the help of a swab. The two petri dishes were placed on top of each other with only common air flow between them and incubated at 25°C, 50 % humidity for 7 days. The percentage inhibition of diameter growth (PIDG) was determined by measuring the difference in diameter of the fungal culture after the two petri dishes were brought together, according to the formula: $PIDG$ (%) = $[(D1-D2) / D1] \times 100$ % where D1 is the diameter of the *Fusarium* in the control plate, D2 is the diameter of *Fusarium* treated with antagonistic bacteria (Toh

et al., 2016). PIDG <30 % indicates low antifungal activity, 30 - <50 % indicates moderate antifungal activity, 50 - <70 % indicates high antifungal activity, and ≥70 % indicates very high antifungal activity. A Petri dish containing only *Fusarium* isolate was used as a negative control. Experiments were performed with 5 technical and 3 biological replicates.

2.4. qPCR for expression of oxidative stress, trichothecene biosynthesis, and apoptosis related genes

Total RNA extraction from *Fusarium* isolates on day 7 of the dual culture assay was performed according to the manufacturer's protocol (NucleoSpin RNA Mini Kit, Macherey Nagel). 30 mg of fungal hyphae was taken and homogenized for 1 min (Retsch MM 400, Germany). The quality and quantity of the total RNAs were determined by agarose gel electrophoresis and spectrophotometer (A260, A280, A230).

Synthesis of cDNA from total RNA was performed according to the manufacturer's protocol (ProtoScript® First Strand cDNA Synthesis BioLABs). 1 µg of total RNA was used and the synthesized cDNAs were diluted to 20 ng μl^{-1}.

qPCR was performed with 1X Sybr Green I mix, 5 pmoL primers, and 40 ng μL^{-1} cDNA in a total volume of 10 µL. The qPCR conditions were 2 min at 95°C, followed by 40 cycles of 5 s at 95 °C, 10 s at 58 °C, and 10 s at 72 °C. At the end of the cycle, melting curve analysis was performed between 65 ºC - 95 ºC by increasing 0.5 ºC at 2-5 sec/step (Bio-Rad, CFX Connect RealTime System). β-tubulin was used as the housekeeping gene while *cat*, *mst20,* and *tri5* were the target genes (Gazdağlı et al., 2018). Gene expression profiles were calculated according to $2^{-\Delta\Delta CT}$ normalization values (Livak and Schmittgen, 2001). Experiments were performed in 3 technical and 2 biological replicates.

2.5. WST-1 cell viability assay

WST-1 assay was performed from *Fusarium* isolates on day 7 of the dual culture assay by optimizing the manufacturer's protocol (Cell Proliferation Reagent WST-1, Roche). 1X PBS (1 L DHSO, 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4) was added onto ~1cm^2 mycelium and homogenized for 40 sec. 1:10 (v/v) WST-1 was added to 100 µL of cell suspension and incubated at 28 °C, 100 rpm for 3 h. Cell viabilities % were calculated by 450/600 nm wavelengths (Teker et al., 2021). The experiments were performed with 2 technical and 2 biological replicates.

2.6. Statistical analysis

Cell viability % assays were performed with a two-way analysis of variance (ANOVA) Bonferroni post-test using GraphPad Prism (Version 5.01) (*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). qPCR analyses were performed using one-way ANOVA with LSD test in R statistical software with RStudio (Version 1.3.1093) and the package agricolae.

3. Results

3.1. Effects of *B. megaterium* strains against *Fusarium* spp.

Antifungal activities of *B. megaterium* CTBmeg1 and HMA5 strains against *F. graminearum* PH-1 and *F. culmorum* UK99 isolates were evaluated by *in vitro* dual culture assay. After 7 days of the assay, morphologically significant differences were observed in the experimental groups compared with the control groups in terms of hyphae color and structure of PH-1 and UK99. The hyphae of the PH-1 control group were looser and cottony, and the color of the hyphae was dark pink. As a result of the antagonistic effect of CTBmeg1 and HMA5 strains, the PH-1 hyphae structure was found to be like the control group, but the color of the hyphae turned white. PH-1 covered the entire petri dish on the 7th day in the control group but did not grow in the experimental groups (Figure 1. a-c). In the UK99 control group, the hyphae structure was looser and cottony, and the color was dark pink. As a result of the antagonistic effect of CTBmeg1 and HMA5 strains in the experimental groups, it was observed that the hyphae structure was more frequent and felt-like, and the color of the hyphae was white (Figure 1. d-f). When the antagonistic effects of *B. megaterium* strains against PH-1 and UK99 were analyzed in terms of the radial growth, it was found that the bacteria showed high *in vitro* antifungal activity against UK99

