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IN VIVO TESTING OF LACTIC ACID BACTERIA AS BIOCONTROL AGENTS AGAINST *B. cinerea* ON WHITE GRAPE BERRIES

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Abstract

The aim of this study was to test three strains of lactic acid bacteria (LAB): Lactobacillus fermentum MI204, Lactobacillus plantarum MI207 and Pediococcus pentosaceus MI213 in order to establish their potential to inhibit the development of the gray mold, Botrytis cinerea, on the surface of white grape berries to extend their shelf life during the storage period. The experiments involved the treatment of grape berries with LABs suspension, followed by their artificial contamination with B. cinerea spores. The berries were than stored and sampled every 24 hours for determining the load of B. cinerea and LABs by culture-based techniques and by qPCR. The L. plantarum MI207 strain was shown to be effective in inhibiting the development of the B. cinerea fungus on the surface of white grapes. Furthermore, all three LAB strains were able to maintain their viability and multiply on the surface of the grape berries during the storage period. The qPCR technique proved to be effective in quantifying the genetic material from both B. cinerea and the three strains of LAB in a shorter time compared to the use of the culture-based methods.

Key words: *lactic acid bacteria, Botrytis cinerea, biocontrol, qPCR.*

INTRODUCTION

Grapes are among the most consumed fruits around the world. The main purpose of the global grape production is wine-making, accounting for 57% of total production, while 36% is used for table grapes and 7% for dried grapes. Furthermore, the demand for grapes and grape products is increasing due to the associated health benefits, including reduced risks of cardiovascular diseases, type 2 diabetes, certain cancers, and other chronic complications (Zhou & Raffoul, 2012; Perestrelo et al., 2014; Rathi & Rajput, 2014; Insanu et al., 2021). Yearly, *B. cinerea* causes disastrous losses in grape production, typically reducing yields by 20-30%, and in severe cases losses can reach up to 50% of the total yield (Shen et al., 2021).

To preserve their freshness and quality, grapes require special storage conditions, and the management of humidity and temperature is crucial to prevent infections with different fungi, among which the most common is *Botrytis cinerea*, also known as the gray mold (Fedele et al., 2020). *B. cinerea* can colonize over 500 plant species (Williamson et al., 2007), the most severely affected being vegetables such as cucumbers, tomatoes and zucchini, but also fruits such as strawberries, grapes or raspberries (Cheung et al., 2020).

Table grapes must be kept at low temperatures (0-2°C) and at a relative humidity of 90-95% to prevent dehydration, maintain texture, slow down metabolic processes and prevent the development of mold and other microorganisms. Adherence to proper storage and hygiene practices is essential to prevent and control *Botrytis* infections (Ky et al., 2012; Zhang et al., 2018).

B. cinerea is a spoilage fungus difficult to control, which is why its chemical control remains the main way of inhibiting its development. Classically, the use of synthetic chemical fungicides such as iprodione, fenheaxmid, pyrimethanil, benzimidazoles, triazoles, dithiocarbamates and strobilurines are among the main management method to prevent gray mold (Diguță et al., 2016; Xu et al., 2021; Boiu-Sicuia et al., 2023). However, due to the risk of fungicide resistance or growing food safety concerns associated with fungicide residues on fresh fruits and crops that could harm consumer health in the long term

(AbuQamar et al., 2017), researchers are making increasing efforts to find alternative and safe methods to combat fungal growth.

Hence, biopreservation refers to the extended shelf life and increased safety of food and raw materials obtained by using natural microflora or by using antimicrobial products produced by certain microorganisms (Schnürer & Magnusson, 2005). Among the microorganisms most often used as biological control agents are various species of *Bacillus*: *B. subtilis*, *B. amyloliquefaciens* and *B. velezensis*; *Trichoderma*: *T. atroviride*, *T. harzianum*, *T. asperellum* and *T. gamsii*; and *Pseudomonas* spp. (Sicuia et al., 2015[a]; Sicuia et al., 2015[b]; Mondello et al., 2018; Proca et al., 2020; Lahlali et al., 2022; Boiu-Sicuia et al., 2023; Toma et al., 2023).

Lactic acid bacteria (LAB), mainly known for their probiotic potential (Diguță et al., 2020; Coulibaly et al., 2023; Kouadio et al., 2024), are used as natural biopreservatives in food, in animal feed, in sauerkraut and silage (Voulgari et al., 2010; Badea et al., 2022). Their preservative effect is mainly due to the formation of organic acids and hydrogen peroxide, competition for nutrients and the production of antimicrobial substances (Stiles, 1996; Pristavu et al., 2022).

