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Modulation of Antimicrobial Activity in Probiotic Lactobacillus Strains by Biowavelength Laser Irradiation

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ABSTRACT: This study investigated the effect of diode laser irradiation on the antibacterial activity and metabolite profile of a local Lactobacillus isolate and evaluated its potential to modulate the production of bioactive antimicrobial metabolites. A local Lactobacillus isolate, designated Lactobacillus sp., was selected based on its inherent antibacterial activity against the tested pathogenic bacteria. De Man, Rogosa and Sharpe (MRS) broth adjusted to pH 5 was used for bacterial cultivation and antimicrobial metabolite production. Cultures were incubated at 40 °C for 60h under shaking conditions. The inoculum was exposed to diode laser irradiation at wavelengths of 532 and 650 nm for 1, 2, and 3 min, with a non-irradiated culture serving as the control. Each treatment was performed in five replicates. The antibacterial activity of the treated and control cultures was evaluated against Escherichia coli and Staphylococcus aureus using an agar-based antimicrobial assay. Furthermore, Gas Chromatography–Mass Spectrometry (GC–MS) was used to characterize the metabolite profiles and identify compounds detected following laser exposure. The results demonstrated that laser irradiation enhanced the antibacterial activity of Lactobacillus sp., with the response varying according to the irradiation wavelength and exposure conditions. Compared with the non-irradiated control, selected irradiation treatments increased the antibacterial activity, as evidenced by the greater inhibition of the tested bacterial pathogens. Furthermore, GC–MS analysis revealed qualitative and quantitative alterations in the detectable metabolite profile, including the appearance or increased abundance of several compounds with reported or potential antimicrobial properties. These findings suggest that laser irradiation may enhance the antibacterial potential of Lactobacillus sp. by modulating its metabolite production. These findings suggest that diode laser irradiation can influence the metabolic behavior of Lactobacillus sp and may serve as a physical stimulus for modulating the production of bioactive antimicrobial metabolites. Further studies are required to elucidate the underlying mechanisms and experimentally confirm the antimicrobial contribution of the individual compounds identified by GC–MS.

INTRODUCTION

Probiotic bacteria are widely recognized as beneficial microorganisms that contribute to the maintenance of host health and the stability of the gastrointestinal microbial ecosystem. Their beneficial effects are mediated through several complementary mechanisms, including modulation of the host immune response, enhancement of intestinal barrier function, regulation of the gut microbiota, and inhibition of potentially pathogenic microorganisms (Mahmood et al., 2026). Among these mechanisms, stimulation and modulation of the immune system represent important biological pathways through which probiotics may enhance host defense and contribute to protection against various gastrointestinal disorders. By interacting with intestinal epithelial cells and immune components, probiotic microorganisms can influence host responses and promote a physiological environment that limits the establishment and proliferation of harmful bacteria (Jadah & Al-Rubaye, 2025). In addition to their immunomodulatory effects, probiotic bacteria can suppress pathogenic microorganisms through several competitive and antagonistic mechanisms. These include competition for available nutrients, production of metabolites that modify the local physicochemical environment, and interference with the adhesion of pathogens to intestinal epithelial surfaces. The ability of probiotics to colonize or transiently associate with the intestinal mucosa may reduce the availability of attachment sites required by pathogenic bacteria, thereby limiting their establishment within the gastrointestinal tract (Ashaolu et al., 2025). Furthermore, many probiotic microorganisms produce a range of biologically active inhibitory substances, including organic acids, hydrogen peroxide, and bacteriocins. These compounds can create unfavorable conditions for competing microorganisms and contribute to the antimicrobial activity of probiotic strains against both Gram-positive and Gram-negative bacteria. Among the antimicrobial substances produced by lactic acid bacteria, bacteriocins have received considerable scientific attention because of their proteinaceous nature, selective antimicrobial activity, and potential applications in food and pharmaceutical systems (Wang et al., 2025). Several species belonging to the genera *Lactobacillus* and other lactic acid bacteria are capable of producing bacteriocin-like inhibitory substances. These antimicrobial peptides or proteins can act against susceptible microorganisms through different mechanisms, depending on their structure and mode of action. One of the best-characterized bacteriocins associated with lactic acid bacteria is nisin, which exhibits antimicrobial activity against a broad range of susceptible Gram-positive bacteria. Nisin interacts with specific components of the target-cell envelope, resulting in membrane permeabilization and disruption of essential cellular processes. Such interactions may lead to loss of membrane integrity, dissipation of the proton motive force, inhibition of cellular energy generation, and, ultimately, bacterial cell death (Darbandi et al., 2022). The antimicrobial properties of lactic acid bacteria and their bacteriocins have generated increasing interest in their use as natural and biologically derived alternatives to conventional chemical preservatives. Foodborne microorganisms remain an important concern for food quality and safety, particularly because microbial contamination can lead to food spoilage and the transmission of foodborne diseases (Zhang et al., 2026). Consequently, there is growing interest in preservation strategies that can effectively inhibit undesirable microorganisms while maintaining the sensory and nutritional characteristics of food products. Antimicrobial metabolites produced by lactic acid bacteria may provide an additional or complementary preservation strategy because they can act directly against target microorganisms and may be produced naturally during fermentation. The application of probiotic and lactic acid bacteria in food preservation is therefore associated with two important objectives: controlling the growth of undesirable microorganisms and maintaining the microbiological safety and quality of food products throughout storage. Their ability to produce organic acids, bacteriocins, and other antimicrobial metabolites makes them promising candidates for incorporation into natural biopreservation systems (Zapašnik et al., 2022). However, the effectiveness of such microorganisms can vary depending on the bacterial strain, food matrix, target microorganism, storage conditions, and concentration of the antimicrobial compounds produced. Therefore, strain-specific evaluation is essential to determine the practical suitability of individual probiotic or lactic acid bacterial isolates for food preservation applications. In this context, The present study aimed to evaluate the antimicrobial potential of a local *Lactobacillus* sp. isolate and investigate whether diode laser irradiation could enhance its antibacterial activity and alter its extracellular metabolite profile. (Uhegwu & Anumudu, 2025). Particular attention is given to the ability of the bacterial strain to inhibit undesirable microorganisms and to its potential as a biological preservation agent. The findings may contribute to the development of safer and more sustainable food preservation approaches based on naturally occurring antimicrobial microorganisms and their metabolites, providing an alternative or complementary strategy to the use of conventional chemical preservatives (Parada Fabián et al., 2025).