(76.4 % and 72.7 % in CTBmeg1 and HMA5, respectively) and PH-1 (77.7 % and 73.7 %, in CTBmeg1 and HMA5, respectively) (Figure 2. a).

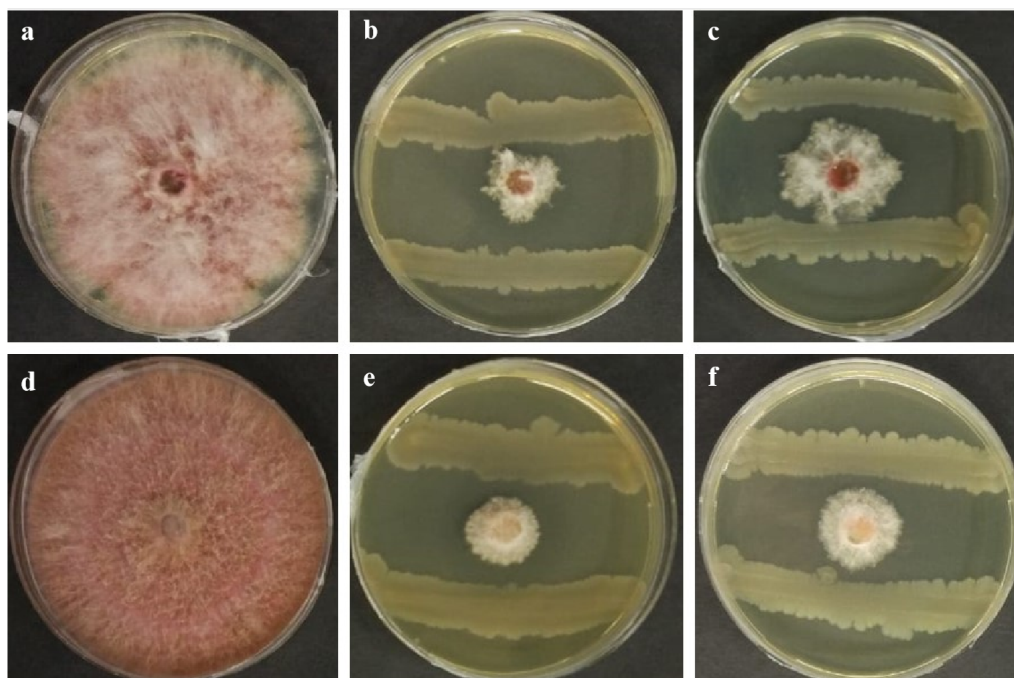

Figure 1. Dual culture assay for the screening of *B. megaterium* CTBmeg1 and HMA5 against *F. graminerarum* PH-1 and *F. culmorum* UK99. a) PH-1 (control), b) PH-1+CTBmeg1, c) PH-1+HMA5, d) UK99 (control), e UK99+CTBmeg1 and f UK99+HMA5.

