

Molecular Identification and Antibiofilm Potential of Bacteria Isolated from *Vanilla planifolia* Beans

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Abstract

The bacterial profile associated with *Vanilla planifolia* beans plays an important role in fermentation processes and the production of bioactive compounds, yet its potential as a source of antibiofilm agents remains poorly understood. This study aimed to isolate bacteria from fermented and dried (unfermented) *V. planifolia* beans using different pH isolation media, characterize their bacterial profiles through molecular identification, and evaluate their antibiofilm potential against two clinically relevant biofilm-forming pathogens. Bacterial strains were isolated on MRS agar adjusted to pH 4 and pH 7, followed by morphological and Gram-staining characterization. Molecular identification was performed using 16S rRNA gene sequencing and BLAST analysis. Antibiofilm activity was assessed using a microtiter plate assay against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. A total of 38 bacterial isolates were obtained, with fermented vanilla beans yielding more isolates (23 isolates) than dried (unfermented) vanilla beans (15 isolates). The bacterial profile was dominated by members of the genera *Bacillus*, *Staphylococcus*, and *Liquorilactobacillus*. Eight isolates exhibited strong antibiofilm activity, with biofilm inhibition reaching 89.9% against *S. aureus* and 99.6% against *E. coli*. Notably, the detection of *Liquorilactobacillus hordei* in fermented vanilla beans expands the known ecological distribution of this genus beyond conventional fermented beverages. These findings demonstrate that bacteria associated with fermented and dried/unfermented *V. planifolia* beans represent promising sources of antibiofilm compounds with potential applications in food safety, healthcare, and biotechnology as sustainable alternatives to conventional antimicrobial agents.

Keywords: *Vanilla planifolia* beans, bacterial diversity, 16S rRNA gene, antibiofilm activity

Introduction

Fermented foods and plant-associated microbiota harbor diverse bacteria capable of producing bioactive metabolites, including bacteriocins, biosurfactants, exopolysaccharides, and antimicrobial peptides (Linares *et al.*, 2017). Those microbiota also have attracted considerable interest as potential sources of antibiofilm compounds (Ghosh *et al.*, 2022; Jiang *et al.*, 2022). Among them, fermented vanilla beans represents a promising yet underexplored source of fermented bean (Cuellar *et al.*, 2022).

The vanilla plant (*Vanilla planifolia*) is considered one of the most important

worldwide flavorings. Once the pod has been harvested, it must undergo a traditional curing process to develop characteristic aroma and flavor (with more than one hundred compounds) of the beans inside the pod. Once the pod have cured, vanilla beans produce a large amount of aromatic, phenolic, and organic acids, and fatty acids and essential oil compounds, which together give it a high antioxidant capacity (Peña-Barrientos *et al.*, 2023).

Previous studies have reported the presence of bacterial genera such as *Bacillus*, *Staphylococcus*, *Halomonas*, and *Pseudoalteromonas* in vanilla beans during the fermentation process (Gu *et al.*, 2015;

Mahadeo *et al.*, 2024). In addition, fermented vanilla beans contain bioactive compounds, including vanillin and phenolic derivatives, that exhibit antimicrobial and antibiofilm activities (Ahmad *et al.*, 2020).

Despite increasing knowledge of vanilla-associated microbiota, studies have primarily focused on microbial diversity and fermentation characteristics, whereas their antibiofilm potential remains poorly understood. Furthermore, the effects of post-harvest processing conditions, such as fermentation and drying, as well as environmental pH, on profil bacteria and bioactivity have received limited attention. Therefore, this study aimed to analyze the profil of bacterial isolated from fermented and dried (unfermented) *V. planifolia* beans under different pH conditions, identify bacterial isolates using 16S rRNA gene sequencing, and evaluate their antibiofilm activities against biofilm producing pathogenic bacteria *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

Materials and Methods

Isolation and Purification of Fermentation Bacteria. Fermented pod sample and unfermented/dried pod sample of *Vanilla planifolia* were obtained from Lombok Island, Indonesia. Pod of fermented or dried vanilla sample were aseptically cut to obtain vanilla beans. The vanilla beans from each sample were homogenized using a sterile mortar with 3 mL of sterile distilled water to obtain a uniform suspension. One mL of the suspension was used for serial dilution in 0.85% NaCl solution up to 10^{-4} . Aliquots (500 μ L) from selected dilutions (10^0 , 10^{-2} , and 10^{-4}) were inoculated using the pour plate method on de Man, Rogosa, and Sharpe agar supplemented with 1% CaCO_3 (MRSA- CaCO_3), adjusted to pH 4 and pH 7. Plates were incubated at 30°C for 24 h. Distinct colonies were purified by repeated streaking on the same medium until pure isolates were obtained. Pure cultures were preserved in 10% glycerol at -80°C .

