Scientific Bulletin. Series F. Biotechnologies, Vol. XXV, No. 2, 2021
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

EFFECTS OF EXTRACTS FROM *PASSIFLORA CAERULEA* LEAVES TREATED WITH A *TRICHODERMA* BIOSTIMULANT CONSORTIUM ON LACTIC ACID BACTERIA

Elena UȚOIU[1, 2], Iulia RĂUT[3], Florin OANCEA[3, 1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, District 1, Bucharest, Romania
[2]National Institute for Research and Development in Biological Sciences,
296 Splaiul Independenței, District 6, 060031, Bucharest, Romania
[3]National Institute for Research & Development in Chemistry and Petrochemistry - ICECHIM,
202 Splaiul Independenței, District 6, 060021, Bucharest, Romania

Corresponding author email: florin.oancea@icechim.ro

Abstract

The alcoholic extracts from Passiflora caerulea leaves protect prebiotic lactic acid bacteria (LAB) against oxidative stress induced by (micro) aerobic conditions. This prebiotic activity is mainly related to the polyphenols accumulated in P. caerulea leaves. Polyphenols are used by prebiotic LAB as electron acceptors of their carbohydrate catabolic chains and promote LAB adaptation to aerobic conditions. Foliar treatment with 10^8 cfu per ml chlamydospores of a Trichoderma plant biostimulants consortium enhances the accumulation of polyphenols in the P. caerulea leaves. The Trichoderma plant biostimulant consortium includes two strains, T. asperellum T36b, and T. harzianum Td50b. Enhanced levels of polyphenols are related to the enhanced prebiotic effect of the extracts from leaves treated with Trichoderma consortium, compared with the control leaves from P. caerulea non-treated with plant biostimulants. The results are discussed in relation to the known physiological effects of P. caerulea leaves extracts on mood and sleep disorders, which could also be explained by the probiotic bacteria's postbiotic effects, promoted by polyphenols from leaves extracts.

Key words*: Passiflora caerulea, Trichoderma-based plant biostimulants, lactic acid bacteria, polyphenols, leaves extracts*

INTRODUCTION

Extracts from plant tissues rich in polyphenols usually exert significant antibacterial effects. There were describes several mechanisms of action of polyphenols on bacteria: disruption of the integrity of cell wall (Nohynek et al., 2006; Zhao et al., 2001) and cellular membranes (Ollila et al., 2002; Perumal et al., 2017); interactions with ion channels and membrane receptors (Alvarez-Martinez et al., 2020; Taylor et al., 2005); inhibition of final stages of catabolism and energy production (Chinnam et al., 2010; Liu et al., 2017); precipitation of the functional proteins (Kang et al., 2006; Nakayama et al., 2013); inhibition of DNA synthesis (Mickymaray et al., 2020; Wang et al., 2017); interferences with quorum-sensing signals and biofilm formation (Gopu et al., 2015; Hengge, 2019), complexation of metabolic substrate and essential metal micronutrients (Daglia, 2012; Rajakovich & Balskus, 2019). However, polyphenols were proven in the last decade to enhance the development of lactic acid bacteria (Chan et al., 2018; Filannino et al., 2016). The main mechanisms of action are related to the lactic acid bacteria (LABs) ability to metabolize polyphenols (Gaur et al., 2020; Ricci et al., 2019). It was demonstrated that lactic acid bacteria could utilize the polyphenols as electron acceptors of their carbohydrate catabolic chains (Filannino et al., 2014). Also, LABs metabolize polyphenols to more bioactive compounds (Rodríguez et al., 2009). Lactic acid bacteria fermented plant materials with high polyphenols contents have an enhanced functionality due to mutual interactions between polyphenols and LABs (Piekarska-Radzik & Klewicka, 2021).

LABs' ability to metabolize the polyphenols differentiate the effects of the polyphenols to

gut microorganisms (Pacheco-Ordaz et al., 2018; Ziarno et al., 2021). Nowadays is primarily accepted that polyphenols promote the development of probiotic bacteria and are included in the new definition of prebiotics (Bindels et al., 2015), as a (metabolic) substrate for probiotic bacteria.

The aerial parts from *Passiflora* genus plants are a functional food (Zeraik et al., 2010), consumed as infusions, extracts, or tinctures. The main active ingredients of *Passiflora* leaves, which are involved in the passion flowers' effects against neuropsychiatric and metabolic disorders, are polyphenols (Angel-Isaza et al., 2021; Saravanan & Parimelazhagan, 2014). Our group recent work demonstrated that treatment with a *Trichoderma* consortium, which has an established plant biostimulant activity, enhances polyphenols' accumulation in the aerial parts of passion flowers (Şesan et al., 2020).

This work investigates the influence of extracts from *Trichoderma* treated and non-treated passionflower leaves on the development of lactic acid bacteria cultivated in aerobic conditions.

MATERIALS AND METHODS

Biological material. *Lactobacillus reuteri* DSM 20016 was cultivated in MRS (de Man, Rogosa, Share) broth and MRS Agar media (Oxoid, Thermo Fischer, Hampshire, UK), at 37°C, in microaerophilic conditions. A *Trichoderma* consortium was used for the treatment of the passionflower. This consortium includes two plant biostimulant strains from the INCDCP-ICECHIM collection, *T. asperellum* T36 NCAIM F 001434 and *T. harzianum* Td50b NCAIM F001412. These strains were selected because they produce bioactive compounds which stimulate plant growth (Oancea et al., 2016; Raut et al., 2014; Răut et al., 2016). *Passiflora caerulea* plants (blue passionflower) were grown in the Hofigal experimental field. This experimental field is established on a reddish preluvosol, and it is located south of Bucharest at 44°25'15" N, 26°1'34" E, altitude 84 m.