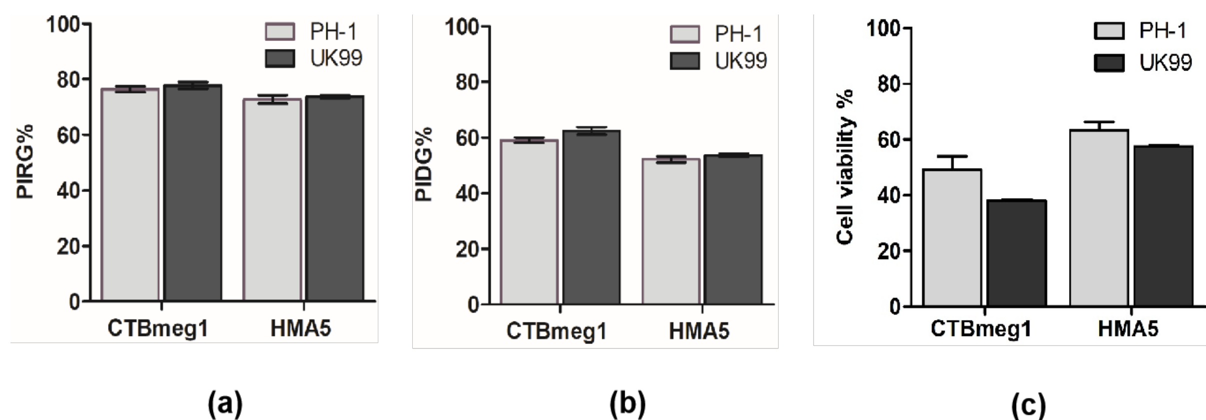

Figure 2. (a) PIRG %, (b) PIDG %, and (c) cell viability % of F. graminerarum PH-1 and F. culmorum UK99 isolates obtained from dual culture assays. Error bars represent ± standard errors (SE) of three replicates.

3.2. VOC analysis

The antifungal activities of CTBmeg1 and HMA5 strains against PH-1 and UK99 isolates were evaluated by *in vitro* VOC analysis. As a result of the analysis, morphologically significant differences were observed in the experimental groups compared with the control groups in terms of hyphae color and structure of both *Fusarium* isolates. In the PH-1 control group, the hyphae structure was looser and cottony, and the color is dark pink, while in the experimental group, the hyphae structure was loose but more fragmented than in the control group. Hyphae growths were longer in all experimental groups compared with the control group and no significant color changes were observed. While the control group grew to cover almost the entire petri dish, it was observed that the diameter growth was

significantly suppressed in the experimental group (Figure 3. a-c). Compared with the control group, it was observed that CTBmeg1 and HMA5 strains also caused morphological differences in the hyphae structure and diameter growth of UK99 isolate. While the hyphae structure was loose and cottony and the color was dark pink in the control group, the group exposed to VOC had a looser hyphae structure than the control group, and the hyphae color was burgundy in the center and the surrounding mycelial growth was white. Although the control group covered the entire petri dish on the 7th day, it was observed that diameter growths were suppressed in the experimental groups (Figure 3. d-f). In terms of PIDG %, bacteria showed high *in vitro* antifungal activity against PH-1 (59.0 % and 52.1 % in CTBmeg1 and HMA5, respectively) and UK99 (62.4 % and 53.6 % in CTBmeg1 and HMA5, respectively). (Figure 2. b).

