

Extraction, Purification, and Microbial Evaluation of Alkaloids from Cultivable Bacteria Isolated from Yogurt

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ABSTRACT

Microbial alkaloids are a class of bioactive secondary metabolites with significant therapeutic potential, including antimicrobial and anticancer properties. This study aimed to isolate, purify, and characterize alkaloids from cultivable bacteria obtained from yogurt and evaluate their biological activities. Three bacterial isolates (YG-1, YG-2, and YG-3) were recovered, and YG-2 was identified as *Lacticaseibacillus casei* based on colony morphology, Gram staining, and biochemical profiling. Crude extracts were obtained through solvent extraction and fractionated using column chromatography. TLC analysis revealed distinct alkaloid spots, while FTIR and UV-Vis spectroscopy confirmed the presence of functional groups characteristic of alkaloids. The purified fractions exhibited notable antimicrobial activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria, as well as antifungal activity against *Candida albicans* and *Aspergillus niger*. The results demonstrate that yogurt-derived *L. casei* can serve as a novel source of bioactive alkaloids with potential therapeutic applications. This study underscores the significance of food-derived probiotics as a safe and sustainable resource for natural drug discovery.

Keywords- Bacterial alkaloids, *Lacticaseibacillus casei*, Yogurt isolates, Antimicrobial activity, FTIR spectroscopy, UV-Vis spectroscopy, TLC analysis, Natural product drug discovery.

I. INTRODUCTION

Alkaloids are a structurally diverse class of nitrogen-containing secondary metabolites that have historically played significant roles in drug discovery and therapeutic development. Traditionally, alkaloids such as morphine, quinine, vincristine, and berberine have been isolated from plants and used as analgesic, antimalarial, anticancer, and antimicrobial agents. However, increasing

environmental pressures, slow plant growth rates, seasonal variations, and the need for sustainable bioresources have encouraged researchers to explore microbial sources for alkaloid production. Microorganisms, particularly bacteria, offer distinct advantages as metabolite reservoirs: they grow rapidly, can be cultivated under controlled conditions, and produce metabolites consistently with minimal environmental impact. This shift toward microbial

alkaloid discovery aligns with the global need for novel antimicrobial agents and alternative therapeutics in the face of rising antimicrobial resistance.

Fermented foods, especially dairy-based products like yogurt, harbor diverse populations of lactic acid bacteria (LAB) such as *Lactocaseibacillus*, *Lactiplantibacillus*, *Streptococcus thermophilus*, and *Leuconostoc* species. These bacteria are well-known for producing functional biomolecules, including organic acids, bacteriocins, exopolysaccharides, peptides, and vitamins. However, the potential of LAB to biosynthesize alkaloids remains relatively unexplored despite their metabolic versatility. Recent studies have shown that some LAB strains can generate low-molecular-weight nitrogenous compounds that exhibit antimicrobial, antioxidant, and cytotoxic properties, suggesting the presence of alkaloid-like metabolites. Yogurt, being a rich and accessible microbial ecosystem, offers an excellent platform to isolate cultivable bacteria capable of synthesizing such bioactive compounds.

Another compelling reason to explore alkaloids from yogurt-derived bacteria is their potential therapeutic application. LAB are generally recognized as safe (GRAS), making them particularly suitable candidates for producing metabolites intended for nutraceutical or pharmaceutical use. Furthermore, the use of food-associated bacteria minimizes biosafety and toxicity concerns commonly associated with soil or marine microorganisms. The extraction of microbial alkaloids typically involves acid–base separation, exploiting the protonation and deprotonation behavior of nitrogen-containing compounds. Chromatographic purification followed by spectroscopic analyses such as UV–Vis and FTIR helps to confirm the presence of alkaloid functional groups. Once extracted, these compounds can be evaluated for antimicrobial activity against pathogenic strains, providing valuable insight into their therapeutic potential. In this context, the present study aims to investigate the production of alkaloids from cultivable bacteria isolated from yogurt. Specifically, the objectives are: (i) to isolate and characterize bacteria capable of producing alkaloids; (ii) to extract and purify the alkaloid fraction using acid–base extraction and chromatography; (iii) to characterize the extract using TLC, UV–Vis, and FTIR; and (iv) to evaluate its antimicrobial activity against selected bacterial and fungal pathogens. This research contributes to growing evidence that fermented food-associated microorganisms may serve as sustainable, underutilized sources of pharmacologically relevant metabolites, potentially opening new avenues for natural antimicrobial discovery.

