



Isolation and Characterization of a New Ferulic-Acid-Biotransforming *Bacillus megaterium* from Maize Alkaline Wastewater (Nejayote)

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Abstract

Nejayote is an alkaline wastewater generated during the nixtamalization process. Nejayote contains high-value compounds such as ferulic acid (FA), which is widely employed as a substrate for the biotechnological production of flavors and aromas. In the present study, the isolation, identification, and characterization of a native strain of *Bacillus megaterium* were performed, and its capacity to produce 4-vinylguaiaicol (4VG) from ferulic acid was evaluated by employing growing cell and resting cell systems. Growing cells of native *B. megaterium* biotransformed 6 mM crude FA in nejayote into 2.1 mM 4VG, reaching a productivity of 0.21 mM h⁻¹ 4VG, while nejayote enriched with FA at 10, 15, and 25 mM resulted in the formation of 2.4, 3.8, and 6.2 mM 4VG and productivities of 0.24, 0.38, and 0.51 mM h⁻¹ 4VG, respectively. In the resting cell system, from 6 and 25 mM pure FA, 3.5 mM 4VG was produced (0.18 mM h⁻¹ 4VG), while at 10 and 15 mM FA, 4.6 and 5.1 mM 4VG (average of 0.24 mM h⁻¹ 4VG) were obtained, respectively. The native *B. megaterium* strain, isolated from nejayote, showed great biotechnological potential to produce 4VG from crude FA contained in this wastewater, in which other *Bacillus* species, such as *B. licheniformis* and *B. cereus*, were unable to grow and biotransform FA into 4VG.

Introduction

Ferulic acid (FA) is a hydroxycinnamic acid found as a component of the plant cell wall in free form and as a homodimer or esterified with lignocellulosic polysaccharides [1]. The extraction and purification of FA from natural sources is important due to its numerous applications in the pharmaceutical, cosmetic, and food industries [2]. The use of FA as a substrate for the biotechnological production of flavors and aromas such as vanillin and 4-vinylguaiaicol (4VG) is well

known [3, 4], and these products can be labeled as “natural flavors” according to USA and EU legislation [5, 6]. 4VG is a highly valued compound with a clove aroma that provides the desirable smoky aroma and flavor to Belgian and German-style wheat beers and soy sauce and contributes to sensory characteristics in food products [7]. The aroma compounds from the styrene family, such as 4VG, are principally synthesized chemically. Currently, consumers demand natural products obtained from plants or by biotechnological processes [4]. Biotechnological production of 4VG comprises the biotransformation of FA, with the *Bacillus* genus one of the most reported biotransforming agents, including *B. coagulans*, *B. cereus*, *B. licheniformis*, *B. aryabhatai*, and *B. methylotrophicus* strains [8–12].

The mechanism proposed for the microbial production of 4VG is the side-chain cleavage of FA by nonoxidative decarboxylation by the enzyme FA decarboxylase (EC 4.1.1.102). FA decarboxylation could be part of a detoxification system that maintains metabolism-inhibitory compounds under a threshold concentration [13]. However, it has been reported that concentrations higher than 5 mM FA inhibit the detoxification system and the simultaneous bioconversion of FA to 4VG [14, 15]. This represents a considerable limitation for biotransformation processes that are relevant to the

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prospection of novel FA-tolerant strains to enhance the productivity of 4VG. An industrial source for recovering FA is nejayote [16], wastewater resulting from the nixtamalization process used in Mexico and other Latin American countries to produce tortillas and other byproducts [17]. In addition to FA, nejayote contains phenolic compounds [18], starch, non-amylaceous polysaccharides, proteins, and sugars [19, 20]. Due to its composition, nejayote has antimicrobial or growth-inhibitory properties [17], and some studies have focused on the isolation of native strains from nejayote for biotechnological applications [21, 22]. However, none of these studies have evaluated the tolerance of the isolated strains to high FA concentrations and their conversion of FA into highly valued compounds, such as 4VG.

In the current study, we report the isolation, identification, and characterization of the native strain *Bacillus megaterium* LBI001 from nejayote. The tolerance to high concentrations of FA and efficiency of biotransformation of crude and pure FA into 4VG in growing and resting cell systems were assessed.

Materials and Methods

Native Strain Isolation from Alkaline Maize Wastewater (Nejayote)

A native strain was isolated from nejayote provided by a local mill located in Hermosillo, Sonora Mexico. Nejayote pH was adjusted (6.5) with H₂SO₄ and incubated at 25 °C for 24–48 h. Then, samples were plated on nutrient agar containing 1 g L⁻¹ of FA and incubated at 37 °C for 24 h. Single colonies were plated again on nutrient agar containing 2 and 3 g L⁻¹ of FA, and plates that developed a clove aroma were stored at 4 °C for further analyses.

Morphological and Biochemical Characterization of the New Ferulic-Acid-Biotransforming Native Strain

The cell morphology of bacteria was examined using optical microscopy (Nikon Type 104c). Gram staining and biochemical characterization of the strain were carried out according to the procedure described by Tindall et al. [23]. The hydrolysis of ethyl ferulate activity was performed as described previously [24].