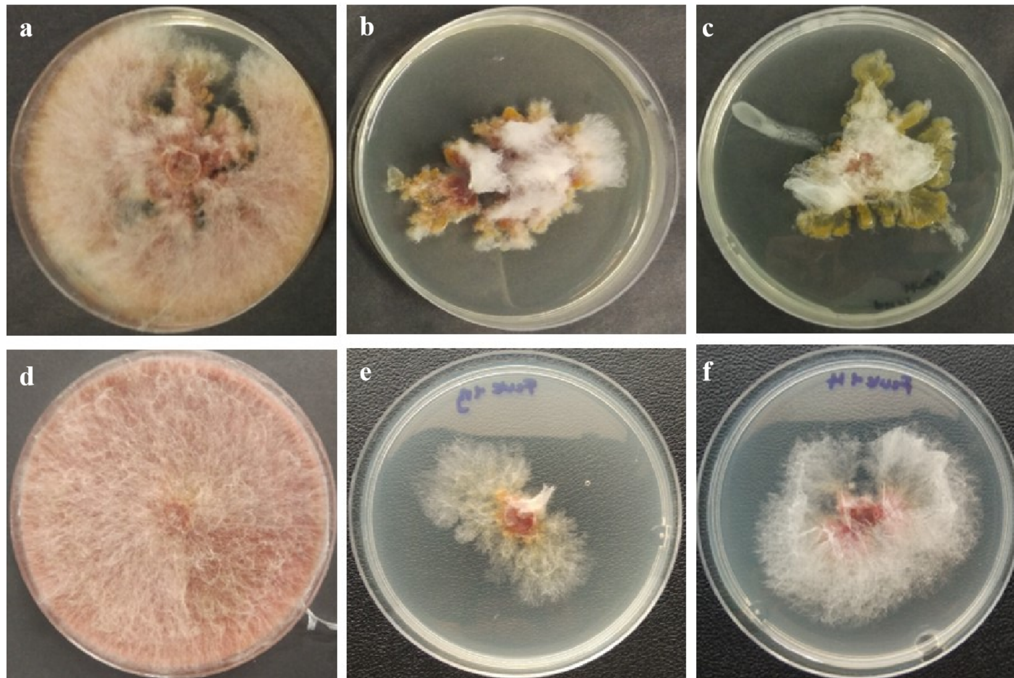

Figure 3. The effects of VOCs produced by CTBmeg1 and HMA5 on the growth of PH-1 and UK99. a) PH-1 (control), b) PH-1+CTBmeg1, c) PH-1+HMA5, d) UK99 (control), e) UK99+CTBmeg1 and f) UK99+HMA5.

3.3. Gene expression analysis

Both in the control and experimental groups, expression levels of *mst20*, *cat*, and *tri5* genes were examined. In each experimental set *tri5* gene decreased, while the decrease in PH-1 + CTBmeg1 (0.15 fold) was significant. *cat* gene increased significantly in all experimental sets (between 3.6 to 4.7 fold). Although there was a significant increase in the *mst20* gene in all experimental sets, these increases were significant in the groups treated with CTBmeg1 strain (2.3 and 3.7 fold in PH-1 and UK99, respectively) (Figure 4).