Application of treatment with the Trichoderma plant biostimulant consortium. A suspension of 10^8 cfu/mL chlamydospores was prepared (Şesan et al., 2020). Briefly, the fungal consortium was cultivated for two weeks days in a medium that promotes the chlamydospore accumulation in the presence of light. The composition of this medium is > 34.2 g/L glucose, 0.37 g/L ammonium sulfate, 0.8 g/L yeast extract, 2.7 g/L soymeal, 1.2 g/L K_2HPO_4, and 1.7 g/L KH_2PO_4 (Zamfiropol-Cristea et al., 2017). After two weeks, the chlamydospores were separated from the culture media in aseptic conditions and quantified by spore counting in a Thoma counting chamber (Aberkane et al., 2002). The experiment was organized with two treatments: Trichoderma 10^8cfu/mL and one treatment with water. Each treatment was applied in four repetitions, and each repetition consisted of 20 passion flowers plant selected to have a similar development.

The *Trichoderma* chlamydospores suspension was applied at the beginning of June 2019. A backpacker spraying unit (Solo SG71, Waiblingen, Germany) with an extension tube and a fan jet brass nozzle TeeJet. The spraying volume for each treatment was equivalent to 400 $L.ha^{-1}$. At the moment of treatment application, the *P. caerulea* plants were in the phenological phase of leaf development and flower primordium formation. After 30 days, 250 grams of leaves and sprouts with leaves (fresh weight) were randomly collected from each repetition.

The multi-annual average values for the experimental area where the experimental field is located are the following: temperature - 11.5°C, total precipitation: 615 mm; wind speed: 3.2 m s^{-1}; daily sunshine duration - 6.8 h. During the experimental period of 2020, the average monthly temperatures and precipitations were the following: May - 19.5°C and 72.4 mm; June - 23.5°C and 88.6 mm; July - 27.2°C and 22.7 mm. The soil was maintained at 80% water capacity by irrigation during the entire experiment.

Plant material extraction and determination of the total polyphenols and flavonoids. The plant leaves and sprouts with leaves were dried at 50°C. The dried passionflower material was ground in a laboratory mill Retsch SM2000 (Retsch GmbH, Haan, Germany) fitted with a 1

mm sieve. The resulting ground plant material was extracted in ethanol, 10 grams of dried, and ground plant material was extracted in 300 mL solution ethanol-water, for 60 min, at room temperature. Two ultrasound treatments (VCX 130, Ultrasonic Processor, Sonics, Newtown, CT, USA) of 5 min were applied, one of the beginnings of extraction treatment and the other after 30 min of extraction. The extract was separated by centrifugation at a relative centrifugal force of 3028 *x* g (Universal 320R, Hettich, Tuttlingen, Germania). The resulting extract was stored at 4°C in dark bottles till further use.

In the ethanolic extracts, we used the Folin-Ciocâlteu method (Huang et al., 2005), with some modifications (Craciunescu et al., 2012), for the total polyphenols determination. Briefly, 750 µL of Folin-Ciocâlteu reagent, 4 mL of 15% Na_2CO_3, and distilled water were added to 150 µL of the sample. The final volume was 15 mL. The incubation was done at room temperature. The optical density was measured after 2 h at λ = 756 nm, in a microplate, by using a multimode microplate reader (CLARIOstar Plus, BMG Labtech, Ortenberg, Germania). The total phenolic compounds (reacting with Folin-Ciocâlteu reagent) were expressed as gallic acid (GA) equivalents based on a calibration curve. This calibration curve was done with known concentrations of gallic acid.

We used the aluminum chloride colorimetric method to determine the total flavonoids (Chang et al., 2002). Briefly, 0.5 mL of sample was mixed with 1.5 mL ethanol, 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminum chloride, and 2.8 mL of distilled water. The incubation was done for 30 min at room temperature. The optical density was determined at λ = 415 nm in a microplate, using a multimode microplate reader (CLARIOstar Plus, BMG Labtech. The flavonoid content was expressed as quercetin (Q) equivalents, using a calibration curve constructed with known quercetin concentrations.

All of the analyses were done in triplicate. The reagents used were analytical-grade reagents purchased from Sigma-Aldrich (Merck Group, Darmstadt, Germany).

Antioxidant activity assay in the extracted plant material. Two different assays determined the antioxidant activity: DDPH• radical (2,2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging and TEAC (Trolox equivalent antioxidant capacity). The alcoholic extract of *P. caerulea* leaves and sprouts with leaves was evaporated while using a Rotavapor® R-300 (Büchi, Flawil, Switzerland). The exact quantities were re-solubilized using absolute ethanol.