Morphological and Gram Characterization. Colony morphology was observed after incubation (30°C, 24–48 h) based on size, shape, margin, elevation, and pigmentation. Gram staining was performed using standard

procedures (crystal violet, iodine, ethanol, and safranin). Slides were observed under a light microscope at 1000 $\times$ magnification using immersion oil. Cell shape, arrangement, and Gram reaction were recorded.

DNA Extraction and 16S rRNA Gene Amplification. Genomic DNA was extracted using a chemical lysis method. Bacterial cells were harvested, washed with 500 μ L TE buffer pH 8.0, and lysed using 300 μ L extraction buffer containing 1 M Tris-HCl pH 8.5, 1 M NaCl, 0.1 M EDTA, and 10% SDS. The mixed solution was centrifuged for 13.000 rpm for 5 min at 4°C. The supernatant was transferred to a new tube, and the DNA was precipitated using 30 μ L of 3 M sodium acetate pH 5.2 and cold absolute ethanol. The pellet of DNA was washed with 70% ethanol, dried, and resuspended in TE buffer pH 8.0. The 16S rRNA gene was amplified using PCR with universal primers 27F and 1492R. The reaction mixture (25 μ L) consisted of DNA template, MyTaq polymerase, primers, DMSO, and nuclease-free water. PCR conditions included initial denaturation (95°C, 2 min), followed by 35 cycles of denaturation (95°C, 30 s), annealing (52°C, 30 s), and extension (72°C, 90 s), with a final extension at 72°C for 7–10 min (Iswanto *et al.*, 2019).

Gel Electrophoresis and Sequencing. PCR products were analyzed using 1% agarose gel electrophoresis in TAE buffer pH 8.0 and visualized under UV light (Iswanto *et al.*, 2019). Sequencing was performed using the Sanger method by a commercial provider. Sequence data were analyzed using BLAST (NCBI) and aligned using BioEdit/ChromasPro.

Antibacterial Activity Assay. Antibacterial activity was evaluated using a dual-layer agar diffusion method (Stachurska *et al.*, 2021). The bottom layer consisted of Mueller-Hinton Agar (2%), while the top layer (0.75%) was inoculated with test bacteria (OD = 0.01). Sterile paper discs were loaded with bacterial isolates or antibiotics (ampicillin, rifampicin, chloramphenicol) as positive controls. Plates were incubated at 37°C for 24–72 h, and inhibition zones were measured.

Biofilm Formation Assay. Biofilm formation was quantified using the microtiter plate assay

(Kirmusaoğlu, 2019) . Bacterial suspensions ($OD_{600} = 0.01$) were inoculated into 96-well plates and incubated at 37°C for 16–24 h. Wells were washed, stained with 0.1% crystal violet, and destained using ethanol. Absorbance was measured at 595 nm. The cut-

off value of optical density (ODc) was calculated as:

$$ODc = \text{mean OD of negative control} + (3 \times SD).$$

Biofilm production was classified as weak, moderate, or strong based on OD values (Table 1).

Table 1. Interpretation of optical density (OD) values from microtiter plate assay results on the level of biofilm formation (Kirmusaoğlu, 2019)

Average OD Value	Biofilm Production Strength
$OD \leq ODc$	No Biofilm production
$ODc < OD \leq 2 \times ODc$	Weak Biofilm Production
$2 \times ODc < OD \leq 4 \times ODc$	Moderate Biofilm Production
$4 \times ODc < OD$	Strong Biofilm Production

$$\% \text{ Biofilm} = ((\text{Sample}_{A595} - \text{SC}_{A595}) / (\text{GC}_{A595} - \text{SC}_{A595})) \times 100 \quad (\text{Formula 1})$$

where, GC = growth control and SC = sterility control.