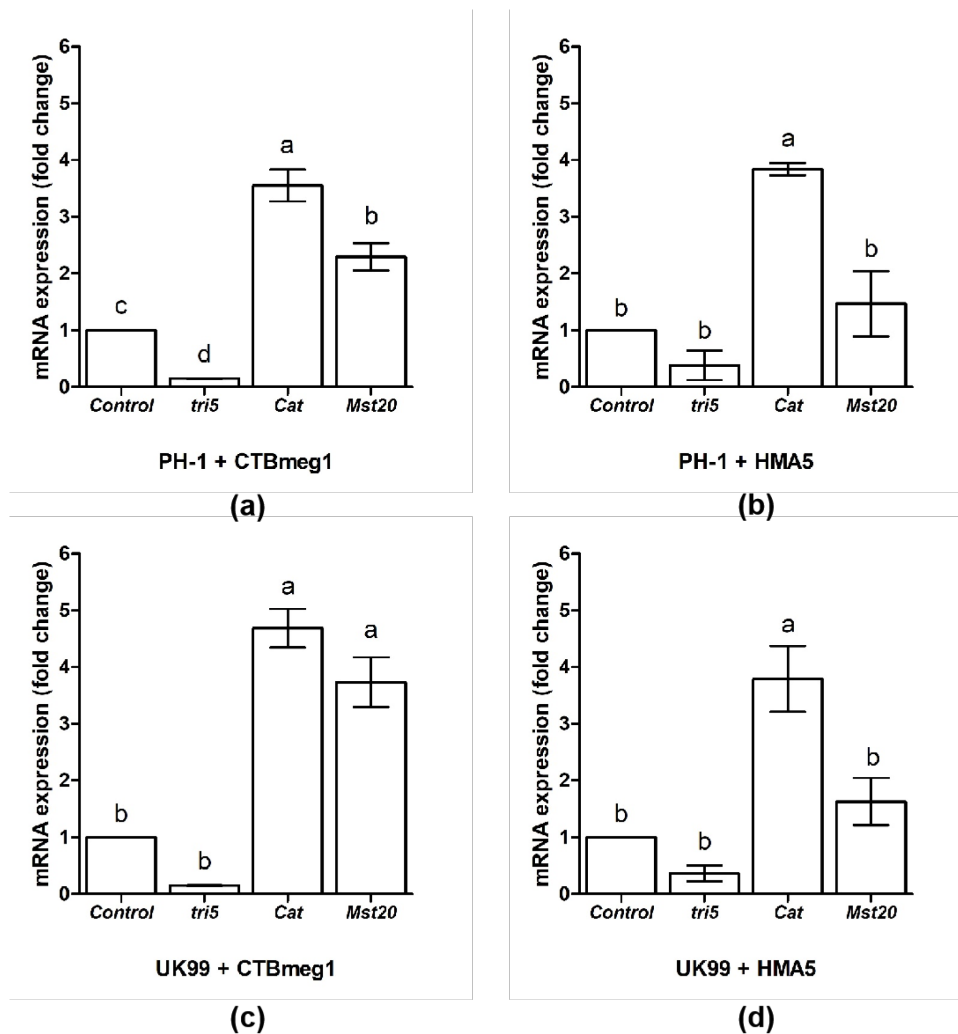

Figure 4. Gene expression analysis of *tri5*, *cat,* and *mst20* genes by means of qPCR. a) PH-1+CTBmeg1, b) PH-1+HMA5, c) UK99+CTBmeg1 and d) UK99+HMA5. Error bars represent ± standard errors (SE) of three replicates.

3.4. Cell viability

Bacillus strains were compared in terms of the cell viability of *Fusarium* isolates. It was observed that both bacteria significantly reduced cell viability (***). Besides, CTBmeg1 suppressed cell viability more than HMA5 in both *Fusarium* species. However, no significant difference was observed between CTBmeg1 and HMA5 in PH-1, while a significant difference was observed between both strains in UK99 (***) (Figure 2. c).

4. Discussion

F. culmorum and *F. graminearum* are the main causative agents of the most destructive *Fusarium* diseases, *Fusarium* head blight and *Fusarium* crown rot. Struggle with these diseases includes several different strategies such as fungicide treatment, disease resistant cultivar development, or plant-derived essential oil usage (Jones, 2000; Bernardo et al., 2007; Özsoy et al., 2020). Each strategy has several disadvantageous. From past to present, common fungicides treatment, in particular triazole group-demethylation inhibiting fungicides, has been the most powerful strategy to manage *Fusarium* diseases (Yörük, 2018). However, fungicide resistance development and ecotoxicological characteristics of fungicides led researchers to find out novel strategies in order to control *Fusarium* diseases. The use of antagonistic microorganisms as biological control agents provide a promising strategy for the management of plant pathogens. *Bacillus* spp. offer several advantages over other

biocontrol microorganisms due to their endospore-forming, antibiotic producing ability, and resistance to extreme conditions (Aksoy et al., 2018; Akcay and Kaya, 2019). There are several reports on the *Bacillus* spp. for biological control of *Fusarium* diseases (Zhao et al., 2014; Grosu et al., 2015; Zalila-Kolsi et al., 2016; Wu et al., 2019; Cantoro et al., 2021). However, there are only limited reports related to the effects of *B. megaterium* on fungal pathogens (Pan et al., 2015; El-Gremi et al., 2017). Also, *B. megaterium* against *F. culmorum* was not used in these studies. Instead, various pieces of research have been studied on the nematicidal and insecticidal effects of *B. megaterium* (Aksoy et al., 2018; Zhou et al., 2020). Studies by Pan et al. (2015) showed that *B. megaterium* BM1 significantly reduced *F. graminearum* growth. Similarly, in our study, both *B. megaterium* CTBmeg1 and HMA5 strains could effectively inhibit both growths of UK99 and PH-1.

Volatile organic compounds produced by *Bacillus* spp. also play an important role in antagonistic activities toward plant pathogens by suppressing the growth and spore germination (Raza et al., 2016; Tahir et al., 2017; Wu et al., 2019). Similarly, in our study, both strains caused high antifungal activity in the VOC analysis. Wu et al. (2014) showed that phenol, toluene, phenol, and benzothiazole VOCs released from the *B. amyloliquefaciens* strain have antifungal effects against *Sclerotinia sclerotiorum*. Gao et al. (2017) found that *B. velezensis* ZSY-1 strain synthesized pyrazine (2,5-dimethyl), benzothiazole, 4-chloro-3-methyl, and phenol-2,4-bis (1,1-dimethylethyl) VOCs with antifungal activity against *Alternaria solani* and *Botrytis cinerea*. Li et al. (2020) showed that *Bacillus velezensis* CT32 synthesized decanal, benzothiazole, 3-undecanone, 2-undecanone, 2-undecanol, undecanal and 2,4-dimethyl-6-tert-butylphenol VOCs with high antifungal activity against *Verticillium dahliae* and *F. oxysporum*.

