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Evaluation of how laser photostimulation at two wavelengths alters the antimicrobial potential of *Streptomycetes* spp

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The purpose of this study was to isolate and identify additional products produced by direct laser irradiation, as well as to ascertain if laser irradiation may stimulate the synthesis of antibiotic compounds in a local *Streptomycetes* (*Strept*). Moreover, we postulate the mechanisms by which lasers function within living bacterial cells and suggest that sequential photochemical reactions may transpire following a designated period of irradiation. Thiophene was found as one of the most significant clinical products with antibacterial and anticancer properties. To accomplish these objectives, we selected two isolates: *Streptomyces thinghirensis* strain S10 (*Strept thin*), which inherently synthesizes an antibacterial agent, and *Streptomyces lienomycini* strain C.P.57 (*Strept. lieno*), which does not generate antimicrobials. The experimental isolates were exposed to identical circumstances as the control isolates, with the exception that the inoculum underwent irradiation with a diode laser for varying durations. We initially assessed the antibacterial efficacy of the irradiation and control *Strept*. Gas chromatography-mass spectrometry (GC-MS) was utilized to discover antibacterial substances. Ultimately, we determined that laser irradiation caused alterations in both antimicrobial-producing and non-producing Strept.; specifically, those that produced antimicrobials ceased to do so post-irradiation, and conversely.

Keywords *Streptomycetes*, Laser, Thiophene, Gas-chromatography, Antibacterial, Antitumor

Streptomyces are Gram-positive filamentous bacteria that are found naturally in organic soil. These bacteria are among the most important bacteria in clinical drug manufacturing, as they are considered a major source of a vast range of antibiotics, and anticancer reagents[1]. The ability of bacteria to produce certain types of products depends on many factors, such as the environment of the isolate, growth conditions, species or genus and the physiological and metabolic status[2]. Therefore, many studies have focused on identifying the optimal conditions to induce the production of desired products in *Strept*. For example, in a study by Al-Rubaye et al. in 2020, different types of antibiotics and bioactive compounds produced by several *Strept* isolates obtained from the Tigris River in Baghdad, were identified by gass chromatoghrphy(GC)[3,4]. Many studies have focused on modulating the secondary metabolites of *Strept* by using fermentation media with limited organic nitrogen contents[5–7]. Furthermore, many scientific publications confirm that the types of secondary metabolites present depend mainly on the particular species of *Strept* owing to the different cell wall structure, and certain genetic modifications found within this genus, which is the largest recognized taxonomic item in the phylum *Actinomycetes*[8].

Among the most significant bioactives compounds produced by certain of *Strept* are thiophenes, which are heterocyclic metabolites commonly produced by both plant species belonging to the Asteraceae family and filamentous *Strept* bacteria[9]. These metabolites have considerable bioactivities, such as antimicrobial, antiviral, anti-inflammatory, antioxidant, insecticidal, and antitumor properties[9,10]. The chemical name of thiophene is thiacyclopentadiene, with a formula of C_4H_4S, where the sulfur atom in the aromatic rings has a significant role in medical chemistry, and can form bonds with different atoms, such as carbon, nitrogen, oxygen, phosphorus

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and halogens. Therefore, sulfur-based functionalities have become important pharmacophores in drug discovery for the design and synthesis of new derivatives and analogues[11].

Numerous experiments have investigated, the production of thiophene derivatives from *Strept* via precursor-based combinatorial biosynthesis. Recently, a thiophene precursor was added to *Strept* fermentation broth, and the product was identified via nuclear magnetic resonance and mass spectrometry[12]. In this study, we aimed to determine if laser irradiation could stimulate the biosynthesis of thiophene in a local *Strept* isolate.

Methods

Microorganisms

The *Strept* isolates were obtained from the Department of Biotechnology/ College of Science at the University of Baghdad[13]. The Isolates were selected, purified and identified biochemically. Both the *Streptomyces lienomycini* strain C.P.57 (*Strept. lieno*) and *Streptomyces thinghirensis* strain S10 (*Strept thin*) were identified at the species level using 16 S rRNA gene sequencing.

In this study we used *S. aureus* and *E. coli* as model Gram-positive and gram-negative bacteria, respectively, which were subjected to antibacterial sensitivity tests with the *Strept* crude extracts.

Media and broth

In this study we used casein starch media as a solid medium for slant storage and *Strept* cultivation prior to colony counting. Casein starch broth, which consisted of 10gm of starch, 3 g of casein, 0.02 g of $CaCO_3$, 0.01 g of $Fe_3SO_4.7H_2O$, 2 g of KNO_3, 0.05 g of $MgSO_4.7H_2O$, 2 g of NaCl in 100 ml distilled water, was used in the antimicrobial activity assays. Sterile broth inoculated with a full loop of *Strept* was inoculated in 20 ml of production broth at pH 7.2 in a 100 ml conical flask, and incubated at 30° C and with shaking at 150 rpm for 10 days [13–15].

