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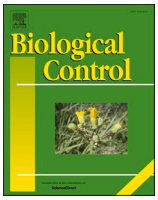

Fermentates of consortia of lactic acid bacteria and a cyanobacterium are effective against toxigenic fungi contaminating agricultural produces

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HIGHLIGHTS

- Antifungal activity of *Arthrospira platensis* and *Lactiplantibacillus plantarum*.
- Enhanced antifungal activity of fermented lactobacilli/cyanobacterium consortia.
- Fermentates of consortia inhibit growth of toxigenic fungi on agricultural produces.
- UHPLC-Q-TOF-MS analysis of metabolites in fermentates of microbial consortia.
- Cell-free supernatants of fermented microbial consortia as promising BCA candidates.

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ABSTRACT

The objectives of this study were to (i) test the *in vitro* inhibitory activity of fermented consortia of the cyanobacterium *Arthrospira platensis* (syn. *Spirulina platensis*) and two selected strains of the *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) against a set of toxigenic fungal plant pathogens, including *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *Fusarium graminearum*, *Giberella zeae*, *Penicillium commune*, and *P. expansum*, (ii) assess the efficacy of consortia fermentates in preventing infections of *F. graminearum* and *P. expansum* on maize cobs and lemon fruits, respectively, and (iii) identify and quantify antifungal compounds and secondary metabolites produced by these consortia. A noticeably inhibitory activity of the fermented consortia, as determined by the agar diffusion test, was observed for all the fungal pathogens tested. *Fusarium graminearum* and *G. zeae* were the most sensitive, while *A. flavus* and *A. niger* were the least sensitive. Cell-free supernatants (CFSs) of fermentates showed the highest antifungal activity after 24 and 48 h of incubation. Their MIC and MFC values ranged from 3.13 (*G. zeae*) to 25 g L^{-1} (*A. niger*) and from 3.13 (*G. zeae*) to 100 g L^{-1} (*Aspergillus* and *Penicillium* species), respectively. CFSs of fermentates were also effective in preventing infections by two very common toxigenic fungi, *F. graminearum* and *P. expansum*, in maize cobs and lemon fruits, respectively. Results of chemical analyses suggest the antifungal activity of fermentates depend, at least in part, on the presence of high levels of lactic acid and significantly higher concentrations of some phenolic compounds, including DL-3-phenallactic acid, benzoic acid and 3–4-dihydroxy hydrocinnamic acid. Among the 66 secondary metabolites detected in fermentates by UHPLC-Q-TOF-MS analysis, only benzoic acid, leucine, oleic acid, and proline possess antifungal activity. Overall, results of this study reveal the potential of fermented microbial consortia as BCAs to prevent post-harvest fungal diseases and contamination by mycotoxins of food and agricultural produces.

1. Introduction

In recent years, there has been a growing interest in food safety issues, prompting the adoption of innovative plant disease management systems based on eco-friendly and sustainable approaches. In line with this trend, the expansion of organic solutions has emerged as a priority in preventing contamination of agricultural produces and food by toxigenic fungi, with the aim of reducing the reliance on synthetic

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chemicals. Therefore, the exploitation of generally recognized as safe (GRAS) natural substances and microorganisms with antimicrobial activity or stimulating plant defense mechanisms has gained prominence (La Spada et al., 2021; Lin et al., 2021; Riolo et al., 2023a; Stracquadanio et al., 2020).

Toxigenic fungi pose significant risks, not only resulting in substantial product losses and economic damages, but also producing mycotoxins and secondary metabolites that have detrimental health effects on humans and animals (Awuchi et al., 2021; Fazekas et al., 2002; Leyva Salas et al., 2017). The genera *Aspergillus, Penicillium* and *Fusarium* are among the most common causal agents of post-harvest rots and major producers of mycotoxins (Moss, 2008; Nazareth et al., 2018; Pitt and Hocking, 2009). For instance, *Penicillium expansum* is recognized as a major toxin producer in food and feed, capable of thriving under various environmental conditions and producing patulin and citrinin (Sanzani et al., 2012; Yu et al., 2020). Similarly, *Fusarium graminearum* and other *Fusarium* species associated with fusarium head blight of wheat and stalk rot of maize produce a wide spectrum of mycotoxins, of which the most important cereal grain contaminants are deoxynivalenol and nivalenol trichothecenes (Senatore et al., 2023; Somma et al., 2014; Visconti and Pascale, 2010; Ward et al., 2008). Synthetic fungicides are commonly employed to prevent fungal contamination of agricultural produces and food. However, their extensive use raises concerns for human and animal health and poses the problem of eliminating their residues from food and environment (Baglieri et al., 2013; Jabłońska-Trypuć et al., 2017; Vanni et al., 2006). In response to these concerns, a number of recent studies have explored the potential of biological control agents (BCAs) or their metabolites, biostimulants, and natural compounds. These are emerging as novel, eco-friendly, and safe approaches to counteract fungal infections (El boumlasy et al., 2022; Hammami et al., 2022; He et al., 2021; Leonardi et al., 2023; Llorens and Agustí-Brisach, 2022; Medina et al., 2017; Parafati et al., 2022; Stracquadanio et al., 2021). Several studies have investigated the potential of LABs to prevent contamination by plant pathogenic and toxigenic fungi (Li et al., 2012; McNair et al., 2018; Riolo et al., 2023b; Volentini et al., 2023; Riolo et al., 2024). The antifungal property of LABs is related to the production of diverse metabolites, such as organic acids and phenolic compounds in the fermented media (Le Lay et al., 2016; Sadiq et al., 2019). Lactic acid is one of the main sources of this antifungal activity (Dopazo et al., 2022). Microalgae/cyanobacteria, being photoautotrophic microorganisms, can fix carbon dioxide (CO_2) and transform widely available natural resources, such as CO_2, H_2O and inorganic salts, into valuable organic products. Due to this characteristic, they are a very promising new source of energy and have numerous biotechnological applications (Sánchez-Bayo et al., 2020). For instance, the cyanobacterium *Arthrospira platensis* (Spirulina), also referred here to as blue-green algae or algae is sold as food-supplement. However, research on the antifungal activity of microalgae/cyanobacteria and their application as food preservatives or to prevent spoilage of agricultural produces due to post-harvest rots or contamination by mycotoxins remain limited (La Bella et al., 2022). In a previous study, it was shown that an extract of seaweed (*Ascophyllum nodosum*), besides having a biostimulant activity, showed a synergistic effect with the synthetic fungicide imazalil in controlling post-harvest rot of citrus fruit incited by *Penicillium digitatum* (La Spada et al., 2021). Although the antifungal activity of *A. platensis* had not been previously reported, studies carried out on other genus of cyanobacteria, such as *Oscillatoria, Aphanocapsa, Trichodesmium*, and *Synechocystis*, proved the antifungal activity of this group of microrganisms (Pawar & Puranik, 2008). Consequently, it was demonstrated that *A. platensis*, which is already produced on an industrial scale, could also be exploited as a biological control agent to prevent fungal infections and mycotoxin contamination in agricultural produces and food. Furthermore, also considering the ability of *A. platensis* to produce organic products with biostimulant activity, it was hypothesized that the incorporation of this microalga into a consortium with other microorganisms, such as LABs, whose antifungal activity has been widely demonstrated, could be a way to obtain a multifunctional bioproduct.

