

Invitro antifungal and probiotic synergy of Lactiplantibacillus derived from tropical fruits: Efficacy against phytopathogen Fusarium oxysporum

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Abstract

Fifty-five putative lactic acid bacteria (LAB) strains were isolated from seven distinct tropical fruits. The highest prevalence of LABs was identified in the *Solanum nigrum* fruit, with *Couroupita guianensis* and *Musa* fruits displaying the lowest counts. Remarkably, two strains, isolated from *Ficus racemosa*, exhibited notable antifungal activity against *Fusarium oxysporum*. Sequencing 16S rDNA confirmed the strain as *Lactiplantibacillus plantarum* MYSVCF3 and *Lpb. argentoratensis* MYSVCF5. The isolates demonstrated adaptability to wide temperatures (10–45°C), pH (pH 2–7), salt (up to 7%), and invitro simulated gastrointestinal conditions. Thus, the strains exhibited characteristic features typical of probiotics. *Lpb. argentoratensis* MYSVCF5 effectively inhibited the growth of *F. oxysporum* and ESKAPE pathogens. 10% cell-free supernatant (CFS) could reduce the biomass yield by 94% and completely inhibit germination of conidia. CFS retained its activity even after long cold storage conditions. LC-MS/MS analysis identified organic acids in CFS, with citric acid as the most abundant component followed by lactic and malic acid. This study showed promising antifungal properties against phytopathogen, making them potential candidates for various applications, including probiotics and antifungal agents in food and agriculture.

Introduction

The contamination of food and agricultural products by *Fusarium oxysporum* poses a formidable challenge, resulting in substantial economic losses, food wastage, and compromised food safety^[1, 2]. *F. oxysporum* is widely distributed in the environment and is particularly endemic to tropical and subtropical areas. Over 120 different plants are listed as hosts for *F. oxysporum*, causing various devastating plant diseases, including vascular wilt, sweet potato wilt, tomato wilt, and Panama disease^[3–5]. In efforts to mitigate such losses, antifungal agents, including fumigants are commonly used for disinfection. However, the effectiveness of these treatments is often inadequate and their usage is limited due to environmental consequences^[6]. Alternatively, applying biocontrol agents such as live microbes, cell-free supernatants, and purified compounds used as fungicides in biocontrol products is gaining prominence as a viable strategy^[7].

The endophytic microorganisms have the potential to produce secondary metabolites that can inhibit the growth of pathogenic fungi. The need for chemical fungicides can be reduced by harnessing natural capabilities while promoting sustainable agricultural practices^[8]. Among these include lactic acid bacteria (LAB), extensively studied and recognized as probiotics due to their numerous health benefits^[9,10]. Traditionally, probiotic bacteria were isolated from fermented foods such as yogurt and consumed as probiotics^[11,12]. Microbiologically, fruits are safer than other raw foods such as milk, meat, etc. Their distinct chemical composition, buffering capacity, and presence of antagonistic compounds create a conducive habitat for bacterial adaptation, including probiotic strains like LAB^[13]. Therefore, fruits serve as continuous and viable sources of probiotic bacteria. Moreover, with the growing demand

for non-dairy alternatives, fruit offers a practical choice for populations with lactose intolerance, dyslipidemia, and those adhering to a vegan lifestyle^[14–16].

Several LAB strains have been successfully isolated from a variety of fruits such as pineapples, oranges, watermelons, papaya, berries, etc^[14,17,18]. Notably, *Lactobacillus rossiae*, *L. brevis*, *Weissella cibaria*, *Weissella paramesenteroides*, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* have been frequently identified in fruits^[17,19]. However, most investigations have only focused on the LAB's presence in fruits or its role in fermentation processes^[19,20].

Given that *F. oxysporum* is native to tropical and subtropical regions, we hypothesize that the LAB derived from tropical fruits possesses antimicrobial properties specifically, antifungal activities that are effective against *F. oxysporum*. By harnessing the antibacterial and antifungal properties of LAB derived from tropical fruits, we could develop natural, probiotic-based solutions to combat *F. oxysporum* infection. Not only would this be a safer option for human consumption, but it would also positively impact the environment by reducing the need for chemical fungicides. With this foreground, this study aimed to identify LAB from fruits having these properties. Accordingly, the study objective was structured to (i) isolate LAB from fruits, (ii) screen and evaluate the antifungal activity of these isolates against *F. oxysporum*, and (iii) identify the antifungal component of the LAB. Seven fruits (see Supplementary Table S1) were used for isolation before testing for antifungal activity against *F. oxysporum*.

Materials and methods

Isolation and preliminary characterization of LAB. LAB was isolated from assorted tropical fruits (Table S1) collected in and around the districts of Mysuru and Mandya (the state of Karnataka, India). LAB was isolated by enrichment technique in de Man, Rogosa, and Sharpe (MRS)^[21]. All isolates were identified primarily based on morphological and biochemical characteristics: Gram stain, and negative catalase reaction (3% v/v H₂O₂).

Screening of LAB isolates for antifungal activity. The antifungal activity was assessed using the agar-overlay method^[22]. LAB strains grown overnight were streaked onto MRS agar plates and incubated at 37°C for 24 h. Molten-cooled PDA (0.7% agar) containing 10⁶ spores per mL was then gently poured onto the agar plates. Spore inoculum of *F. oxysporum* was prepared from potato dextrose agar (PDA) using a 0.1M PBS NaCl solution with 0.2% tween 80. After solidification, plates were incubated at room temperature for 5 days to observe any inhibition zones around LAB colonies. The zone of inhibition was measured as the distance between LAB and *F. oxysporum* colonies.

Molecular identification of LAB with antifungal activity. Selected LAB strains were identified by sequencing their 16S rDNA. Overnight cultures in MRS broth were centrifuged and washed with PBS (0.1M, pH 7.4). Genomic DNA was extracted using the GeNei™ kit following the manufacturer's recommendations. The 16S rRNA region was amplified using universal primers 8F and 1391R^[9].

Amplicons were sequenced, and sequences were matched using the BLAST tool of NCBI. A phylogenetic tree was constructed using MEGA11 software^[23].

In vitro characterization of selected LABs for probiotic attributes. The overnight grown isolates on MRS broth at 37°C for 24 h were harvested by centrifugation at 8000 rpm for 10 min and used in the following assays. After incubation at the desired conditions, the cells were serially diluted and plated on MRSA. The bacterial viability was determined as logCFU/mL^[21,25].

Tolerance to low pH, bile salts, and phenol. For the tolerance test at low pH, the cell pellet was resuspended in 5 mL of pH-adjusted MRS broth. The pH was adjusted to pH 2, pH 3, and pH 4 using 1 N HCl^[24]. For the bile salt and phenol tolerance tests, the cell pellet was resuspended in MRS broth containing 0.1 to 0.6% (w/v) ox gall or 0.4 to 0.6% (v/v) phenol respectively. The isolates were incubated at 37°C up to 4 and 24 h.

Growth at different temperatures and NaCl concentrations. 100 µL of active cell culture was inoculated into 5 mL sterile MRS broth containing NaCl (3 to 7% (w/v)). To evaluate the growth at different temperatures, 100 µL of active cell culture was inoculated into sterile MRS broth and incubated at 4 to 60°C. The cultures were incubated for 24–48 h before determining the viability.