Soybean casein digest broth (HIMEDIA) was used to activate the pathogenic bacteria via 18–24 h of incubation at 37° C.

Irradiation procedure

In this experiment, we used two devices equipped with diode lasers (JD-R303, HUONJE 114 TM/ China) one laser emitted at a wavelength of 650 nm and the other at a wavelength of 532 nm. The other parameters of the two devices were the same, as shown in Table 1 :

The laser apparatus setup and irradiation procedure were described previously (Jadah 2022)[16].A fresh culture of *Strept* was incubated for 10 days at 30° C in a rotating incubator; under sterile conditions. Each sample was diluted to 10^3 cells/ml before irradiation, in the irradiated and nonirradiated (control) groups to ensure that all the cells and spores received an equal dose of laser irradiation.

The samples were divided into 7 groups, with 5 replicates in each group, as follows:

1. Non-irradiated group(control) (5 flasks).
2. Three groups of *Strept* irradiated at 650 nm three times (15 flasks).
3. Three groups of *Strept* at 532 nm three times (15 flasks).

Laser irradiation of 1 ml of cell suspension (10^3 cells/ml), was performed in a dark room at a power of 100 mW at 650 nm for 1 min (for example), and then immediately inoculated into a 100 ml flask containing 20 ml of production broth. The inoculated flasks were transferred immediately to a shaker incubator, for incubation at temperature 30° C and 150 rpm for 10 days.

Crude extraction

After 10 days of shaking incubation, 10 ml of liquid bacterial culture was transferred to test tubes and centrifuged at 8000 rpm for 10 min. Then, a portion of the supernatant was used for antimicrobial sensitivity testing via the well diffusion method. Another 10 ml of the supernatant was lyophilized with a bench-top freeze dryer (LYO60B-1PT, Infitek Company) to produce a solid powder for GC-MS analysis.

Antibacterial sensitivity test

Mueller–Hinton agar plates were divided into two groups; the *S. aureus* group and the *E. coli* group. Each plate was inoculated with 0.1 ml of pathogenic bacteria (10^3 cells/ml) by spreading with sterile cotton swabs for 18 h of incubation. Then, wells (6 mm in diameter) were made in each plate with sterile micropipette tips. The crude supernatant (0.1 ml) was added to each well, and the plates were incubated for 24 h at 37 °C.

The parameters	Dose
Laser power	100mW
Type of proliferation	CW
Time of exposure	1 min, 2 min, 3 min
Diameter of laser beam	0.7 mm

Table 1. Irradiation device specifications.

Statistical analysis

The results indicate the diameters of radius (in cm) of the inhibitory growth zones surrounding the wells in the pathogenic bacterial cultures (*E. coli* and *S. aureus*). The collected data were analyzed using GraphPad Prism version 10.5.0. The comparisons were conducted between the control and irradiated groups using standard One-Way ANOVA to demonstrate the significant differences among means ($p < 0.05$) of the maximum values of antibacterial potentials in the irradiation groups. The maximal antibacterial potentials were assessed by T-test analysis by comparing the means of irradiated and non-irradiated groups for each *Strept* strain.

GC-MS analysis

The supernatants were examined utilizing GC–MS. (7820 A GC system, Agilent Technologies) (Table 2). The mass spectra of the lyophilized crude materials were compared with spectra of known compounds in the National Institute Standard and Technology (NIST) database to determine the name, molecular formula and weight, and the area under the curve of the compounds[17]. The specification of GC-Mass system that were used in identifying produced compounds were listed in Table 2.

Results

Antibacterial potential of the isolates

The *Strept. lieno* isolates exhibited smaller inhibition zones with the test pathogenic bacteria before laser irradiation. These isolates exhibited a lighter pink color compared to their non-irradiated counterparts in the casein starch medium following laser irradiation, whereas the *Strept thin* isolates lost their pigmentation after irradiation. First, the antimicrobial potential of the *Strept.* isolates against *S. aureus and E. coli* was examined via the well diffusion method and measurement the radius of the inhibition zone. These data were analyzed by GraphPad Prism, as shown in Fig. 1.

Figure 1 indicates that the antibacterial efficacy of the non-irradiated group, *Strept thin*, exceeds that of *Strept lieno*, albeit not significantly. The effect of laser irradiation on the antibacterial properties of *Strept thin* occurred at a wavelength of 532 nm, whereas *Strept lieno* responded to both wavelengths at the same exposure duration. The antibacterial impact of irradiated *Strept. lieno* on *E. coli* and *S. aureus* is significantly greater than that of irradiated *Strept. thin*. The inhibitory effect of *Strept. lieno* following 532 nm irradiation was effective against both bacterial strains. The peak inhibitory impact against *E. coli* was almost 95% at a wavelength of 650 nm, whereas the efficacy against *S. aureus* was approximately 86% following irradiation at 532 nm.

GC–MS

To identify the antibacterial compounds produced by the *Strept* strains before and after laser irradiation that inhibited the growth of *S. aureus* and *E. coli*, we utilized GC–MS. Considering the NIST library, the GC–MS peaks are shown in Figs. 2 and 3. The area percentages of the peaks allowed semiquantitative analysis of metabolite abundance, as shown in Tables 3 and 4.