The objectives of the present study were to (i) evaluate the *in vitro* antagonistic activity of cell-free supernatants from fermented consortia of LABs and a cyanobacterium against toxigenic fungal plant pathogens, (ii) assess their potential in preventing infections of *F. graminearum* and *P. expansum* on maize cobs and lemon fruits, respectively, and (iii) identify and quantify antifungal metabolites produced by these fermented consortia.

2. Materials and methods

2.1. Chemicals

Unless indicated otherwise, all chemicals were purchased from Thermo Fischer Scientific Inc. (Oxoid, Limited, Basingstoke, Hampshire, UK), VWR Chemicals (Radnor, PA, USA), Sigma-Aldrich (St. Louis, MO, USA), and Millipore Corp. (Bedford, MA, USA) and were of analytical grade or higher. Ultrapure water was obtained from a Milli-Q water purification system (Millipore Corp.).

2.2. Bacterial and fungal strains

The lactic acid bacteria (LABs) used in this study were isolated from corn (*Zea mays*) and identified as *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) DMS 1055 (FR47) and *L. plantarum* DMS 2601 (FR48). The isolation and characterization of bacterial strains were carried out following the methodology outlined by Riolo et al., (2023a).

The cyanobacterium used in this study was *Arthrospira platensis* (syn. *Spirulina platensis*), in the form of a lyophilized powder purchased by Herbolario Navarro (Valencia, Spain).

The antifungal activity of culture-free supernatant (CFS) of LABs and their fermented consortia with *A. platensis* was tested *in vitro* against the plant pathogenic fungi *Alternaria alternata* (CECT 646), *Aspergillus niger* (CECT 153), *A. flavus* (CECT 146), *F. graminearum* (CECT 836), *Giberella zeae* (CECT 103), *P. commune* (CECT 151), and *P. expansum* (CECT 95). All bacterial and fungal isolates used in this study were sourced from the Colección Española de Cultivos Tipo (CECT) at Valencia University, Spain.

2.3. Preparation of microbial consortia

Several consortia formulations containing LABs and different concentrations of *A. platensis* were prepared. The procedure for the preparation of the consortia is described below. Initially, the two LAB strains (FR47 and FR48) of *L. plantarum*, after defrosting and recovery, were suspended in sterile distilled water. The suspensions of the two bacterial strains were inoculated separately in Man-Rogosa-Sharpe broth (MRS-B) (Liofilchem, Teramo, Italy) in a proportion of 5 % (v/v) of the final volume. The cultures were incubated at 37 °C for 12 h and then, depending on the consortia to be made, were treated differently as described later. In total, three types of consortia differing in the length of incubation period following the addition of bacterial suspensions or their cell-free supernatants (CFs) to the basal medium (T0, T1 and T2) were prepared for each bacterial strain (FR47 and FR48) and concentration of *A. platensis* (1 % or 5 % w/v). All consortia media consisted of a basal medium, here referred to as minimal medium (MM) composed of glucose (10 g), yeast extract (10 g), tryptone (10 g), $(NH_4)_2SO_4$ (2.5 g), NaCl (6.25 g), $MgSO_4$, K_2HPO_4 (2.5 g), $MnSO_4$ (0.06 g) per litre of deionised water (<18 MΩ cm^{-1}) produced with a Milli-Q water purification system (Milli-pore Corp.). The mixtures of MM plus the suspension of *A. platensis* (at 0.1 or 0.5 %, v/v) were pasteurised according to the method described by Dopazo et al. (2021). For preparing the first consortium (T0), the two LAB strains were cultured separately. The bacterial cultures were incubated at 37° C for 24 h on MRS-B; after incubation, the suspensions were centrifuged at 4000 g for 10 min in an Eppendorf 5810 R centrifuge (Eppendorf, Hamburg, Germany). The

biomass was discarded and the supernatant was recovered. This cell-free supernatant (CFS) was added in a proportion of 5 % (v/v) to the pasteurised mixed suspensions of MM and *A. platensis* (1 or 5 %).

For the second and third consortia (T1 and T2, respectively), each bacterial strain was fermented in the pasteurised mixed suspensions of MM plus the two concentrations (1 or 5 %) of *A. platensis*. After 24 and 48 h of incubation at 37 °C, the cultures thus obtained were centrifuged, as mentioned above. The recovered CFSs were frozen at −80 °C, freeze-dried (FreeZone 2.5 L, Labconco, MI, USA) and stored at −20 °C for further analysis. To be tested for their antifungal activity, the lyophilized CFSs were resuspended in SDW and tested *in vitro* and *in vivo*. The pH value was, then, measured to ensure that the bacteria had fermented the culture medium. Finally, as a control, MM consisting exclusively of pasteurised 1 or 5 % *A. platensis* was prepared (1 or 5 % Ctrl - T0) and incubated at 37 °C for 24 h (1 or 5 % Ctrl – T1) or 48 h (1 or 5 % Ctrl – T2) to evaluate the effect of *A. platensis* alone at different incubation time.

2.4. Identification and quantification of organic acids in fermented consortia

Lactic acid, the main organic acid produced by LABs, was determined as described by Xu et al., (2021), with few modifications. Samples of not-lyophilised consortia were diluted 1:20 with MilliQ water and filtered with a 0.22 μm pore filter. The high-performance liquid chromatography (HPLC) was equipped with a Jasco PU-4180 pump (Maryland, USA), a Jasco MD-4015 diode array detector (Maryland, USA) and a 20-μL sample injection loop. The mobile phase was an isocratic solution of water and sulphuric acid 0.005 M, flowing at 0.8 mL/min and a temperature of 40 °C. The detector was set at a wavelength of 214 nm for the quantification. Calibration curve was performed using lactic acid, at the concentrations from 0 to 1000 mg/L. Results were expressed in g/L and software used for the data analyses was ChromNAV 2.0 HPLC (Jasco, Maryland, USA).

2.5. Total antioxidant capacity

The Oxygen Radical Absorbance Capacity (ORAC) activity, a widely used method for analyzing total antioxidant capacity of compounds, was determined following the procedure described by Barba et al., (2013). The ORAC assay was carried out on plates with 96 flat-bottomed wells at 37 °C, since the reaction is initiated by thermal decomposition of AAPH in phosphate buffer solution (PBS) due to the sensitivity of fluorescein (FL) to pH. Daily working solution of fluorescein (FL) (0.015 mg/mL), Trolox standard (100 μM), and AAPH radical (221 mM) were prepared in neutralized PBS (75 mM). Due to the sensitivity of the assay, samples were diluted 1:350 (V/V) in PBS. For the reaction, 50 μL of FL and 50 μL of PBS (blank), Trolox (standard) or sample were placed in each well. Then, 25 μL of AAPH was added and fluorescence measurements were recorded every 5 min for one hour, until the reaching of intensity of the FL was less than 5 % of the starting absorbance value. ORAC values, expressed as μM Trolox equivalent, were calculated by applying the following formula:

$$\text{ORAC}\,(\mu\text{M Trolox equivalent}) = \frac{\text{Ct} \times (\text{AUCs} - \text{AUCb}) \times k}{(\text{AUCt} - \text{AUCb})}$$

Where Ct is the concentration of Trolox, k is the dilution factor, and AUC is the area under the curve of the fluorescence drop of the sample, blank, and Trolox, respectively.