In recent years, numerous studies have turned their attention to the use of lactic acid bacteria as biological control agents (De Simone et al., 2021; Dopazo et al., 2022; Wafula et al., 2022). LABs are known for their ability to produce numerous antifungal compounds, such as organic acids (lactic, acetic, formic, citric and succinic acid, phenyllactic acid) (Zalán et al., 2010; Yoo et al., 2016; Badea et al., 2022), hydroxyl-fatty acids (Kanauchi, 2019), various protein compounds (Schnürer & Magnusson, 2005) and reuterin (Ortiz-Rivera et al., 2017).

In this context, this study involved the *in vivo* evaluation of three strains of LAB for their potential to inhibit the development of the fungus *B. cinerea* in white grape berries.

MATHERIALS AND METHODS

Microorganisms and growth conditions

Three strains of LAB were used in this study: *Lactobacillus fermentum* MI204, *Lactobacillus plantarum* MI207, and *Pediococcus pentosaceus* MI213. These three strains of LAB have previously been shown by *in vitro* tests to have activity against *B. cinerea*. All of them are belonging to the microorganism collection of UASVM Bucharest. The strains were stored under freezing at -20°C, in MRS broth medium (Man, Rogosa and Sharpe, Oxoid, Limited, Hampshire, United Kingdom) containing 20% glycerol. For the revitalization, the LABs were cultivated in MRS broth medium at 37°C for 24 hours. The fungal strain used was *B. cinerea* MI Aligote Husi, grown on potato dextrose agar (PDA, Alliance Bio Expertise, France) for 2 weeks at 25°C.

Sample preparation

Studied LAB strains were tested for antifungal potential against *B. cinerea* on white grape berries, according to the following protocol.

White table grapes were purchased from a local supermarket, and the berries were separated from the grape brunches by cutting the pedicel, ensuring that it remained attached to the berry. The surface sterilization of the berries was performed by immersion in a 1% sodium hypochlorite solution for 5 minutes, followed by 3 washes with sterile distilled water (Lazo-Javalera et al., 2016). The berries were than sprayed with a sterile suspension of 0.2% sodium carboxymethylcellulose (CMC) and dried under sterile conditions in a laminar flow hood to form a protective coating (Li et al., 2020). Afterwards, the grapes were sprayed with suspensions of viable LABs, and one of the samples was left untreated, representing the control. The berries were then distributed in sterile polypropylene boxes, and a cutting was made on the epicarp area in which *B. cinerea* spores with a concentration of 2.3×10^3 spores mL^{-1} were inoculated. The samples were distributed according to Table 1.

In order to quantify the amount of *B. cinerea* and LABs on the surface of artificially contaminated grapes, three berries were taken in 3 mL of sterile distilled water, after which they were vigorously shaken to retrieve the LABs cells and *B. cinerea* spores and hyphae from their surface. The suspension thus obtained was used in subsequent analyses. Samples were taken at time 0 and after 24, 48 and 72 hours.

Table 1. Working protocol for artificial inoculation with LABs and *B. cinerea* of grape berries

Sample code	MI204	MI207	MI213	*Botrytis cinerea*
		log_{10} cells mL^{-1}		log_{10} spores mL^{-1}
204	8.29	-	-	-
207	-	8.31	-	-
213	-	-	8.49	-
204Bc	8.29	-	-	3.36
207Bc	-	8.31	-	3.36
213Bc	-	-	8.49	3.36
Bc	-	-	-	3.36

Culture-based method to quantify microorganisms

The quantification of microorganisms by classical techniques involves the use of specific culture media. For lactic acid bacteria, we used MRS Agar medium supplemented with cycloheximide, applying the incorporation technique detailed by Diguță & Matei (2020). The plates were incubated at 30°C for 48 hours. The results were expressed as decimal logarithms of colony-forming units per milliliter of sample (log_{10} CFU mL^{-1}).