Genotypic Characterization and Sequence Analysis of the New Ferulic-Acid-Biotransforming Native Strain

After growth at 37 °C for 24 h, a single colony was used directly to amplify a 1.4 kb fragment of the 16S rRNA gene

using primers 63F and 1387R at 500 nM in 20 µL [25]. Both strands of the PCR product were sequenced (Macrogen USA Corp), and the corresponding consensus sequence was assembled with CLC Main Workbench 5.5 (CLC BIO) and aligned with those available in GenBank using the BLAST algorithm [26]. The sequence was deposited in GenBank.

A phylogenetic tree was constructed using the UPGMA method [27]. The analysis involved 19 nucleotide sequences. The codon positions included were 1st + 2nd + 3rd + Non-coding. All positions containing gaps and missing data were eliminated. There were a total of 488 positions in the final dataset. Reference sequences were retrieved from GenBank under the accession numbers indicated on the trees.

Nejayote Production

2 kg of white maize was cooked at 95 °C for 45 min in 4 L of lime solution (Nixtocal® provided by Calidra). Supernatant (nejayote) was collected and treated according to Asaff and Reyes [18]. The effluent free of suspended solids, containing 6 mM crude FA, was used for biotransformation into 4VG in a growing cell system.

Inoculum Preparation for Biotransformation Assays

A 250-mL Erlenmeyer flask with 100 mL of TSB broth (BD Difco, USA) was inoculated using a single colony from freshly streaked agar plates containing bacterial cultures and incubated at 37 °C and 120 rpm. Samples were taken every 2 h, the microbial culture was stopped at 12 h when the stationary phase reached OD_{600nm} = 2.136, and 4 mg mL⁻¹ of biomass was obtained.

Resting Cell System

A 10 mL aliquot of the inoculum at the stationary phase was centrifuged (15,300×g, 4 °C, 20 min), and the recovered pellets were washed three times with 50 mM phosphate buffer (PB) at pH 7. The washed pellets were resuspended in 10 mL of 50 mM PB with 5, 10, 15, and 25 mM FA (Minkab), containing 4 mg cells mL⁻¹, and then transferred to 20 mL glass bottles, which were incubated at 37 °C and 120 rpm for 12 h. Samples were taken every 2 h and stored at -10 °C for further analysis.

Growing Cell System

Biotransformation was carried out in 250-mL Erlenmeyer flasks containing 100 mL of nejayote with 6 mM crude FA and enriched nejayote with pure FA to obtain concentrations of 10, 15, and 25 mM. The flasks were inoculated with 5 mL of inoculum of the *B. megaterium* LBI001 strain (~0.2 mg cells mL⁻¹ solution) and incubated at 37 °C and 120 rpm for

12 h. Samples were taken every 2 h and stored at -10°C for further analysis.

For the biotransformation efficiency assays, 125-mL Erlenmeyer flasks containing 30 mL of nejayote were inoculated with 1 mL of inocula of *B. megaterium* LBI001, *B. licheniformis* FD36, and *B. cereus* MS15, cultured for 24 h. The flasks were incubated at 37°C and 120 rpm for 24 h.

Analytical Techniques

Biomass

The biomass concentration during biotransformation was measured according to AOAC, 986.35 [28]. A Neubauer hemocytometer chamber was used for bacterial cell counting.

Quantitation of FA and 4VG

The biotransformation samples were centrifuged ($15,300\times g$, 4°C , 15 min). The supernatant was filtered with a $0.22\text{-}\mu\text{m}$ PTFE membrane. FA and 4VG were measured by HPLC Varian 920-LC and PDA detector. A Phenomenex ODS-25 μm $250\times 4.6\ \mu$ column at 35°C was used with a linear gradient elution of acetonitrile:water (60:40) acidified with trifluoroacetic acid (0.01%) at a flow rate of $1\ \text{mL}\ \text{min}^{-1}$ and wavelength 254 and 320 nm.

Biotransformation and Growth Parameters

The estimated biotransformation parameters were the molar yield ($Y_{p/s}$) of product formation (Eq. 1), the molar substrate consumption (Eq. 2), and the productivity (Eq. 3).

$$\frac{Y_p}{s} = \frac{P - P_0}{S_0 - S} \times 100 \quad (1)$$

$$\text{FA} = \frac{S_0 - S}{S_0} \times 100 \quad (2)$$

$$\text{Productivity} = \frac{[4\text{VG}]_{\text{max}}}{t}, \quad (3)$$

where P and P_0 are the final and initial 4VG molar concentrations; S and S_0 are the final and initial FA molar concentrations, respectively. The molar productivity was expressed as the maximum molar concentration of 4VG over time (t).

The growth kinetic parameters (X_{max} and μ_{max}) were estimated by fitting the experimental data to three-parameter logistic models (Eq. 4).

$$X(t) = \frac{X_{\text{max}}}{1 + \left[\left(\frac{X_{\text{max}} - X_0}{X_0} \right) \right] e^{-\mu t}}, \quad (4)$$

where X represents the biomass concentration ($\text{g}\ \text{L}^{-1}$), and X_0 and X_{max} are the initial and maximal biomass concentrations ($\text{g}\ \text{L}^{-1}$), respectively, μ is the specific growth rate (h^{-1}), and t is the biotransformation time.