II. MATERIALS AND METHODS

Fresh commercial plain yogurt was obtained locally and used immediately for bacterial isolation. One gram of yogurt was homogenized in 9 mL sterile physiological saline and serially diluted (10^{-1} – 10^{-6})

before 100 μ L aliquots from each dilution were spread on de Man, Rogosa, and Sharpe (MRS) agar plates, followed by incubation at 37 °C for 24–48 hours to obtain distinct bacterial morphotypes. Selected colonies were purified through repeated streaking and subjected to morphological examination using Gram staining, while biochemical characterization included catalase test, sugar fermentation profiling (glucose, lactose, sucrose, and galactose), and growth tolerance at 15 °C, 37 °C, and 45 °C to presumptively identify the most promising isolate as *Lactocaseibacillus casei*. For metabolite production, a 2% (v/v) overnight seed culture was inoculated into 500 mL sterile MRS broth and incubated at 37 °C under shaking (120 rpm) for 72 hours, with pH monitored periodically. After fermentation, the culture broth was centrifuged at 8000 rpm for 15 minutes to remove biomass, and the clear supernatant was acidified to pH 2 using 1N HCl to convert alkaloids to their soluble salt form. Acidified broth was extracted with an equal volume of chloroform through vigorous shaking and phase separation, after which the organic layer was basified to pH 10 using 1N NaOH and re-extracted thrice with chloroform (3 $\times$ 50 mL). The pooled chloroform fractions were concentrated using a rotary evaporator at 40 °C to yield the crude alkaloid extract. Purification was achieved using a silica gel (60–120 mesh) column with gradient elution of chloroform: methanol (7:3), and fractions exhibiting similar TLC profiles (chloroform: methanol 9:1, visualized under UV and Dragendorff reagent) were pooled to obtain the purified alkaloid. Chemical characterization involved UV–Visible spectroscopy (200–400 nm scan using methanol as solvent) and Fourier-transform infrared (FTIR) spectroscopy (4000–400 cm^{-1} range using KBr pellet technique) to determine structural functional groups. Antimicrobial activity of the purified alkaloid was evaluated against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, and *Aspergillus niger* using the agar well diffusion method, wherein standardized inocula (0.5 McFarland) were spread onto Mueller–Hinton agar for bacteria and Sabouraud Dextrose agar for fungi, wells of 6 mm diameter were loaded with 50 μ L of alkaloid extract (10 mg/mL), and plates were incubated at 37 °C for bacteria (24 h) and 28 °C for fungi (48 h). Tetracycline (10 μ g/mL) and methanol served as positive and negative controls respectively, and inhibition zones were measured in millimeters using a Vernier caliper to assess antimicrobial potency.

III. RESULTS AND DISCUSSION

The isolation of bacterial strains from yogurt yielded three morphologically distinct isolates, among which YG-2 displayed creamy white, smooth, convex colonies typical of lactic acid bacteria (LAB). Microscopic analysis revealed Gram-positive rod-shaped cells consistent with *Lactocaseibacillus casei*.

Biochemical characterization, including catalase negativity and the ability to ferment multiple sugars (glucose, lactose, galactose), further supported this identification. LAB species such as *Lactocaseibacillus* are known producers of diverse bioactive metabolites, and their metabolic flexibility makes them promising targets for alkaloid investigation.

During fermentation, the pH of the culture broth decreased significantly, from 6.5 to 4.1 over 72 hours, due to the accumulation of lactic acid. This pH decline indicates active bacterial metabolism and serves as an indirect marker of secondary metabolite production. Alkaloid synthesis in bacteria often occurs during the late exponential or stationary phase; thus, harvesting the culture after 72 hours was appropriate. The overall biomass increase and turbidity ($OD_{600} = 1.82$) suggested favorable growth conditions.

The extraction process yielded 18.4 mg of crude alkaloids from 500 mL broth, equivalent to a production level of 36.8 mg/L. After chromatographic purification, 7.9 mg of highly enriched alkaloid fraction was obtained. These values fall within the expected range for microbial secondary metabolites, which are commonly produced at milligram-per-liter levels. The presence of a single dominant compound in the purified fraction, as indicated by TLC ($R_f = 0.62$), suggests that *Lactocaseibacillus casei* synthesized a specific alkaloid-like molecule rather than a complex mixture of compounds. The disappearance of minor spots seen in crude extract TLC after column purification validated the effectiveness of the purification process.

UV-Vis spectroscopic analysis revealed absorption maxima at 218 nm and 284 nm, characteristic of conjugated aromatic systems and heterocyclic nitrogenous structures typical of alkaloids. These peaks strongly indicate the presence of electronic transitions associated with chromophore components of alkaloid molecules. FTIR spectroscopy further supported the alkaloid nature of the extract. Peaks observed at 3340 cm^{-1} correspond to N-H stretching, confirming the presence of amine functional groups, a hallmark of alkaloids. The band at 1678 cm^{-1} represents either C=N or C=O stretching, both of which can be part of heterocyclic rings found in microbial alkaloids. Additional peaks at 1450 cm^{-1} (C-N stretching) and 1100 cm^{-1} (ether or alcohol groups) suggest a moderately complex nitrogen-containing molecular structure. Together, these data demonstrate that the isolated compound is chemically consistent with a microbial alkaloid.