The peaks in Fig. 2 indicate each biomolecule in the crude extract. The GC–MS data of the twenty biomolecules in the crude material are presented in Table 3. The peaks with the highest abundance were identified as fatty acids.

Table 3 illustrates that the lyophilized powder of the *Strept lieno* culture supernatant comprises several significant metabolites employed in the production of valuable pharmaceuticals[18–21]. This study aimed to assess the impact of laser irradiation after 10 days of incubation on the metabolism of *Strept. lieno* by analyzing extracellular metabolite content; furthermore, genetic alterations in *Strept. lieno* may be investigated in subsequent research. This study identified novel products, including thiophene. The findings demonstrated a complete reversal of the activity of the non-antibacterial *Strept. lieno* strain following irradiation at a wavelength

No.	Parameter	descriptions
1	Gas Chromatograph	Agelint Technologies(7820 A)
2	GC Mass Spectrometer	(5977E) USA
3	Analytical Column	Agelint HP-5ms Ultra lneit (30 m length x 250 µm inner diameter x 0.25 µm film thickness)
4	Injection volume	1 µl
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.

Table 2. Instrument conditions of GC-MS analysis.

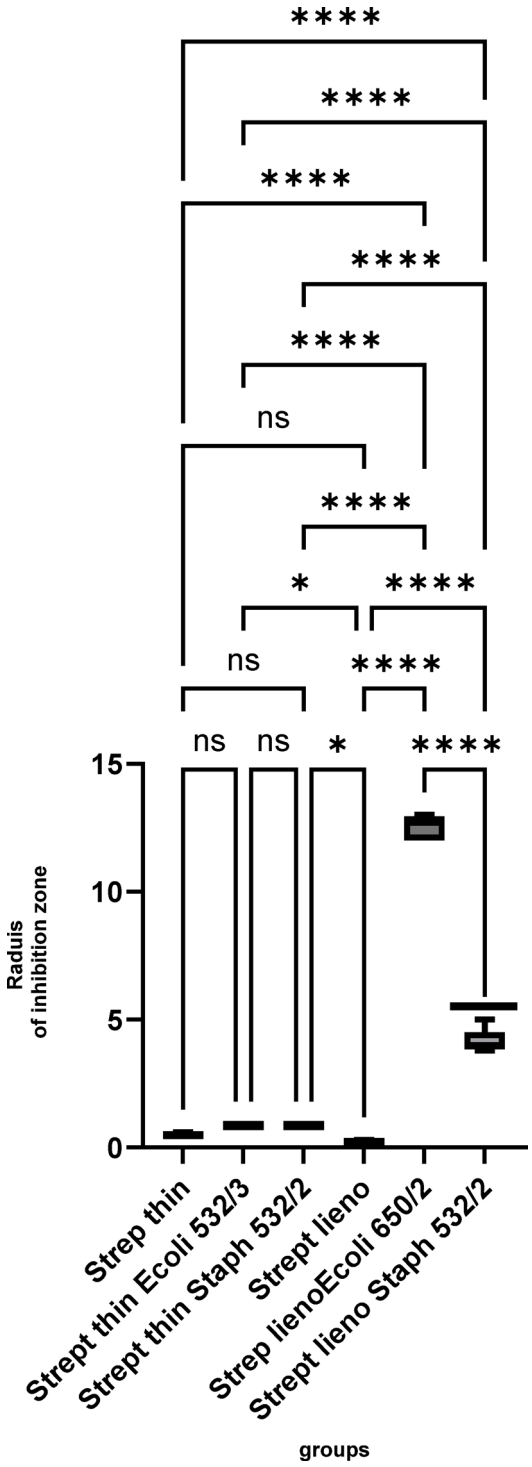

Fig. 1. Comparisons of the mean values between non- irradiated and irradiated *Strept*. groups; where the non-irradiated groups (control) include; ***Strept thin***: *Streptomyces thinghirensis* strain S10, and ***Strept. lieno***: *Streptomyces lienomycini* strain C.P.57. ***Strept thin E coli532/3***: antibacterial potential of the irradiated *Strept thin* with 532 nm laser wavelength for 3 min of exposure time against *E coli*. ***Strept thin S. aureus532/2***: antibacterial potential of the irradiated *Strept thin* with 532 nm laser wavelength for 2 min of exposure time against *S.aureus*; ***Strept. lieno E coli650/2***: antibacterial potential of the irradiated *Strep. lieno* with 650 nm laser wavelength for 2 min of exposure time against *E coli*; ***Strept. lieno S. aureus532/2***: antibacterial potential of the irradiated *Strept lieno* with 532 nm laser wavelength for 2 min of exposure time against *S.aureus*.