Negative control, positive control (antibiotics), media control, and solvent control (DMSO) were calculated.

Production of Secondary Metabolites.

Selected bacterial isolates were cultured in 50 mL MRS broth pH 4.0 or 7.0 at 30°C for 72 h with shaking. Cultures were centrifuged, and supernatants were extracted using 50 mL ethyl acetate (1:1, v/v). The organic phase was evaporated using a rotary evaporator (40°C) to obtain crude extracts.

Biofilm Inhibition and Eradication Assays.

Antibiofilm activity was evaluated using a modified microtiter plate method (Haney *et al.*, 2021) . For inhibition assays, extracts were added prior to biofilm formation, while for eradication assays, preformed biofilms were treated with extracts. Biofilm biomass was quantified using crystal violet staining and measured the absorbance (A) at 595 nm. Biofilm inhibition and eradication was calculated as in Formula 1.

Results

Isolation of Bacteria

A total of 38 bacterial isolates were obtained from *Vanilla planifolia* beans, comprising 23 isolates from fermented pod sample and 15 isolates from dried/unfermented pod sample. From fermented pod sample, 13 isolates were isolated on MRS agar medium at pH 7.0 and 10 isolates were isolated on MRS agar medium at pH 4.0. Further, from unfermented or dried pod sample, 8 isolates

were isolated on MRS agar medium at pH 7.0 and 7 isolates were isolated on MRS agar medium at pH 4.0 (Table 2). Based on their colony and cellular morphology, the predominant isolates exhibited punctiform, circular colonies with an entire margin, convex elevation, and white pigmentation (22 isolates). Other colony characteristics included large, moderate, and small sizes with varying margins and elevations, such as curled or undulate margins and raised, pulvinate, umbonate, or convex elevations. All isolates exhibited white pigmentation. Gram staining revealed that all 38 isolates were Gram-positive, comprising 26 bacilli and 12 cocci. These morphological characteristics provided preliminary phenotypic data for characterizing and identifying the bacterial isolates prior to molecular identification.

Based on 16S rRNA gene sequencing, totally the 38 isolates were identified as *Staphylococcus epidermidis* (7 isolates), *Liquorilactobacillus hordei* (6 isolates), *Bacillus subtilis* (6 isolates), *Bacillus wiedmannii* (3 isolates), *Bacillus altitudinis* (3 isolates), *Bacillus amyloliquefaciens* (2 isolates), *Staphylococcus warneri* (2 isolates), *Bacillus inaquosorum* (1 isolate), *Bacillus siamensis* (1 isolate), *Bacillus cereus* (1 isolate), *Bacillus circulans* (1 isolate), *Lysinibacillus fusiformis* (1 isolate), *Staphylococcus aureus* (1 isolate), *Staphylococcus hominis* (1 isolate), *Staphylococcus saprophyticus* (1 isolate), and

Clostridium sartagoforme (1 isolate). Sequence similarity ranged from 94.22% to 100%, indicating high genetic relatedness to reference strains (Table 2).

The results revealed distinct bacterial community profiles among fermentation and non-fermentation conditions, characterized by the occurrence of condition-specific taxa. Under fermentation at pH 7, the bacterial community was uniquely dominated by *L. hordei* (6 isolates). In contrast, fermentation at pH 4 was characterized by the occurrence of *S. epidermidis*, indicating their adaptation to acidic environments. Non-fermented samples at pH 7 were specifically associated with *B. subtilis* and *B. altitudinis*. Meanwhile, non-fermented samples at pH 4 exhibited a distinct bacterial composition, with the exclusive detection of *S. warneri*. These findings demonstrate that each combination of processing condition and pH

generated a unique bacterial assemblage, reflecting the selective pressures imposed by differences in nutrient availability, water activity, and environmental acidity (Table 2).

The bacterial community composition differed according to both processing condition and pH. Fermented vanilla beans were dominated by *Liquorilactobacillus hordei* (6 isolates), whereas unfermented beans were primarily dominated by *B. subtilis* and *B. altitudinis* (3 isolates each) (Table 2). Species richness was higher at pH 7, where the community was dominated by *Bacillus*, *Liquorilactobacillus*, and *Staphylococcus* species. In contrast, pH 4 supported fewer species and was characterized mainly by acid-tolerant bacteria, including *S. epidermidis*, *S. hominis*, *S. saprophyticus*, and several *Bacillus* species (Table 2).