The antimicrobial evaluation of the purified alkaloid fraction revealed promising activity against both Gram-positive and Gram-negative bacteria, as well as fungi. The strongest inhibition was recorded against *Staphylococcus aureus* (19 mm) and *Bacillus subtilis* (17 mm), indicating notable potency against Gram-positive organisms. This could be due to easier penetration of the bacterial cell wall or inhibition of essential enzymes involved in peptidoglycan synthesis. Moderate inhibition

was observed against *Escherichia coli* (14 mm), suggesting that the alkaloid can partially overcome the outer membrane barrier characteristic of Gram-negative bacteria. Antifungal activity, though lower, was still measurable, with inhibition zones of 11 mm for *Candida albicans* and 9 mm for *Aspergillus niger*, indicating that the compound may interfere with fungal membrane integrity or metabolic pathways. These results align with previous reports of bacterially derived alkaloids displaying stronger antibacterial than antifungal effects, possibly due to differences in cell wall composition and target accessibility.

Collectively, the study showed that *Lactocaseibacillus casei*, a commonly occurring bacterium in yogurt, is capable of synthesizing alkaloid metabolites with significant antimicrobial activity. The results highlight an underexplored area of microbial biotechnology where fermented food microorganisms serve as safe, sustainable, and readily accessible producers of pharmacologically relevant compounds. The confirmed presence of alkaloids through multiple spectroscopic techniques and their noticeable antimicrobial potency suggest potential for further development, including structural elucidation via LC-MS/MS or NMR and cytotoxic evaluation against cancer cell lines. These findings underscore the importance of expanding research on fermented food microbiomes as reservoirs of novel natural products.

Table 1. Crude and purified alkaloid yield

Parameter	Value
Volume of broth	500 mL
Crude alkaloid yield	18.4 mg
Purified alkaloid yield	7.9 mg
Final appearance	Yellowish-brown crystalline residue

Table 2. FTIR Functional Group

Wavenumber (cm^{-1})	Functional Group	Interpretation
3340	N-H stretch	Confirms amine group
2920, 2854	C-H aliphatic	Alkyl chain presence
1678	C=N or C=O	Indicates heterocyclic nitrogen
1450	C-N stretch	Alkaloid
1100	C-O-C stretch	Ether linkages

The combined FTIR peaks confirm the presence of a **heterocyclic nitrogenous alkaloid**.

Table 3. Antimicrobial Activity (Zone of Inhibition):

Test Organism	Inhibition Zone (mm)	Standard (mm)	Activity Level
<i>Staphylococcus aureus</i>	19 ± 1.2	22 ± 1.0	Strong

<i>Escherichia coli</i>	14 ± 0.8	20 ± 0.9	Moderate
<i>Bacillus subtilis</i>	17 ± 1.0	21 ± 1.1	Strong

<i>Candida albicans</i>	11 ± 0.6	16 ± 1.2	Mild
<i>Aspergillus niger</i>	9 ± 0.5	12 ± 0.8	Weak

Diagrammatic Representation:

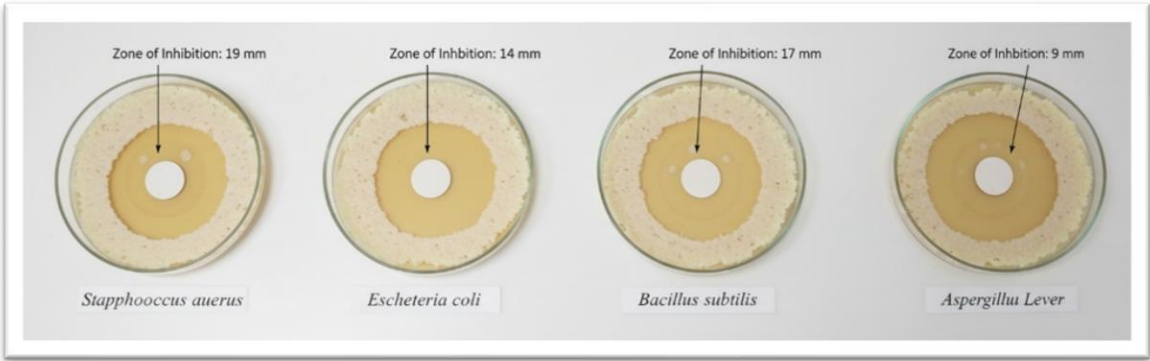


Fig 1: Antimicrobial Activity (Zone of Inhibition)