Statistical Analysis

The biotransformations were performed in duplicate, and kinetic parameters were reported as the mean \pm standard deviation and compared with the Tukey–Kramer test at the 95% confidence limit. The analysis was carried out using the statistical package NCSS version 7.0 (Number Cruncher Statistical System for Windows, Utah, USA).

Results

Isolation, Characterization, and Identification of the Native Strain

Axenic cultures had the same colonial macroscopic morphology with round to irregular colonies that were white in color and had undulate margins. The phenotypal and biochemical characterization of the isolated native strain is shown in Table 1. The 16S rRNA gene sequence of the strain LBI001 showed 99% similarity to *B. megaterium* GMB4002-a and *B. megaterium* GMB40025003 with NCBI Accession Numbers AB738968.1 and AB738973.1, respectively. Sequence data were deposited in GenBank under Accession Number MH729583. Figure 1 shows the phylogenetic tree.

Table 1 Phenotypic characteristic the native strain from nejayote

Characteristic	Nejayote native strain
Colony shape	Entire, round and flat
Gram stain	Positive
Motility	Positive
Catalase	Positive
Ornithine decarboxylase	Positive
Utilization of citrate	Negative
Acetoin production	Negative
Acid production	
Fructose, glucose, mannitol, xylose, galactose, maltose	Positive
Lactose, inositol, arabinose, mannose	Negative
Hydrolysis	
Ethyl ferulate	Positive
Casein	Positive
Starch	Positive

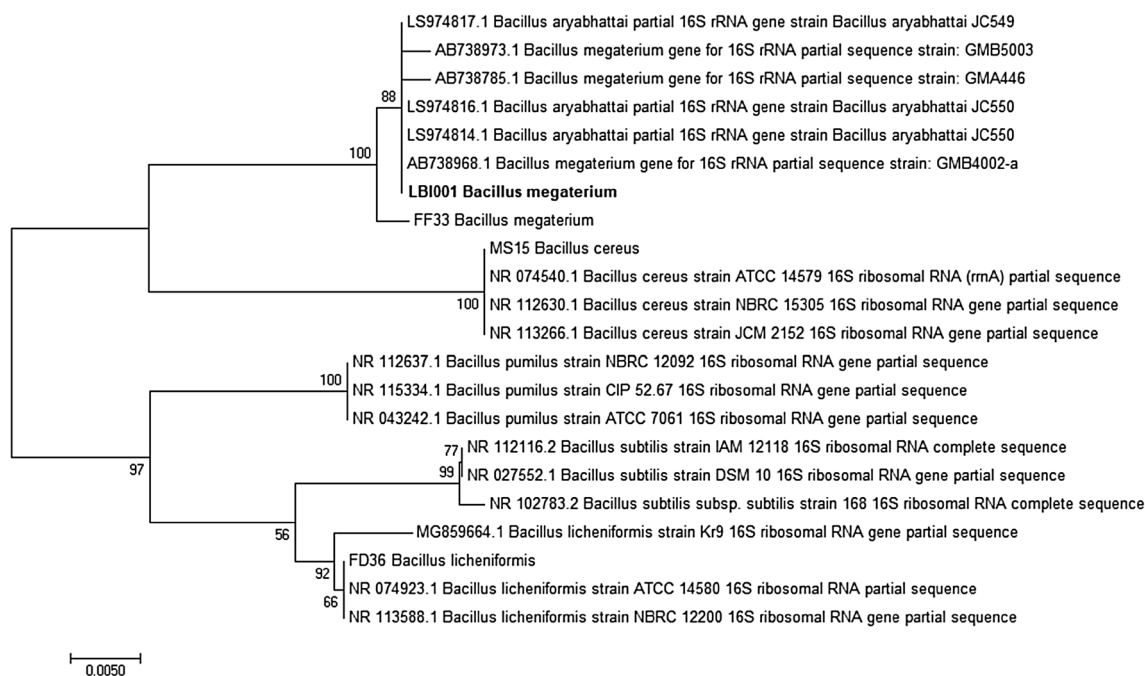


Fig. 1 Phylogenetic tree based upon on 16S rRNA gene sequences showing the branching of strain LBI001 and closely related various *Bacillus* species. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree

Biotransformation of Crude FA by Three *Bacillus* Species

The crude FA in nejayote was biotransformed by *B. licheniformis* FD36, *B. cereus* MS15, and *B. megaterium* LBI001 into 4VG, achieving concentrations of 0.006, 0.012, and 2.4 mM, respectively, after 24 h, while the consumption of FA was 10.9, 19.5, and 96%, respectively. Only the culture of the *B. megaterium* LBI001 strain showed biomass growth (1.9×10^9 cells mL⁻¹) because the *B. licheniformis* FD36 and *B. cereus* MS15 cultures maintained the cell number at the inoculum level (1×10^7 cells mL⁻¹).