Quantification of *B. cinerea* and LABs by qPCR

DNA extraction

The microbial DNA was extracted from *B. cinerea* MI Aligote Husi and *L. fermentum* MI204, using a commercial Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Germany) according to the protocol described by the manufacturer. The DNA quantification was performed using a SpectraMax® QuickDrop™ Micro-Volume Spectrophotometer (Molecular Devices, San Jose, CA, USA), then diluted tenfold and quantified by a qPCR Rotor Gene 6000 (Corbett Research, Australia).

qPCR amplification

To create both standard curves, the DNA was diluted tenfold, ranging from 5.50 µg mL^{-1} to 550 fg mL^{-1} for *B. cinerea* and 12.40 µg mL^{-1} to 124 pg mL^{-1} for *L. fermentum*. All dilutions were made in duplicate. Specific primers for *B. cinerea* (Bc3F and Bc3R, according to Carisse et al., 2014) and specific primers or LABs (LacF and LacR, according to Ritchie et al., 2010) were used in this study. The qPCR was performed in a final volume of 25 µL with 5 µL DNA sample, 0.075 µL (0.3 mM) of each specific primers for *B. cinerea* or LABs, 12.5 µL of Maxima SYBER GREEN/ROX qPCR MasterMix (2X, Thermo-Scientific, Baltics, UAB, Vilnius, Lithuania) and water. The qPCR reactions were performed in qPCR Rotor Gene 6000 (Corbett Research, Australia) following the programs: 3 min at 95°C, followed by 40 cycles of 15 s at 95°C and 30 s at 62°C (Al Zaidi et al., 2023). At the end, a melting curve was created by dropping the temperature from 90°C by 0.5°C every 10 s. To quantify the DNA from LAB strains, the program recommended by Uțoiu et al. (2018) was used: 10 min at 95°C for the initial denaturation, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. The efficiency (E) of the qPCR assay was calculated using the formula $E = (10^{-1/slope} - 1) \times 100$, where the slope was extracted from the curve Ct = f (log Q0), where Ct is the cycle where the fluorescence first occurs, and Q0 is the amount of initial DNA. The efficiency was expressed as a percentage. Subsequently, DNA samples of grapes were submitted to qPCR, and the quantity of *B. cinerea* and LABs DNA was calculated in accordance with the standard curve that was generated for this purpose.

Statistical analysis

The results were obtained by averaging three independent experiments. The results were represented as mean ± standard deviation.

RESULTS AND DISCUSSIONS

Culture-based method to detect LABs

Classical microbial quantification techniques offer a simple and cost-effective means of determining the number of viable microorganisms in a sample by cultivating and counting them on specific culture media. In order to observe the evolution of the number of viable LABs on the surface of grape berries during storage, we utilized the classical method with MRS Agar

culture medium, applying the incorporation technique.

The macroscopic differences between the grape samples treated with LABs suspensions compared to the untreated ones after 72 hours of storage (Figure 1) suggest that the LABs suspensions had an inhibitory effect on *B. cinerea*, the fungus being slightly visible on the surface of the artificially contaminated samples, treated with the LABs suspensions (204Bc, 207Bc and 213Bc) after 72 hours of storage, compared to the untreated control sample (Bc), where the fungus grew at an accelerated rate.

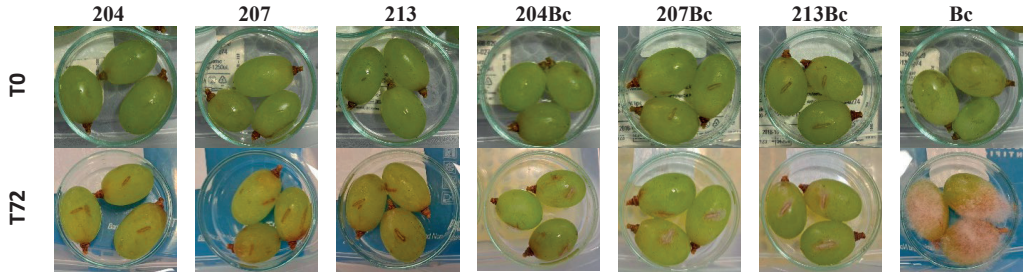

Figure 1. Macroscopic differences between the grape samples treated with LABs suspensions and uncontaminated with *B. cinerea* (204, 207, 213), the grape samples treated with LABs suspensions and artificially contaminated with *B. cinerea* (204Bc, 207Bc, 213Bc) and the grape sample untreated with LABs suspensions and artificially contaminated with *B. cinerea* (Bc), in the initial moment (T0) and after 72 hours (T72)

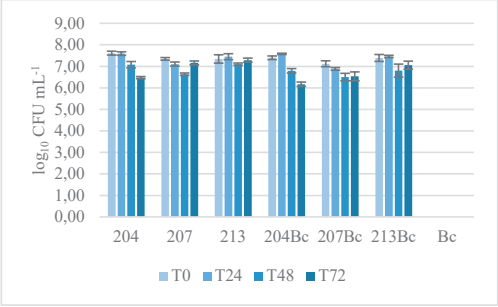

Figure 2. Quantification of LABs by the culture-based method on artificially contaminated grapes

The LABs successfully adapted on the surface of white grapes (Figure 2), maintaining a concentration around 7 $\log_{10}$ CFU mL^{-1} throughout the storage period, grapes being a native source of LABs, thus providing a favorable environment for their development (Franco et al., 2021). Comparable results were obtained by Yin et al. (2022) in a similar experiment on strawberries, where the LABs population remained constant on the surface of the fruits during the 7 days of storage.