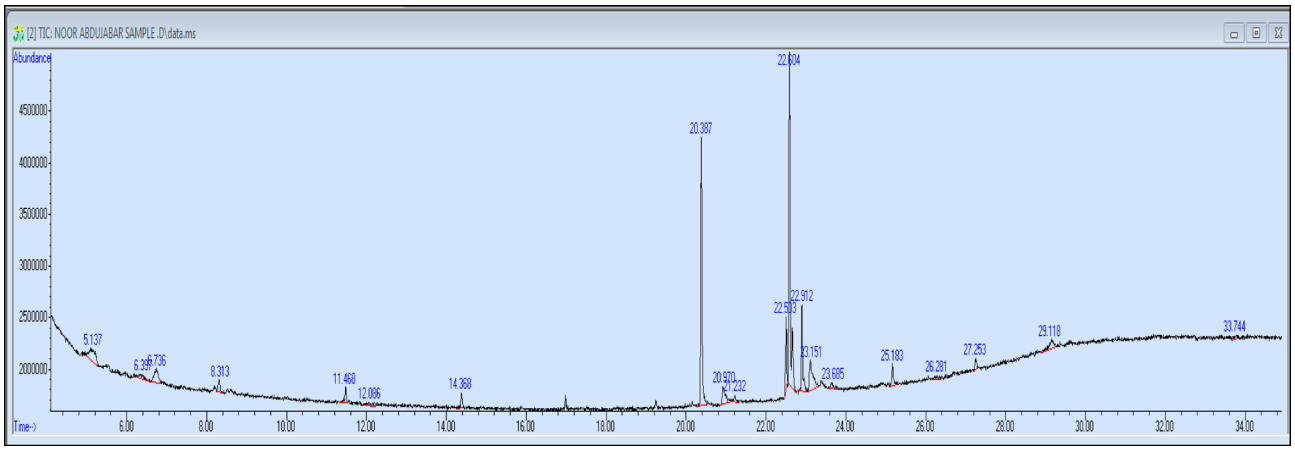

Fig. 2. Curve of secondary metabolites produced by *Strept. lieno* (control) as it obtained from software of GC-mass spectroscopy machine. The y-axes represent the abundance of magnetic field that will change relatively according to different biomolecules in lyophilized extracellular crude of *Strept. Lieno*, that pass through heated thin coil-canals. The x-axes is the time (-second) for biomolecules respond to magnetic field.

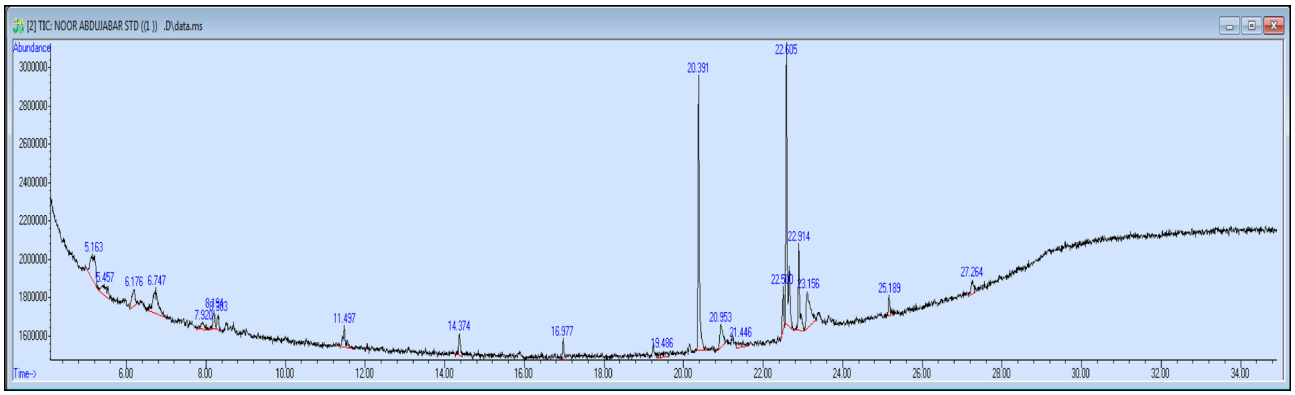

Fig. 3. Curve of secondary metabolites produced by *Strept. lieno* (irradiated with 650 nm for 2 min) as it obtained from software of GC-mass spectroscopy machine.

of 650 nm for 2 min, which displayed a significant bactericidal impact post-irradiation. GC-MS was conducted to identify the biomolecules generated after 650 nm irradiation, with findings presented in Table 4; Fig. 3.

The curve shape distinctly transitions from the 600–1200 s^{-1} range (of non-irradiated Streptomyces lieno, exhibiting a light pink hue) to the 2200–2800 s^{-1} range, where the irradiated strain culture displays a dark brown coloration.

Table 4 prominently displays numerous significant bioactive chemicals, including thiophene derivatives, hormones and neurotransmitters such as; epinephrine, derivatives of norepinephrine, vitamin B6 precursors, toxins, hydrogen bond donors, anti-hypertension reagents and so on.