Table 2. Bacterial diversity isolated from *Vanilla planifolia* under fermentation and drying conditions based on molecular analysis

Condition	pH	Number of isolate	Species (Number of isolate)
Fermented vanilla beans	4.0	10	<i>Bacillus subtilis</i> (1), <i>Bacillus amyloliquefaciens</i> (1), <i>Bacillus wiedmannii</i> (1), <i>Staphylococcus epidermidis</i> (5), <i>Staphylococcus hominis</i> (1), <i>Bacillus cereus</i> (1),
	7.0	13	<i>Liquorilactobacillus hordei</i> (6), <i>Bacillus inaquosorum</i> (1), <i>Bacillus subtilis</i> (2), <i>Bacillus amyloliquefaciens</i> (1), <i>Lysinibacillus fusiformis</i> (1), <i>Bacillus wiedmannii</i> (1), <i>Staphylococcus epidermidis</i> (1)
Un-fermented or Dried vanilla beans	4.0	7	<i>Bacillus wiedmannii</i> (1), <i>Staphylococcus epidermidis</i> (1), <i>Staphylococcus saprophyticus</i> (1), <i>Bacillus circulans</i> (1), <i>Clostridium sartagoforme</i> (1), and <i>Staphylococcus warneri</i> (2)
	7.0	8	<i>Bacillus subtilis</i> (3), <i>Staphylococcus aureus</i> (1), <i>Bacillus siamensis</i> (1), <i>Bacillus altitudinis</i> (3)

Biofilm Inhibition and Eradication Assays

All isolates were tested for antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The results showed that 8 isolates exhibited strong activity, with clear zone diameters > 5 mm. These eight selected bacteria, *Bacillus subtilis* (W57-F-7), *Staphylococcus aureus* (W64-D-7), *Bacillus amyloliquefaciens* (W52-F-7 and W121-F-4), *Bacillus altitudinis* (W66-D-7), *Bacillus wiedmannii* (W126-D-4), and *Staphylococcus epidermidis* (W118-F-4 and W131-D-4), were evaluated for their anti-biofilm activity using ethyl acetate extracts of secondary metabolites.

All isolates exhibited inhibitory activity against biofilm formation in both *E. coli* ATCC 25922 (Figure 5) and *S. aureus* ATCC 25923 (Figure 4), although the magnitude and

consistency of inhibition differed between the two test organisms.

Against *E. coli* ATCC 25922, inhibition was more variable and concentration-dependent. At 500 µg/mL, inhibition ranged widely from 38.5% to 99.6%, with the highest activity observed in *B. amyloliquefaciens* (W121-F-4 and W52-F-7). However, most isolates showed a marked decline in activity with decreasing concentration of extract. For instance, *B. altitudinis* (W66-D-7) decreased sharply from 87.4% at 500 µg/mL to 1.9% at 0.98 µg/mL (Figure 3).

In contrast, inhibition *S. aureus* ATCC 25923 was consistently high across all concentrations. At 500 µg/mL, inhibition ranged from 77.0% to 89.9%, with the highest activity observed in *B. amyloliquefaciens* W121-F-4 (89.9%), followed closely by *S.*

aureus (W64-D-7) (81.8%) (Figure 4). Overall, inhibitory activity was higher and more stable against *S. aureus* ATCC 25923 than *E. coli* ATCC 25922. In addition, isolates derived from fermented samples generally exhibited more consistent inhibition profiles compared to those from dried samples.

All isolates were further evaluated for their ability to eradicate pre-formed biofilms (Figures 5 and 6). In general, eradication activity was strongly influenced by extract concentration and was lower at reduced concentrations.

For *E. coli* ATCC 25922, eradication activity ranged from 65.8% to 94.3% at 500 µg/mL. The highest activity was recorded in *S. epidermidis* isolates W118-F-4 (94.3%) and W131-D-4 (93.2%), as well as *B. altitudinis* (W66-D-7) (90.4%). Unlike the inhibition assay, certain isolates—particularly W118-F-4 and 66-D-7—retained relatively high activity

even at lower concentrations, indicating greater persistence of eradication effects.