2.6. Total polyphenolic compounds in the consortia

Total polyphenolic compounds in the consortia were quantified through the Folin–Ciocâlteu method, as described in Kschonsek et al. (2018), with some modifications. For the reaction, the consortia samples were diluted (1:4) with MilliQ water. Afterward, 130 μL of each diluted sample were mixed with 780 μL of MilliQ water and 130 μL Folin–Ciocâlteu reagent and vortexed for 1 min. Then, 130 μL of a solution of 20 % (w/V) $NaCO_3$ were added to stop the reaction, and the solution was vortexed for 1 min and placed in the dark at room temperature for 2 h. Samples were read at 750 nm, and total phenolic compounds were obtained from a standard calibration curve prepared by plotting the change in absorbance versus a gradient of concentrations of gallic acid (from 8.5 to 140 mg/L).

2.7. Identification of phenolic compounds in microbial consortia

Phenolic compounds in microbial consortia were identified using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method. For this purpose, an aliquot of 10 mL of the consortium sample was combined with a pre-prepared solution of 4 g MgSO4, 1 g NaCl, 1 % formic acid (v/v), and 10 mL ethyl acetate in 50 mL tubes. The mixture was vortexed for 1 min and then incubated on ice for 1 min. Afterward, the samples were centrifuged at 4 °C and 3000 g for 10 min. The resulting supernatant was carefully withdrawn and mixed with 150 mg C18 and 900 mg MgSO4. The mixture was vortexed for 1 min and centrifuged under the same conditions. Subsequently, the supernatant was evaporated under a nitrogen flow. For analysis, the purified samples were reconstituted in 1 mL of a mixture of water and acetonitrile (90:10 v/v). Chromatographic determination was carried out using an Agilent 1200 system (Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler, a binary pump, and a vacuum degasser. A Gemini C18 column (50 mm x 2 mm, 100 Å, 3 μm particle size; Phenomenex) was utilized. Prior to the analysis, the column was equilibrated.

The mobile phase flow rate was set at 0.3 mL/min. The solvents used were water and acetonitrile, with an elution gradient of 0 min, 5 % B; 30 min, 5 % B; and 35 min, 5 % B. Both solvents were acidified with 0.1 % formic acid.

The analysis was performed using a UHPLC-Q-TOF-MS (6540 Agilent ultra-high-definition accurate mass spectrometer) equipped with an Agilent Dual Jet Stream electrospray ionization interface in negative ionization mode. The following conditions were applied: drying gas flow (N2), 8.0 L/min; nebulizer pressure, 30 psig; gas drying temperature, 350 °C; capillary voltage, 3.5 kV; fragmentor voltage, 175 V; and scan range, *m/z* 20–380. Collision energies of 10, 20, and 40 eV were employed. Masshunter Qualitative Analysis Software B.08.00 (Denardi-Souza et al., 2018) was used for integration and data elaboration. The results were expressed as mg/L.

2.8. Extraction and characterization of the secondary metabolites of consortia

The extraction and the analysis of the secondary metabolites content in the consortia was carried out according to Riolo et al., (2023b) with some modifications. The extraction was performed transferring 1 mL of each sample into 15 mL Eppendorf tubes and adding methanol (MeOH) in a 1:5 (V/V) ratio. The tubes were incubated overnight at room temperature and continuous stirring (150 rpm). After incubation, the samples were centrifuged at 4000 rpm for 15 min, and the supernatant was filtered through a 0.22 μm filter and injected for UHPLC-Q-TOF-MS analysis. All the analyses were performed in triplicate. The chromatographic determination was performed using the Agilent 1200 system, which comprised an automatic sampler, a binary pump, and a vacuum degasser. The injection volume was set at 5 mL, and the analysis was completed within 25 min. Separation of the analytes was achieved using a Gemini C18 column (50 mm × 2 mm, 110 Å, and particle size 3 mm) from Phenomenex (Palo Alto, CA, USA). The mobile phases consisted of water (solvent A) and acetonitrile (solvent B), both containing 0.1 % formic acid. The elution flow rate was maintained at 0.3 mL/min. The elution gradient followed this pattern: 0 min, 5 % B; 30 min, 95 % B; and 35 min, 5 % B. Mass spectrometry analyses were conducted using a

QTOF (6540 Agilent Ultra High-Definition Accurate Mass) system, coupled with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface operating in positive ion mode. Optimized mass spectrometry parameters included a capillary voltage of 3.5 kV, fragment voltage of 175 V, drying gas flow (N2) of 8 L/min at a temperature of 350 °C, collision energies of 10, 20, and 40 eV, and a nebulizer pressure of 30 psi. Data analysis was performed using MassHunter Qualitative Analysis Software B.08.00 from Agilent Technologies (Santa Clara, CA, USA).

2.9. Qualitative and quantitative assay of in vitro antifungal activity of consortia

The antifungal activity of the consortia was evaluated using the agar diffusion test. Inoculum consisted in a conidial suspension (10^4 conidia/mL) of each isolate. The concentration of the suspension was adjusted using a Neubauer chamber (Puglisi et al., 2017). The test was performed according with the method described by Bauer (1966) with slight modifications. The lyophilized consortia were re-suspended in sterile distilled water to achieve a final concentration of 400 g/L. Dishes containing potato dextrose agar (PDA) were inoculated with the conidium suspension using sterile swabs to spread the suspension over the agar medium surface. Wells were made in the agar using sterile 1000 μL tips, and 100 μL of the diverse consortium suspensions were added to each well. Dishes were incubated at 25 °C for 72 h. After the incubation period, the inhibition halo around each well was measured, and the antifungal activity was rated as follows: (+) indicating an inhibition zone of 4–8 mm between the well edge and the front of mycelium growth, (++) indicating an inhibition zone of 8.1–12 mm, and (+++) indicating an inhibition zone >12 mm.

Both the minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC) were determined according with the method described by De Melo Nazareth et al. (2019) to quantitatively measure the antifungal activity of consortia. MIC, defined as the minimum concentration of the test substance that completely inhibits visible growth (Stracquadanio et al., 2020), was determined using a microdilution method. In 96-well plates, 100 μL of a fungal suspension containing 10^5 spores/mL were mixed with 100 μL of sterile potato dextrose broth (PDB) in a 1:1 (v/v) ratio, resulting in a series of 10 serial dilutions of the test substance ranging from 0.1 to 200 g/L. A negative control (200 μL of sterile PDB) and a positive control (100 μL of sterile PDB mixed with 100 μL of the fungal suspension containing 10^5 spores/mL) were included. The plates were then incubated at 25 °C for 3 days. At the end of the incubation period, the MIC was determined as the lowest concentration where no cloudiness or visible growth was observed in the wells, indicating complete inhibition of pathogen growth. Each test was performed in four replicates to ensure accuracy and reliability.

To evaluate the MFC, 10 μL of the MIC and higher concentrations were transferred onto potato dextrose agar (PDA) dishes. The dishes were incubated at 25 °C for 3 days. The MFC was determined as the lowest concentration of the substance required to completely eradicate fungal pathogens, resulting in the absence of any mycelial growth.

2.10. In vivo efficacy of consortia

Based on the chemical characterization of the consortia and the results of *in vitro* assays, the consortia 1 % FR47 T1 and T2 were selected for *in vivo* testing. The *in vivo* antagonist efficacy test of the consortia followed the protocol reported by Siahmoshteh et al. (2017) with minor modifications. Each experimental trial consisted of ten replications per treatment, and the experiments were repeated three times to ensure accuracy and reliability.

Maize cobs and lemon fruits, obtained from a biological commercial market in Valencia (Spain) were carefully surface disinfected by dipping in a 0.5 % solution of NaCl for 5 min, followed by rinsing in sterile distilled water (sdw). Subsequently, the samples were immersed in 70 % ethanol for 5 min and rinsed again in sdw for 15 min. Afterward, the seeds and fruits were air-dried under sterile conditions in a laminar flow hood.