Discussion

The local *Strept.lieno* isolate examined in this study exhibited minimal antibacterial activity when compared to the antibacterial efficacy of *Strept thin*; specifically, *Strept.lieno* produced a narrow inhibition zone with a diameter of less than 5 mm in a sensitivity test against *S. aureus* and showed no effect on *E. coli*. The GC-Mass data (Table 3) indicated the absence of antibiotic compounds.

In the preliminary study, the isolate with notable antibacterial potential, *Strept thin*, showed a slight increase in activity post-laser irradiation, while *Strept.lieno*, which exhibited reduced inhibitory effects, displayed significant efficacy in the production of antimicrobial compounds following laser irradiation.The *Strept lieno* groups irradiated at a wavelength of 650 nm exhibited a significant enhancement in antibacterial activity. In the second group, the *Strept lieno* subjected to 532 nm irradiation were likewise impacted, albeit to a lesser degree than those exposed to the red wavelength.

In general, inducing changes in biological components requires the absorption of laser photon energy by specific biomolecules, such as cytochrome c oxidase, acetyl carrier protein and acetyl-CoA. The precursors for fatty acid biosynthesis are derived from the acetyl-CoA pool[9]. This energy stimulates several photochemical reactions and changes the redox potential inside bacterial cells[26]. If energy is transferred to biomaterials via

Peak no	Retention time	Area (%)	Library (ID)	Reference peak	CAS number	Qualitative (magnetic susceptibility)	Description and importance
1	5.134	4.64	Ethyl formate	806	000109-94-4	43	Secondary metabolite that is an ester formed when ethanol reacts with formic acid (18)
			Ethylene oxide	73	000075-21-8	12	Secondary metabolite isomer of acetaldehyde and vinyl alcohol[18]
			Ethylene oxide	75	000075-21-8	16	Secondary metabolite isomer of acetaldehyde and vinyl alcohol[18]
2	6.398	1.42	(+/−)-2-Amino-1-propanol	905	006168-72-5	12	Contains both amine (−NH2) and alcohol (−OH) groups and exhibits characteristics of both[19,20]
			(+/−)-2-Amino-1-propanol	904	006168-72-5	12	Contains both amine (−NH2) and alcohol (−OH) groups and exhibits characteristics of both[19,20]
			Cyclobutanol	663	002919-23-5	14	Hydrogen bond donor and starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer[19]
3	6.736	3.51	4-Methyl-1,3-dioxolane	2052	001072-47-5	27	No application was reported
			Octanoic acid methyl ester	30,223	000111-11-5	25	Extracellular membrane fatty acid[18,21]
			Octanoic acid methyl ester	30,233	000111-11-5	14	Extracellular membrane fatty acid[18,21]
4	8.311	1.52	N-Methylglycine	2163	000107-97-1	16	Amino acid also known as sarcosine[18]
			Propanamide	728	000079-05-0	14	No application was reported
			Propanamide	726	000079-05-0	14	
5	11.470	1.51	(3-Hydroxy-5-methyl-2-oxo-2,3-dihydro-1 H-indole-3-carbonyl) urea	101,141	1000296-21-6	9	No application was reported
			Hexamethyl disilathiane	44,259	003385-94-2	14	Flammable, highly toxic irritant[18,22]
			3-(2 Benzoxazolylthio)-1-phenyl-propenone	128,273	299461-72-6	14	Cyclooxygenase-2 (COX-2) inhibitory activity[19,23]
6	12.084	1.13	Cyclobutanol	655	002919-23-5	58	Hydrogen bond donor and starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer[19]
			(+/−)-2-Amino-1-propanol	906	006168-72-5	42	Contains both amine (−NH2) and alcohol (−OH) groups and exhibits characteristics of both[10,20]
			Cyclobutanol	663	002919-23-5	52	Hydrogen bond donor and starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer[19]
7	14.369	1.56	N-[3,5-Dinitropyridin-2-yl] proline	128,542	003264-09-3	4	No application was reported
			Sarcosine, N- valeryl pentadecyl ester	204,024	1000321-56-8	4	No application was reported
			Sarcosine, N-valeryl pentadecyl ester	204,024	1000321-56-8	4	No application was reported
8	20.385	22.44	Hexadecanoic acid methyl ester	119,400	000112-39-0	96	Fatty acid methyl ester[18–24]
			Hexadecanoic acid methyl ester	119,407	000112-39-0	95	Fatty acid methyl ester[24]
			Hexadecanoic acid methyl ester	119,408	000112-39-0	91	Fatty acid methyl ester[18,24]
9	20.385	22.44	Propanamide	728	000079-05-0	47	No application was reported
			N,2-Dimethyl-1-propanamine	1935	000625-43-4	46	No application was reported
			4-Chloro-alpha methyl-benzeneethanamine	37,816	000064-12-0	38	Irritant and environmental hazard, secondary metabolite[18,25]
10	21.233	0.9	Chloroamphetamine	37,813	1000248-75-1	38	Replacement monoamine and amphetamine releaser neurotoxic to serotonergic neurons only[20,21]
			4-Chloro-alpha methyl-benzeneethanamine-	37,816	000064-12-0	47	No application was reported
			DL-Phenylephrine	36,227	001477-63-0	49	Alpha-1 adrenergic agonist and irritating substance that is typically given in conjunction with anesthesia during surgery to treat hypotension[26]
11	22.505	4.6	(Z, Z)-9,12-Octadecadienoic acid	127,648	000060-33-3	91	Doubly unsaturated fatty acid[18]
			Trans-10-Methyl-12-cis octadecadienoate	139,709	1000336-44-2	94	Fatty acid[18]
			9,12-Octadecadienoic acid methyl ester	139,708	002462-85-3	96	Fatty acid[18]
12	22.601	30.05	(Z)-9-Octadecenoic acid methyl ester	141,302	000112-62-9	93	Fatty acid[18]
			11-Octadecenoic acid methyl ester	141,290	052380-33-3	91	Fatty acid[18]
			9-Octadecenoic acid methyl ester, (E)-	141,310	001937-62-8	99	Fatty acid[18]
13	22.912	7.69	Methyl stearate	143,126	000112-61-8	99	Fatty acid[18]
			Methyl stearate	143,128	000112-61-8	95	Fatty acid[18]
Continued							