We used for the DPPH• radical scavenging activity the method of Re et al. [55], with slight modifications. Briefly, 150 µL DPPH ethanolic solution (0.25 mM) was vigorously mixed with 15 µL of the sample (re-solubilized in absolute ethanol) and 90 µL of 0.1 M Tris-HCl buffer. We incubated in the dark the resulting mixture at 37 °C for 30 min. Butylated hydroxytoluene (BHT) was used as a positive control. The sample absorbance (A_{sample}) was read using a microplate multimode reader (CLARIOstar Plus, BMG Labtech) at λ = 520 nm, against a blank with ethanol (A_{blank}). DPPH inhibition (%) was calculated using the following equation:

$$\% \, Inhibition = (1 - A_{sample} / A_{blank}) * 100. \quad (1)$$

We measured the antioxidant capacity (TEAC) using the method of Re et al. [55], with slight modifications. Briefly, the ABTS [(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical was generated by the reaction of 7 mM 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) solution with a 2.45 mM potassium persulfate solution (1:1, *v*/*v*). We incubated the mixture in the dark at room temperature for 16 h. The initial optical density of the ABTS radical solution was equilibrated to a value of 0.7 ± 0.02 at λ = 734 nm. (CLARIOstar Plus, BMG Labtech). Next, a 0.1 mL test sample was mixed with 1 mL of the ABTS radical solution and then incubated for 6 min. After incubation, the optical density was measured at λ = 734 nm (CLARIOstar Plus, BMG Labtech). A calibration curve of Trolox (0–250 µM) was used to convert the absorbance into the equivalent activity of Trolox per mL sample (µg Trolox/mL).

All the assays were performed in triplicate. All of the reagents used were analytical-grade reagents purchased from Sigma-Aldrich (Merck Group).

Influence of passion flower extracts on L. reuteri growth. We inoculated 10^7 ufc/ml of *L. reuteri* in 5 mL test tubes with 2 mL MRS broth. Each of the inoculated tubes contained 5% ethanolic extracts from aerial parts of *P. caerulea*, treated or not treated with *Trichoderma*. The extracts were sterilized by filtration on a 0.2 µm filter before being added to the MRS broth. The test tubes were incubated for 48 h at 37°C, in (micro)aerobic conditions. The number of bacteria was determined by a cultural method. Serial dilutions were done in fresh MRS broth, and 0.1 mL aliquots were aseptically spread in MRS agar plates. MRS agar plated were incubated at 37°C for 48 hours, and the number of colonies was counted. We used the following controls: MRS medium without extract and bacteria, MRS medium with corresponding quantities of alcohol and bacteria, MRS medium and 5% ethanolic extracts from *Trichoderma* treated and not treated aerial parts of the passion flowers without bacteria.

Statistical analysis. The statistical analysis was performed using SigmaStat (Systat Software, San José, CA, USA). Averages and standard deviations of the data from each set of replicates were calculated. The results were expressed as means ± SE (standard error). The Student's t-test tested statistical differences between groups. The means are considered to be significantly different at $P < 0.05$.

RESULTS AND DISCUSSIONS

Foliar Treatments with chlamydospores from our *Trichoderma* plant biostimulant consortium stimulated the accumulation of polyphenols and flavonoids from passion flowers aerial parts (Figure 1).

This result confirms our group's previous report, which demonstrated a concomitant chloroplast proliferation and polyphenols accumulation in *P. caerulea* aerial parts treated with the *Trichoderma* plant biostimulant consortium (Şesan et al., 2020).

This effect of enhanced polyphenols accumulation is related to the plant defense's activation and related secondary metabolism pathways - i.e., the phenylpropanoid pathway (Sharma et al., 2019).

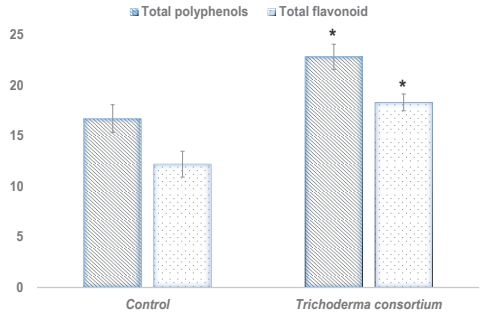

Figure 1. Enhanced accumulation of polyphenols and flavonoids following treatment with the plant biostimulant consortium *T. asperellum* T36 NCAIM F 001434 and *T. harzianum* Td50b NCAIM F001412

Activation of this phenylpropanoid pathway after *Trichoderma* treatment was demonstrated in the last years for bread wheat, *Triticum aestivum* (Singh et al., 2019), tea, *Camellia sinensis* (Shang et al., 2020), tomatoes, *Solanum lycopersicum* (Coppola et al., 2019; Yan et al., 2021), strawberry, *Fragaria x ananassa*, and corn, *Zea mays* (Agostini et al., 2021).

A higher level of polyphenols accumulated in the passionflower aerial parts following the *Trichoderma* plant biostimulant consortium is related to an enhanced antioxidant activity (Table 1).

Table 1. The antioxidant activity of the passionflower leaves treated with the *Trichoderma* plant biostimulant consortium

Specification	Control	Treated with the *Trichoderma* consortium
Antioxidant activity, DPPH method, %	72.24±6.24	54.32±4.82*
Antioxidant activity, TEAC method, µg Trolox equiv./mL	22.69±2.74	30.74±3.53

The higher level of the polyphenols and flavonoids from passionflower l is also related to an enhanced prebiotic activity, i.e., stimulating the growth of lactic acid bacteria in aerobic conditions (Figure 2).