Biotransformation of FA into 4VG by *B. megaterium* LBI001 in a Growing Cell System

During the biotransformation of FA, the maximum biomass concentration (X_{\max}) was achieved at 12 h in all cases (Fig. 2a–d). The estimated values for X_{\max} were 3.15, 2.51, 1.40, and 1.12 mg mL⁻¹ at 6, 10, 15, and 25 mM FA, respectively, but the last two values were statistically similar ($\alpha < 0.05$). The values for μ_{\max} at 6, 10, 15, and 25 mM FA were 0.51, 0.44, 0.32, and 0.22 h⁻¹, respectively; however, nonstatistically significant differences were found among them ($\alpha < 0.05$). The correlation coefficients (R^2) for the experimental values obtained at 6, 10, 15, and 25 mM FA fitted to the three parameters logistic model were 0.95, 0.97, 0.95, and 0.92, respectively.

During the first 2 h of culture, FA was not consumed, nor was 4VG detected, as this time corresponded to the adaptation phase of *B. megaterium* in nejayote. Between 2 and 4 h, at 6 and 10 mM FA (Fig. 2a, b, respectively), the FA concentration diminished but without 4VG production. At 15 and 25 mM (Fig. 2c, d), a similar phenomenon was observed between 2 and 6 h, suggesting that at these periods, cells started to internalize FA. During the exponential phase of growth and stationary phase, similar profiles of FA consumption and 4VG production were observed at any initial FA concentration.

The biotransformation parameters estimated at the maximal 4VG concentrations (10 h for 6, 10, and 15 mM FA and 12 h for 25 mM) are shown in Fig. 3a–d. The production of 4VG was 2.1, 2.4, 3.8, and 6.2 mM 4VG at 6, 10, 15, and 25 mM FA, respectively (Fig. 3a), while the percentage of FA consumption was 97, 90, 71, and 32%, respectively, at those concentrations (Fig. 3b). Molar yields of 4VG ($Y_{p/s}$) were statistically similar at 6, 10, and 15 mM FA (average 33%) but increased up to 76% at 25 mM (Fig. 3c). Finally, at 6 and 10 mM FA, 4VG productivities were statistically similar (average of 0.23 mM h⁻¹), increasing up to 0.38 and 0.51 mM h⁻¹ at 15 and 25 mM FA, respectively (Fig. 3d).

Biotransformation of FA into 4VG by *B. megaterium* LBI001 in a Resting Cell System

Unlike the growing cell system in which biotransformation did not start immediately, in the resting cell system,

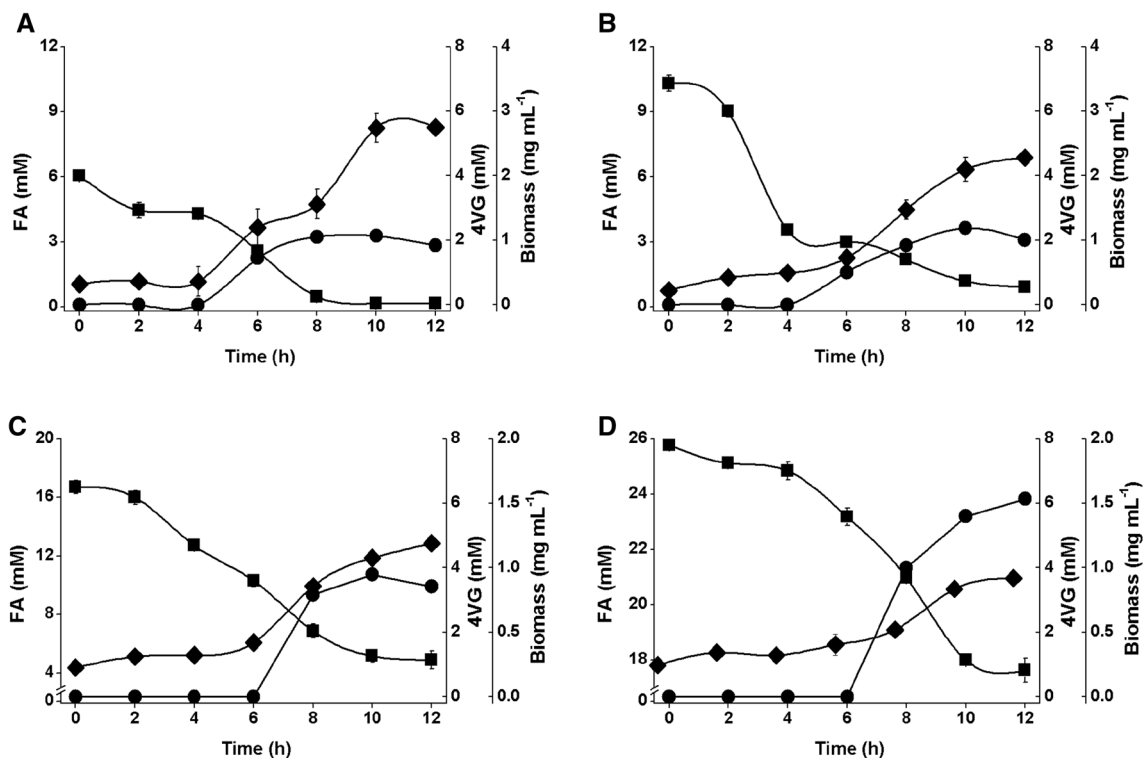
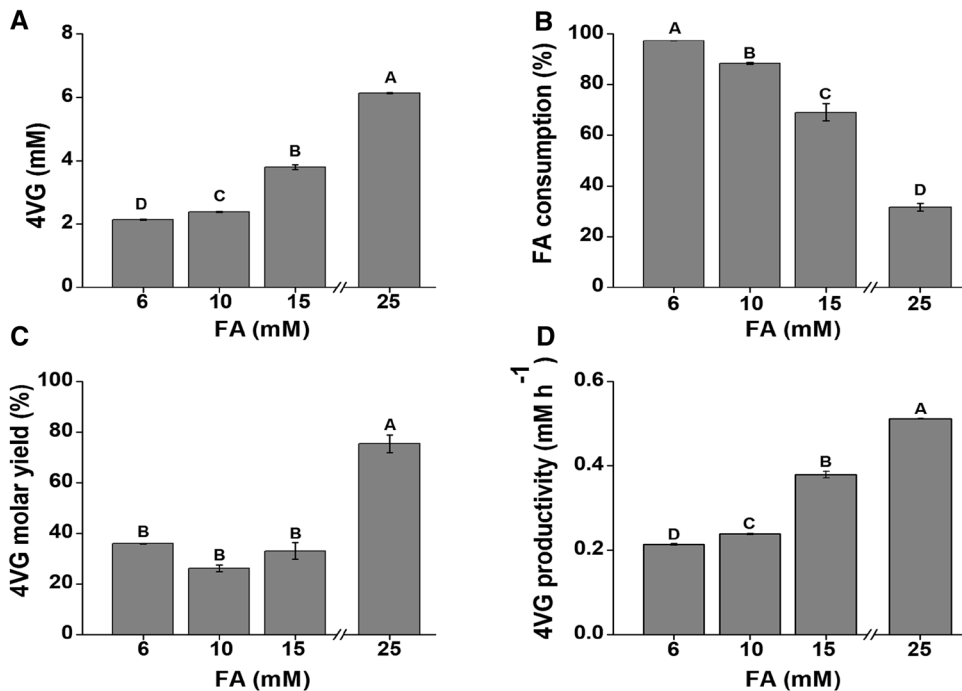