In the next step, we aimed to detect potential alterations in *Fusarium* spp. in response to *B. megaterium* treatment. Apoptosis related (*mst20*) and oxidative stress related (*cat*) genes were increased while the deoxynivalenol biosynthesis related gene (*tri5*) was decreased. Similarly, significant differences were reported by previous studies Gazdağlı et al., 2018; Yörük, 2018; Cantoro et al. 2021; Teker et al., 2021). Moreover, both *B. megaterium* strains reduced significantly the cell viability of *Fusarium* isolates.

Conclusion

In this study, *B. megaterium* CTBmeg1 and HMA5 strains significantly reduced the mycelial growth of *F. culmorum* UK99 and *F. graminearum* PH-1 isolates. VOC could affect the inhibition of mycelial growth. At the molecular level, a decrease in the gene associated with toxin production and increases in genes associated with antioxidants and apoptosis were detected in *Fusarium* isolates treated with *Bacillus* strains. To our knowledge, this is the first report to reveal transcripts analysis in *B. megaterium* strains against *Fusarium* spp. However, detailed and comprehensive investigations such as in planta tests and more *Fusarium* species would be useful in providing more detailed data in disease management.

Acknowledgements

This work is a part of the master thesis of the author Esra Nur Keleş. The authors thank Dr. Yılmaz Kaya and Prof. Hasan Murat Aksoy, Ondokuz Mayıs University for the *Bacillus* strains and their valuable discussions.

References

Akcay, K., & Kaya, Y. (2019). Isolation, characterization and molecular identification of a halotolerant *Bacillus megaterium* CTBmeg1 able to grow on halogenated compounds. *Biotechnology & Biotechnological Equipment*, *33*(1), 945–953. https://doi.org/10.1080/13102818.2019.1631717

Aksoy H. M., Saruhan I., Kaya, Y., & Ozturk, M. (2018). Morphological changes caused by *Bacillus megaterium* on adult emergence of fall webworm's pupa, *Hyphantria cunea* (Drury)(Lepidoptera: Erebidae). *Journal of Agricultural Sciences*, *24*(4), 539–546. https://doi.org/10.15832/ankutbd.349474

Bernardo, A., Bai, G., Guo, P., Xiao, K., Guenzi, A. C., & Ayoubi, P. (2007). *Fusarium graminearum*-induced changes in gene expression between *Fusarium* head blight-resistant and susceptible wheat cultivars. Functional & Integrative Genomics, 7, 69-77. https://doi.org/10.1007/s10142-006-0028-1

Bivi, M. R., Farhana, M. S. N., Khairulmazmi, A., & Idris, A. (2010). Control of *Ganoderma boninense*: A causal agent of basal stem rot disease in oil palm with endophyte bacteria *in vitro*. *International Journal of Agriculture and Biology*, *12*(6), 833–839.

Bolivar-Anillo, H. J., González-Rodríguez, V. E., Cantoral, J. M., García-Sánchez, D., Collado, I. G., & Garrido, C. (2021). Endophytic bacteria *Bacillus subtilis*, isolated from *Zea mays*, as potential biocontrol agent against *Botrytis cinerea*. Biology, *10*(6), 492. https://doi.org/10.3390/biology10060492

Cantoro, R., Palazzini, J. M., Yerkovich, N., Miralles, D. J., & Chulze, S. N. (2021). *Bacillus velezensis* RC 218 as a biocontrol agent against *Fusarium graminearum*: effect on penetration, growth and TRI5 expression in wheat spikes. *BioControl*, *66*(2), 259–270. https://doi.org/10.1007/s10526-020-10062-7

El-Gremi, S. M., Draz, I.S., & Youssef, W. A. E. (2017). Biological control of pathogens associated with kernel black point disease of wheat. *Crop Protection*, *91*, 13–19. https://doi.org/10.1016/j.cropro.2016.08.034