PCR-based method to detect *B. cinerea* and LABs

In order to be able to measure the amount of DNA present in a sample using the qPCR technique, it was necessary to create standard curves by diluting the DNA extracted from *B. cinerea*, respectively from *L. fermentum* and amplifying the dilutions by qPCR, following that the Ct values thus obtained, together with the complementary logarithm of the DNA concentrations, to be transformed into standard curves (Figures 3 and 4).

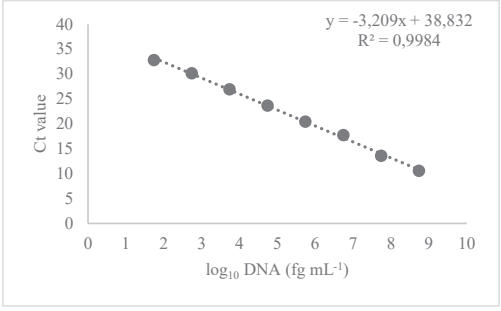

Figure 3. Standard curve generated from the amplification of *B. cinerea* DNA

Both standard curves show a high linearity due to the R^2 values close to 1 (0.999 and 0.995), resulting in a low inter-assay variability. The slopes of the standard curves were -3.209 and -3.454, corresponding to an amplification efficiency of 104.94% and 94.77% respectively, both values falling within the range of 90-110%, limit values established by the literature (Broeders et al., 2014).

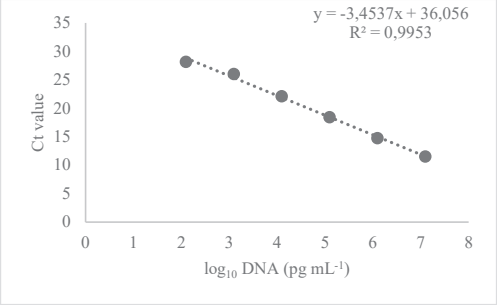

Figure 4. Standard curve generated from the amplification of *L. fermentum* DNA

Under these conditions, the maximum C_t value that could be used for *B. cinerea* was 38, corresponding to a DNA concentration of 1.81 fg, and for LABs, the maximum C_t number was 36, corresponding to a DNA concentration of 1.03 pg.

The qPCR method is a fast and efficient way to quantify DNA, through which we can determine even the lowest concentrations of DNA in a sample. In the present study we applied this method to determine the amount of DNA present on the artificially contaminated grape berries from both *B. cinerea* and the three LAB strains, the results being presented in the following.

The concentration of *B. cinerea* DNA in each grape sample is presented in Figure 5. The results indicate that the LAB strains effectively inhibited the development of *B. cinerea* on the surface of the artificially contaminated grape berries, the DNA concentrations being lower on the artificially contaminated grapes samples treated with LAB strains (204Bc, 207Bc and 213Bc) compared to the untreated control sample (Bc).

It can also be seen that the strain of *L. plantarum* MI207 showed the best efficiency in inhibiting the development of the *B. cinerea* fungus on the surface of the grape berries, the DNA concentration value after 72 hours of storage being $\log_{10}$ 1.32 fg mL^{-1} DNA, compared to $\log_{10}$ 6.49 fg mL^{-1} DNA in the case of the control sample, being also more efficient compared to the other two strains of LAB used in this study, where the DNA concentration after 72 hours of storage was around $\log_{10}$ 3.5 fg mL^{-1} DNA. Recently, similar results were obtained by Chen et al. (2022), who conducted the study in which they demonstrated the effectiveness of the *L. plantarum* CM-3 strain to inhibit the development of the fungus *B. cinerea* on the surface of black grape berries. In another study, the authors demonstrated the effectiveness of the same *L. plantarum* CM-3 strain against *B. cinerea* development on strawberries surface (Chen et al., 2020).

The qPCR method was also used to determine the amount of DNA from lactic acid bacteria on the surface of artificially contaminated grapes to see if they managed to remain viable during storage.