Once the rind surface was dried, the lemon fruits were gently wounded using a sterile needle (a single hole per fruit). Then, 20 μL of each consortium (400 g/L) were injected into each hole. After air-drying, 10 μL of a *P. expansum* conidial suspension (3×10^4 conidia/mL) was inoculated into each hole. The efficacy of the consortia was evaluated by calculating the disease severity (DS) using the scale proposed by Chen et al. (2019). The scale ranged from 0 (no decay, lesion diameter = 0 mm) to 3 (lesion diameter > 10 mm). The average lesion diameter was measured 7 and 10 days post inoculation (dpi), and the disease severity was calculated using the formula:

$$DS(\%) = \frac{\Sigma \text{ disease scale } \times \text{ number of fruit in each scale}}{\text{highest disease scale } \times \text{ number of total fruit}} \times 100$$

For the maize cobs, ten random wounds were made on each cob. Each wound was treated with 1 mL of the antagonistic consortia (200 g/L) using a spray application. The treated samples were air-dried before the fungal pathogen (*F. graminearum*, 8×10^4 conidia/mL) was applied. Controls for both trials consisted of inoculating the samples only with the pathogen. Subsequently, all samples were placed in sterile plastic containers and incubated at 25 °C. The incubation period lasted until all positive control samples showed visible fungal growth, with daily monitoring.

The efficacy of the consortia was evaluated by calculating the sporulation capacity of *F. graminearum*, expressed as the number of spores produced (Evangelista et al., 2021). Specifically, at the end of the 4-day incubation period, 10 g of maize from each treatment were added to 90 mL of 0.1 % H2O tween solution (1:10 ratio) and homogenized. Serial dilutions were performed, and 100 μL of each dilution was inoculated onto potato dextrose agar (PDA) dishes. The dishes were then incubated for 3 days at 25 °C, and the fungal population was counted as log (CFU/g) following the method described by Nazareth et al. (2018).

2.11. Statistical analysis

Data from *in vitro* and *in vivo* experiments were analysed separately by using the Statistical package software Minitab version 16.0, and the arithmetic means were calculated and analysed by using one-way analysis of variance (ANOVA). Fischer's least significant difference test was used to compare the significance of differences among *in vitro* and *in vivo* data at $p < 0.05$. The metabolites obtained from consortia LAB x *S. platensis* were analysed with Pearson's correlation analysis, and MetaboAnalyst 5.0 software (Pang et al., 2021) was used for Heatmap using log10 transformed data.

3. Results

3.1. pH measurement

In Table 1, the pH values of the consortia after 24 and 48 h of

Table 1
Ph values of pasteurized consortia fermented by labs after 24 and 48 h incubation at 37 °C, respectively.[a]

CFS sample	T1 (24 h of fermentation)	T2 (48 h of fermentation)
1 % Ctrl	5.05	5.03
1 % FR47	3.62	3.53
1 % FR48	3.55	3.55
5 % Ctrl	5.15	5.11
5 % FR47	3.82	3.78
5 % FR48	3.79	3.76

[a] Controls were incubated under the same conditions without LAB inoculation.

fermentation at 37 °C are shown. As expected, pH values of the LAB-fermented consortia were significantly lower than non-inoculated controls. The controls showed a pH from 5.03 to 5.15 while the pH values of the consortia ranged from 3.53 to 3.82, probably due to the production of metabolites, such as lactic acid, acetic acid, and phenyllactic acid, which cause the reduction of pH.

3.2. Antifungal activity assays

In laboratory assays, a noticeably inhibitory activity of the fermented consortia, as determined qualitatively by the agar diffusion test, was observed for all seven fungal pathogens tested (*A. alternata*, *A. niger*, *A. flavus*, *F. graminearum*, *G. zeae*, *P. commune*, and *P. expansum*) (Table 2). Conversely, none of the control suspensions showed inhibitory activity on mycelial growth.

Fermentates of consortia showed similar inhibitory activity against all tested fungi (Table 2). Among these fungi, *F. graminearum* and *G. zeae* were the most sensitive and the most noticeable inhibitory activity was shown by 1 % FR47 T1 and T2 and 1 % FR48 T1 and T2. On the contrary, *A. flavus* and *A. niger* were the least sensitive to the inhibitory activity of the CFSs of consortia.

Fermentates were further tested to determine MIC and MFC values. Fermentates 1 % FR47 T1 and T2 and 1 % FR48 T1 and T2 were again those with the highest inhibitory activity on all tested fungi (Table 3). The values of MIC of fermentates 1 % FR47 T1 and T2 and 1 % FR48 T1 and T2 for all the fungi tested ranged from 3.13 to 25 g L^{-1}. The lowest values (3.13 g L^{-1}), indicating a high inhibitory activity, were recorded in tests on *G. zeae*. Values of MFC of these four fermentates were higher than MIC values, ranking from 3.13 for *G. zeae* to a maximum of 100 g L^{-1} for *Aspergillus* and *Penicillium* species.

3.3. Identification of antifungal compounds

Lactic acid, that is one of the major compounds responsible for the antifungal activity of LABs, was quantified in the CFSs from consortia fermented by *L. plantarum* DMS 1055 and *L. plantarum* DMS 2601. The lactic acid concentration in these CFSs varied from 8.48 to 10.65 g L^{-1} while lactic acid was not detected in non-fermented and control samples (Table 4).

Phenolic compounds found in CFSs of the fermented consortia are reported in Table 5. In total, seven different compounds were identified (DL-3-phenyllactic acid, benzoic acid, 3–4-dihydroxyhydrocinnamic acid, hydroxicinnamic acid, p-coumaric acid, salicylic acid, and sinapic acid). DL-3-phenyllactic acid, benzoic acid, and 3–4-dihydroxyhydrocinnamic acid were the most abundant, with concentrations varying significantly among the diverse fermentate samples (Table 5). As regards the other four phenolic acids no significant differences were detected between diverse fermentates (Table 5).

3.4. Antioxidant activity and total polyphenols

The results of ORAC and Folin- Ciocâlteu assays are shown in Table 6. All fermented samples showed significant decreases ($p < 0.05$) in ORAC activity compared to non-fermented samples, with values ranging from 968 to 1380. Contrarily, the quantification of total polyphenol content revealed an increasing trend in the fermented samples. The highest contents were found in 1 % FR47 T2 and 5 % FR48 T2.

3.5. Efficacy of fermented consortia in preventing fungal growth on plant matrices

Treatments with CFSs of both tested consortia significantly reduced sporulation and disease incidence in comparison to the controls (Figs. 1 and 2). In particular, the treatment with CFSs of fermented consortia reduced significantly the sporulation of *F. graminearum* on maize cobs (Fig. 3A). The antagonistic activity of the two microbial consortia against *F. graminearum* did not differ significantly, while both consortia reduced significantly the sporulation of this fungal pathogen compared with the control. The amount of conidia (log g^{-1}) produced on inoculated maize cobs used as controls was of two orders of magnitude higher than that observed in cobs treated with CFSs of microbial consortia. Similarly, as regards the assays on lemon fruits, both consortia showed a comparable antifungal activity, being able to suppress the mycelial growth and sporulation of *P. expansum* and leading to a significant reduction of disease severity compared to the non-treated control at both time intervals after inoculation (Fig. 3B).