Auto-aggregation and hydrophobicity index. For auto-aggregation, overnight-grown cell culture in PBS was monitored for absorbance at 600 nm at regular intervals to calculate aggregation (%) using the formula^[26]: $[(A_t - A_0) / A_t] \times 100$. For cell surface hydrophobicity, bacterial suspension in xylene was assessed for absorbance at 600 nm, with hydrophobicity (%) computed as $[(1 - A_t / A_0) \times 100]$, where A_t and A_0 represent absorbance at 600 nm at any given time interval and at time 0 h.

Antibacterial activity. LAB isolates and their cell-free supernatants (CFS) were tested against indicator microorganisms: *Pseudomonas aeruginosa* (ATCC15422), *Salmonella typhi* (ATCC27870), *Staphylococcus aureus* (ATCC6538), and *Escherichia coli* (ATCC25922). The crude CFS was obtained by centrifuging 48-h-old LAB suspension at 8000 rpm for 10 min. and filtered using a 0.2-micron pore syringe filter (Millipore). A portion of the CFS was neutralized to pH 7 (n-CFS) using 1N NaOH to eliminate acidic component's effects^[27].

Agar-well diffusion using CFS of LAB. Indicator organisms grown overnight in nutrient broth were spread-plated onto nutrient agar plates. Wells of 6 mm diameter were aseptically made on the agar plates using a cork-borer, and 50 µL of CFS or nCFS was loaded. After incubation at 37°C for 24–48 h, clear zone formation around the wells was examined, and antagonistic activity was calculated as the zone of inhibition.

Safety profiling

Hemolytic activity. Hemolytic activity was assessed by plating overnight-grown LAB isolates on blood-agar plates^[21]. Hemolysis was classified as β (complete), α (partial), or γ (none) based on zone characteristics. *Staphylococcus aureus* (ATCC6538) served as the positive hemolysis reference strain^[21].

Antibiotic susceptibility. The susceptibility of LAB to common antibiotics was assessed using the disc diffusion method^[28] (Table 2). After incubation, the zone of inhibition was calculated as described and classified for potential *Lactobacillus* according to Charteris et al. ^[28].

Table 2
Antibiotic susceptibility of selected LAB isolated from tropical fruits.

Antibiotics			LAB isolates*		Interpretive criteria
		Concentration ($\mu\text{g}/\text{disc}$)	MYSVCF3	MYSVCF5	
Ampicillin	Amp	10	S	S	$\geq 16/ \leq 12$
Clindamycin	Cli	2	S	S	$\geq 12/ \leq 8$
Chloramphenicol	Chl	30	S	R	$\geq 18/ \leq 13$
Erythromycin	Ery	15	S	S	$\geq 18/ \leq 13$
Kanamycin	Kan	30	R	S	$\geq 18/ \leq 13$
Streptomycin	Ste	10	S	MS	$\geq 15/ \leq 11$
Tetracycline	Tet	30	S	S	$\geq 19/ \leq 14$
Vancomycin	Van	30	R	R	15
*R: resistance; S: sensitive; MS: moderately sensitive. The interpretive for sensitivity/resistance is expressed as the zone of inhibition (mm) of disc diffusion for potential <i>Lactobacillus</i> species (Charteris et al., 1998).					

Antifungal activity by co-inoculation assay. Erlenmeyer flasks containing 50 mL sterile MRS were inoculated with 100 μL of LAB and *F. oxysporum* (10^6 spores/mL) ^[21] and incubated for 3 to 14 days at room temperature. Mycelial biomass was harvested on 3, 7, 10, and 14th day by filtration and dried at 80°C for 2 h. The cell viability was determined as detailed above.

Antifungal activity using CFS of LAB strain.

Microdilution method of LAB. To a sterile 24-well microtiter plate, 1.9 mL/well of CFS in various ratios (v/v) was prepared by mixing CFS directly with PDB. The extract concentrations ranged from 0.6 to 1.5 mg/mL (w/v). Each well received 100 μL of conidial suspension (10^6 spores/mL). Controls included CFS or spores alone. After incubation at 30°C, absorbance was measured at 600 nm. Minimum fungicidal

concentration (MFC) was determined as the minimum concentration of crude CFS extract needed to inhibit *F. oxysporum* growth.

Biomass inhibition. Erlenmeyer flasks containing 50 mL of crude CFS at varying ratios (5–25%, v/v) were prepared by mixing CFS directly with PDB. *F. oxysporum* fungal discs (7.5 mm diameter) were added, and flasks were incubated at room temperature. Control flasks contained only PDB. After 10 days, fungal mats were harvested, and dry weights were compared between CFS-treated and control flasks^[21].

Conidia germination inhibition. To a 24-well microtiter plate, each well contained a mixture of 100 µL of LAB (~ 10⁶ CFU/mL) and *F. oxysporum* (10⁶ spores/mL) in 1 mL of 0.1M PBS, pH 7.4. For CFS assays, 200 µL of 10% CFS (v/v) and *F. oxysporum* (10⁶ spores/mL) were combined. Control wells contained only spores in PBS (0.1 M, pH 7.4). Plates were incubated at 30°C for 24–48 h to evaluate conidial germination. The conidial germination was evaluated using a hemocytometer and expressed as percentage germination.

Stability and activity of CFS of LAB strain. The CFS was treated with proteinase K (1 mg/mL), pH neutralization from pH 4.2 to pH 7 using 1 N NaOH, and high-temperature treatments by heating 100 °C for 15 min. The residual antifungal activity was assessed following the microdilution method^[29]. Additionally, a shelf-life test assessed stability and inhibitory ability following > 8 months of freezing at -20°C, followed by antifungal experiments using the stored CFS.

Identification and quantification of organic acids. The organic acid content of CFS was quantified by Liquid chromatography with tandem mass spectrometry (LC-MS) using UPLC BEH-amide (Waters) column using 10mM ammonium acetate and acetonitrile (1:1), pH 8.5 as mobile phase. Complete details can be found elsewhere^[30].

Statistical analysis. The data in the graph represents mean ± SD (standard deviation) derived from at least three replicates (n = 3). The effect of LAB on *F. oxysporum* was determined using the t-test in Microsoft Excel 2019 (Microsoft Corporation) software. The statistical significance was determined at $p \leq 0.05$.

Results

Isolation of LAB. Seven tropical fruits were sampled during a dry season with an average temperature of 30°C and humidity exceeding 50% (see Supplementary Table S1). LAB counts ranged from 9–10 log CFU/g across the fruits, with *Solanum nigrum* and *Annona muricata* showing the highest counts at 10 log CFU/g each, followed by *Tinospora cordiofolia*, *Ficus benghalensis*, *Couroupita guianensis*, and *Musa*, each with 9 log CFU/g. Interestingly, the morphological distribution of LAB varied among the fruits. *Solanum nigrum*, *Musa*, and *Annona muricata* predominantly harbored rod-shaped cells, while *Ficus racemose* exhibited mostly cocci-shaped cells. Among the rods, *Annona muricata* predominantly had

short-rod cell types, while *Tinospora cordiofolia* contained a uniform combination of cell types, including rods, short rods, and cocci.

Preliminary screening for antifungal activity. Out of fifty-five LAB strains (see Supplementary Table S1) screened against *F. oxysporum*, varied inhibition was observed: three with zones of 7–8 mm, two with 9–11 mm, four with 12–17 mm, and one with > 18 mm (Fig. 1) after 5 days. An additional incubation was conducted on the selected ten strains with a zone of inhibition > 8 mm to assess stability and sustained inhibition. After 10 days, seven strains showed reduced inhibition ($p < 0.001$), while two (MYSVCF3 and MYSVCF5) maintained > 15 mm inhibition (Fig. 1). These strains, isolated from *Ficus glomerata* fruit, maintained stable inhibition zones > 15 mm, prompting further investigation into their combined probiotic and antifungal properties.