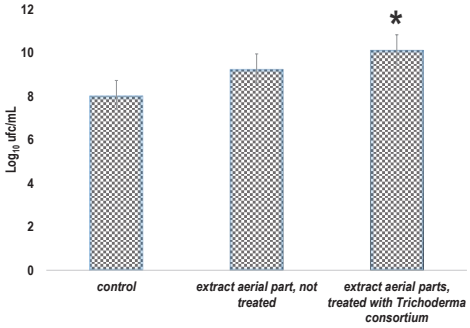

Figure 2. Stimulation of the growth of *L. reuteri* DSM 20016 by the extract of *P. caeruluea* leaves, treated and not treated with the *Trichoderma* plant biostimulant consortium

Increased content of polyphenols and flavonoids and a higher level of antioxidant activity after treatment with *Trichoderma* plant biostimulant preparations were also reported for others cultivated plants. Treatment with *Trichoderma* strains was demonstrated to increase the polyphenolic content of various plants-grape (Pascale et al., 2017), artichoke (Rouphael et al., 2017), and tomatoes (Alwhibi et al., 2017). Flavonoids and polyphenols contents were shown to increase in the edible parts of onions (Ortega-Garcia et al., 2015) and cucumbers (Nawrocka et al., 2018) following treatments with *Trichoderma* strains. Foliar application of *T. harzianum* T22 strain on grape leaves increased polyphenols content and antioxidant activity in grape fruits (Pascale et al., 2017). The same plant biostimulant strain, *T. harzianum* T22, enhances the antioxidant activity of plum tomatoes fruits (Carillo *et al.*, 2020). Foliar stimulation with the *T. atroviride* P1 strain increased the level of flavonoids, lignans, and oleuropein from olive leaves (Dini et al., 2020).

Treatment with *Trichoderma* consortium plant biostimulant strains has multilevel effects, leading to agronomic benefits (plant more resistant to biotic and abiotic stress, higher marketable yield) and enhanced health effects. Such enhanced health effects are also due to the increased prebiotic effect of the polyphenols on probiotic bacteria, including lactic acid bacteria (Figure 3).

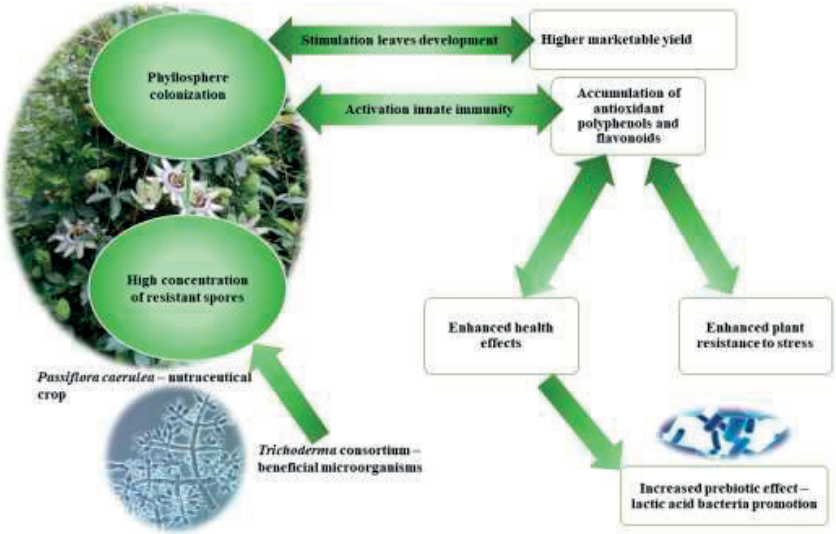

Figure 3. Multilevel effects of foliar treatments with *Trichoderma* consortium plant biostimulant strains. Modified from our previous *open-access* paper related to the effect of *Trichoderma* consortium on *P. caerulea* (Șesan et al., 2020). Licensee MDPI AG, Bassel, Switzerland

The activation of the phenylpropanoid pathway determines not only polyphenol accumulation, but also enlargement and reinforcement of the plant cell walls. Our group also demonstrated enlargement of the passionflower cell wall, especially in the palisade tissue, after treatment with *Trichoderma* consortium spores (Sârbu et al., 2018). This effect results from enhanced lignin biosynthesis (Vogt, 2010) and the accumulation of hydroxycinnamic acids

(Carrington et al., 2018). Hydroxycinnamic acids are polyphenols that anchor the hydrophilic components of the cell wall (i.e., cellulose and hemicellulose) to the hydrophobic lignin (Mnich et al., 2020).

Accumulation of the polyphenols enhances plant resistance to biotic and abiotic stress. At the same time, polyphenols from edible parts of the cultivated plants, especially from nutraceutical plants, are related to proven health benefits. (Bendini et al., 2006; da Silva et al., 2013; Lugato et al., 2014). Such health benefits also result from the mutual interactions between polyphenols and probiotic lactic acid bacteria (Piekarska-Radzik & Klewicka, 2021). The phenolic acids present in mango fruits (catechin and gallic, vanillic, ferulic, and protocatechuic acids) were reported to stimulate the growth of two probiotics, *Lactobacillus rhamnosus* GG ATCC 53103 and *Lactobacillus acidophilus* NRRLB 4495 (Pacheco-Ordaz et al., 2018). Grape pomace polyphenols induced a significant biomass increase on *Lactobacillus acidophilus* CECT 903 (Hervert-Hernández et al., 2009). Resveratrol isolated from grape pomace promotes biofilm formation and adhesion of the probiotic strain *Lacticaseibacillus paracasei* subsp. *paracasei* ATCC334 (Al Azzaz et al., 2020).