MATERIALS AND METHODS

Microorganisms

The *Lactobacillus* isolate used in this study was obtained from the College of Science, Al-Nahrain University. The isolate was purified and subjected to preliminary characterization using standard biochemical tests, followed by molecular identification based on 16S rRNA gene sequencing. *Staphylococcus aureus* and *Escherichia coli* were included in the experimental design as representative Gram-positive and Gram-negative bacterial strains, respectively, for the assessment of the antibacterial activity of the *Lactobacillus* crude extract, following the approach described by Meradji et al. (2023).

Optimal Conditions for the Production of antibacterial

Optimal Culture Media

M17broth, Brain Heart Infusion (BHI) broth, Nutrient Broth (NB), and de Man–Rogosa–Sharpe (MRS) broth were comparatively evaluated to determine the most suitable culture medium for antibacterial production by the *Lactobacillus* isolate. Briefly, 30 mL of each sterile medium was inoculated with 5 mL of an actively growing, previously activated *Lactobacillus* culture and incubated overnight under the appropriate growth conditions. Following incubation, the cultures were centrifuged at 15,000 rpm for 10 min to remove bacterial cells and cellular debris. The resulting supernatants were carefully collected and passed through sterile 0.22- μ m Millipore membrane filters to obtain cell-free culture supernatants (CFCs), which were subsequently used for the assessment of antibacterial production (Zhang et al., 2020; Sakoui et al., 2024).

pH Value

Aliquots of 30 mL of MRS broth were prepared in separate sterile tubes and adjusted to five different pH levels (3, 4, 5, 6, and 7). Each pH-adjusted medium was subsequently inoculated with 5 mL of a freshly prepared bacterial culture under aseptic conditions (Jadah & Al-Rubaye, 2025).

Temperature

An activated culture of the *Lactobacillus* isolate (5 mL) was used to inoculate flask (100 ml) containing 30 mL of MRS broth. The inoculated cultures were divided into five groups and incubated for 68 h under controlled temperature conditions of 30, 35, 40, 45, and 50°C. Following incubation, the cultures were evaluated to determine the effect of temperature on the growth and/or antimicrobial activity of the *Lactobacillus* isolate (Agbankpe et al., 2019).

Period of Incubation

To determine the optimal incubation period, five culture flasks containing 30 mL of MRS broth were prepared, inoculated, and statically incubated under controlled conditions at 40°C and pH 5.0. To assess the effect of duration on growth, the cultures were sampled across five discrete time intervals: 12, 24, 36, 48, and 60 hours (Gökmen et al., 2024).