3.6. Metabolites

A total of 66 metabolites from CFSs of consortia and the control were identified and quantified (Fig. 4). They encompassed a wide spectrum of compounds, including amino acids, fatty acids, organic compounds, and hormone-like substances. Several of the metabolites detected were compounds involved in physiological and metabolic processes, able to

Table 2
Inhibitory activity of fermented consortia (T1 and T2) of *Lactiplantibacillus plantarum,* FR 47 or FR48 strain, plus 1 or 5 % *Arthrospira platensis* in MM, on potential toxigenic fungal contaminants of agricultural produces and food, as determined by the agar diffusion assay. Diameter of Inhibition Halos (DIH) was rated as follows: '−', no inhibition; '+', DIH from 4 to 8 mm; '++', DIH from 8.1 to 12 mm; '+++', DIH >12 mm. T0: non-fermented consortia; T1 and T2: consortia fermented for 24 and 48 h, respectively. 1 or 5 % Ctrl - T0: *A. platensis* in MM; 1 or 5 % Ctrl – T1, 1 or 5 % Ctrl – T2: *A. platensis* in MM incubated at 37 °C for 24 h or 48 h, respectively.

CFS sample	Fungi						
	A. alternata	*A. flavus*	*A. niger*	*F. graminearum*	*G. zeae*	*P. commune*	*P. expansum*
1 % Ctrl - T0	–	–	–	–	–	–	–
1 % Ctrl - T1	–	–	–	–	–	–	–
1 % Ctrl - T2	–	–	–	–	–	–	–
1 % FR47 - T0	–	–	–	–	–	–	–
1 % FR47 - T1	++	++	+	+++	++	+++	++
1 % FR47 - T2	++	+	+	+++	+++	++	++
1 % FR48 - T0	–	–	–	–	–	–	–
1 % FR48 - T1	++	+	+	+++	++	++	++
1 % FR48 - T2	++	+	+	+++	+++	+	++
5 % Ctrl - T0	–	–	–	–	–	–	–
5 %Ctrl - T1	–	–	–	–	–	–	–
5 % Ctrl - T2	–	–	–	–	–	–	–
5 % FR47 - T0	–	–	–	–	–	–	–
5 % FR47 - T1	+	+	+	++	++	+	+
5 % FR47 - T2	+	+	+	+	++	+	+
5 % FR48 - T0	–	–	–	–	–	–	–
5 % FR48 - T1	+	+	+	++	++	+	+
5 % FR48 - T2	+	+	+	+	+	+	+

Table 3
Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of fermented consortia (T1 and T2) of *Lactiplantibacillus plantarum,* FR 47 or FR48 strain, plus 1 or 5 % *Arthrospira platensis* in MM tested against diverse potential toxigenic fungal contaminants of agricultural produces and food. T0: non fermented consortia; T1 and T2: consortia fermented for 24 and 48 h, respectively. 1 or 5 % Ctrl - T0: *A. platensis* in MM; 1 or 5 % Ctrl – T1, 1 or 5 % Ctrl – T2: *A. platensis* in MM incubated at 37 °C for 24 h or 48 h, respectively.

	Fungi													
	A. alternata		*A. flavus*		*A. niger*		*F. graminearum*		*G. zeae*		*P. commune*		*P. expansum*	
CFS sample	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
1 % Ctrl - T0	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
1 % Ctrl - T1	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
1 % Ctrl - T2	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
1 % FR47 - T0	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
1 % FR47 - T1	6.25	6.25	12.5	50	12.5	50	6.25	25	3.13	3.13	6.25	25	6.25	25
1 % FR47 - T2	6.25	6.25	12.5	50	12.5	50	6.25	25	3.13	3.13	6.25	25	6.25	25
1 % FR48 - T0	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
1 % FR48 - T1	25	25	12.5	50	12.5	50	6.25	25	3.13	3.13	12.5	100	12.5	100
1 % FR48 - T2	6.25	12.5	12.5	50	12.5	50	6.25	12.5	3.13	3.13	12.5	50	12.5	50
5 % Ctrl - T0	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
5 % Ctrl - T1	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
5 % Ctrl - T2	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
5 % FR47 - T0	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
5 % FR47 - T1	6.25	6.25	25	50	25	50	6.25	50	3.13	12.5	25	100	25	100
5 % FR47 - T2	6.25	6.25	25	50	25	50	12.5	25	3.13	25	25	100	25	100
5 % FR48 - T0	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
5 % FR48 - T1	25	25	12.50	100	12.5	100	12.5	50	6.25	25	12.5	100	12.5	100
5 % FR48 - T2	6.25	12.5	25	100	25	100	12.5	25	6.25	6.25	12.5	100	12.5	100

Table 4
Quantification of lactic acid in fermented consortia (T1 and T2) of *Lactiplantibacillus plantarum,* FR 47 or FR48 strain, plus 1 or 5 % *Arthrospira platensis* in MM [abcd]. T0: non fermented consortia; T1 and T2: consortia fermented for 24 and 48 h, respectively. 1 or 5 % Ctrl - T0: *A. platensis* in MM; 1 or 5 % Ctrl – T1, 1 or 5 % Ctrl – T2: *A. platensis* in MM incubated at 37 °C for 24 h or 48 h, respectively.[abcd.]

CFS sample	**Lactic acid**
1 % Ctrl - T0	nd
1 % Ctrl - T1	nd
1 % Ctrl - T2	nd
1 % FR47 - T0	nd
1 % FR47 - T1	8.48 ± 0.05c
1 % FR47 - T2	9.38 ± 0.11b
1 % FR48 - T0	nd
1 % FR48 - T1	10.52 ± 0.05 a
1 % FR48 - T2	10.65 ± 0.62 a
5 % Ctrl - T0	nd
5 % Ctrl - T1	nd
5 % Ctrl - T2	nd
5 % FR47 - T0	nd
5 % FR47 - T1	8.70 ± 0.03 bc
5 % FR47 - T2	9.08 ± 0.11bc
5 % FR48 - T0	nd
5 % FR48 - T1	9.43 ± 0.32b
5 % FR48 - T2	8.68 ± 0.51 bc

[a]Results are expressed in gL^{-1}. [b] Means ± SE (n = 3). [c] nd = data under the limit of detection. [d]Values followed by different letters in the same column are significantly different according to Fisher's least significance difference test ($p < 0.05$).

positively affect the growth, improve the vigor and enhance the tolerance of the plant to stress conditions. Only the metabolite profile of the consortium 1 % FR47, compared with the control, showed a significant higher amount of metabolites known for their potential antifungal activity, such as benzoic acid, leucine, oleic acid, and proline, which are able to interfere with fungal cellular processes or to inhibit enzyme activity, leading to mycelium growth inhibition or cell death.