Our previous work demonstrated that treatment with plant biostimulant *Trichoderma* consortium enhances polyphenols accumulation. The findings from this paper confirm such enhanced accumulation in another year, with different climatic conditions, and demonstrates an increase in the prebiotic effect of extracts from leaves and sprouts treated with the *Trichoderma* consortium. This prebiotic effect is correlated with the enhanced polyphenols accumulation in the leaves of treated *P. caerulea* plants. Polyphenols were recognized in the last years as prebiotics (Alves-Santos et al., 2020; Bindels et al., 2015; Moorthy et al., 2020).

Prebiotic effects of the polyphenols from *P. caerulea* plants could also be related to the effect of the preparation from this plant on mood disorders. Probiotic microorganisms from digestive systems were described as a "neglected endocrine organ" (Clarke et al., 2014), with a contribution to the normal brain function – "melancholic microbes" (Dinan et al., 2019).

CONCLUSIONS

Foliar treatment with a suspension of chlamydospores from a *Trichoderma consortium* with plant biostimulant properties enhance polyphenols accumulation and antioxidant activity

Enhanced polyphenol accumulation and antioxidant activity are correlated with stimulating lactic acid bacteria *L. reuteri* DSM 20016 in aerobic conditions. Such a prebiotic effect could be related to the known efficiency of passionflower extract on mood disorders. Probiotic bacteria produce short-chain fatty acids, which assure proper brain function.

ACKNOWLEDGEMENTS

The authors thank Geta Negru and Gelu Vasilescu for their support during field experiments. This work was supported by the Romanian Ministry of Research and Innovation, CCCDI– UEFISCDI, through the following research projects: PN-III-P1-1.2-PCCDI-2017-0569 PRO-SPER, "Closing the bioeconomy value chains by manufacturing market-driven innovative bioproducts" (contract 10 PCCDI) and ERA.NET FACCE.SURPLUS DEBUT "Production of ferulic acid, 2,3 Butanediol, and microbial plant biostimulants from lignocellulosic biomass by a two-step cascading process" (contract 43/2018), within PNCDI IIII

REFERENCES

Aberkane, A., Cuenca-Estrella, M., Gomez-Lopez, A., Petrikkou, E., Mellado, E., Monzon, A., & Rodriguez-Tudela, J. (2002). Comparative evaluation of two different methods of inoculum preparation for antifungal susceptibility testing of filamentous fungi. *Journal of Antimicrobial Chemotherapy, 50*(5), 719-722.

Agostini, R. B., Rius, S. P., Vargas, W. A., & Campos-Bermudez, V. A. (2021). Proteome impact on maize silks under the priming state induced by *Trichoderma* root colonization. *Planta, 253*(5), 1-17.

Al Azzaz, J., Al Tarraf, A., Heumann, A., Da Silva Barreira, D., Laurent, J., Assifaoui, A., Rieu, A., Guzzo, J., & Lapaquette, P. (2020). Resveratrol favors adhesion and biofilm formation of *Lacticaseibacillus paracasei* subsp. *paracasei* Strain ATCC334. *International Journal of Molecular Sciences, 21*(15), 5423.

Alvarez-Martinez, F. J., Barrajon-Catalan, E., Encinar, J. A., Rodriguez-Diaz, J. C., & Micol, V. (2020). Antimicrobial Capacity of Plant Polyphenols against Gram-positive Bacteria: A Comprehensive Review. *Current Medicinal Chemistry, 27*(15), 2576-2606. doi:10.2174/0929867325666181008115650

Alves-Santos, A. M., Sugizaki, C. S. A., Lima, G. C., & Naves, M. M. V. (2020). Prebiotic effect of dietary polyphenols: A systematic review. *Journal of Functional Foods, 74*, 104169.

Alwhibi, M. S., Hashem, A., Abd Allah, E. F., Alqarawi, A. A., Soliman, D. W. K., Wirth, S., & Egamberdieva, D. (2017). Increased resistance of drought by *Trichoderma harzianum* fungal treatment correlates with increased secondary metabolites and proline content. *Journal of Integrative Agriculture, 16*(8), 1751-1757. doi:10.1016/s2095-3119(17)61695-2

Angel-Isaza, J., Carmona-Hernandez, J. C., Narváez-Solarte, W., & Gonzalez-Correa, C. H. (2021). Polyphenols from Passiflora ligularis Regulate Inflammatory Markers and Weight Gain. *Biomolecular Concepts, 12*(1), 36-45.

Bendini, A., Cerretani, L., Pizzolante, L., Toschi, T. G., Guzzo, F., Ceoldo, S., Marconi, A. M., Andreetta, F., & Levi, M. (2006). Phenol content related to antioxidant and antimicrobial activities of *Passiflora* spp. extracts. *European Food Research and Technology, 223*(1), 102-109. doi:10.1007/s00217-005-0150-7

Bindels, L. B., Delzenne, N. M., Cani, P. D., & Walter, J. (2015). Towards a more comprehensive concept for prebiotics. *Nature reviews Gastroenterology & hepatology, 12*(5), 303-310.