Molecular identification. The strains were identified after 16S rDNA sequencing as *Lactiplantibacillus* (*Lpb.*) *plantarum* MYSVCF3 and *Lpb. argentoratensis* MYSVCF5, with NCBI accession numbers **OL347999** and **OR435844**, respectively. Phylogenetic analysis revealed a high level of similarity with other known strains of *Lactiplantibacillus* species, particularly *Lpb. pentoses* suggesting they may have evolved from a common ancestor, providing valuable insights into its genetic lineage and adaptation of these LAB strains.

Probiotic attributes

Growth parameters. Both *plantarum* and *argentoratensis* were Gram-positive and catalase-negative, rod-shaped, and non-spore-forming bacteria. Except for D-arabinose for *argentoratensis*, both could ferment all the sugars tested (Table 1). Optimal growth for *plantarum* occurred at 37°C (Fig. 3), reaching counts as high as $10^9 \log \text{CFU.mL}^{-1}$, whereas *argentoratensis* thrived between 30 and 37°C, with counts ranging from 10^9 to $10^{11} \log \text{CFU.mL}^{-1}$. However, temperatures above 37°C or below 10°C significantly reduced *argentoratensis* growth by at least 2 logs ($p < 0.01$). Although a similar growth pattern was observed at 10°C ($p < 0.5$), the temperature impact was less pronounced than *plantarum*.

Table 1
The phenotypic traits and fermentation ability of the
selected LAB isolates from tropical fruits.

Test	MYSVCF3	MYSVCF5
Gram character	+	+
Shape	rod	rod
Catalase	-	-
Hydrophobicity (%)	27 ± 2	42 ± 4
Carbohydrate fermentation		
Glucose	+	+
Lactose	+	+
Sucrose	+	+
Xylose	+	+
Maltose	+	+
D-Arabinose	+	-
Sorbitol	+	+
D-Raffinose	+	+

The strains *plantarum* and *argenteratensis* were characterized by rapid acidification during growth attributed to organic acid production and sugar fermentation. Despite initial lag phases of 7–8 hours, both strains entered exponential growth lasting approximately 8 hours before reaching the stationary phase (Fig. 2). Growth kinetics were similar, with division rates of 0.38-1.0 and 0.35 h⁻¹, and generation times of 2.6 and 2.4 h, respectively. Maximum acidification (pH 4.5 ± 0.3) occurred between 10–17 hours, aligning with the exponential growth phase characterized by specific growth rates of 0.24 and 0.26 h⁻¹ at 37°C for *plantarum* and *argenteratensis*, respectively. Both strains demonstrated sustained osmotic stress tolerance up to 7% NaCl concentration, with *argenteratensis* showing decreased growth beyond this threshold.

Survival ability to gastrointestinal conditions. Figure 4 illustrates the viability of LABs under simulated gastrointestinal conditions. Both *plantarum* and *argenteratensis* exhibited high tolerance to pH 2, with *argenteratensis* maintaining > 95% and > 88% cell viability after 4 hours (Fig. 4). Compared to the initial cell density (10⁹ logCFU.mL⁻¹), a decrease in cell viability by a logCFU.mL⁻¹ after 4 h in pH 2 was observed, which is statistically insignificant ($p > 0.05$). In contrast, the viability of *argenteratensis* declined significantly when phenol was added for concentrations greater than 0.4% phenol (Fig. 4). while, even with 0.6% phenol, *plantarum* demonstrated > 90% cell viability with 10⁹ log.CFU.mL⁻¹. Further, the strains exhibited tolerance to bile salts simulating the enteric phase (Fig. 4). Both *plantarum*, and

argenteratensis showed excellent tolerance with *argenteratensis* having better tolerance even after 4 h of exposure. The viability of *plantarum* decreased by 15% ($p < 0.05$) after 3h to the exposure at 0.3% bile (Fig. 4). Overall, the isolated LAB strains showed high tolerance to harsh simulated gastro-intestinal conditions making them ideal probiotic isolates.

Cell surface properties of LAB. The cell surface hydrophobicity of both isolates was evaluated, showing slightly higher hydrophobicity for *argenteratensis* ($42\% \pm 4$) compared to *plantarum* ($27\% \pm 2$) (Table 1). Over time, the percent aggregation increased, with strain *argenteratensis* reaching 50% and *plantarum* at 27% after 5 hours (see Supplementary Fig. S1). By 24 hours, *argenteratensis* showed a marginal increase to 52% aggregation, while *plantarum* increased to 57%.

Antibiotic susceptibility. Differences in antibiotic response were observed between *plantarum* and *argenteratensis* (Table 2). Both isolates were sensitive to ampicillin, clindamycin, erythromycin, streptomycin, and tetracycline, offering potential treatment options. While *plantarum* was resistant to kanamycin, *argenteratensis* exhibited resistance to chloramphenicol. Despite variations, the susceptibility of *plantarum* and *argenteratensis* to antibiotics did not differ significantly. Interestingly, both isolates exhibited resistance to vancomycin-specific antibiotics, suggesting inherent resistance mechanisms.

Antibacterial activity of LAB and its CFS. Both *plantarum* and *argenteratensis* exhibited comparable inhibition to selected pathogens (including ESKAPE pathogens) against *E. coli*, *P. aeruginosa*, *S. paratyphi*, and *S. aureus* (see Supplementary Fig. S2). The CFS displayed antagonistic activity against all tested pathogens, with inhibition ranging from 70–80% for *plantarum* and *argenteratensis*. However, *S. aureus* and *P. aeruginosa* exhibited lower inhibition by *argenteratensis* (74%) compared to other strains. Moreover, adding just 25% CFS was adequate to completely inhibit the pathogen growth, but the activity notably decreased after neutralization (nCFS) to 22–25% from the original 80% by both strains.

Hemolytic activity. *Lpb. argenteratensis* did not exhibit any red blood cell lysis in the hemolytic test. The clear yellow coloration surrounding the colony of *S. aureus* indicated that the organism had completely lysed red blood cells (β -hemolytic) (see Supplementary Fig. S3). Additionally, phenotypic tests for coagulase activity confirmed *argenteratensis* as non-hemolytic and non-pathogenic.

Antifungal activity of *Lpb. argenteratensis* MYSVCF5

Inhibition by bacterial cells of MYSVCF5. Figure 5A shows a steady growth of *F. oxysporum* in 14 days, characterized by a dense, white mat-like appearance. The biomass content progressively increased from $< 0.01\text{g}$ on day 1 to 0.9 g (by dry weight) on day 14 (Fig. 5). In contrast, when viable cells of *argenteratensis* were introduced, and complete inhibition ($p < 0.01$) of *F. oxysporum* growth was observed from day 1 onward (Fig. 5). Meanwhile, *argenteratensis* showed excellent viability throughout the incubation period, with an exponential phase lasting 3 days and a gradual decline as time progressed (Fig. 5). These results indicate that *argenteratensis* is effective in inhibiting the growth of *F. oxysporum*. The viability of the LAB cells was not affected by *F. oxysporum*.