4. Discussion

This study demonstrated the antifungal activity of CFSs of fermented consortia of the cyanobacterium *A. platensis* and either one or the other of two selected strains of the lactobacillus *L. plantarum*. Moreover, it investigated the chemical nature and properties of CFSs of fermented consortia in order to contribute to a better understanding of the mechanisms underlying their antifungal efficacy. Interestingly, fermentates of microbial consortia showed a significant *in vitro* inhibitory activity against a wide range of fungal plant pathogens and food contaminants and were effective in preventing infections by two very common toxigenic fungi, *F. graminearum* and *P. expansum*, in specific plant matrices such as maize cobs and lemon fruits, respectively. Fusarium graminearum is one of the most damaging fungal pathogens of cereals, including wheat, barley, oats, maize and rice (Bentivenga et al., 2021; Del Ponte et al., 2022; Senatore et al., 2023; Zhou et al., 2018). Infections by this pathogen not only result in severe yield losses but also in grain contamination with various mycotoxins, such as the trichotecene deoxynivalenol and the estrogenic mycotoxin zearalenone (Chen et al., 2019; Foroud et al., 2019; Munkvold et al., 2021). The fungus *P. expansum*, though mainly known as the causal agent of blue mold of apples, can infect a broad range of hosts including citrus, which were repeatedly demonstrated by diverse Authors to be susceptible to artificial inoculations by this pathogen (Chen et al., 2022; Luciano-Rosario et al., 2020). *Penicillium expansum* produces an array of mycotoxins, in particular the polyketide patulin, a common contaminant of pome fruits, both apples and pears, and their processed products (Hocking, 2014). The presence and maximum tolerable levels of this toxin in fruits and foodstuffs is regulated in several countries (Aslam et al., 2021; EUR-Lex, 2023; Hussain et al., 2020). Recently, it has been reported that also *P. digitatum*, the causal agent of green mold of citrus fruits, can produce patulin in infected sweet orange fruits (Rovetto et al., 2023). Interestingly, CFSs from fermented consortia of *A. platensis* and *L. plantarum* tested in this study showed a comparable *in vitro* inhibitory activity against different fungus species and genera, indicating the antifungal activity was not species-specific. The results of chemical analysis suggest that the fermentates from the microalgae/LABs consortium showed a higher concentration of certain phenolic compounds, including DL-3-phenylactic acid, benzoic acid, and 3,4-dihydroxyhydrocinnamic acid, compared with control fermentates of *A. platensis* (Ctrl 1–5 %), thus

Table 5
Identification and quantification of phenolic compounds in fermented consortia (T1 and T2) *of Lactiplantibacillus plantarum*, FR 47 or FR48 strain, plus 1 or 5 % *Arthrospira platensis* in MM[abcd]. T0: non fermented consortia; T1 and T2: consortia fermented for 24 and 48 h, respectively. 1 or 5 % Ctrl - T0: *A. platensis* in MM; 1 or 5 % Ctrl – T1, 1 or 5 % Ctrl – T2*: A. platensis* in MM incubated at 37 °C for 24 h or 48 h, respectively.

	Phenolic acids						
CFS sample	**DL-3-Phenyllactic acid**	**Benzoic acid**	**3–4-Dihydroxy hydrocinnamic acid**	**Hydroxycinnamic acid** [e]	**P-Coumaric acid** [e]	**Salicylic acid** [e]	**Sinapic acid** [e]
1 % Ctrl - T0	nd	nd	nd	0.06 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
1 % Ctrl - T1	0.44 ± 0.17 e	nd	0.02 ± 0.01b	0.02 ± 0.01	nd	0.01 ± 0.01	nd
1 % Ctrl - T2	0.48 ± 0.18 e	nd	0.04 ± 0.01b	0.02 ± 0.01	0.10 ± 0.02	0.02 ± 0.01	0.09 ± 0.01
1 % FR47 - T0	0.06 ± 0.03 e	nd	0.02 ± 0.01b	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
1 % FR47 - T1	4.42 ± 0.06 ab	0.51 ± 0.16 ab	0.49 ± 0.19 a	0.03 ± 0.01	0.10 ± 0.03	nd	0.03 ± 0.01
1 % FR47 - T2	3.15 ± 0.71 bcd	0.54 ± 0.08 ab	0.43 ± 0.07 a	0.02 ± 0.01	0.09 ± 0.04	0.04 ± 0.01	0.05 ± 0.01
1 % FR48 - T0	3.17 ± 1.03 bcd	0.23 ± 0.19c	0.40 ± 0.04 a	0.04 ± 0.01	0.08 ± 0.06	0.03 ± 0.01	0.04 ± 0.01
1 % FR48 - T1	0.10 ± 0.01 e	nd	nd	0.01 ± 0.01	0.09 ± 0.01	0.03 ± 0.01	0.06 ± 0.01
1 % FR48 - T2	4.58 ± 1.13 a	0.61 ± 0.05 a	0.48 ± 0.18 a	0.02 ± 0.01	0.08 ± 0.06	0.03 ± 0.01	0.08 ± 0.02
5 % Ctrl - T0	0.05 ± 0.02 e	nd	nd	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
5 % Ctrl - T1	2.21 ± 0.14 d	0.25 ± 0.14c	0.33 ± 0.08a	nd	0.07 ± 0.02	0.02 ± 0.01	0.04 ± 0.01
5 % Ctrl - T2	4.09 ± 0.02 abc	0.37 ± 0.01 bc	0.36 ± 0.01a	nd	0.07 ± 0.02	0.03 ± 0.01	0.05 ± 0.01
5 % FR47 - T0	0.56 ± 0.25 e	nd	0.04 ± 0.01b	0.05 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
5 % FR47 - T1	0.15 ± 0.02 e	nd	nd	0.02 ± 0.01	0.10 ± 0.01	0.03 ± 0.01	0.08 ± 0.01
5 % FR47 - T2	0.13 ± 0.01 e	nd	nd	nd	nd	nd	nd
5 % FR48 - T0	2.77 ± 0.73 cd	0.30 ± 0.01c	0.29 ± 0.02a	0.01 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	nd
5 % FR48 - T1	0.26 ± 0.01 e	nd	nd	0.01 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
5 % FR48 - T2	3.90 ± 0.24 abc	0.42 ± 0.01 abc	0.43 ± 0.03 a	0.03 ± 0.01	0.08 ± 0.01	0.03 ± 0.01	0.07 ± 0.02

[a]Results are expressed in mg mL^{-1}. [b] Means ± SE (n = 3). [c] nd = under the limit of detection. [d] Values followed by different letters are significantly different according to Fisher's least significance test ($p < 0.05$). [e] No significant differences between means.

Table 6
Total polyphenol content (mg GAE L^{-1}) and ORAC (μM Trolox eq mL-1) of fermented microbial consortia[ab] T0: non fermented consortia; T1 and T2: consortia fermented for 24 and 48 h, respectively. 1 or 5 % Ctrl - T0: *A. platensis* in MM; 1 or 5 % Ctrl – T1, 1 or 5 % Ctrl – T2*: A. platensis* in MM incubated at 37 °C for 24 h or 48 h, respectively.

Sample	**ORAC (μmoles Trolox eq mL^{-1})**	**Total polyphenol content (mg GAE L^{-1})**
1 % Ctrl - T0	18.42 ± 0.81 bc	1102.22 ± 25.02 ghi
1 % Ctrl - T1	11.32 ± 0.80 de	968.15 ± 78.91 j
1 % Ctrl - T2	12.51 ± 2.48 cde	1194.07 ± 54.13 efg
1 % FR47 - T0	15.60 ± 0.25 bcd	1380.00 ± 22.96b
1 % FR47 - T1	11.48 ± 2.71 de	1058.52 ± 52.11 hij
1 % FR47 - T2	11.90 ± 5.38 de	1352.59 ± 63.49 bc
1 % FR48 - T0	31.66 ± 2.76 a	1150.37 ± 30.55 efgh
1 % FR48 - T1	11,0.69 ± 3.10 de	1254.07 ± 13.60 cde
1 % FR48 - T2	14.99 ± 1.96 bcd	1586.67 ± 27.59 a
5 % Ctrl - T0	11.26 ± 0.80 de	1229.63 ± 29.71 def
5 % Ctrl - T1	19.06 ± 1.13b	1028.15 ± 27.61 ij
5 % Ctrl - T2	15.01 ± 0.87 bcd	1193.33 ± 40.58 efg
5 % FR47 - T0	15.19 ± 0.90 bcd	1354.81 ± 45.78 bc
5 % FR47 - T1	12.22 ± 3.13 cde	1248.89 ± 10.29 cde
5 % FR47 - T2	14.36 ± 2.22 bcde	1666.67 ± 19.30 a
5 % FR48 - T0	20.12 ± 1.29b	1339.26 ± 18.64 bcd
5 % FR48 - T1	13.89 ± 1.11 bcde	1128.15 ± 44.52 fghi
5 % FR48 - T2	8.24 ± 0.04 e	1208.89 ± 50.22 efg