Fig. 2 Biotransformation in a growing cell system with the ferulic acid in nejayote **a** 6 mM and nejayote enriched with FA **b** 10 mM, **c** 15 mM, and **d** 25 mM into 4-vinylguaiacol by native *B. megaterium* LBI001. Biomass production (filled diamond), 4VG (filled circle), FA (filled square)

Fig. 3 Biotransformation parameters in the growing cell system with the ferulic acid in nejayote (6 mM) and enriched nejayote (10, 15, and 25 mM FA) into 4-vinylguaiacol by native *B. megaterium* LBI001. **a** 4VG production (mM), **b** FA consumption (%), **c** 4VG molar yield (%), and **d** 4VG productivity (mM h⁻¹)



biotransformation of FA into 4VG at any FA concentration started immediately, followed by an exponential decay of FA and formation of 4VG until 12 h, stabilizing at 16 to 20 h, as depicted in Fig. 4a–d.

The biotransformation parameters estimated at the maximal 4VG concentrations (20 h) are shown in Fig. 5a–d. At 6 and 25 mM FA (Fig. 5a), 4VG production was statistically similar (average of 3.5 mM), while at 10 and 15 mM FA, higher concentrations (4.6 and 5.1 mM 4VG, respectively) were achieved, although those values were statistically similar.

The FA consumption (%) at 6, 10, 15, and 25 mM FA (Fig. 5b) was 88, 64, 25, and 24%, respectively. The highest yield of 4VG (Y_p/s) (Fig. 5c) was obtained at 6 mM FA with a value of 82%, while at 10 and 15 mM FA, the yields were lower (80%), although statistically similar. The lowest yield (51%) was obtained at 25 mM FA.

Regarding productivity (Fig. 5d), the highest values were obtained at 10 and 15 mM FA (average of 0.24 mM h^{-1}). At 6 and 25 mM FA, the productivities were lower (average of 0.18 mM h^{-1}).

Discussion

Isolation, Characterization, and Identification of the Native Strain

The phenotypical and biochemical characteristics of the LBI001 strain isolated from nejayote correspond to the *Bacillus* genus [29]. Phylogenetic analysis of the 16S rRNA gene sequence of the strain LBI001 (1196 bp) indicated that its closest relatives were *Bacillus megaterium* GMB4002-a (99% similarity), *Bacillus megaterium* GMB5003 (99% similarity), and *Bacillus aryabhatai* B8W22 (99%). However, the degree of similarity between the amino acid sequence of LBI001 (data not shown) indicated that its closest relative was *Bacillus megaterium* GMB4002-a (100% similarity). UPGMA phylogenetic tree (Fig. 1) and the maximum-parsimony tree (not shown) revealed that the strain LBI001 clustered most closely with these strains.