Inhibition by CFS of MYSVCF5. Up to 5% of CFS, the inhibition of *F. oxysporum* was weak since the reduction of only 17% of the biomass was reached. However, by increasing the concentration to 10% CFS a significant reduction ($p < 0.01$) of 94% in fungal biomass yield was achieved, dropping from 1.1g to < 0.1g (Fig. 6). Further increasing the CFS concentration to 20% led to a > 98% decrease in biomass yield (Fig. 6). Therefore, the inhibitory effects of CFS of *argentoratensis* are considerably strong in inhibiting the growth of *F. oxysporum*, with a requirement of just 10% of crude extract. Moreover, the CFS retained its activity even after storage for 8 months at -20 or 4°C suggesting a strong and stable shelf-life.

The minimum fungicidal concentration (MFC) of CFS. A CFS/media ratio of 5–10% was sufficient to completely inhibit the growth of *F. oxysporum*. At 0.6 mg/mL crude CFS, a 90% reduction was observed (see Supplementary Fig. S4). At this concentration, no visible growth of *F. oxysporum* was observed. An increase in CFS concentration increased activity and, as a result, an increase in inhibition. This clearly shows that 0.4–0.6 mg/mL CFS is effective at inhibiting *F. oxysporum* germination and growth, indicating CFS potential as a strong antifungal agent.

Conidial germination inhibition. The presence of viable *argentoratensis* cells significantly inhibited conidial germination and mycelial development. Conidial germination was impeded, with no germ tube formation observed even after 48 hours in the presence of active LAB cells or their CFS (Fig. 7). In contrast, conidia germinated rapidly in the absence of LAB cells or CFS, with germ tube formation within 4 hours and subsequent mycelial growth by 12 hours, reaching 50% germination (10^6 spores/mL) from apical cells (Fig. 7). By 24 hours, over 90% of the conidia had germinated with extensive mycelial growth (Fig. 7). The germination of conidia and formation of mycelia was much faster (< 24 h) when nutrient-rich, PDB medium was used.

Characterization of CFS. The CFS was subjected to heat, proteinase enzyme, and pH neutralization treatments to assess the stability and nature of its antifungal activity. The heat treatment preserved the inhibitory properties, but pH neutralization and proteinase K treatment resulted in the loss of antifungal activity allowing the fungi to sporulate and grow (see Supplementary Fig. S5). This indicates that the antifungal compound in the CFS is likely acidic, such as organic acids, and possibly contains heat-stable proteinaceous substances. However, the protein component of the CFS remains unidentified at this stage.

The chromatographic analysis of the CFS revealed a mixture of organic acids, with citric acid being the most abundant, constituting 67% of the total identified acids. This was followed by lactic acid at 16% and malic acid at 10%. The concentration of citric acid ranged from 34–36 µg/mL, while lactic acid ranged from 8–9 µg/mL in the CFS (Table 3).

Table 3
Major organic acids composition of soluble microbial products (CFS) of strain
Lpb. argentoratensis MYSVCF5

Organic acids	Molecular weight (g.mol ⁻¹)	CFS (µg.mL ⁻¹)	% distribution
Citric acid	192.12	34.9 ± 0.3	66.9
Lactic acid	90.08	8.3 ± 0.003	16.5
Malic acid	116.1	5.2 ± 0.2	9.9
Succinic acid	118.09	1.7 ± 0.1	3.3
Pyruvic acid	88.06	1.3 ± 0.04	2.5

Discussion

Microbial isolation from seven different fruits revealed LAB counts ranging from 9–10 log CFU/g, with notably high counts in *Solanum nigrum* and *Annona muricata*. The abundance of viable LABs was comparable to previous studies^[17]. Pineapple, papaya, grapes, blackberries, and kiwis showed counts ranging from 10²-10⁶ CFU/g, while grape and Musa had lower counts (< 10² CFU/g)^[31]. Fifty-five LABs strains were obtained after several screening stages, differing from previous reports. LAB species varied among fruits, with *Lactobacilli* predominating^[32]. Previous studies have reported *L. plantarum*, *Weisella cibaria*, *Weisella paramesenteroides*, *Leuconostoc mesenteroides*, *Leuconostoc citreum* in ripe mulberries, watermelons, peaches, and coffee cherries. Yet, *plantarum*, *brevis fermentum* and *paracasei* are the most common species of *Lactobacillus* isolated from fruits^[18,32,33]. Both strains were identified as hydrophobic, catalase-negative Gram-positive rods, likely belonging to the *Lactiplantibacillus* or *Lactobacillus* genus. 16S rDNA analysis revealed a close relationship to *plantarum* and *argentoratensis*, both known for their probiotic properties. Despite their taxonomic distinction, these strains exhibited high homology and potential as beneficial microorganisms.

Both *plantarum* and *argentoratensis* displayed sensitivity to common antibiotics, suggesting safe and effective use in probiotic formulations. They also demonstrated resistance to vancomycin-specific antibiotics, indicating potential resistance mechanisms or unique cell wall characteristics. In vitro assays confirmed their tolerance to acidic conditions and bile contents typical of the gastrointestinal (GI) tract, enhancing their ability to colonize the host's gut. The GI environment, known for its acidity and bile presence, poses challenges to bacterial viability. Both strains survived pH 2 and tolerated human bile up to 0.5%, with *plantarum* exhibiting slightly better tolerance. Actual stomach pH variations, buffered by meals and gastric juices, may offer protection against bacterial viability. Therefore, the resilience of probiotic strains depends on inherent resistance and the food matrix.

In screening for antimicrobial activity, the strains exhibited both antibacterial and antifungal properties. Their antibacterial effects were observed against various pathogenic and indicator bacteria, including *S. aureus*, *P. aeruginosa*, *S. typhi*, and *E. coli*, albeit with varying potency. Interestingly, both LAB cells and CFS revealed a positive inhibition of these pathogens. One of the functional attributes of probiotics lies in the ability to produce antimicrobial compounds, encompassing a combination of organic acids, short-chain fatty acids, and other bacteriocin toxins^[32]. The synthesis of these antimicrobial metabolites by LAB bears potential benefits not only for food preservation but also for mitigating the proliferation of pathogens^[34].

The proliferation of *Fusarium* sp. is influenced by environmental factors such as agricultural practices and storage conditions, including temperature and moisture content. LAB is an important commensal microorganism with documented antifungal attributes and bio-preservation capabilities^[22,35,36]. Among LABs, *Lactobacillus* species, particularly *L. plantarum*, are known for effectively suppressing *Fusarium* growth^[37]. While *plantarum* is recognized for inhibiting *Aspergillus*, *Listeria monocytogenes*, etc., the antifungal activity of *argentoratensis* isolated from tropical fruits is yet to be studied but has great potential. The microconidia germination ceased completely in the presence of cells or CFS of *argentoratensis*. A minimal fungicidal concentration of 1 mg/mL of CFS could effectively inhibit both mycelial and conidial germination of *F. oxysporum*.