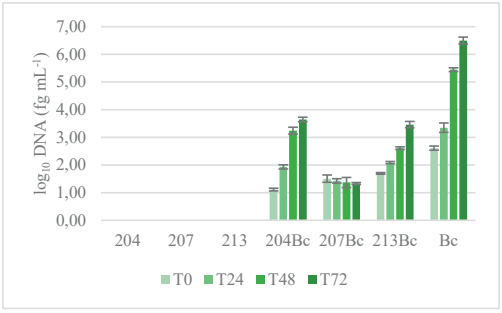

Figure 5. *B. cinerea* DNA concentration detected on the grape samples by the qPCR method, expressed in $\log_{10}$ DNA (fg mL^{-1})

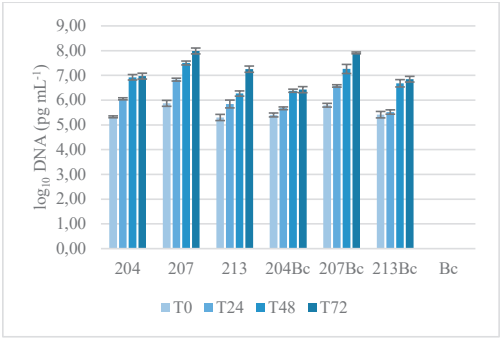

Figure 6. LAB DNA concentration detected on the grape samples by the qPCR method, expressed in $\log_{10}$ DNA (pg mL^{-1})

Compared to the results obtained by quantification using the culture-based method (Figure 2), in the case of DNA quantification by qPCR (Figure 6) we can observe an increase in the concentration of DNA from the LAB strains in the samples, an increase that could be due to the fact that the culture-based method can only quantify cells in a viable state, while the qPCR method quantifies both DNA from viable and non-viable cells.

However, the increase in the concentration of LABs DNA in the samples during the storage period suggests that, indeed, the LAB strains were able to survive and multiply on the surface of the grape berries, producing organic compounds with antifungal effect against *B. cinerea*. According to multiple studies, the qPCR method has been shown to be effective for the detection of LABs in several food matrices (Zwielehner et al., 2008; Kántor et al., 2014; Al-Zaidi et al., 2022; Jérôme et al., 2022). To improve the method and quantify only the amount of DNA from viable cells, in the following studies we can use photoactive dyes such as propidium monoazide (PMA), ethidium monoazide (EMA) or PEMAX, substances capable of binding to the nucleic acids of damaged or dead cells, thus allowing qPCR quantification of only viable cells (Daranas et al., 2018; Shi et al., 2022; Wang et al., 2023).

CONCLUSIONS

Maintaining the nutritional values and sensory properties of perishable fruits susceptible to fungal attack during storage is a significant challenge. The growing interest in organic and unprocessed food products has sparked the interest of scientists in seeking new methods of extending the shelf life of food products, thus avoiding chemical preservation methods. This study demonstrated that the *L. plantarum* MI207 strain effectively inhibited the growth of the fungus *B. cinerea* on the surface of white grapes while also maintaining its viability and multiplying on the grape berries during storage. Furthermore, the qPCR technique proved to be effective in quantifying the genetic material of both *B. cinerea* and the three LAB strains in a shorter time compared to culture-based methods. In this context, LABs offer a promising approach to be used as biocontrol agents, as they are naturally found in foods like fresh vegetables and fruits, are considered safe for human health, are widely utilized in the food industry, and also are producing antimicrobial compounds.

REFERENCES

AbuQamar, S., Moustafa, K., & Tran, L. S. (2017). Mechanisms and strategies of plant defense against *Botrytis cinerea*. *Critical Reviews in Biotechnology*, *37*(2), 262-274.

Al Zaidi, Q., Badea, F., Diguță, C. F., & Matei, F. (2023). Comparison of classical versus qPCR methods in the detection of *Botrytis cinerea* in raspberries. *Scientific Bulletin Series F. Biotechnologies*, *27*(1).

Al-Zaidi, Q., Diguță, C. F., Dopcea, G., & Matei, F. (2022). Conventional versus modern techniques used for the detection of pathogens in food matrices: a review. *Scientific Bulletin Series F. Biotechnologies*, *26*(1), 79-88.

Badea, F., Diguță, C. F., & Matei, F. (2022). The use of lactic acid bacteria and their metabolites to improve the shelf life of perishable fruits and vegetables. *Sci. Bull. Series F. Biotechnologies*, *26*(1), 117-125.