Against *S. aureus* ATCC 25923, eradication activity at 500 µg/mL ranged from 78.6% to 91.0%. The highest activity was observed in *S. epidermidis* isolates (W131-D-4) (91.0%) and (W118-F-4) (90.7%), followed by *B. wiedmannii* (W126-D-4) (89.8%). However, activity declined substantially at lower concentrations, with several isolates showing minimal effects at 0.98 µg/mL (Figure 6).

Compared to *S. aureus* ATCC 25923, eradication activity against *E. coli* ATCC 25922 showed greater variability across isolates and concentrations. Nevertheless, several isolates demonstrated stable performance across a wide concentration range. Consistent with inhibition results, isolates obtained from fermented samples tended to exhibit more stable and sustained eradication activity than those derived from dried samples.

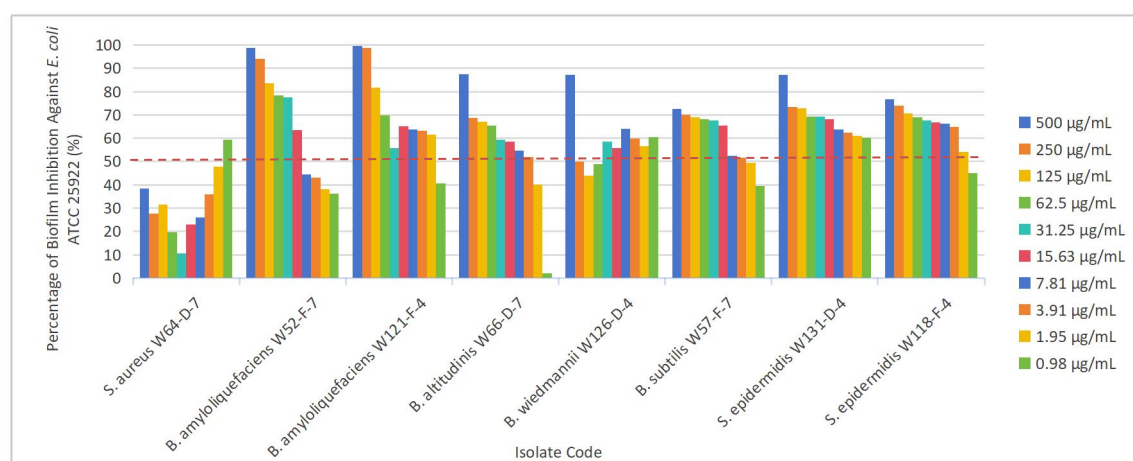


Figure 3. Biofilm inhibition activity against *Escherichia coli* ATCC 25922 by secondary metabolites of bacterial isolates with various ethyl acetate extract concentration ((µg/mL)