Carillo, P., Woo, S. L., Comite, E., El-Nakhel, C., Rouphael, Y., Fusco, G. M., Borzacchiello, A., Lanzuise, S., & Vinale, F. (2020). Application of *Trichoderma harzianum,* 6-pentyl-α-pyrone and plant biopolymer formulations modulate plant metabolism and fruit quality of plum tomatoes. *Plants, 9*(6), 771

Carrington, Y., Guo, J., Le, C. H., Fillo, A., Kwon, J., Tran, L. T., & Ehlting, J. (2018). Evolution of a secondary metabolic pathway from primary metabolism: shikimate and quinate biosynthesis in plants. The Plant Journal, 95(5), 823-833..

Chan, C.-L., Gan, R.-Y., Shah, N. P., & Corke, H. (2018). Polyphenols from selected dietary spices and medicinal herbs differentially affect common food-borne pathogenic bacteria and lactic acid bacteria. *Food control, 92*, 437-443.

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis, 10*(3).

Chinnam, N., Dadi, P. K., Sabri, S. A., Ahmad, M., Kabir, M. A., & Ahmad, Z. (2010). Dietary bioflavonoids inhibit *Escherichia coli* ATP synthase in a differential manner. *International journal of biological macromolecules, 46*(5), 478-486.

Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. F., & Dinan, T. G. (2014). Minireview: gut microbiota: the neglected endocrine organ. *Molecular endocrinology, 28*(8), 1221-1238.

Coppola, M., Diretto, G., Digilio, M. C., Woo, S. L., Giuliano, G., Molisso, D., Pennacchio, F., Lorito, M., & Rao, R. (2019). Transcriptome and Metabolome Reprogramming in Tomato Plants by *Trichoderma harzianum* strain T22 Primes and Enhances Defense Responses Against Aphids. *Frontiers in Physiology, 10*(745). doi:10.3389/fphys.2019.00745

Craciunescu, O., Constantin, D., Gaspar, A., Toma, L., Utoiu, E., & Moldovan, L. (2012). Evaluation of antioxidant and cytoprotective activities of *Arnica montana* L. and *Artemisia absinthium* L. ethanolic extracts. *Chemistry Central journal, 6*(1), 97. doi:10.1186/1752-153x-6-97

da Silva, J. K., Cazarin, C. B. B., Colomeu, T. C., Batista, Â. G., Meletti, L. M. M., Paschoal, J. A. R., Bogusz Júnior, S., Furlan, M. F., Reyes, F. G. R., Augusto, F., Maróstica Júnior, M. R., & de Lima Zollner, R. (2013). Antioxidant activity of aqueous extract of passion fruit (*Passiflora edulis*) leaves: *In vitro* and *in vivo* study. *Food Research International, 53*(2), 882-890. doi:https://doi.org/10.1016/j.foodres.2012.12.043

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current opinion in biotechnology, 23*(2), 174-181.

Dinan, T. G., Stanton, C., Long-Smith, C., Kennedy, P., Cryan, J. F., Cowan, C. S., Cenit, M. C., van der Kamp, J.-W., & Sanz, Y. (2019). Feeding melancholic microbes: MyNewGut recommendations on diet and mood. *Clinical Nutrition, 38*(5), 1995-2001.

Dini, I., Graziani, G., Fedele, F. L., Sicari, A., Vinale, F., Castaldo, L., & Ritieni, A. (2020). Effects of *Trichoderma* Biostimulation on the Phenolic Profile of Extra-Virgin Olive Oil and Olive Oil By-Products. *Antioxidants, 9*(4), 284. Retrieved from https://www.mdpi.com/2076-3921/9/4/284

Filannino, P., Di Cagno, R., Addante, R., Pontonio, E., & Gobbetti, M. (2016). Metabolism of fructophilic lactic acid bacteria isolated from the *Apis mellifera* L. bee gut: Phenolic acids as external electron acceptors. *Applied and environmental microbiology, 82*(23), 6899-6911.

Filannino, P., Gobbetti, M., De Angelis, M., & Di Cagno, R. (2014). Hydroxycinnamic acids used as external acceptors of electrons: an energetic advantage for strictly heterofermentative lactic acid bacteria. *Applied and environmental microbiology, 80*(24), 7574-7582.

Gaur, G., Oh, J.-H., Filannino, P., Gobbetti, M., Van Pijkeren, J.-P., & Gänzle, M. G. (2020). Genetic determinants of hydroxycinnamic acid metabolism in heterofermentative lactobacilli. *Applied and environmental microbiology, 86*(5), e02461-02419.

Gopu, V., Meena, C. K., & Shetty, P. H. (2015). Quercetin influences quorum sensing in food borne bacteria: in-vitro and in-silico evidence. *PLOS ONE, 10*(8), e0134684.

Hengge, R. (2019). Targeting bacterial biofilms by the green tea polyphenol EGCG. *Molecules, 24*(13), 2403.

Hervert-Hernández, D., Pintado, C., Rotger, R., & Goñi, I. (2009). Stimulatory role of grape pomace polyphenols on *Lactobacillus acidophilus* growth.

International journal of food microbiology, 136(1), 119-122.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of agricultural and food chemistry, 53*(6), 1841–1856. doi:10.1021/jf030723c

Kang, S. S., Kim, J.-G., Lee, T.-H., & Oh, K.-B. (2006). Flavonols inhibit sortases and sortase-mediated *Staphylococcus aureus* clumping to fibrinogen. *Biological and Pharmaceutical Bulletin, 29*(8), 1751-1755.