Boiu-Sicuia, O. A., Toma, R. C., Diguță, C. F., Matei, F., & Cornea, C. P. (2023). *In vitro* evaluation of some endophytic *Bacillus* to potentially inhibit grape and grapevine fungal pathogens. *Plants*, *12*(13), 2553.

Broeders, S., Huber, I., Grohmann, L., Berben, G., Taverniers, I., Mazzara, M., ... & Morisset, D. (2014). Guidelines for validation of qualitative real-time PCR methods. *Trends in Food Science & Technology*, *37*(2), 115-126.

Carisse, O., Tremblay, D. M., & Lefebvre, A. (2014). Comparison of *Botrytis cinerea* airborne inoculum progress curves from raspberry, strawberry and grape plantings. *Plant Pathology*, *63*(5), 983-993.

Chen, C., Cao, Z., Li, J., Tao, C., Feng, Y., & Han, Y. (2020). A novel endophytic strain of *Lactobacillus plantarum* CM-3 with antagonistic activity against *Botrytis cinerea* on strawberry fruit. *Biological Control*, *148*, 104306.

Chen, C., Zhang, X., Wei, X., Zhu, Y., Chen, W., & Han, Y. (2022). Postharvest biological control of *Botrytis cinerea* and the mechanisms underlying the induction of disease resistance in grapes by *Lactobacillus plantarum* CM-3. *Biological Control*, *172*, 104982.

Cheung, N., Tian, L., Liu, X., & Li, X. (2020). The destructive fungal pathogen *Botrytis cinerea*—Insights from genes studied with mutant analysis. *Pathogens*, *9*(11), 923.

Coulibaly, W. H., Kouadio, N. G. R., Camara, F., Diguță, C., & Matei, F. (2023). Functional properties of lactic acid bacteria isolated from Tilapia (*Oreochromis niloticus*) in Ivory Coast. *BMC Microbiology*, *23*(1), 152.

Daranas, N., Bonaterra, A., Francés, J., Cabrefiga, J., Montesinos, E., & Badosa, E. (2018). Monitoring viable cells of the biological control agent *Lactobacillus plantarum* PM411 in aerial plant surfaces by means of a strain-specific viability quantitative PCR method. *Applied and Environmental Microbiology*, *84*(10), e00107-18.

De Simone, N., Capozzi, V., de Chiara, M. L. V., Amodio, M. L., Brahimi, S., Colelli, G., ... & Russo, P. (2021). Screening of lactic acid bacteria for the bio-control of *Botrytis cinerea* and the potential of *Lactiplantibacillus plantarum* for eco-friendly preservation of fresh-cut kiwifruit. *Microorganisms*, *9*(4), 773.

Diguță, C. F., & Matei, F. (2020). *Microbiologie Generală - Tehnici de laborator*. Bucharest, RO: Ex Terra Aurum.

Diguță, C. F., Matei, F., & Cornea, C. P. (2016). Biocontrol perspectives of *Aspergillus carbonarius*, *Botrytis cinerea* and *Pencillium expansum* on grapes using epiphytic bacteria isolated from Romanian vineyards. *Romanian Biotechnological Letters*, *21*(1), 11126-11132.

Diguță, C. F., Nițoi, G. D., Matei, F., Luță, G., & Cornea, C. P. (2020). The biotechnological potential of *Pediococcus* spp. isolated from Kombucha microbial consortium. *Foods*, *9*(12), 1780.

Dopazo, V., Luz, C., Quiles, J. M., Calpe, J., Romano, R., Mañes, J., & Meca, G. (2022). Potential application of lactic acid bacteria in the biopreservation of red grape from mycotoxigenic fungi. *Journal of the Science of Food and Agriculture*, *102*(3), 898-907.

Fedele, G., Brischetto, C., & Rossi, V. (2020). Biocontrol of *Botrytis cinerea* on grape berries as influenced by temperature and humidity. *Frontiers in Plant Science*, *11*, 1232.

Franco, W., Benavides, S., Valencia, P., Ramírez, C., & Urtubia, A. (2021). Native yeasts and lactic acid bacteria isolated from spontaneous fermentation of seven grape cultivars from the maule region (Chile). *Foods*, *10*(8), 1737.

Insanu, M., Karimah, H., Pramastya, H., & Fidrianny, I. (2021). Phytochemical compounds and pharmacological activities of *Vitis vinifera* L.: An Updated Review. *Biointerface Research in Applied Chemistry, 11*(5), 13829–13849.

Jérôme, M., Passerini, D., Chevalier, F., Marchand, L., Leroi, F., & Macé, S. (2022). Development of a rapid qPCR method to quantify lactic acid bacteria in cold-smoked salmon. *International Journal of Food Microbiology*, *363*, 109504.