Note: code F = fermented, D = dried, 4 = pH 4, and 7 = pH 7

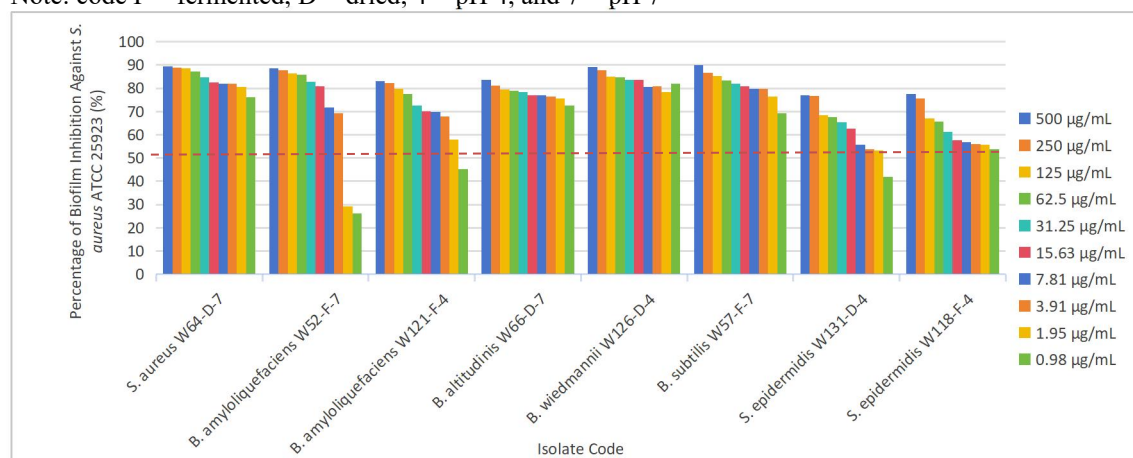


Figure 4. Biofilm inhibition activity against *Staphylococcus aureus* ATCC 25923 by secondary metabolites of bacterial isolates with various ethyl acetate extract concentration ((µg/mL).

Note: code F = fermented, D = dried, 4 = pH 4, and 7 = pH 7

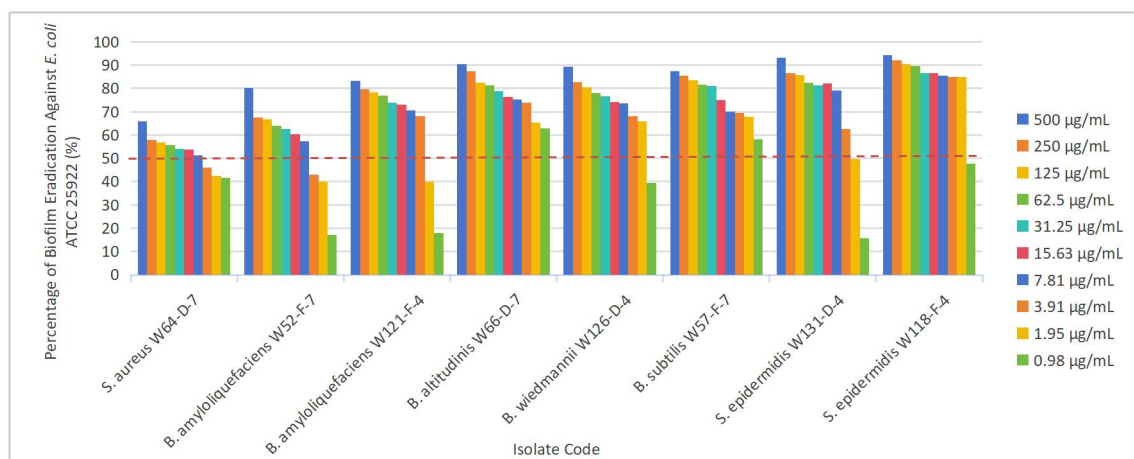


Figure 5. Biofilm eradication activity against *Escherichia coli* ATCC 25922 by secondary metabolites of bacterial isolates with various ethyl acetate extract concentration ((µg/mL)

Note: code F = fermented, D = dried, 4 = pH 4, and 7 = pH 7

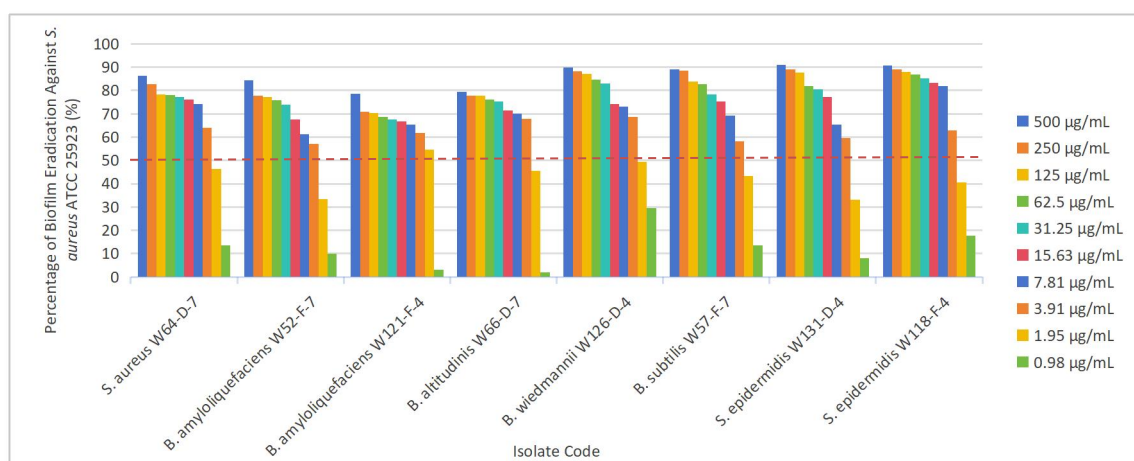


Figure 6. Biofilm eradication activity against *Staphylococcus aureus* ATCC 25923 by secondary metabolites of bacterial isolates with various ethyl acetate extract concentration ((µg/mL).