Irradiation procedure

In this experiment, two diode laser devices (JD-R303, HUONJETTM, China), operating at wavelengths of 650 and 532 nm, were employed, respectively, while the remaining laser parameters were kept constant as indicated in Table 1. The laser irradiation setup and exposure procedure were conducted following the methodology previously reported by Flórez et al. (2023). Briefly, a fresh culture of the *Lactobacillus* isolate was incubated at 40 °C for 60 h under aseptic conditions. The resulting bacterial culture was subsequently diluted to a final concentration of 10³ cells/mL to provide a standardized and uniform bacterial suspension for laser treatment. The prepared bacterial suspension was randomly allocated into seven experimental groups, with five independent replicates for each group. The experimental design consisted of one non-irradiated control group and six laser-treated groups, comprising three groups irradiated with a 650 nm diode laser and three groups irradiated with a 532 nm diode laser. For each treatment, 1 mL of the standardized bacterial suspension (10³ cells/mL) was subjected to laser irradiation at a power output of 100 mW under dark-room conditions, using the exposure conditions specified in (Table 1). Immediately after irradiation, the treated suspension was transferred into 100-mL Erlenmeyer flasks containing 20 mL of the production medium. The inoculated cultures were subsequently incubated in a shaking incubator at 30 °C and 150 rpm for 3 days. After the incubation period, the cultures were collected and subjected to subsequent analyses to assess the effect of diode laser irradiation on the antimicrobial activity of the *Lactobacillus* isolate (Jadah & Al-Rubaye, 2025; Wang et al., 2025).

Crude extraction

After 60 hours of dynamic incubation, 15 mL of the bacterial broth culture was collected and subjected to centrifugation at 15,000 rpm for 10 min to yield a cell-free supernatant. The recovered supernatant was partitioned into two portions: one portion was employed to assess antibacterial activity using the agar well diffusion method, whereas the remaining 15 mL portion was freeze-dried via a bench-top lyophilizer (Model LYO60B-1PT, Infitek Co.) to produce a powder extract designated for GC–MS characterization (Mahmood et al., 2026).

Antibacterial sensitivity test

Mueller–Hinton agar plates were prepared and separately inoculated with the test pathogens *Staphylococcus aureus* and *Escherichia coli*. Bacterial suspensions were first prepared from fresh cultures and adjusted to the turbidity of a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. The suspensions were then serially diluted with sterile physiological saline to obtain a final bacterial concentration of 10^3 cells/mL. The concentration was confirmed by standard plate-counting procedures. For each assay, 100 μ L of the adjusted bacterial suspension (10^3 cells/mL) was uniformly spread over the surface of the Mueller–Hinton agar using sterile cotton swabs to ensure homogeneous inoculation. Subsequently, wells measuring 6 mm in diameter were aseptically prepared in the agar using sterile micropipette tips. An aliquot of 100 μ L of the crude culture supernatant was carefully dispensed into each well. The inoculated plates were incubated at 37 °C for 18 h, after which the diameters of the resulting inhibition zones were measured in millimeters to evaluate the antimicrobial activity of the crude supernatant against the tested bacterial pathogens (Lemos et al., 2010; Al Aboghaubar et al., 2025).

Table 1. Irradiation device specifications

Parameter	Value
Laser wavelength	532 nm and 650 nm
Laser output power	100 mW
Operation mode	Continuous wave (CW)
Exposure duration	1 min, 2 min, 3 min
Beam diameter	0.7 mm
Target sample	Bacterial suspension culture

STATISTICAL ANALYSIS

The results are presented as mean inhibition zone diameters (cm) measured around the wells against the pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. Statistical analyses were performed using GraphPad Prism software, version 10.5.0. Differences among the control and laser-irradiated groups were evaluated using one-way analysis of variance (ANOVA), with statistical significance set at $p < 0.05$. In addition, an independent-samples t-test was used to compare the mean inhibition zone diameters between irradiated and non-irradiated groups to determine the effect of laser irradiation on the antibacterial activity of the *Lactobacillus* isolate. The treatment producing the highest mean inhibition zone was considered to represent the maximum antibacterial activity under the experimental conditions (Marianelli et al., 2025).

GC-MS ANALYSIS

The lyophilized crude supernatants were subjected to Gas Chromatography–Mass Spectrometry (GC–MS) analysis using a 7820A GC System (Agilent Technologies). The resulting mass spectra were compared with reference spectra available in the National Institute of Standards and Technology (NIST) database for the identification of the detected compounds. The detected compounds were tentatively identified by comparing their mass spectra with those available in the NIST library. The operating conditions and analytical parameters of the GC–MS system used for compound identification are presented in (Table 2) (Swanson-Mungerson et al., 2019).

RESULTS

Optimal Culture Media

The results (Figure 1) showed that the highest antibacterial production was 1.08 mg/mL using MRS broth, while lower amounts were obtained with M17 broth, trypton soya broth TSB, and nutrient broth (0.62, 0.38, and 0.46 mg/mL, respectively). This may be due to the presence of Tween 80 and potassium phosphate, as well as the rich composition of MRS in glucose, amino acids, and vitamins, which enhance cell growth and antibacterial synthesis.