Biotransformation of Crude FA by Three *Bacillus* Species

Although nejayote is reported as a suitable substrate for some bacterial growth [21, 30], its high phenolic content [17, 31] usually inhibits the growth of other bacteria. Indeed,

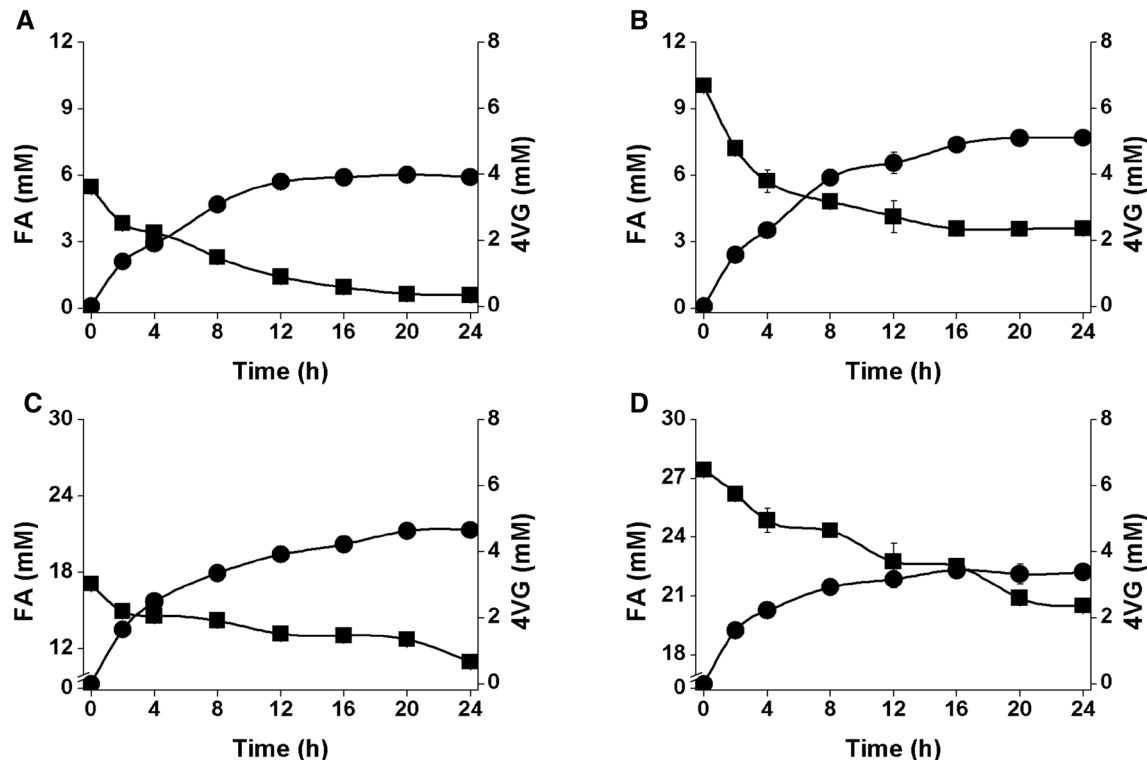
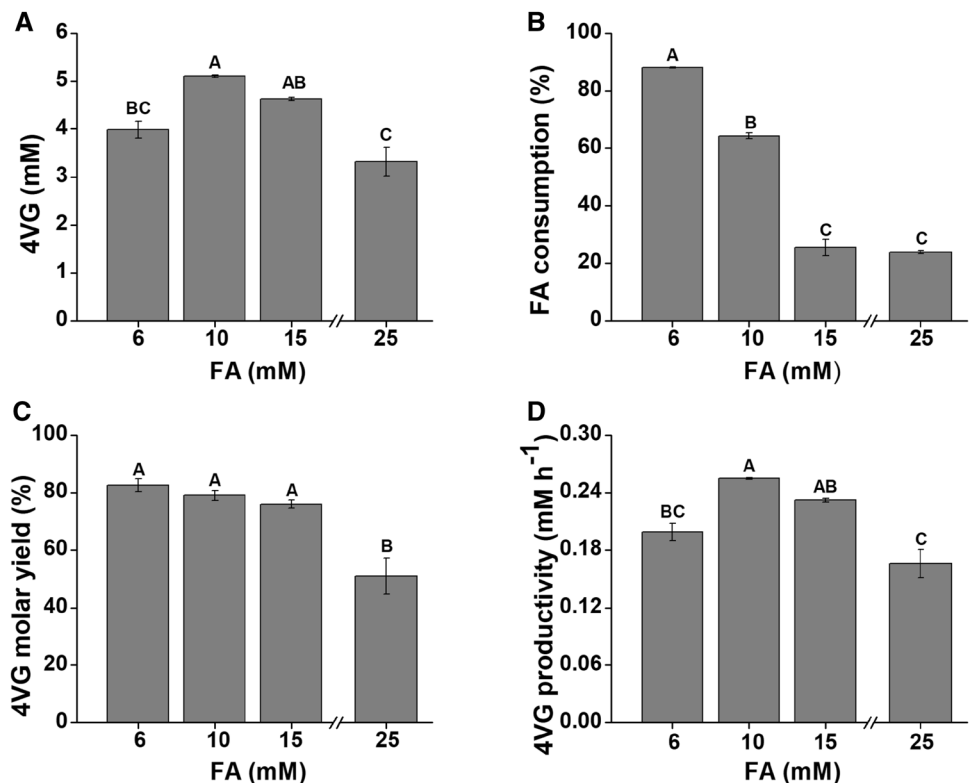