Given that the LABs are known to produce organic acids^[36,38], it was anticipated that the organic acids were primarily responsible for the observed antifungal effect, as pH may have a significant impact as an antifungal agent. Organic acids produced by LABs can disrupt microbial metabolic activity by penetrating cell membranes in their undissociated form, lowering intracellular pH. While previous studies have identified organic acids like acetic acid and propionic acid in *plantarum*^[38], similar investigations on *argentoratensis* are lacking. Thus, this study is the first evidence of the antifungal activity of *argentoratensis*. The CFS after neutralization to pH 7 or treatment with proteinase K resulted in the loss of inhibition. However, sustained inhibition was observed after heat treatment. Thus, it was inferred that the antifungal metabolite produced by *argentoratensis* comprised both organic acids and thermally stable protein substance. Subsequent analysis of the organic acid profile confirmed the presence of several known organic acids. Citric acid and lactic acid together accounted for 82% of the organic acid pool. Citric acid, in particular, was identified as the most relevant antifungal compound, as it accumulated to concentrations matching the minimum fungicidal concentration against fungal spores. Although organic acids likely contribute to this activity, the presence of any protein component in the cell-free supernatant has yet to be confirmed. Additional studies are needed to better comprehend the mechanisms through which *Lpb. argentoratensis* inhibits *Fusarium oxysporum*. Nonetheless, this study marks the first report on the isolation of a novel LAB strain from tropical fruit *Solanum nigrum*, with exceptional antifungal activity against *F. oxysporum*.

Implications

The effective management of fungal plant diseases caused by *Fusarium* species, including *F. oxysporum* has presented a persistent challenge since its adverse impact on agricultural yields was recognized^[39]. Environmental factors such as cool, damp weather, particularly during growth necessitate alternative, economic, and environmentally sustainable solutions. Despite this challenge, the potential use of LABs as probiotics and antifungal agents offers promise. The key hurdles such as identifying optimal probiotic strains, elucidating their mechanisms of action, and refining delivery methods need to be addressed. In this context, LABs present advantages including economic viability, safety, ease of production and handling, and probiotic properties beneficial for human consumption, making them attractive biocontrol agents for crop protection.

The diverse population of LAB in fruits offers a valuable resource for creating various probiotic products. Future studies must focus on assessing the in vitro efficacy of fruit-derived LABs and their cell-free supernatants as potential biocontrol agents. This approach could promote sustainable and environmentally friendly agricultural practices while utilizing the probiotic benefits of LABs.

Conclusion

In this study, multiple LAB strains were isolated from various tropical fruits, with the highest counts observed in *Solanum nigrum*. Two strains, *Lactiplantibacillus plantarum* MYSVCF3 and *Lpb. argentoratensis* MYSVCF5, both isolated from cluster fig, exhibited promising probiotic properties in vitro. Both strains showed antibacterial activity and antifungal activity against *Fusarium oxysporum*, with *Lpb. argentoratensis* demonstrating stable inhibition. Moreover, the specific ability of *Lpb. argentoratensis* to inhibit *F. oxysporum*, indicating the possible role of organic acids in this process. Citric acid was identified to be the most abundant organic acid synthesized. Overall, the study enhances the knowledge of LAB diversity in fruits and explores their applications in probiotic and biocontrol agents against *F. oxysporum*.

Declarations

Competing interests

The author(s) declare no competing interests.

Author Contribution

RV: Conceptualization, data curation, formal analysis, Writing-original draft; MS: data curation, Writing-original draft, Writing-review-editing; SD and ND: Methodology; Writing-review-editing, PNA: Writing-review-editing. MYS: Conceptualization, Supervision; Writing-review-editing.

Data Availability

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files). The sequences of bacterial isolates from this study can be found in the NCBI GenBank repository under NCBI accession numbers OL347999

(<https://www.ncbi.nlm.nih.gov/nuccore/OL347999.1/>) and OR435844

(<https://www.ncbi.nlm.nih.gov/nuccore/OR435844>)

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Figures

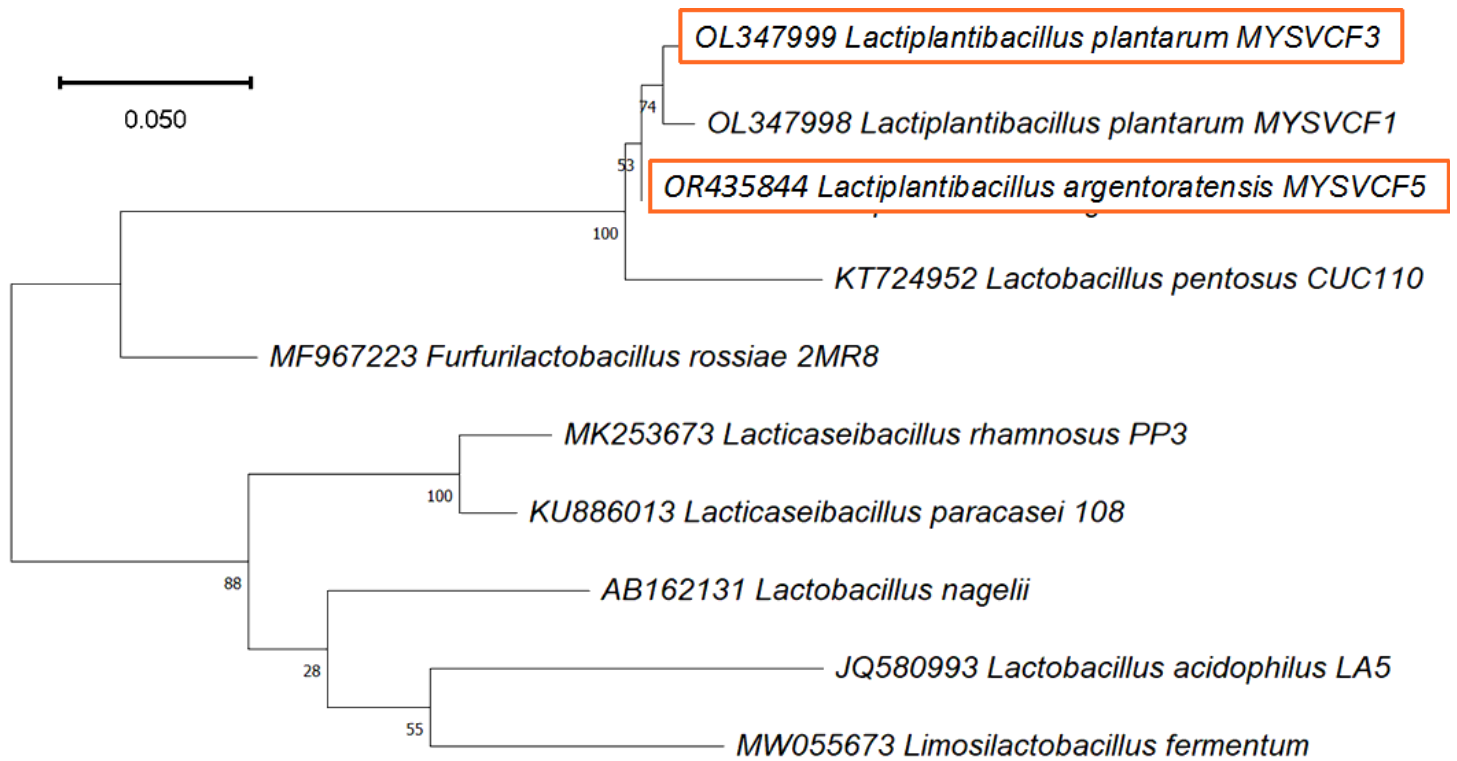


Figure 2

Phylogenetic tree showing the relative positions of LAB (MYSVCF3, MYSVCF5) obtained from fruits to that of reference strains.