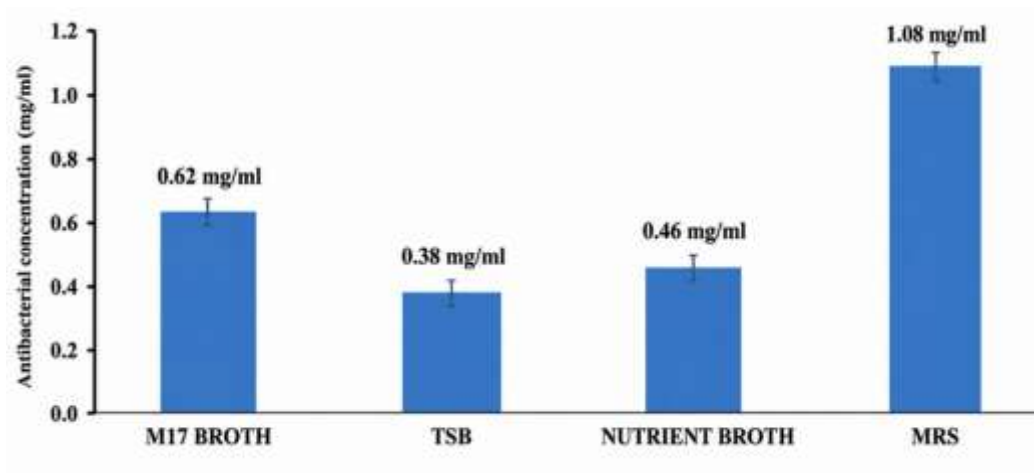


Figure 1. The best medium in the production of antibacterial

PH Value

The results showed that the highest antibacterial production occurred at pH 5, while the lowest production was observed at pH 3 (Figure 2). This indicates that pH significantly affects antibacterial synthesis, likely by influencing the ionic environment and the cell envelope, with optimal pH improving production.

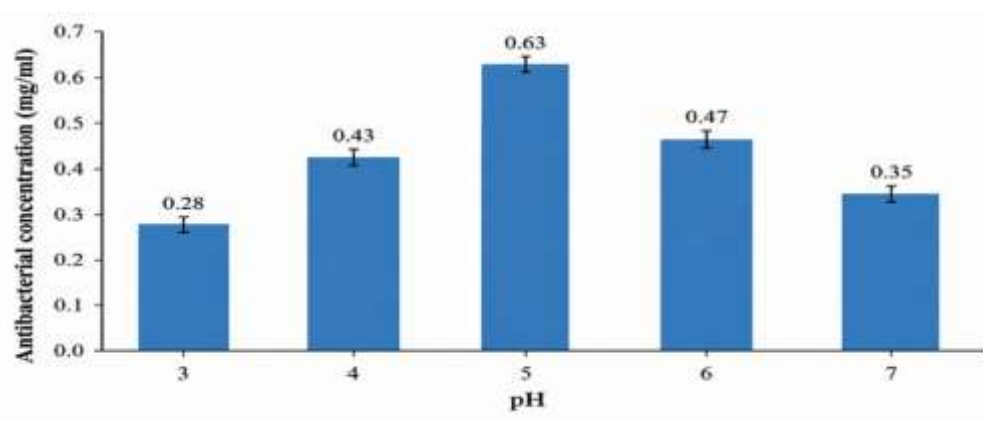


Figure 2. The optimal pH in the production of antibacterial

TEMPERATURE

The effect of incubation temperature on the yield of antibacterial bacteriocin produced by the *Lactobacillus* isolate in MRS medium (pH 5.0) is presented in Figure 3. The highest production was observed at 40 °C, reaching 1.25 mg/mL. Deviating from this optimal temperature resulted in a gradual decrease in yield, recording 0.95 mg/mL at 35 °C, 0.85 mg/mL at 45 °C, 0.65 mg/mL at 30 °C, and 0.40 mg/mL at 50 °C. These results indicate that 40 °C provides the optimal physiological environment for maintaining the enzymatic and metabolic activity required for bacteriocin biosynthesis. In contrast, higher temperatures appear to suppress production, likely due to thermal inactivation of the involved biosynthetic enzymes or reduced

bacterial growth.