Peak no	Retention time	Area (%)	Library (ID)	Reference peak	CAS number	Qualitative (magnetic susceptibility)	Description and importance
			Methyl stearate	143,130	000112-61-8	83	Fatty acid[18]
14	23.155	6.1	Metaraminol	36,216	000054-49-9	38	Secondary metabolite sympathomimetic that is used to prevent and treat hypotension, which is a common side effect of anesthesia[26]
			Racepinephrine	48,249	000329-65-7	38	Short-term remedy for moderate asthma symptoms[18]
			6-Methyl-2-piperidinone	6942	004775-98-8	41	Can be utilized to synthesize vitamin K1, vitamin A, vitamin E, and a variety of molecules with various tastes and scents[25]
15	23.683	1.3	Metaraminol	36,216	000054-49-9	46	Secondary metabolite sympathomimetic that is used to prevent and treat hypotension, which is a common side effect of anesthesia[26]
			Meglumine	57,369	006284-40-8	43	Glucose-derived sugar alcohol[18]
			Meglumine	57,368	006284-40-8	43	Glucose-derived sugar alcohol[18]
16	25.180	2.42	Methyl 18-methylnonadecanoate	166,215	1000352-20-6	99	Fatty acid[18]
			Eicosanoic acid methyl ester	166,219	001120-28-1	92	Fatty acid[18]
			Eicosanoic acid methyl ester	166,218	001120-28-1	90	Fatty acid[18]
17	26.279	0.85	Metaraminol	36,216	000054-49-9	49	Secondary metabolite sympathomimetic that is used to prevent and treat hypotension, which is a common side effect of anesthesia[26]
			L-Alanine-4-nitroanilide	68,810	001668-13-9	49	Secondary metabolite used as a substrate for alanine aminopeptidase (AAP) activity determination[24]
			Phenylephrine	36,221	000059-42-7	49	Alpha-1 adrenergic agonist and irritant that is typically given in conjunction with anesthesia during surgery to treat hypotension[26]
18	27.257	1.07	N-Methyl-1-octanamine	20,375	002439-54-5	38	Fatty acid[18]
			Cotinine	23,581	071031-15-7	25	Monoamine alkaloid monoamine alkaloid found in the shrub Catha edulis (khat)[26]
			Cyclobutanol	655	002919-23-5	38	Hydrogen bond donor and starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer[19]
19	29.118	2.62	Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	113,997	1000283-54-9	16	Anti-inflammatory, antioxidant and antibacterial Compound[19]
			2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	102,104	1000161-21-8	22	Antioxidant, anticancer[19]
			Trimethyl[4-(1-methyl-1-methoxyethyl)phenoxy]silane	92,131	1000283-54-8	12	Anti-inflammatory, antioxidant and antibacterial compound[19]
20	33.740	0.9	Ala-.beta.-Ala, trimethylsilyl ester	87,953	1000333-69-0	27	No application was reported
			Fluoxetine	151,963	054910-89-3	22	Utilized to treat panic disorder, bulimia nervosa, depression, premenstrual dysphoric disorder (PMDD), and obsessive-compulsive disorder (OCD)[26]
			1-Octadecanamine, N-methyl-	130,249	002439-55-6	30	No application was reported

Table 3. The molecular components of extracellular crude powder of non-irradiated *Strept. lieno*[18–26].

electron excitation, this leads to the formation of covalent bonds and the release of reactive oxygen species (ROS). Consequently, these ROS cause direct damage to bacterial enzymes, denature proteins in some organelles, and increase the synthesis of antioxidants, including oxidoreductases, as a defence mechanism to protect RNA from total damage[27].

According to our findings in this work, irradiation of the *Strept lieno* isolates at 650 nm led to the production of more antibacterial molecules that irradiation at 532 nm did.