Note: code F = fermented, D = dried, 4 = pH 4, and 7 = pH 7

Discussion

The higher number of isolates obtained from fermented *Vanilla planifolia* pod sample compared to dried (unfermented) pod sample indicates that fermentation plays an important role in shaping the bacterial profile of vanilla beans. Fermentation provides moist and nutrient-rich conditions that support active microbial growth and succession, resulting in a more complex bacterial composition than that observed in dried sample. During fermentation, the degradation of plant tissues releases simple sugars, amino acids, and other organic compounds that serve as substrates for microbial growth. At the same time, microbial metabolism generates organic acids and antimicrobial compounds that create selective pressures, leading to the establishment of

characteristic bacterial communities. In contrast, drying reduces water activity and nutrient availability, limiting bacterial growth and favoring the persistence of only stress-tolerant species(Xiang *et al.*, 2019).

The bacterial profile observed in this study was dominated by *Bacillus* sp. and *Staphylococcus* sp., which is consistent with previous reports on the microbiota associated with *Vanilla planifolia* (Chen *et al.*, 2015; Cuan-Escobar *et al.*, 2025; Gu *et al.*, 2015; Lalanne-Tisné *et al.*, 2023; Mahadeo *et al.*, 2024; Manyatsi *et al.*, 2024). These genera are widely recognized for their ecological adaptability and ability to produce various bioactive compounds, which likely contribute to their persistence in vanilla-associated environments.

A notable finding of this study was the detection of *Liquorilactobacillus hordei* among bacterial isolates recovered from fermented *Vanilla planifolia*. Members of the genus *Liquorilactobacillus* are commonly associated with water kefir and other sugar-rich fermented beverages (Lynch *et al.*, 2021; Zannini *et al.*, 2022). To the best of our knowledge, the occurrence of *Liquorilactobacillus* in fermented vanilla has rarely been reported, highlighting the potential novelty of this finding and suggesting that vanilla fermentation may provide a previously unexplored ecological niche for this genus.

The presence of *L. hordei* may be attributed to the conditions generated during vanilla fermentation. Vanilla beans inside the pods contain fermentable carbohydrates that provide suitable substrates for lactic acid bacteria, while fermentation creates acidic and microaerophilic conditions favorable for the growth of acid-tolerant species such as *Liquorilactobacillus*. Furthermore, the use of MRS agar supplemented with CaCO₃ facilitated the selective isolation of acid-producing bacteria (Eiteman & Ramalingam, 2015; Renschler *et al.*, 2020). These findings suggest that fermented *V. planifolia* pod provides a suitable ecological niche for *Liquorilactobacillus*, extending its known occurrence beyond conventional fermented beverages.

Morphological and Gram-staining analyses revealed a predominance of Gram-positive bacteria, mainly *Bacillus*, *Staphylococcus*, and lactic acid bacteria. Bacterial profiles also varied with pH, with a greater number of species detected at pH 7, where *Bacillus* sp. predominated, whereas pH 4 favored acid-tolerant taxa such as *Staphylococcus* sp. and selected *Bacillus* species. These results indicate that both fermentation and pH act as key drivers shaping the bacterial profile of *Vanilla planifolia*.

Building upon these ecological findings, the present study further demonstrates that bacterial isolates from *Vanilla planifolia* produce secondary metabolites with significant antibiofilm activity, both in inhibiting biofilm formation and disrupting mature biofilms. The observed differences in susceptibility between *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 may be related to structural differences in their cell envelopes, particularly the presence of an outer

membrane in Gram-negative bacteria that can reduce the penetration of antimicrobial compounds.