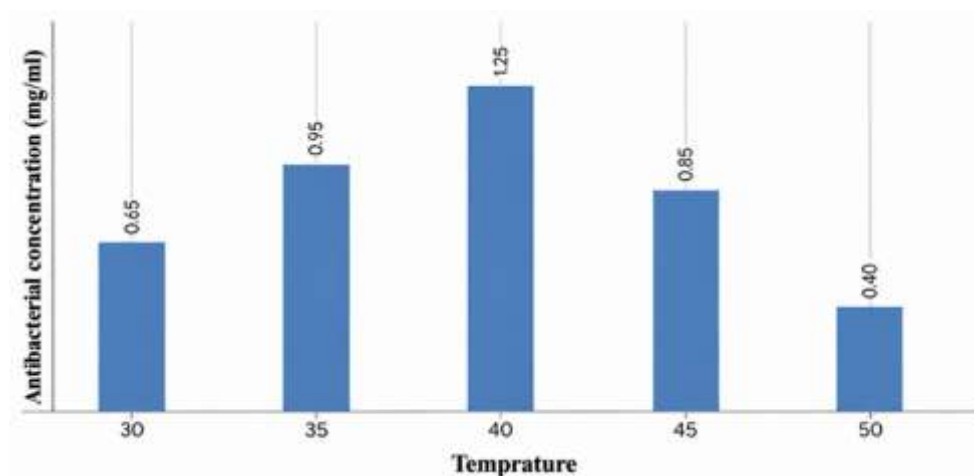


Figure 3. The optimum temperature in the production of antibacterial

INCUBATION PERIOD FOR HIGHER PRODUCTION OF ANTIBACTERIAL

The effect of incubation time on antibacterial production was evaluated under anaerobic conditions using MRS broth at pH 5.0 and 40 °C. As illustrated in Figure 4, antibacterial yield exhibited a time-dependent increase, reaching a maximum concentration of 1.09 mg/mL at 60 hours. Lower yields were observed at shorter incubation intervals, recording 0.11, 0.29, 0.47, and 0.89 mg/mL at 12, 24, 36, and 48 hours, respectively. This progressive accumulation indicates that antibacterial biosynthesis is strongly linked to the late-logarithmic and early-stationary growth phases, where metabolic activity and energy status favor antibacterial secretion. However, extended incubation beyond the optimal duration may cause a yield decline due to nutrient exhaustion or enzymatic degradation by endogenous proteases.

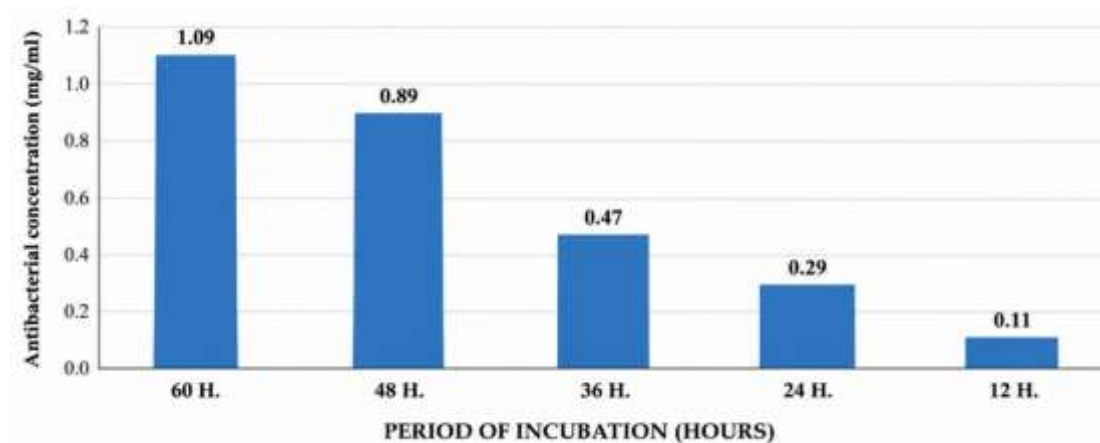


Figure 4. Optimal incubation period in the production of antibacterial

Antibacterial potential of the isolate

The *Lactobacillus* isolate exhibited smaller inhibition zones against the tested pathogenic bacteria before laser irradiation. After exposure to laser light, the colonies in the MRS medium showed a lighter pink color compared to the non-irradiated control. The antimicrobial potential of the *Lactobacillus* isolate against *Staphylococcus aureus* and *Escherichia coli* was evaluated using the well diffusion method, and the inhibition zone diameters were analyzed using GraphPad Prism, as illustrated in Figure 6. As shown in Figure 6, laser irradiation significantly enhanced the antibacterial activity of the *Lactobacillus* isolate compared with the non-irradiated control. The optimal antibacterial response was observed at a wavelength of 532 nm, where the inhibitory effect against *S. aureus* reached approximately 89%, while the maximum inhibition against *E. coli* was recorded at 650 nm, approaching

93%. These results suggest that specific laser wavelengths can effectively stimulate the antibacterial capability of *Lactobacillus*, improving its inhibitory efficiency against both Gram-positive and Gram-negative bacteria.