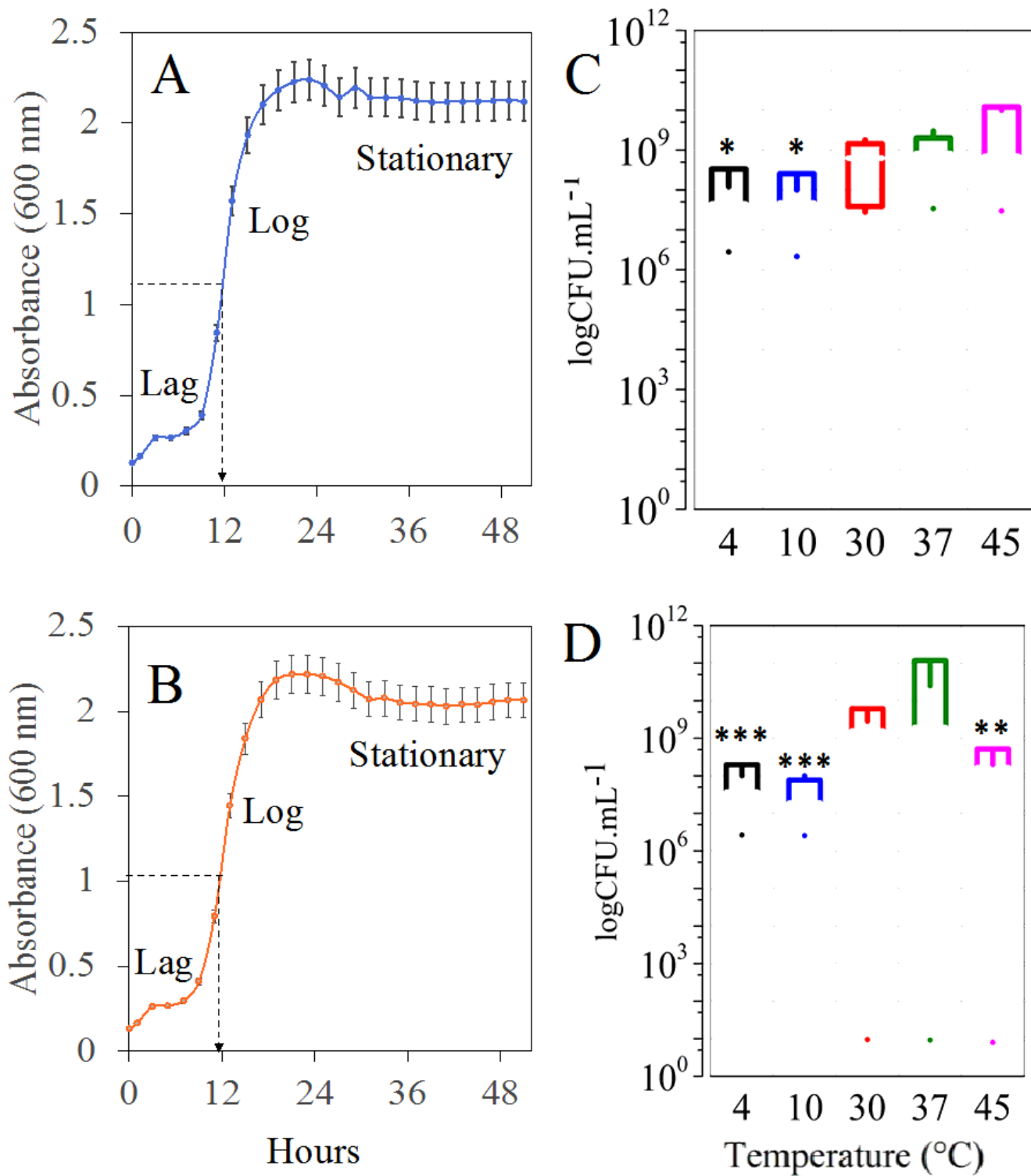


Figure 3

Growth curves of LAB (A and B) *Lpb. plantarum* MYSVCF3 (A, C) and *Lpb. argentoratensis* MYSVCF5 (B, D) at 37 °C. Viability and growth at different temperatures ranging from 4 to 45 °C (C and D) are shown. Statistical significance at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

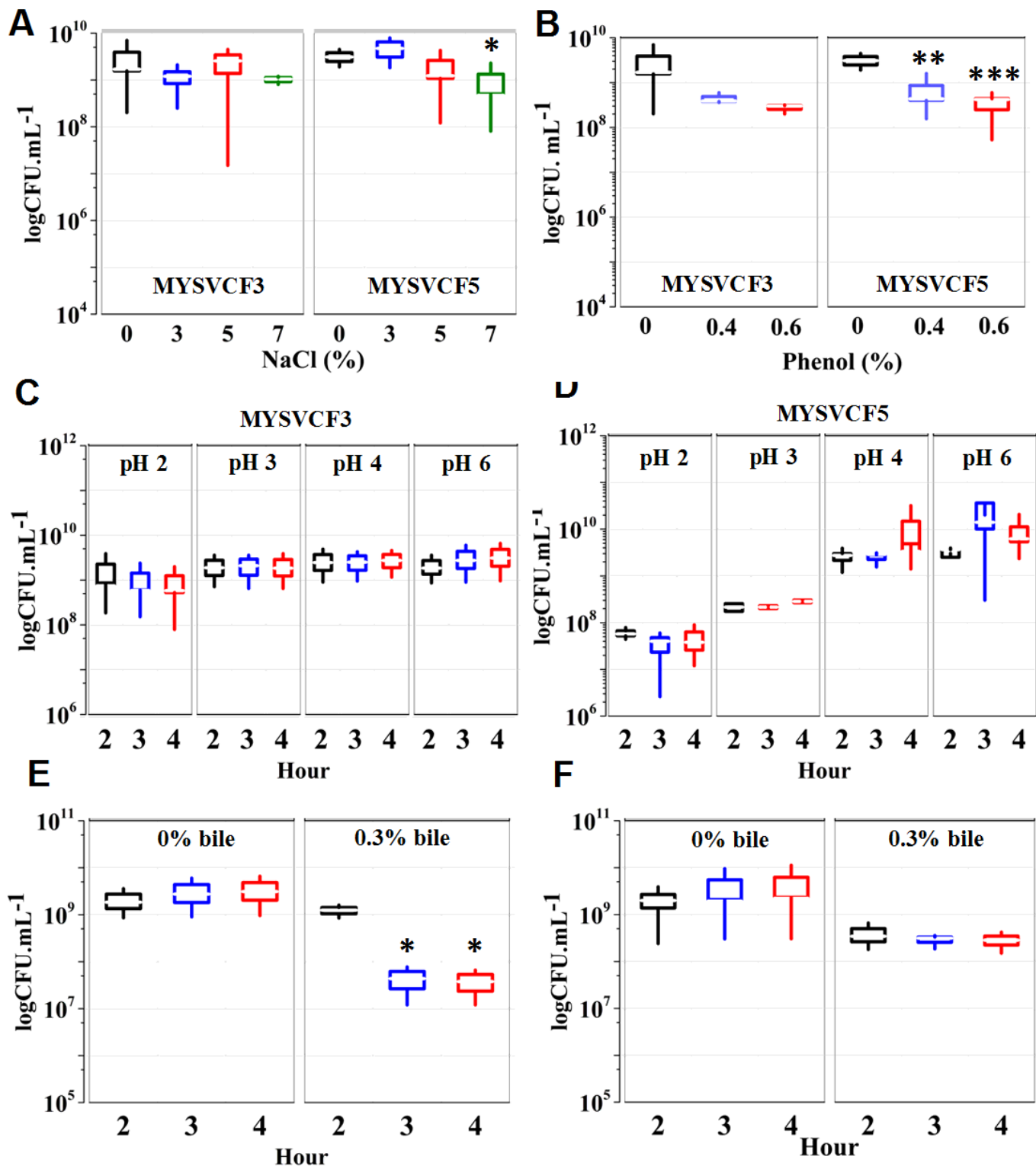


Figure 4

Tolerance of LAB at different conditions of osmotic stress (A), phenol (B), pH (C-D), and bile (E-F). The tolerance assay for *Lpb. plantarum* MYSVCF3 (A, C, and E) and *Lpb. argentoratis* MYSVCF5 (B, D, F) are shown. Statistical significance at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