Gao, Z., Zhang, B., Liu, H., Han, J., & Zhang, Y. (2017). Identification of endophytic *Bacillus velezensis* ZSY-1 strain and antifungal activity of its volatile compounds against *Alternaria solani* and *Botrytis cinerea*. Biological Control, 105, 27–39. https://doi.org/10.1016/j.biocontrol.2016.11.007

Gazdağlı, A., Sefer, Ö., Yörük, E., Varol, G. İ., Teker, T., & Albayrak, G. (2018). Investigation of camphor effects on *Fusarium graminearum* and *F. culmorum* at different molecular levels. *Pathogens*, *7*(4), 90. https://doi.org/10.3390/pathogens7040090

Grosu, A. I., Sicuia, O. A., Dobre, A., Voaideş, C., & Cornea, C. P. (2015). Evaluation of some *Bacillus* spp. strains for the biocontrol of *Fusarium graminearum* and *F. culmorum* in wheat. *Agriculture and Agricultural Science Procedia*, *6*, 559–566. https://doi.org/10.1016/j.aaspro.2015.08.085

Jones, R. K. (2000). Assessments of *Fusarium* head blight of wheat and barley in response to fungicide treatment. Plant Disease, 84(9), 1021-1030. https://doi.org/10.1094/PDIS.2000.84.9.1021

Khan, N., Maymon, M., & Hirsch, A. (2017). Combating *Fusarium* infection using *Bacillus*-based antimicrobials. *Microorganisms*, *5*(4), 75. https://doi.org/10.3390/microorganisms5040075

Legrand, F., Picot, A., Cobo-Díaz, J. F., Chen, W., & Le Floch, G. (2017). Challenges facing the biological control strategies for the management of Fusarium head blight of cereals caused by *F. graminearum*. *Biological Control. 113*, 26–38. https://doi.org/10.1016/j.biocontrol.2017.06.011

Li, X., Wang, X., Shi, X., Wang, B., Li, M., Wang, Q., & Zhang, S. (2020). Antifungal effect of volatile organic compounds from *Bacillus velezensis* CT32 against *Verticillium dahliae* and *Fusarium oxysporum*. Processes, *8*(12), 1674. https://doi.org/10.3390/pr8121674

Livak, K.J., & Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Matny, O. N. (2015). Fusarium head blight and crown rot on wheat & barley: losses and health risks. *Advances in Plants & Agriculture Research*, 2(39), 10-15406. ol

Moya-Elizondo, E. A., & Jacobsen, B.J. (2016). Integrated management of Fusarium crown rot of wheat using fungicide seed treatment, cultivar resistance, and induction of systemic acquired resistance (SAR). *Biological Control*, 92: 153–163. https://doi.org/10.1016/j.biocontrol.2015.10.006

Nalam, V.J., Sarowar, S., & Shah, J. (2016). Establishment of a *Fusarium graminearum* infection model in *Arabidopsis thaliana* leaves and floral tissues. Bio-protocol *6*(14), e1877. https://doi.org/10.21769/BioProtoc.1877

Nayak, S. K., Nayak, S., & Mishra, B. B. (2017). Antimycotic role of soil *Bacillus* sp. against rice pathogens: a biocontrol prospective. Microbial Biotechnology: Volume 1. Applications in Agriculture and Environment, 29-60. https://doi.org/10.1007/978-981-10-6847-8_2

Özsoy, E., Kesercan, B., & Yörük, E. (2020). Antifungal activity of specific plant essential oils against *Fusarium graminearum*. *Journal of Plant Science and Phytopathology*, *4*, 060-062. https://doi.org/10.29328/journal.jpsp.1001052

Pan, D., Mionetto, A., Tiscornia, S., & Bettucci, L. (2015). Endophytic bacteria from wheat grain as biocontrol agents of *Fusarium graminearum* and deoxynivalenol production in wheat. *Mycotoxin Research*, *31*(3), 137–143. https://doi.org/10.1007/s12550-015-0224-8