GC–MS

To identify the antibacterial compounds produced by *Lacto* strains before and after laser irradiation that inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*, gas chromatography–mass spectrometry (GC–MS) analysis was performed. Based on comparison with the NIST library, the GC–MS chromatographic peaks are presented in Figs. 5 and 6. The relative area percentages of the detected peaks enabled a semi quantitative estimation of metabolite abundance, as summarized in Tables 3 and 4. The chromatogram shown in Fig. 5 illustrates the presence of individual biomolecules in the crude extract. GC–MS analysis revealed a total of fifteen biomolecules in the crude extract, which are listed in Table 3. The most abundant compounds were identified as fatty acids. As shown in Table 3, the lyophilized powder obtained from the culture supernatant of *Lacto* contains several important metabolites that are widely utilized in the production of valuable pharmaceutical compounds. The primary objective of this study was to evaluate the effect of laser irradiation after 60h of incubation on the metabolic profile of *Lacto* by analyzing extracellular metabolites. Additionally, future studies should investigate whether the observed effects are associated with transcriptional or metabolic changes induced by laser exposure. Notably, this study reported the formation of novel metabolites, including Reuterin (3-hydroxypropionaldehyde) derivatives. The results demonstrated a complete transformation in the biological activity of the originally non-antibacterial *Lacto* strain following laser irradiation at a specific wavelength, leading to the acquisition of pronounced antibacterial activity.

Table 2. Instrument conditions of GC-MS analysis.

No.	Parameter	descriptions
1	Gas Chromatograph	Agilent Technologies(7820A)
2	GC Mass Spectrometer	(5977E) USA
3	Analytical Column	Agilent HP-5ms Ultra Inert (30 m length x 250 µm inner diameter x 0.25 µm film thickness)
4	Injection volume	1µl
5	Pressure	11.933 psi
6	GC Inlet Line Temperature	250 °C
7	Aux heateres Temperature	300 °C
8	Carrier Gas	He 99.99%
9	Injector Temperature	250 °C
10	Scan Range	m/z 25-1000
11	Injection Type	Splitless
12	Oven Program	Temperature Ramp 1 60 °C hold to 3 min. Ramp 2 60 °C to 180 °C 7 °C/min Ramp 3 180 °C to 280 °C 8 °C/min Ramp 4 280 °C hold to 5 min.

Laser irradiation at a wavelength of 650 nm for 2 minutes induced a pronounced bactericidal effect in the treated *Lactobacillus* isolate. To identify the biomolecules produced following this treatment, GC–MS analysis was conducted, with the results

detailed in Table 4 and Fig. 6. A significant shift in the spectral profile was observed: the non-irradiated strain, characterized by a light pink coloration, displayed a chromatographic pattern in the 600–1200 s^{-1} range, whereas the irradiated culture shifted to the 2200–2800 s^{-1} range and developed a distinct dark brown pigmentation. As indicated in Table 4, the irradiation triggered the synthesis of diverse biologically active compounds, including Reuterin (3-hydroxypropionaldehyde) derivatives, hormones and neurotransmitters such as epinephrine and norepinephrine derivatives, vitamin B6 precursors, toxins, hydrogen bond-donating compounds, antihypertensive agents, and various other bioactive metabolites.

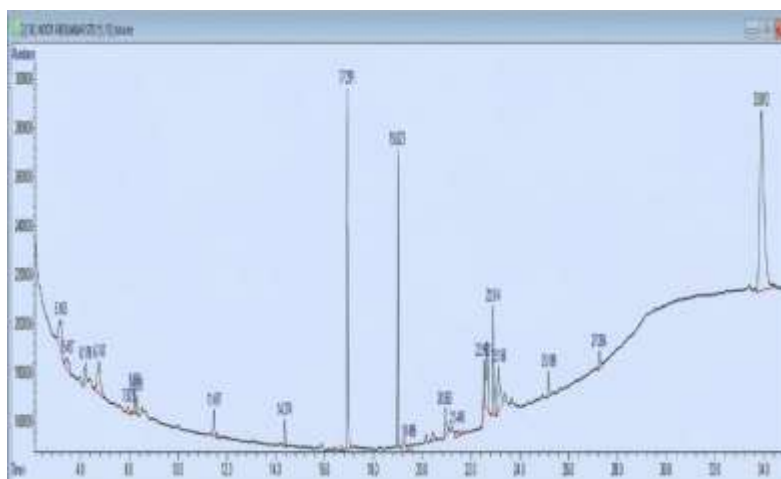


Figure 5. GC–MS Profile of Secondary Metabolites in Lacto (Control).



Figure 6. Curve of secondary metabolites produced by *Lactobacillus* sp. (irr. 650 nm, 2 min) obtained from GC–MS software.