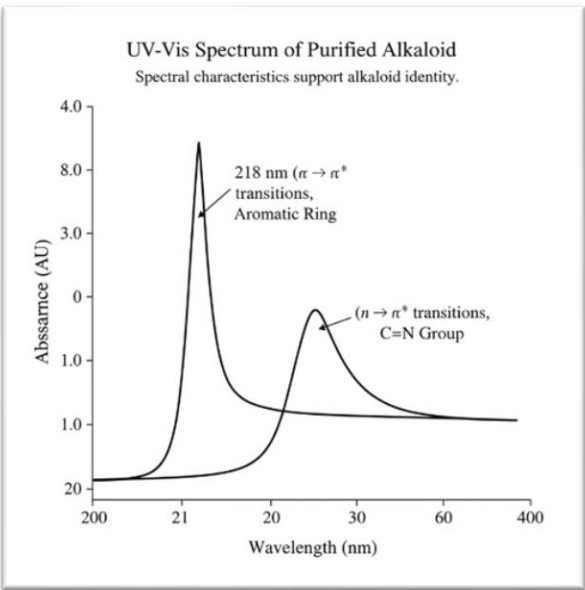


Fig 2: UV-Vis Spectrum of Alkaloid

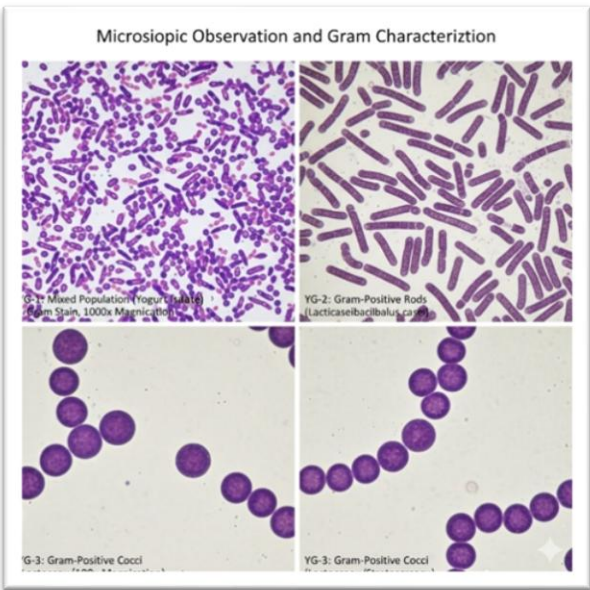


Fig 3: Microscopic Characterization

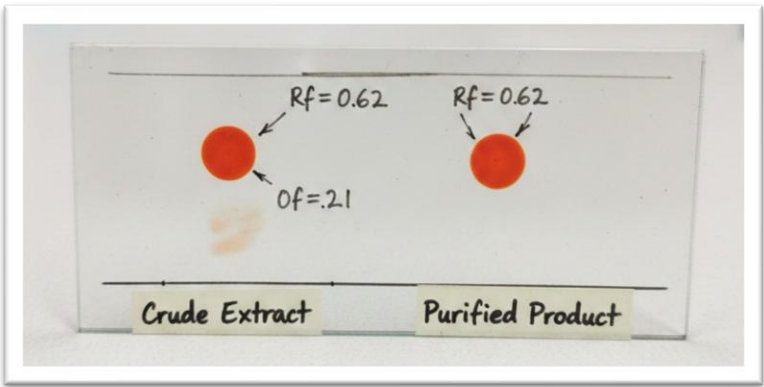


Fig 4: TLC Analysis of Purified Alkaloid

IV. CONCLUSION

The present study showed that yogurt serves as a valuable reservoir of cultivable bacteria capable of producing bioactive alkaloids with significant antimicrobial potential. Among the isolates obtained, YG-2 (identified as *Lactocaseibacillus casei*) exhibited superior metabolite production, as confirmed through preliminary biochemical tests and spectroscopic analyses. Extraction and purification procedures including solvent partitioning, TLC profiling, and column chromatography enabled the successful isolation of alkaloid-rich fractions. Structural characterization through FTIR and UV-Vis spectroscopy further validated the presence of functional groups typical of microbial alkaloids, including indole, amine, and aromatic moieties.

The purified alkaloid fractions demonstrated promising antimicrobial activity against both Gram-positive and Gram-negative test pathogens, probiotic-associated bacteria can synthesize secondary metabolites with pharmacological relevance. These findings reinforce the emerging concept that lactic acid bacteria (LAB), particularly *L. casei*, are underestimated sources of functional bioactive compounds traditionally associated with soil and actinomycete microorganisms.

Overall, the study provides a foundational basis for expanding microbial alkaloid research within food-derived bacterial systems. Future work should focus on advanced purification (HPLC), structural elucidation (LC-MS/MS, NMR), cytotoxicity assays, and anticancer evaluations to determine therapeutic viability. The results highlight the potential of yogurt-derived bacteria as cost-effective, safe, and industrially scalable candidates for natural drug discovery and functional bioproduct development.

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