Fig. 4 Biotransformation in the resting cell system at several concentrations of ferulic acid: **a** 6 mM, **b** 10 mM, **c** 15 mM, and **d** 25 mM into 4-vinylguaiacol by native *Bacillus megaterium* LBI001. 4VG (filled circle), FA (filled square)

Fig. 5 Biotransformation parameters in the resting cell system at several concentrations of ferulic acid into 4-vinylguaiacol by native *B. megaterium* LBI001. **a** 4VG production (mM), **b** FA consumption (%), **c** 4VG molar yield (%), and **d** 4VG productivity (mM h^{-1})



the ability of the strain LBI001 to grown on nejayote and transform crude FA into 4VG suggests an adaptive tolerance to FA and other phenolics because other *Bacillus* species, such as *B. licheniformis* and *B. cereus*, were unable to grow despite both species being reported as effective agents for biotransforming FA from other sources into 4VG. [10, 22].

The sequence analysis of ferulic acid decarboxylases of *B. pumilus* (AC050701.1), *B. subtilis* (ARW33292.1), *B. licheniformis* (AVI46886.1), and *B. amyloliquefaciens* (ARW40653.1) from NCBI and alignment with Clustal W 2.1 showed that the sequences are highly conserved with approximately 83% similarity or higher. Despite the homology between them, some variations at the level of the catalytic site could cause sufficient changes to modify the efficiency of FA conversion among species [32]. Matte et al. [33] reported the crystal structure of phenolic decarboxylase from *Bacillus pumilus* UI 670, which showed high overall similarity to the enzyme from *L. plantarum* and *B. subtilis*. A conformational change in the $\beta 1$ – $\beta 2$ loop between the *B. pumilus* and *L. plantarum* structures was observed; this region influences the shape and size of the cavity that defines the active site. On the other hand, the broad range of specificity shown by phenolic acid decarboxylases could be explained by the presence of certain amino acid sequence motifs that are absent in enzymes showing more restricted substrate specificity [34]. The highest amino acid sequence variability among the bacterial phenolic acid decarboxylases

was found in a region of 18 amino acids adjacent to the C-terminal region, proposed to be involved in enzyme–substrate specificity [34]. Since nejayote contains several phenolic acids, the ability of the strain LBI001 to grow on nejayote and transform crude FA suggests that several phenolic acid decarboxylases could be involved in its detoxification system.

Biotransformation of FA into 4VG by *B. megaterium* LBI001 in a Growing Cell System

The X_{max} at 15 and 25 mM FA was lower than the X_{max} at 6 and 10 mM FA (Fig. 2). This finding suggests that there is an inhibitory effect of FA and/or 4VG on bacterial growth. Lee et al. [35] reported that the FA decarboxylase of *B. pumilus* was inhibited at concentrations higher than 3 mM 4VG. Additionally, during the production of 4-vinylphenol by *E. coli* expressing a decarboxylase from *L. plantarum*, inhibition was observed at concentrations higher than 4 mM [36]. As depicted in Fig. 3a, at 15 and 25 mM FA, 3.8 and 6.2 mM 4VG, respectively, were obtained; this concentration is higher than those of the inhibitory limits. On the other hand, the μ_{max} of *B. megaterium* at any initial FA concentration was statistically similar, showing that FA had no inhibitory effect on growth until 4VG accumulation occurred. The μ_{max} of *B. megaterium* grown on nejayote (0.22–0.51 h^{-1}) was higher than the μ_{max} of *B. subtilis* grown on minimal

medium with several carbon sources ($0.05\text{--}0.20\text{ h}^{-1}$) [37], revealing that nejayote is an appropriate culture medium for the *B. megaterium* LBI001 strain.