DISCUSSION

The local *Lactobacillus* isolate investigated in this study exhibited minimal antibacterial activity compared with another *Lactobacillus* isolate showing strong antibacterial performance. Specifically, the local isolate produced a narrow inhibition zone of less than 7 mm in sensitivity assays against *Staphylococcus aureus* and showed no inhibitory effect against *Escherichia coli* (Soltani et al., 2022). GC-MS analysis did not detect the specific compounds reported in (Table 3) as antimicrobial metabolites under the analytical conditions used. During the preliminary screening, the *Lactobacillus* isolate with pronounced antibacterial potential demonstrated only a slight enhancement in activity following laser irradiation. In contrast, the local isolate, which initially exhibited weak inhibitory properties, showed a remarkable increase in antimicrobial compound production after laser exposure (Sehim et al., 2023). Notably, cultures irradiated at a wavelength of 650 nm displayed a significant enhancement in antibacterial activity. Similarly, irradiation at 532 nm also resulted in increased activity, although to a lesser extent than that observed with red-light irradiation. Overall, the induction of biological and metabolic alterations depends on the absorption of

laser photon energy by specific intracellular biomolecules, including cytochrome c oxidase, acetyl carrier protein, and acetyl-CoA. The precursors required for fatty acid biosynthesis originate from the intracellular acetyl-CoA pool. Laser energy absorption stimulates a series of photochemical reactions and modulates the intracellular redox state of bacterial cells, thereby activating metabolic pathways involved in the biosynthesis of antimicrobial compounds (Leanse et al., 2022); Jadah et al. 2022).

Table 3. The molecular components of extracellular crude powder of non-irradiated *Lactobacillus* isolate.

No.	Compound Name	Chemical Class	Antibacterial Significance	Area %
1	Phenyllactic acid (3-PLA)	Aromatic hydroxy acid / Phenylpropanoid-related organic acid	Reported antimicrobial	21.7
2	Caproic acid (Hexanoic acid)	Medium-chain fatty acid (MCFA)	Inhibits Gram+ and Gram- bacteria	18.2
3	3-Phenyllactic acid	3-PLA	Reported antimicrobial activity	16.4
4	Stearic acid	Saturated fatty acid	Bacterial growth inhibition	14.2
5	Lactic acid	Organic acid	Suppresses pathogenic bacteria	12.8
6	Methyl myristate	Fatty acid ester	Antimicrobial lipid compound	8.9
7	Acetoin (3-hydroxy-2-butanone)	Ketone	Antimicrobial fermentation metabolite	7.4
8	Butyric acid	Short-chain fatty acid	Disrupts bacterial membranes	5.1
9	Benzyl alcohol	Aromatic alcohol	Reported antimicrobial agent	4.9
10	Butyl acetate	Ester	Reported Antibacterial volatile compound	3.6
11	Methyl laurate	Fatty acid ester	Reported antibacterial activity	3.2
12	Citronellyl acetate	Terpene ester	Effective against pathogenic bacteria	2.7
13	Hexanal	Aldehyde	Reported Antibacterial action	1.9
14	Vanillin	Phenolic compound	Reported Natural antimicrobial agent	1.8
15	1-Butanol	Alcohol	Reported Inhibits bacterial metabolism	1.5

Electron excitation induces the formation of covalent bonds accompanied by the generation of reactive oxygen species (ROS).

These ROS can directly damage bacterial enzymes, promote protein denaturation within specific cellular components, and stimulate the synthesis of antioxidant defense systems, including oxidoreductases, in order to protect RNA from extensive oxidative damage (Juan et al., 2021). According to the findings of the present study, irradiation of the local *Lactobacillus* isolate at a wavelength of 650 nm resulted in the production of a higher number of antibacterial compounds compared with irradiation at 532 nm. The cellular response observed at 532 nm may be attributed to the presence of various intracellular chromophores, particularly haem-containing molecules such as protoporphyrin IX, which exhibit strong absorption of green light. This absorption mainly leads to photosignal transduction and amplification processes that may persist for up to 10 days following incubation of the bacterial culture (Bazukyan et al., 2022). In contrast, several studies have reported that red and infrared laser wavelengths are predominantly absorbed by cytochrome c oxidase. Moreover, water molecules within the cytoplasm may participate in laser-induced redox reactions, resulting in the generation of ROS such as H_2O_2 , $OH^\cdot$, and NO through photodissociation processes associated with cytochrome c oxidase activity. In the present investigation, the local *Lactobacillus* isolate initiated the production of Reuterin (3-hydroxypropionaldehyde) only after exposure to 650 nm irradiation; this compound was neither detected by GC–MS analysis nor associated with antibacterial activity in sensitivity assays prior to laser treatment (Tafur and Mills 2008).

Table 4. The molecular components of extracellular crude powder of irradiated *Lactobacillus* sp.