Kanauchi, M. (2019). Screening the lactic acid bacteria converting hydroxy fatty acid from unsaturated fatty acid. *Methods in Molecular Biology*, *1887,* 119-127.

Kántor, A., Kačániová, M., Petrová, J., Medo, J., Hleba, L., & Rovná, K. (2014). Identification of lactic acid bacteria isolated from red wine samples by RT-qPCR. *Journal of Microbiology, Biotechnology and Food Ssciences*, *3*(1), 235-237

Kouadio, N. J., Zady, A. L. O., Kra, K. A. S., Diguță, F. C., Niamke, S., & Matei, F. (2024). *In vitro* probiotic characterization of *Lactiplantibacillus plantarum* strains isolated from traditional fermented dockounou paste. *Fermentation*, *10*(5), 264.

Ky, I., Lorrain, B., Jourdes, M., Pasquier, G., Fermaud, M., Gény, L., ... & Teissedre, P. L. (2012). Assessment of grey mould (*Botrytis cinerea*) impact on phenolic and sensory quality of Bordeaux grapes, musts and wines for two consecutive vintages. *Australian Journal of Grape and Wine Research*, *18*(2), 215-226.

Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., ... & Barka, E. A. (2022). Biological control of plant pathogens: A global perspective. *Microorganisms*, *10*(3), 596.

Lazo-Javalera, M. F., Troncoso-Rojas, R., Tiznado-Hernández, M. E., Martínez-Tellez, M. A., Vargas-Arispuro, I., Islas-Osuna, M. A., & Rivera-Domínguez, M. (2016). Surface disinfection procedure and in vitro regeneration of grapevine (*Vitis vinifera* L.) axillary buds. *SpringerPlus*, *5*, 1-9.

Li, S., Ma, Y., Ji, T., Sameen, D. E., Ahmed, S., Qin, W., ... & Liu, Y. (2020). Cassava starch/carboxymethylcellulose edible films embedded with lactic acid bacteria to extend the shelf life of banana. *Carbohydrate Polymers*, *248*, 116805.

Mondello, V., Songy, A., Battiston, E., Pinto, C., Coppin, C., Trotel-Aziz, P., ... & Fontaine, F. (2018). Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Disease*, *102*(7), 1189-1217.

Ortiz-Rivera, Y., Sánchez-Vega, R., Gutiérrez-Méndez, N., León-Félix, J., Acosta-Muñiz, C., & Sepulveda, D. R. (2017). Production of reuterin in a fermented milk product by *Lactobacillus reuteri*: Inhibition of pathogens, spoilage microorganisms, and lactic acid bacteria. *Journal of Dairy Science, 100*(6), 4258-4268.

Perestrelo, R., Silva, C., Pereira, J., & Câmara, J. S. (2014). Healthy effects of bioactive metabolites from *Vitis vinifera* L. grapes: A review. In J. S. Câmara (Ed.). *Grapes: Production, phenolic composition and potential biomedical effects* (pp. 305-338). Nova Science Publishers

Pristavu, M. C., Diguță, C., Coulibaly, W. H., Fanche, S. A. Y., Dopcea, G., & Matei, F. (2022). A review of postbiotics as new health promoters. *AgroLife Scientific Journal*, *11*(2), 142-152.

Proca, I. G., Diguță, C. F., Cornea, C. P., Jurcoane, S., & Matei, F. (2020). Halotolerant *Bacillus amyloliquefaciens* 24.5. *Romanian Biotechnological Letters*, *25*(4), 1744-1753.

Rathi, P., & Rajput, C. S. (2014). Antioxidant potential of grapes (Vitis vinifera): A review. *Journal of Drug Delivery and Therapeutics*, *4*(2), 102–104.

Ritchie, L. E., Burke, K. F., Garcia-Mazcorro, J. F., Steiner, J. M., & Suchodolski, J. S. (2010). Characterization of fecal microbiota in cats using universal 16S rRNA gene and group-specific primers for *Lactobacillus* and *Bifidobacterium* spp. *Veterinary Microbiology*, *144*(1-2), 140-146.

Schnürer, J., & Magnusson, J. (2005). Antifungal lactic acid bacteria as biopreservatives. *Trends in Food Science & Technology*, *16*(1-3), 70-78.

Shen, F., Wu, W., Han, X., Wang, J., Li, Y., & Liu, D. (2021). Study on the occurrence law and green control of grape gray mold from the perspective of ecological balance. *Bioengineered*, *12*(1), 779-790.