The molar yields ($Y_{P/S}$) and productivities (P) of 4VG at 6 and 10 mM FA (Fig. 3c, d) suggest that FA was consumed as a carbon source by the strain LBI001. This phenomenon was also reported in *Amycolatopsis* sp. ATCC 39116, which can use vanillin, the product of the FA biotransformation process, as a carbon source for cell growth [3]. In the case of 4VG, the mechanism proposed for its incorporation into metabolism involves the excision of the aromatic ring and its incorporation into the β -ketoadipate pathway for the formation of aliphatic molecules [38], maintaining inhibitory compounds under a minimal concentration [39]. The improvement of 4VG productivities at 15 and 25 mM FA suggests that the LBI001 strain acts as a whole-cell biocatalyst, avoiding the use of FA and 4VG as carbon sources. Similar behavior was reported by Laforgue and Lonvaud-Funel [40] in the yeast *B. bruxellensis*, which retains decarboxylase activity during wine fermentation. However, more studies are necessary to thoroughly understand the changes in the metabolism of *B. megaterium* cultivated at high concentrations of FA. Additionally, the bioconversion parameters ($Y_{P/S}$ and P) obtained at the highest FA concentrations (0.38 and 0.51 mM h^{-1} 4VG at 15 and 25 mM FA, respectively) show that the strain LBI001 converts FA into 4VG as the main biotransformation product (Supplementary Figure), as one of the most efficient members of the *Bacillus* genus for biotransforming FA into 4VG. For instance, a productivity of 0.03 mM h^{-1} 4VG from 5 mM pure FA, with vanillin as a byproduct, was reported employing *B. aryabhatai* [39]. The strain *B. methylotrophicus* showed a 0.09 mM h^{-1} concentration of 4VG from 2.5 mM FA, and trace amounts of vanillin, vanillic acid, and protocatechuic acid were also produced. Mishra et al. [10] reported a 0.044 mM h^{-1} 4VG productivity from 2.5 mM FA employing *B. cereus* SAS-3006. Low 4VG productivities are mostly associated with multiple FA conversion pathways and the degradation of 4VG into other phenolic compounds, such as vanillin, vanillic acid, and protocatechuic acid [41]. Thus, the regulation and expression of the microbial enzymes involved in FA degradation can modulate the production of several metabolites and reduce the efficiency of 4VG production. However, the absence of the formation of byproducts may not be sufficient since even when 4VG is obtained as the only product, very low productivities were obtained, possibly due to the low efficiency of ferulate decarboxylase. For instance, Adamu et al. [14] obtained a productivity of 0.001 mM h^{-1} 4VG from FA employing *Lactobacillus farciminis*, which was 510 times lower than the highest productivity reported in the current study.

Although our results demonstrate that the strain LBI001 converts FA into 4VG at elevated FA concentrations (15

and 25 mM FA) acting as a whole-cell biocatalyst, it is evident that the system must be optimized to avoid the inhibition phenomenon by the product (4VG) since the amount of residual FA was very high (e.g., 69% at 25 mM FA).

Biotransformation of FA into 4VG in a Resting Cell System

Unlike the growing cell system in which 4VG production is growth-associated (Fig. 2), in the resting cell system, the lag phase was not observed (Fig. 4). In this case, bioconversion started very quickly once FA was internalized in the cells present at a high concentration (4 mg mL^{-1}). Similar results were obtained by Sun et al. [42] with immobilized cells of *B. licheniformis*.

In the resting cell system, at 6 and 25 mM FA, the amounts of 4VG produced were statistically similar (3.5 mM), increasing slightly up to 4.85 mM 4VG at 10 and 15 mM FA, whereas in the growing cell system, 4VG increased from 2.1 to 6.2 mM as the concentration of FA increased. The $Y_{P/S}$ of the resting cell system at concentrations of 6, 10, and 15 mM FA remained above 83% (Fig. 5), whereas in growing cell system at the same concentrations, the $Y_{P/S}$ values were below 36% (Fig. 2), while at 25 mM FA, the $Y_{P/S}$ increased up to 75% (Fig. 3d). However, at the highest FA concentration in the resting cell system, the $Y_{P/S}$ decreased (51%) (Fig. 5c). As shown in Fig. 4d, the maximum concentration of 4VG was reached at 8 h of culture and remained almost constant, while FA decreased linearly. This finding could be related to the formation of other molecules from FA and 4VG that were not excreted and were employed to generate maintenance energy. It is possible that a dynamic equilibrium between the formation and the degradation speeds of 4VG was achieved. Chai et al. [43] reported the capacity of the strain *Cupriavidus* sp. B-8 to biotransform FA; however, the produced 4VG was rapidly metabolized into vanillic and protocatechuic acids.

Finally, productivity (P) in the resting cell system was between 0.20 and 0.25 mM h^{-1} 4VG, whereas in growing cell system, the productivity was between 0.38 and $0.51\text{ h}^{-1}\text{ mM}$ 4VG, more than twice that observed in the resting cell system. This suggests that a nutrient-rich environment such as nejayote allows the generation of enough maintenance energy to support some metabolic pathways properly [39], particularly those related to the biotransformation of FA into 4VG, making it more efficient. Additionally, it has been reported that phenolic decarboxylases are inducible enzymes [44, 45]. Nonetheless, the ferulic acid decarboxylase of *B. megaterium* LBI001 showed constitutive expression of the enzyme, although it was five times lower than that under induction conditions (data not shown), which would explain the lower productivity of 4VG in the resting cells compared to growing cells.

In conclusion, the current study shows that the native *B. megaterium* LBI001 strain, isolated from the biological niche of the wastewater “nejayote,” possesses great biotechnological potential to produce 4VG, a highly valued product in the aroma and flavor industries. The FA and other phenolics contained in nejayote suggest that the native strain developed an adaptive mechanism to support high concentrations of phenolics and an efficient detoxification system that drives 4VG formation from crude FA and could reduce production costs by avoiding expenses related to the purification of raw materials.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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