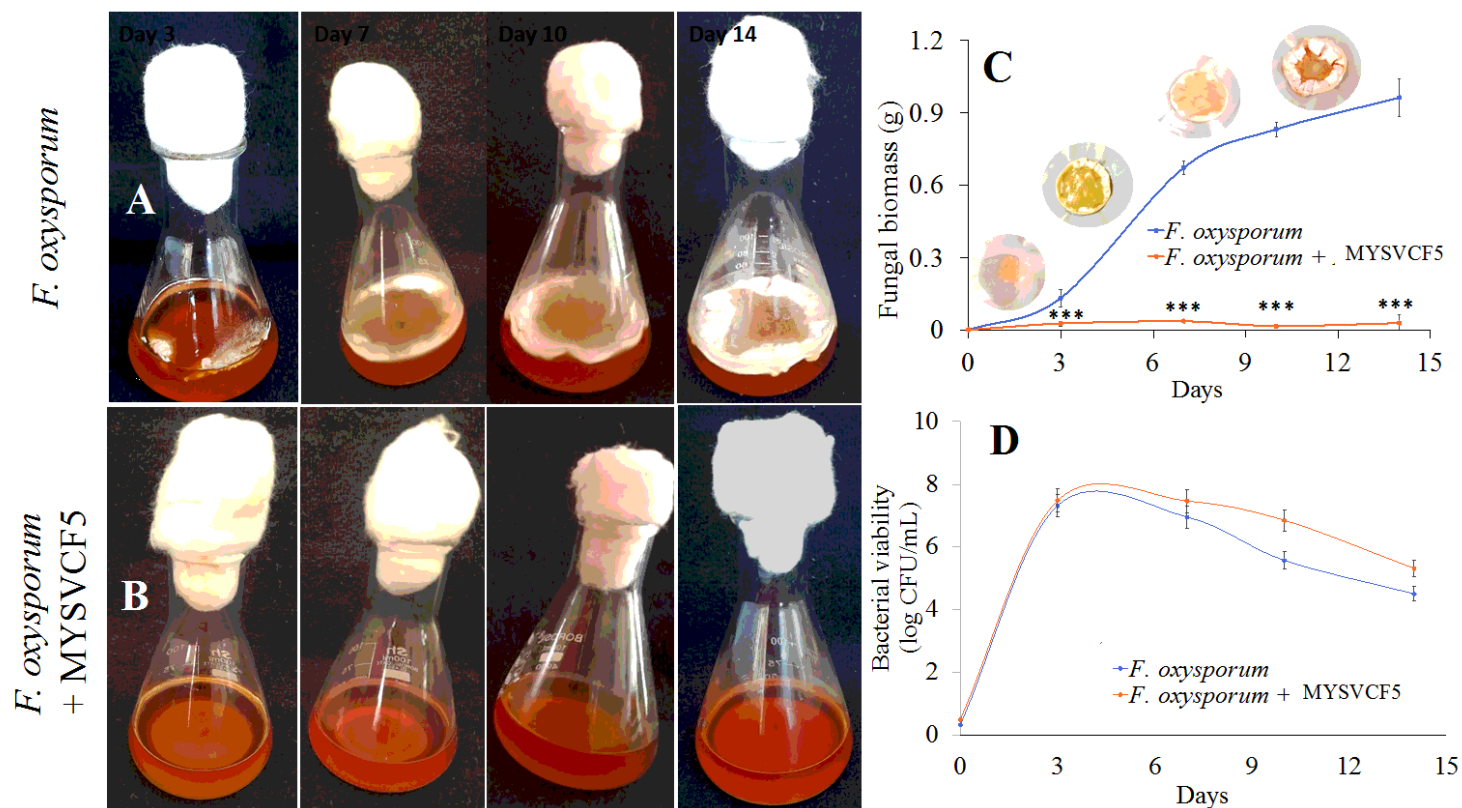
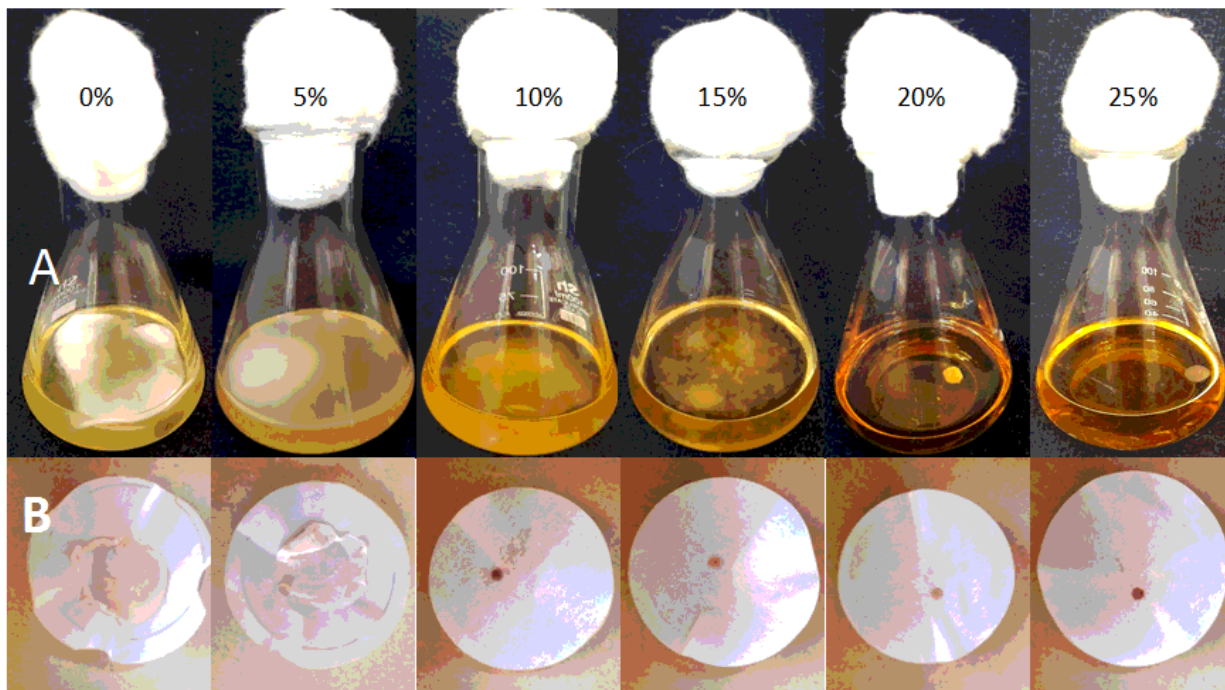


Figure 5

Time course growth of mycelial mat of *F. oxysporum* (control) (A, C) and *F. oxysporum* with cells of MYSVCF5 (test) (B, D). The growth of biomass of *F. oxysporum* under different conditions (C). The growth pattern of strain MYSVCF5 in the presence of *F. oxysporum* (D). Inset in C shows the fungal biomass obtained after filtration at different periods of the experiment. Statistical significance at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.