Pasquali M., & Migheli, Q. (2014). Genetic approaches to chemotype determination in type B-trichothecene producing Fusaria. *International Journal of Food Microbiology*, *189*, 164–182. https://doi.org/10.1016/j.ijfoodmicro.2014.08.011

Raza, W., Wang, J., Wu, Y., Ling, N., Wei, Z., Huang, Q., & Shen, Q. (2016). Effects of volatile organic compounds produced by *Bacillus amyloliquefaciens* on the growth and virulence traits of tomato bacterial wilt pathogen *Ralstonia solanacearum*. *Applied Microbiology and Biotechnology*, *100*, 7639–7650. https://doi.org/10.1007/s00253-016-7584-7

Tahir, H. A. S., Gu, Q., Wu, H., Raza, W., Hanif, A., Wu, L., Colman, M. V., & Gao, X. (2017). Plant growth promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. *Frontiers in Microbiology*, *8*, 171. https://doi.org/10.3389/fmicb.2017.00171

Teker, T., Sefer, Ö., Gazdağlı, A., Yörük, E., Varol, G. İ., & Albayrak, G. (2021). α-Thujone exhibits an antifungal activity against *F. graminearum* by inducing oxidative stress, apoptosis, epigenetics alterations and reduced toxin synthesis. *European Journal of Plant Pathology*, *160*(3), 611–622. https://doi.org/10.1007/s10658-021-02269-w

Toh, S. C., Samuel, L., & Awang, A. S. A. H. (2016). Screening for antifungal-producing bacteria from Piper nigrum plant against Phytophthora capsici. *International Food Research Journal*, *23*(6), 2616-2622.

Tufan, F., Uçarlı, C., Tunalı, B., & Gürel, F. (2017). Analysis of early events in barley (*Hordeum vulgare* L.) roots in response to *Fusarium culmorum* infection. *European Journal of Plant Pathology*, *148*, 343–355. https://doi.org/10.1007/s10658-016-1087-3

Uluhan, E., Keleş, E. N., & Tufan, F. (2019). Analysis of WRKY transcription factors in barley cultivars infected with *Fusarium culmorum*. *International Journal of Life Sciences and Biotechnology*, *2*, 165–174. https://doi.org/10.38001/ijlsb.588730

Wu, Y., Yuan, J., Raza, W., Shen, Q., & Huang, Q. (2014). Biocontrol traits and antagonistic potential of *Bacillus amyloliquefaciens* strain NJZJSB3 against *Sclerotinia sclerotiorum*, a causal agent of canola stem rot. Journal of Microbiology and Biotechnology, 24(10), 1327–1336.

Wu, Y., Zhou, J., Li, C., & Ma, Y. (2019). Antifungal and plant growth promotion activity of volatile organic compounds produced by *Bacillus amyloliquefaciens*. *MicrobiologyOpen*, *8*(8), e813. https://doi.org/10.1002/mbo3.813

Yörük, E. (2018). Tetraconazole leads to alterations in *Fusarium graminearum* at different molecular levels. *Applied Ecology and Environmental Research*, *6*, 6155–6167. http://dx.doi.org/10.15666/aeer/1605_61556167

Zalila-Kolsi, I., Mahmoud, A. B., Ali, H., Sellami, S., Nasfi, Z., Tounsi, S., & Jamoussi, K. (2016) Antagonist effects of *Bacillus* spp. strains against *Fusarium graminearum* for protection of durum wheat (*Triticum turgidum* L. subsp. durum). *Microbiology Research*, *192*, 148–158. https://doi.org/10.1016/j.micres.2016.06.012

Zhao, Y., Selvaraj, J. N., Xing, F. et al. (2014) Antagonistic action of *Bacillus subtilis* strain SG6 on *Fusarium graminearum*. *PLoS ONE*, *9*(3), e92486. https://doi.org/10.1371/journal.pone.0092486

Zhou, Y., Chen, J., Zhu, X., Wang, Y., Liu, X., Fan, H,, Duan, Y., & Chen, L. (2020). Efficacy of *Bacillus megaterium* strain Sneb207 against soybean cyst nematode (*Heterodera glycines*) in soybean. *Pest Management Science*, *77*(1), 568–576. https://doi.org/10.1002/ps.6057