The response to the 532 nm wavelength may arise from the presence of various chromophores in the cytoplasm of cells, particularly those containing haem groups, such as protoporphyrin IX, which demonstrates significant absorption of green light. This absorption leads to mainly the transduction and amplification of photosignals, which is maintained after 10 days of incubation of the bacterial culture[28]. On the other hand, many studies have reported that red and infrared laser light is absorbed mainly by cytochrome c oxidase and that water molecules in the cytoplasm stimulate redox reactions by releasing reactive oxygen species (ROS), such as H_2O_2, OH^-, and NO owing to photodissociation from cytochrome c oxidase[29,30]. In this research, the local *Strept. lieno* isolate began producing thiophene only after 650 nm irradiation, as this compound was not detected by GC–MS and this isolate was not active in the sensitivity test before irradiation. GC–MS provided information about the

Peak no.	Ratio	Area%	Library/ID	Beilsten refrence	CAS number	Magnetic susceptibility	Description and importance
1	5.160	6.91	Hydrazinecarboxamide	908	000057-56-7	9	Known as Aminourea it is irritate and acute toxic compound (toxin) [18].
			Ethylene oxide	75	000075-21-8	12	Secondary metabolites that is isomeric with acetaldehyde and vinyl alcohol[18].
			Cyacetacide	3437	000140-87-4	9	Known as Cyanoacetohydrazide it is irritate and acute toxic compound(toxin) [18].
2	5.454	3.30	1-Propanol, 2-amino-, (.+/-.)-	663	002919-23-5	10	The compound possesses both the amine group -NH2 and the alcohol group -OH, thus exhibiting characteristics of both groups[18].
			1-Propanol, 2-amino-, (.+/-.)-	289	000071-23-8	10	
			1-Propanol, 2-amino-, (.+/-.)-	90	006168-72-5	10	
3	6.173	2.96	Cyclobutanol	663	002919-23-5	10	Cyclobutanol serves as a hydrogen-bond donor and is used as a starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer [19].
			1-Propanol	289	000071-23-8	10	Alcohol used as a solvent and chemical intermediate[18].
			Cyclobutanol	655	002919-23-5	11	Cyclobutanol serves as a hydrogen-bond donor and is used as a starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer [19].
4	6.744	6.45	Propanamide, N-(1cyclohexylethyl)	47,937	1000142-14-3	38	No application was reported
			Guanidine, N,N-dimethyl-	1860	006145-42-2	18	No application was reported
			Cyclobutanol	655	002919-23-5	11	Cyclobutanol serves as a hydrogen-bond donor and is used as a starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer [19].
5	7.921	1.61	Cyclobutanol	655	002919-23-5	46	
			Cyclobutanol	663	002919-23-5	43	
			2-Hexanamine	4135	005329-79-3	47	No application was reported
6	8.198	1.72	Cyclotetrasiloxane, octamethyl-	141,481	000556-67-2	38	Organo-silicon substance cause hazard for human and environment (toxin) [18].
			Cyclotetrasiloxane, octamethyl-	141,484	000556-67-2	78	
			1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol	196,332	1000364-61-2	35	Organo-silicon compound [18].
7	8.302	1.46	Cyclobutanol	663	002919-23-5	14	Cyclobutanol serves as a hydrogen-bond donor and is used as a starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer[19].
			2-Aminononadecane	130,243	031604-55-4	10	No application was reported
			Cyclobutanol	655	002919-23-5	11	Cyclobutanol serves as a hydrogen-bond donor and is used as a starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer[19].
8	11.496	3.18	Thiophene, 2-(bromoacetyl)-5-[bis(dimethylamino)phosphinoyl]-	174,743	1000142-86-5	33	antiviral, antioxidant, antibacterial, anti-inflammatory, insecticidal, and anti-tumor [10,19].
			Acetamide, 2-cyano-	1335	000107-91-5	4	The precursor reagent for the synthesis of vitamin B6 is cyanoacetamide[20].
			2-(4,5-Dihydro-3-methyl-5-oxo-1-ph enyl-4-pyrazolyl)-5-nitrobenzoic acid	194,638	020307-76-0	4	Acute toxin[18].
9	14.378	1.80	N-[3,5-Dinitropyridin-2-yl]proline	2,128,542	003264-09-3	2	Bioactive compound [21].
			Sarcosine, N-valeryl-, pentadecyl ester	204,024	1000321-56-8	4	Amino acid derivatives[18].
			Sarcosine, n-hexanoyl-, pentadecyl ester	210,813	1000321-13-0	2	Amino acid derivatives[18].
10	16.974	1.38	4-Pyridinecarboxamide, 6-chloro-4, 5-dicyano-2-[(cyclohexylidenamino) oxy]-1,2,3,4-tetrahydro-3,3-dimeth yl-	183,188	1000350-38-4	2	derivative of isonicotinic acid [18].
			N-[3,5-Dinitropyridin-2-yl]proline	128,542	003264-09-3	1	Bioactive compound [21].
			3,6-Bis-dimethylaminomethyl-2,7-di hydroxy-fluoren-9-one	165,814	1000318-33-0	12	No application was reported