Shi, Z., Li, X., Fan, X., Xu, J., Liu, Q., Wu, Z., & Pan, D. (2022). PMA-qPCR method for the selective quantitation of viable lactic acid bacteria in fermented milk. *Frontiers in Microbiology*, *13*, 984506.

Sicuia, O. A., Grosu, I., Constantinescu, F., Voaideș, C., & Cornea, C. P. (2015[a]). Enzymatic and genetic variability in *Bacillus* spp. strains with plant beneficial qualities. *AgroLife Scientific Journal*, *4*(2).

Sicuia, O.A., Constantinscu, F., & Cornea, C.P. (2015[b]). Biodiversity of *Bacillus subtilis* group and beneficial traits of Bacillus species useful in plant protection. *Romanian Biotechnological Letters*, *20*(5), 10737-10750.

Stiles, M. E. (1996). Biopreservation by lactic acid bacteria. *Antonie van Leeuwenhoek*, *70*, 331-345.

Toma, R. C., Boiu-Sicuia, O. A., Diguță, F. C., Ciucă, M., Matei, F., & Cornea, C. P. (2023). Selected plant protection *Bacillus* strains increase food safeness by inhibiting human pathogenic bacteria. *Romanian Agricultural Research*, *40*, 36.

Uțoiu, E., Matei, F., Toma, A., Diguță, C. F., Ștefan, L. M., Mănoiu, S., ... & Oancea, F. (2018). Bee collected pollen with enhanced health benefits, produced by fermentation with a Kombucha Consortium. *Nutrients*, *10*(10), 1365.

Voulgari, K., Hatzikamari, M., Delepoglou, A., Georgakopoulos, P., Litopoulou-Tzanetaki, E., & Tzanetakis, N. (2010). Antifungal activity of non-starter lactic acid bacteria isolates from dairy products. *Food Control*, *21*(2), 136-142.

Wafula, E. N., Muhonja, C. N., Kuja, J. O., Owaga, E. E., Makonde, H. M., Mathara, J. M., & Kimani, V. W. (2022). Lactic acid bacteria from african fermented cereal-based products: Potential biological control agents for mycotoxins in Kenya. *Journal of Toxicology*, *2022*(1), 2397767.

Wang, J., Wei, B., Chen, Z., Chen, Y., Liu, S., Zhang, B., ... & Ye, D. (2023). A rapid and reliable method for the determination of *Lactiplantibacillus plantarum* during wine fermentation based on PMA-CELL-qPCR. *Frontiers in Microbiology*, *14*, 1154768.

Williamson, B., Tudzynski, B., Tudzynski, P., & Van Kan, J. A. (2007). *Botrytis cinerea*: the cause of grey mould disease. *Molecular plant pathology*, *8*(5), 561-580.

Xu, Y., Wang, L., Liang, W., & Liu, M. (2021). Biocontrol potential of endophytic *Bacillus velezensis* strain QSE-21 against postharvest grey mould of fruit. *Biological Control*, *161*, 104711.

Yin, H. B., Chen, C. H., Colorado-Suarez, S., & Patel, J. (2022). Biocontrol of Listeria monocytogenes and Salmonella enterica on fresh strawberries with lactic acid bacteria during refrigerated storage. *Foodborne Pathogens and Disease*, *19*(5), 324-331.

Yoo, J. A., Lim, Y. M., & Yoon, M. H. (2016). Production and antifungal effect of 3-phenyllactic acid (PLA) by lactic acid bacteria. *Journal of Applied Biological Chemistry*, *59*(3), 173-178.

Zalán, Z., Hudáček, J., Štětina, J., Chumchalová, J., & Halász, A. (2010). Production of organic acids by *Lactobacillus* strains in three different media. *European Food Research and Technology*, *230*, 395-404.

Zhang, Y., Li, X., Shen, F., Xu, H., Li, Y., & Liu, D. (2018). Characterization of *Botrytis cinerea* isolates from grape vineyards in China. *Plant Disease*, *102*(1), 40-48.

Zhou, K., & Raffoul, J. J. (2012). Potential anticancer properties of grape antioxidants. *Journal of Oncology*, *2012*(1), 803294.

Zwielehner, J., Handschur, M., Michaelsen, A., Irez, S., Demel, M., Denner, E. B., & Haslberger, A. G. (2008). DGGE and real-time PCR analysis of lactic acid bacteria in bacterial communities of the phyllosphere of lettuce. *Molecular Nutrition & Food Research*, *52*(5), 614-623.