[a]Means ± SE (n = 3). [b] Values followed by different letters are significantly different according to Fisher's least significance test ($p < 0.05$).

confirming the crucial and synergistic role of this microalga in the production of antifungal fermentates. The antifungal activity of these compounds and their role in preventing post-harvest spoilage of agricultural products have been previously documented by several studies, although there are some discrepancies between the effective doses of these compounds reported by diverse Authors (Dalié et al., 2010; Gerez et al., 2009; Omedi et al., 2019; Vilanova et al., 2012). No significant differences between fermentates and controls were observed for other phenolic compounds, such as hydroxycinnamic, p-cumaric and salycilic acids. Moreover, in fermented microbial consortia total polyphenol content showed a tendency to increase, while there was a significant decrease of ORAC, which is a measure of total antioxidant capacity (Schaich et al., 2015; Zulueta et al., 2009). No obvious correlation was noticed between the antifungal activity and the secondary metabolite profile of fermented microbial consortia as determined by UHPLC-Q-TOF-MS analysis. Most of the 66 metabolites detected do not possess inherent antifungal or biocidal properties. The only exception was the metabolite profile of the consortium of *A. platensis* and 1 % *L. plantarum* strain FR47, which showed a high relative amount of compounds known for their antifungal activity such as benzoic acid, leucine, oleic acid, and proline (Levashina et al., 1995; López et al., 2002; Taniguchi et al., 2016; Walters et al., 2004). While the presence of lactic acid, phenolic compounds and secondary metabolites with antifungal activity is sufficient per se to explain the *in vitro* inhibitory activity of fermented microbial consortia, it cannot ruled out that the efficacy of CFSs from consortia fermentates in preventing fungal infections on plant matrices was also the result of priming effects on plant immune system, as demonstrated for other BCAs or abiotic elicitors in diverse pathosystems (La Spada et al., 2020; Orozco-Mosqueda et al., 2023; Pršić and Ongena,

Fig. 1. Performance of microbial consortia (1% FR47 – T1 and 1% FR47 – T2) in inhibiting colonization of maize cobs by *Fusarium graminearum* compared with untreated control.

Fig. 2. Performance of microbial consortia (1% FR47 – T1 and 1% FR47 – T2) in inhibiting *Penicillium expansum* infection on lemon fruits compared with untreated control.

2020; Saijo and Loo, 2020).

Bacteria and cyanobacteria or their consortia may be a sustainable alternative to synthetic fungicides for the management of post-harvest rots and contamination by mycotoxigenic fungi of agricultural products. Previously, numerous evidences of the antifungal properties of LABs have been provided and the potential of LABs to prevent spoilage caused by toxigenic fungi has been exploited in a variety of agricultural products, feed- and foodstuffs (Sadiq et al., 2019). For instance, selected strains of *L. plantarum* preserved cheese from contamination by diverse *Penicillium* species and grape from post-harvest contamination by both *Aspergillus ochraceus* and *Botrytis cinerea* (Dopazo et al., 2021). In another study, selected strains of *L. plantarum* and *Pediococcus pentosaceus* isolated from olive drupes showed a noticeable *in vitro* inhibitory activity against *Fusarium oxysporum*, *Penicillium nordicum* and diverse *Colletotrichum* species (Riolo et al., 2023a). Luz et al. (2020a) evaluated the antifungal properties of a whey-based medium (WM) fermented by LABs and experienced that the use of fermented WM for dough preparation reduced significantly the contamination by *P. expansum* and prolonged the shelf life of bread. Ouiddir et al. (2019) screened a large set of LABs recovered from both milk and a traditional fermented milk product for antifungal activity and selected *L. plantarum*, *L. paracasei* and *Leuconostoc mesenteroides* strains as candidate food bio-preservatives. Very recently, Volentini et al. (2023) demonstrated the *in vitro* inhibitory activity of CFSs from *L. paraplantarum*, *L. fermentum*, *L. casei* and *L. reuteri* against *P. digitatum* and *P. italicum*. The treatment with the CFS of the *L. paraplantarum* strain, which showed the strongest *in vitro* antifungal activity, reduced substantially the severity of Penicillium mold on lemon fruits. De Melo Nazareth et al. (2019) reported that the treatment with the CFS from liquid cultures of a *L. plantarum* strain, selected for its high *in vitro* antifungal activity, reduced significantly aflatoxin B_1 and fumonisin B_1 content in kernels and ears of corn contaminated by *A. flavus* and *F. verticillioides*, respectively. Yépez et al.

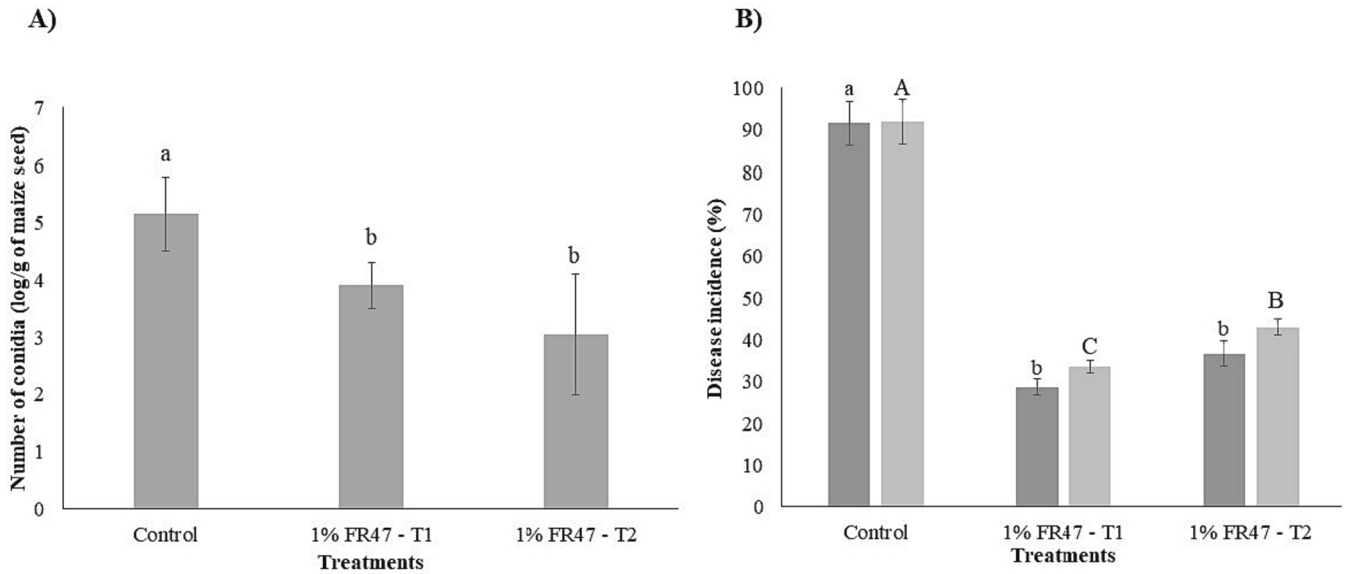

Fig. 3. A. *Fusarium graminearum* sporulation after 4 days of incubation expressed as number of conidia per gram of plant matrix (log g^{-1}) produced in infected wounds (means ± SE). Different letters indicate statistical significance according to Fisher's protected LSD test ($p < 0.05$). Fig. 3**B.** *Penicillium expansum* disease severity (%) after 7 and 10 days (dark gray and light gray, respectively) of incubation (means ± SE). Different letters indicate statistical significance according to Fisher's protected LSD test ($p < 0.05$). The lowercase letters refer to the significance of treatments at 7 days, while the uppercase letters indicate significance after 10 days. The trials on corn and lemon (A and B) were repeated 2 times.