The variability in anti-biofilm activity among isolates is closely related to differences in secondary metabolite production. Biofilms are composed of extracellular polymeric substances (EPS), including polysaccharides, proteins, lipids, and extracellular DNA (eDNA), which collectively provide structural stability and protection (Panlilio & Rice, 2021; Sharma & Singh, 2018). Disruption of these components is therefore a key mechanism underlying anti-biofilm activity.

Hydrolytic enzymes such as protease, lipase, and amylase contribute significantly to EPS degradation. Amylase breaks down polysaccharides, reducing cell adhesion (Rather *et al.*, 2021), while lipase disrupts lipid components and compromises membrane integrity (Yassein *et al.*, 2021). Protease targets adhesion proteins essential for early biofilm formation (Amin *et al.*, 2025). In addition, DNase-mediated degradation of eDNA weakens biofilm structure and reduces biomass (Liu *et al.*, 2020; Sharma & Singh, 2018).

Besides enzymatic activity, biosurfactants such as phenol-soluble modulins (PSMs) produced by *Staphylococcus* sp. can reduce surface tension, inhibit adhesion, and promote biofilm (Baldry *et al.*, 2020). Similarly, bacteriocins contribute by disrupting cell membranes and interfering with quorum sensing systems that regulate biofilm formation (Algburi *et al.*, 2017; Kim *et al.*, 2023).

Moreover, *Bacillus* sp. produce lipopeptides such as surfactin, iturin, and fengycin, which play a major role in antibiofilm activity. These compounds disrupt membrane integrity and inhibit adhesion, with surfactin reported to reduce biofilm formation by up to 90% in a dose-dependent manner (Gutiérrez-Chávez *et al.*, 2021; Jardak *et al.*, 2024; Shan *et al.*, 2021).

The composition of the biofilm matrix may also influence the antibiofilm activity observed against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. *S. aureus* biofilms are mainly composed of polysaccharide intercellular adhesin (PIA/PNAG), adhesin proteins, and extracellular DNA (eDNA), whereas *E. coli* biofilms possess a more complex matrix

containing curli fimbriae, cellulose, lipopolysaccharides (LPS), exopolysaccharides, and eDNA (Hung *et al.*, 2013; Schilcher & Horswill, 2020). The greater structural complexity of *E. coli* biofilms may reduce the accessibility and effectiveness of secondary metabolites, which could explain the higher antibiofilm activity observed against *S. aureus*, particularly at lower concentrations

Consistent with these mechanisms, the observed anti-biofilm activity generally followed a dose-dependent pattern, indicating that metabolite concentration is a critical factor. However, the presence of non-linear responses suggests that anti-biofilm effects are not solely linked to antibacterial activity. Instead, some metabolites may specifically target biofilm formation pathways, such as adhesion and quorum sensing, without fully inhibiting planktonic growth.

Overall, this study highlights the strong potential of bacterial isolates from *Vanilla planifolia* as sources of natural anti-biofilm agents, with greater effectiveness observed against *Staphylococcus aureus* ATCC 25923 than *Escherichia coli* ATCC 25922.

Conclusions

This study successfully demonstrated that fermentation bacteria isolated from *Vanilla planifolia* beans can be identified through molecular analysis of the 16S rRNA gene and possess significant antibiofilm activity against pathogenic bacteria. The isolates obtained showed diverse morphological and molecular characteristics, indicating the presence of various bacterial taxa associated with the fermentation process. Molecular identification using PCR and sequence analysis with BLAST confirmed the taxonomic affiliation of the isolates, while phylogenetic analysis using MEGA supported their evolutionary relationships. Functional assays revealed that several isolates and their secondary metabolite extracts exhibited effective inhibition and destruction of biofilm formed by *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, indicating their potential as sources of antibiofilm agents. These findings highlight the importance of fermentation-derived bacteria as promising candidates for the development of alternative antimicrobial strategies. However, further studies are

recommended to explore the purification and characterization of active compounds, elucidate the molecular mechanisms underlying antibiofilm activity, and evaluate their efficacy.

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Ethical approval

No endangered and/or protected species were involved in this study.

Author contribution

All authors contributed equally to this work. All authors contribute to writing, review, and editing of the manuscript.

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