Liu, M. S., Amini, A., & Ahmad, Z. (2017). Safranal and its analogs inhibit *Escherichia coli* ATP synthase and cell growth. *International journal of biological macromolecules, 95*, 145-152. doi:10.1016/j.ijbiomac.2016.11.038

Lugato, D., Simo, M. J., Garcia, R., Mansur, E., & Pacheco, G. (2014). Determination of antioxidant activity and phenolic content of extracts from *in vivo* plants and *in vitro* materials of *Passiflora alata* Curtis. *Plant Cell Tissue and Organ Culture, 118*(2), 339-346. doi:10.1007/s11240-014-0486-4

Mickymaray, S., Alfaiz, F. A., & Paramasivam, A. (2020). Efficacy and mechanisms of flavonoids against the emerging opportunistic nontuberculous *Mycobacteria*. *Antibiotics, 9*(8), 450.

Mnich, E., Bjarnholt, N., Eudes, A., Harholt, J., Holland, C., Jørgensen, B., Larsen, F. H., Liu, M., Manat, R., & Meyer, A. S. (2020). Phenolic cross-links: building and de-constructing the plant cell wall. Natural product reports, 37(7), 919-961.

Moorthy, M., Chaiyakunapruk, N., Jacob, S. A., & Palanisamy, U. D. (2020). Prebiotic potential of polyphenols, its effect on gut microbiota and anthropometric/clinical markers: A systematic review of randomised controlled trials. *Trends in Food Science & Technology, 99*, 634-649.

Nakayama, M., Shimatani, K., Ozawa, T., Shigemune, N., Tsugukuni, T., Tomiyama, D., Kurahachi, M., Nonaka, A., & Miyamoto, T. (2013). A study of the antibacterial mechanism of catechins: Isolation and identification of *Escherichia coli* cell surface proteins that interact with epigallocatechin gallate. *Food control, 33*(2), 433-439.

Nawrocka, J., Szczech, M., & Malolepsza, U. (2018). *Trichoderma atroviride* Enhances Phenolic Synthesis and *Cucumber* Protection against *Rhizoctonia solani*. *Plant Protection Science, 54*(1), 17-23. doi:10.17221/126/2016-pps

Nohynek, L. J., Alakomi, H.-L., Kähkönen, M. P., Heinonen, M., Helander, I. M., Oksman-Caldentey, K.-M., & Puupponen-Pimiä, R. H. (2006). Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. *Nutrition and cancer, 54*(1), 18-32.

Oancea, F., Raut, I., Sesan, T. E., Cornea, P. C., Badea-Doni, M., Popescu, M., & Jecu, M. L. (2016). Hydro-gelified and film forming formulation of microbial plant biostimulants for crop residues treatment on conservation agriculture systems. *Studia Universitatis" Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series), 26*(2), 251.

Ollila, F., Halling, K., Vuorela, P., Vuorela, H., & Slotte, J. P. (2002). Characterization of flavonoid–biomembrane interactions. *Archives of biochemistry and biophysics, 399*(1), 103-108.

Ortega-Garcia, J. G., Montes-Belmont, R., Rodriguez-Monroy, M., Ramirez-Trujillo, J. A., Suarez-Rodriguez, R., & Sepulveda-Jimenez, G. (2015). Effect of *Trichoderma asperellum* applications and mineral fertilization on growth promotion and the content of phenolic compounds and flavonoids in onions. *Scientia Horticulturae, 195*, 8-16. doi:10.1016/j.scienta.2015.08.027

Pacheco-Ordaz, R., Wall-Medrano, A., Goñi, M. G., Ramos-Clamont-Montfort, G., Ayala-Zavala, J. F., & González-Aguilar, G. (2018). Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. *Letters in applied microbiology, 66*(1), 25-31.

Pascale, A., Vinale, F., Manganiello, G., Nigro, M., Lanzuise, S., Ruocco, M., Marra, R., Lombardi, N., Woo, S. L., & Lorito, M. (2017). *Trichoderma* and its secondary metabolites improve yield and quality of grapes. *Crop Protection, 92*, 176-181. doi:https://doi.org/10.1016/j.cropro.2016.11.010

Perumal, S., Mahmud, R., & Ismail, S. (2017). Mechanism of action of isolated caffeic acid and epicatechin 3-gallate from *Euphorbia hirta* against *Pseudomonas aeruginosa*. *Pharmacognosy magazine, 13*(Suppl 2), S311.

Piekarska-Radzik, L., & Klewicka, E. (2021). Mutual influence of polyphenols and *Lactobacillus* spp. bacteria in food: a review. *European Food Research and Technology, 247*(1), 9-24. doi:10.1007/s00217-020-03603-y

Rajakovich, L. J., & Balskus, E. P. (2019). Metabolic functions of the human gut microbiota: the role of metalloenzymes. *Natural product reports, 36*(4), 593-625.

Raut, I., Badea-Doni, M., Calin, M., Oancea, F., Vasilescu, G., Sesan, T. E., & Jecu, L. (2014). Effect of volatile and non-volatile metabolites from *Trichoderma* spp. against important phytopathogens. *Revista de Chimie, 65*(11), 1285-1288.