No .	Compound Name	Chemical Class	Antibacterial Bioactive Role	Area % (typical)	Change after Laser
1	Phenyllactic acid (3-PLA)	Aromatic hydroxy acid / Phenylpropanoid-related organic acid	Reported antimicrobial	22.1	↑
2	Caproic acid (Hexanoic acid)	Medium-chain fatty acid (MCFA)	Inhibits Gram+ and Gram– bacteria	18.7	↑
3	3-Phenyllactic acid	3-PLA	Reported antibacterial activity	17.2	↑
4	Stearic acid	Saturated fatty acid	Inhibits bacterial growth	15.2	↑
5	Lactic acid	Organic acid	Lowers pH; suppresses pathogens	13.4	↑
6	Butyric acid	Short-chain fatty acid	Antibacterial and anti-inflammatory	9.1	↑
7	Acetoin (3-hydroxy-2-butanone)	Ketone	Bioactive fermentation metabolite	8.2	↑
8	Reuterin	3-hydroxypropionaldehyde	Antibacterial flavor compound	6.7	↑
9	Benzyl alcohol	Aromatic alcohol	Broad-spectrum antibacterial agent	5.1	↑
10	Hexanal	Aldehyde	Antibacterial via lipid oxidation	4.4	↑
11	Butyl acetate	Ester	Antibacterial volatile compound	3.9	↑

12	Citronellyl acetate	Terpene ester	Antimicrobial compound	3.2	↑
13	Vanillin	Phenolic compound	Natural antimicrobial and antioxidant	2.2	↑
14	1-Butanol	Alcohol	Inhibits bacterial metabolism	1.9	↑
15	Methyl myristate	Medium-chain fatty acid ester	Antibacterial lipid metabolite	1.7	↑

The alterations in the extracellular components of the culture supernatant may contribute to a better understanding of how laser irradiation stimulates the biosynthesis of Reuterin (3-hydroxypropionaldehyde) and potentially its metabolic precursors. Figure 2 illustrates the compounds identified by GC–MS through comparison with spectra from the NIST database for the control *Lactobacillus* isolate, Figure 3 presents the GC–MS profile of the *Lactobacillus* isolate culture irradiated at a wavelength of 650 nm for 2 min (Li et al., 2025).

As shown in Figs. 5 and 6, the chromatograms of the control and irradiated *Lactobacillus* samples displayed a comparable number of peaks, representing the biomolecular compounds detected in the fermentation broth supernatant. However, several changes in peak retention times and intensities were observed following laser irradiation. For example, the first peak appeared at a retention time of 5.137 min in the control sample (Fig. 5), whereas it was detected at 5.163 min in the irradiated sample (Fig. 6). These variations indicate that laser irradiation influenced the metabolic profile of the *Lactobacillus* isolate, resulting in changes in the relative abundance and/or chromatographic behavior of some detected metabolites. To further investigate the effect of laser irradiation on metabolite production, the presence and relative abundance of reuterin (3-hydroxypropionaldehyde) and its derivatives before and after irradiation were specifically examined, as summarized in Table 4 (Ianni et al., 2021).

Reuterin (3-hydroxypropionaldehyde) is a well-known antimicrobial metabolite biosynthesized by the *Lactobacillus* isolate through glycerol metabolism and is closely linked to fatty acid–related metabolic pathways. Reuterin belongs to a class of low-molecular-weight bioactive compounds that exhibit broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. Laser irradiation induces oxidative stress within the bacterial cells, leading to metabolic rearrangements that enhance fatty acid turnover and related biosynthetic pathways. These stress-mediated photochemical reactions may indirectly affect DNA integrity through the formation of photoproducts, such as cyclobutane–pyrimidine dimers and pyrimidine-(6–4)-pyrimidone lesions. Such cellular responses can activate secondary metabolic routes, ultimately promoting the formation of reuterin and its precursors. This proposed mechanism is supported by the increased abundance of cyclobutane-related compounds and fatty acid derivatives detected in the culture supernatants of laser-irradiated *Lactobacillus* isolate samples (Table 4), suggesting that laser-induced oxidative stress plays a key role in stimulating antimicrobial metabolite biosynthesis (Schaefer et al., 2010; Ju et al., 2021).

CONCLUSION

Laser irradiation was associated with the detection of a compound tentatively identified as reuterin (3-hydroxypropionaldehyde). This finding highlights laser light as a promising, eco-friendly approach for activating antimicrobial metabolite production in bacteria. Reuterin exhibits broad-spectrum antibacterial potential, supporting its relevance in antimicrobial drug development. The enhanced antibacterial activity observed after irradiation underscores the importance of optimizing laser wavelength and exposure time. Further studies are required to elucidate the molecular mechanisms and assess the safety and stability of the induced metabolite..

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