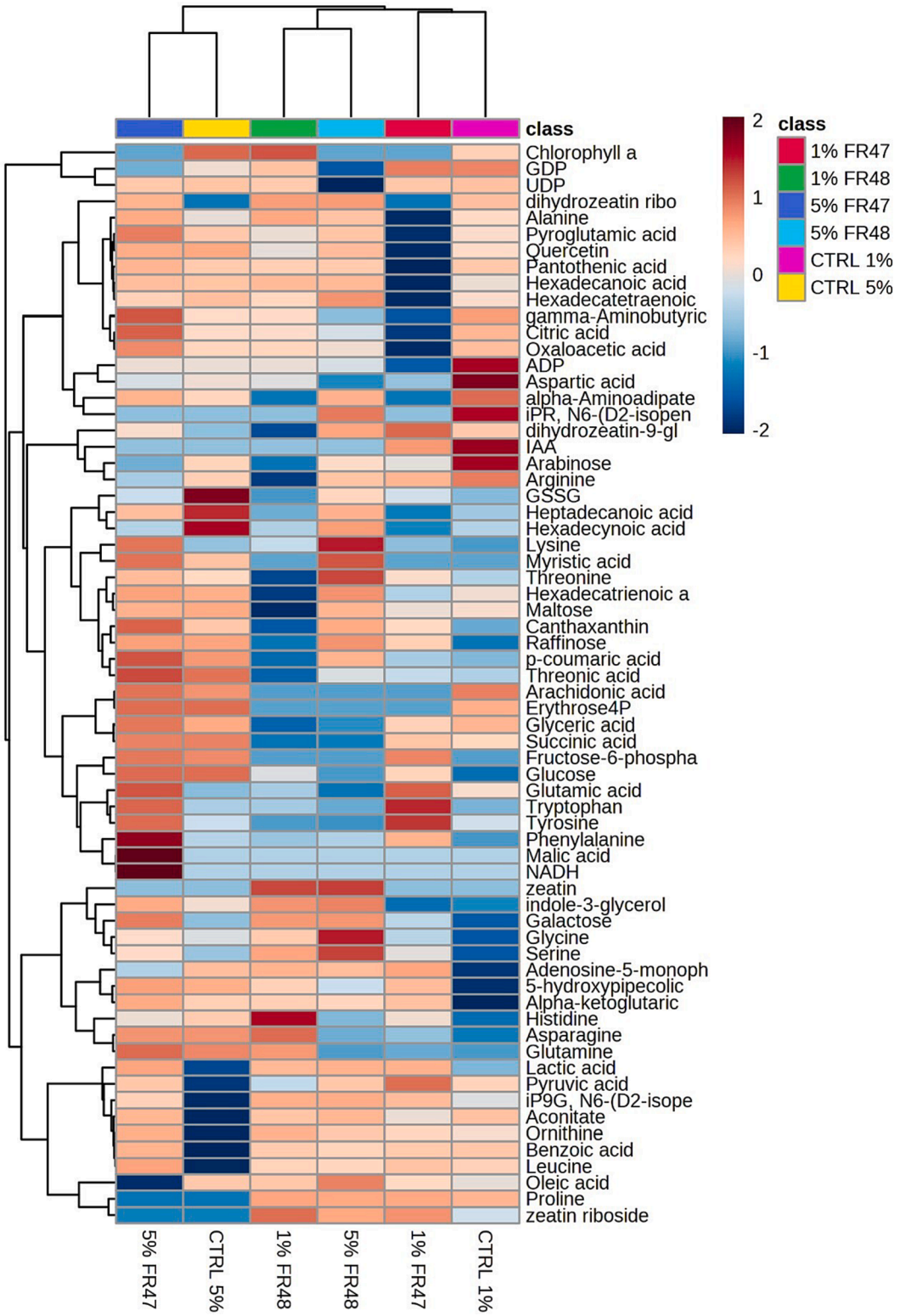

Fig. 4. Heat map of metabolites contained in fermented consortia characterised in this study. Colours indicate the relative amount of metabolites (logarithmic scale): red represents high abundance and blue low abundance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(2017) recovered a total of 130 LAB strains, representing nine species of four diverse genera, from typical fermented plant products of Andean origin and screened them for the antimicrobial activity against a set of food contaminant fungi, including *Meyerozyma guilliermondii* (syn. *Pichia guilliermondii*), *P. roqueforti*, *A. oryzae* and *A. niger*, as well as the foodborne human pathogens *Escherichia coli*, *Listeria innocua* and *Salmonella enterica* subsp. *enterica* serovar Typhi. Inhibitory activity of CFSs from seven selected strains grown in MRS liquid medium was confirmed against the toxigenic fungi *A. parasiticus*, *P. expansum* and *F. verticilloides*, as well as on the three foodborne bacteria included in the

aforementioned study. Phenyllactic and 3,5-Di-O-caffeoylquinic acids were identified as the predominant bioactive compounds in CFSs. Four strains, including two strains of *L. plantarum*, a strain of *L. fermentum* and a strain of *Lc. mesenteroides*, showed high potential as food bio-preservatives. Conversely, regarding the use of microalgae and cyanobacteria as bio-fungicides fewer studies can be found in literature. For instance, Pawar and Puranik (2008) screened 40 cyanobacteria strains isolated from terrestrial and freshwater habitats for antifungal activity against five phytopathogenic fungi.

Consistently with the literature, results of the present study further confirm that LABs produce a large array of antifungal metabolites including volatile compounds (Dopazo et al., 2021; Riolo et al., 2023a) and the antimicrobial activity of these metabolites depend on their ability of lowering the pH of culture medium (Leyva Salas et al., 2017; Luz et al., 2020b; Volentini et al., 2023). Beside the antifungal activity some LABs are able to adsorb, degrade or detoxify fungal mycotoxins (Sadiq et al., 2019). The rationale of using fermented consortia of LABs and cyanobacteria/microalgae is to broaden the spectrum of antifungal metabolites and to exploit synergisms between diverse metabolites as well as plant growth promoting and resistance eliciting properties of some algal metabolites.

Overall, results of this study unveil the potential of CFSs from fermented microbial consortia to prevent fungal post-harvest diseases and contamination by mycotoxins of agricultural produces. However further experiments, including the evaluation of the effects on the quality of products, are needed to confirm the feasibility of large-scale application of these consortia as biofungicide in food supply chains.

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CRediT authorship contribution statement

Emanuele La Bella: Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Mario Riolo:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Carlos Luz:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Andrea Baglieri:** Writing – review & editing, Visualization, Validation, Methodology, Conceptualization. **Ivana Puglisi:** Writing – review & editing, Visualization, Validation, Methodology, Conceptualization. **Giuseppe Meca:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization. **Santa Olga Cacciola:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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