Răut, I., Sesan, T. E., Macias, R., Doni, M., Oancea, F., Calin, M., Arsene, M. L., Vasilescu, G., & Jecu, L. (2016). Study on the effectiveness of antagonistic *Trichoderma* spp. on the growth of some vegetables under laboratory and greenhouse conditions. *Revista de Chimie, 67*, 1504-1507.

Ricci, A., Cirlini, M., Calani, L., Bernini, V., Neviani, E., Del Rio, D., Galaverna, G., & Lazzi, C. (2019). In vitro metabolism of elderberry juice polyphenols by lactic acid bacteria. *Food chemistry, 276*, 692-699.

Rodríguez, H., Curiel, J. A., Landete, J. M., de las Rivas, B., de Felipe, F. L., Gómez-Cordovés, C., Mancheño, J. M., & Muñoz, R. (2009). Food phenolics and lactic acid bacteria. *International journal of food microbiology, 132*(2-3), 79-90.

Rouphael, Y., Colla, G., Graziani, G., Ritieni, A., Cardarelli, M., & De Pascale, S. (2017). Phenolic composition, antioxidant activity and mineral profile in two seed-propagated artichoke cultivars as affected by microbial inoculants and planting time. *Food*

Chemistry, 234, 10-19. doi:10.1016/j.foodchem.2017.04.175

Saravanan, S., & Parimelazhagan, T. (2014). *In vitro* antioxidant, antimicrobial and anti-diabetic properties of polyphenols of *Passiflora ligularis* Juss. fruit pulp. *Food science and human wellness, 3*(2), 56-64.

Sârbu, A., Paraschiv, M. A., Oancea, F., & Sesan, T. E. (2018). Passiflora caerulea L. treated with Trichoderma plant biostimulants consortium. Morpho-anatomical considerations. Journal of Plant Development, 25, 3-14.

Şesan, T. E., Oancea, A. O., Ştefan, L. M., Mănoiu, V. S., Ghiurea, M., Răut, I., Constantinescu-Aruxandei, D., Toma, A., Savin, S., & Bira, A. F. (2020). Effects of foliar treatment with a *Trichoderma* plant biostimulant consortium on *Passiflora caerulea* L. yield and quality. *Microorganisms, 8*(1), 123.

Shang, J., Liu, B., & Xu, Z. (2020). Efficacy of *Trichoderma asperellum* TC01 against anthracnose and growth promotion of *Camellia sinensis* seedlings. *Biological Control, 143*, 104205. doi:https://doi.org/10.1016/j.biocontrol.2020.104205

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., & Zheng, B. S. (2019). Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants under Abiotic Stress. *Molecules, 24*(13), 22. doi:10.3390/molecules24132452

Singh, U. B., Malviya, D., Singh, S., Kumar, M., Sahu, P. K., Singh, H. V., Kumar, S., Roy, M., Imran, M., Rai, J. P., Sharma, A. K., & Saxena, A. K. (2019). *Trichoderma harzianum*- and Methyl Jasmonate-Induced Resistance to Bipolaris sorokiniana Through Enhanced Phenylpropanoid Activities in Bread Wheat (*Triticum aestivum* L.). *Frontiers in Microbiology, 10*, 19. doi:10.3389/fmicb.2019.01697

Taylor, P. W., Hamilton-Miller, J. M., & Stapleton, P. D. (2005). Antimicrobial properties of green tea catechins. *Food science and technology bulletin, 2*, 71.

Vogt, T. (2010). Phenylpropanoid biosynthesis. Molecular plant, 3(1), 2-20.

Wang, M. Q., Firrman, J., Zhang, L. Q., Arango-Argoty, G., Tomasula, P., Liu, L. S., Xiao, W. D., & Yam, K. (2017). Apigenin Impacts the Growth of the Gut Microbiota and Alters the Gene Expression of *Enterococcus*. *Molecules, 22*(8). doi:10.3390/molecules22081292

Yan, Y., Mao, Q., Wang, Y., Zhao, J., Fu, Y., Yang, Z., Peng, X., Zhang, M., Bai, B., & Liu, A. (2021). *Trichoderma harzianum* induces resistance to root-knot nematodes by increasing secondary metabolite synthesis and defense-related enzyme activity in *Solanum lycopersicum* L. *Biological Control, 158*, 104609.

Zamfiropol-Cristea, V., Răut, I., Șeșan, T. E., Trică, B., & Oancea, F. (2017). Surface response optimization of submerged biomass production for a plant biostimulant *Trichoderma* strain. *Scientific Bulletin. Series F. Biotechnologies, 21*, 56-65.

Zeraik, M. L., Pereira, C. A. M., Zuin, V. G., & Yariwake, J. H. (2010). Passion fruit: a functional food? *Revista Brasileira De Farmacognosia-Brazilian Journal of Pharmacognosy, 20*(3), 459-471. doi:10.1590/s0102-695x2010000300026

Zhao, W.-H., Hu, Z.-Q., Okubo, S., Hara, Y., & Shimamura, T. (2001). Mechanism of synergy between epigallocatechin gallate and β-lactams against methicillin-resistant *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy, 45*(6), 1737-1742.

Ziarno, M., Kozłowska, M., Ścibisz, I., Kowalczyk, M., Pawelec, S., Stochmal, A., & Szleszyński, B. (2021). The Effect of Selected Herbal Extracts on Lactic Acid Bacteria Activity. *Applied Sciences, 11*(9), 3898.

FOOD BIOTECHNOLOGY