F. oxysporum ————— *F. oxysporum* + MYSVCF5 CFS —————

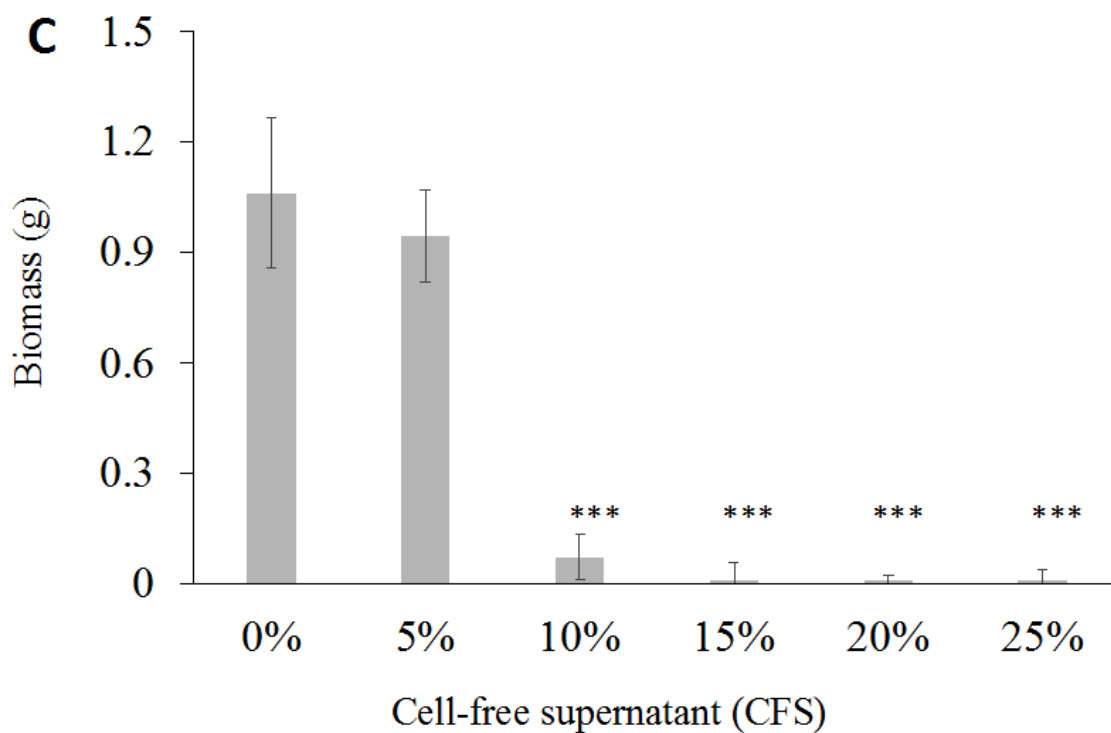


Figure 6

Antifungal activity as a function of inhibition of fungal biomass by cell-free supernatant (CFS) extracted from *Lpb. argentoratensis* MYSVCF5 (A-C). Experiments were performed with different concentrations (%) of CFS. Flasks showing growth of *F. oxysporum* with CFS of strain MYSVCF5 (A). Biomass yield obtained at different CFS treatments (B and C). Statistical significance at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

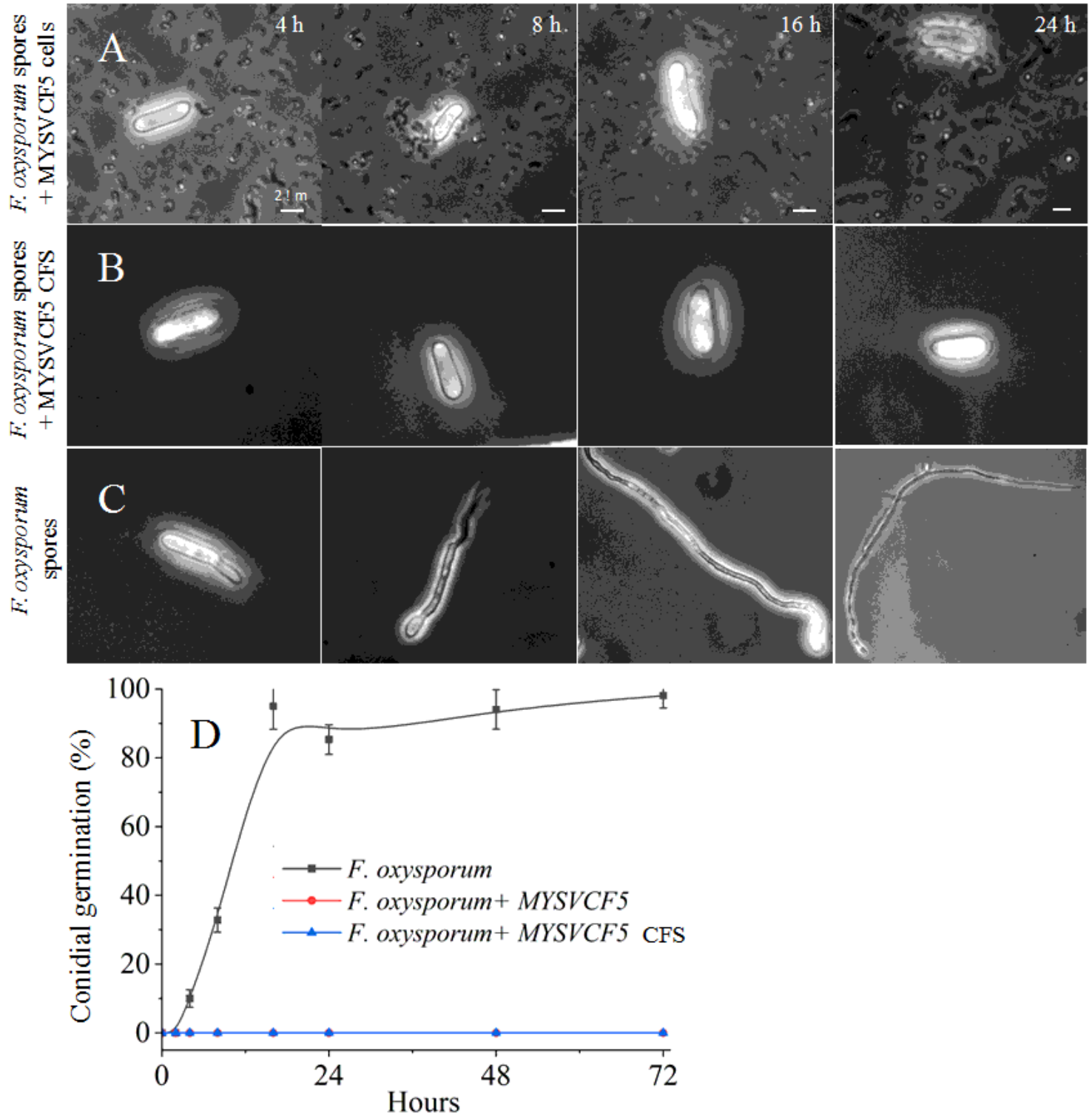


Figure 7

Microscopic observation of conidial germination inhibition by *Lpb. argentoratensis* MYSVCF5 cells (A), its CFS (B), and percent inhibition (D). Conidial germination in the absence of *Lpb. argentoratensis* MYSVCF5 cells or its CFS (C).

Supplementary Files

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