Continued

Peak no.	Ratio	Area%	Library/ID	Beilsten refrence	CAS number	Magnetic susceptibility	Description and importance
11	19.485	1.42	Cyclobutanol	663	002919-23-5	53	Cyclobutanol serves as a hydrogen-bond donor and is used as a starting material for the production of pharmaceuticals, including antimicrobial agents and drugs for treating cancer [19].
			2-Butanamine, 3-methyl-	1914	000598-74-3	50	Acute toxic[18].
			2-Ethoxyamphetamine	44,804	135014-84-5	64	Biomolecule similar to a drug of the amphetamine class[22].
12	20.393	19.53	Hexadecanoic acid, methyl ester	119,400	000112-39-0	99	Type of fatty acids, used as Hepatoprotective; hypocholesterolemic; antifungal; antiarthritic; antitumor; anticancer; anticoronary; anti-inflammatory compound[23].
			Hexadecanoic acid, methyl ester	119,407	000112-39-0	95	
			Hexadecanoic acid, methyl ester	119,408	000112-39-0	86	
13	20.956	3.59	Propanamide	727	000079-05-0	43	No application was reported
			1,3-Dioxolane-4-methanol	4690	005464-28-8	38	No application was reported
			1,2-Benzenediol, 4-[2-(methylamino)ethyl]-	36,259	000501-15-5	38	No application was reported
14	21.449	1.65	Ala-gly, trimethylsilyl ester	76,119	1000333-70-1	45	No application was reported
			Benzeneethanamine, 2,5-difluoro-.b eta.,3,4-trihydroxy-N-methyl-	76,728	152434-78-1	53	No application was reported
			1,3-Dioxolane-4-methanol	4690	005464-28-8	53	No application was reported
15	22.497	2.33	Methyl 5,12-octadecadienoate	139,679	1000336-43-1	42	bioactive compounds toxin [24].
			9,12-Octadecadienoic acid (Z, Z)-, methyl ester	139,727	000112-63-0	38	A doubly unsaturated fatty acid [18].
			9,12-Octadecadienoic acid, methyl ester	139,708	002462-85-3	97	A doubly unsaturated fatty acid[18].
16	22.609	22.46	9-Octadecenoic acid, methyl ester, (E)-	141,310	001937-62-8	83	No application was reported
			11-Octadecenoic acid, methyl ester	141,291	052380-33-3	95	Bioactive compound has antidiarrhoeal activity [24].
			trans-13-Octadecenoic acid, methyl ester	141,314	1000333-61-3	91	fatty acid has a role as a human metabolite [18].
17	22.912	6.90	Methyl stearate	143,126	000112-61-8	99	fatty acid [18].
			Heptadecanoic acid, 16-methyl-, me thyl ester	143,185	005129-61-3	99	methyl-branched fatty acid [18].
			Methyl stearate	143,130	000112-61-8	74	fatty acid has a role as metabolite [18].
18	23.154	7.40	Epinephrine	48,264	006539-57-7	38	a hormone and a neurotransmitter [25].
			1,2-Benzenediol, 4-(2-amino-1-hydroxypropyl)	48,264	006539-57-7	32	a derivative of norepinephrine that constricts blood vessels [18,25].
			1,8-Octanediamine, N,N'-dimethyl-	39,684	033563-54-1	38	No application was reported
19	25.188	1.79	1,3-Dioxolane-4-methanol	4690	005464-28-8	47	No application was reported
			3-Amino-2-ethyl-butyric acid	13,898	121006-12-0	35	Bioactive compound used to determine the volatile fatty acids [18].
			Metaraminol	36,214	000054-49-9	43	prevention and management of hypotension, or low blood pressure, especially when it arises as an anesthetic side effect [25].
20	27.266	2.16	Ala-gly, trimethylsilyl ester	76,119	1000333-70-1	38	No application was reported
			12-Methylaminododecanoic acid, t-butyl ester	132,029	1000194-27-6	35	No application was reported
			dl-3-Aminoisobutyric acid, N-methy l-, methyl ester	13,921	1000332-87-9	35	No application was reported

Table 4. The molecular components of extracellular crude powder of irradiated *Strept. lieno*[18–26].

components in the supernatant, which may help us to understand how laser light stimulates the biosynthesis of thiophene and possibly its precursors.

Figure 2 shows the compounds identified by GC–MS according to spectra in the NIST database from the control *Strept. lieno* sample (Fig. 3) and the *Strept. lieno* sample irradiated at a wavelength of 650 nm nm for 2 min.

Figures 2 and 3 show that both curves have the same number of peaks, which represent the biomolecules released into the fermentation broth supernatant of *Strept.* However, in Fig. 3, the values for some peaks changed markedly; for example, the first peak at 5.137 in Fig. 2 appeared at 5.163 in Fig. 3, which indicates that the structures of the compounds changed by consecutive photochemical reactions in the bacterial cells. To identify the compounds released before and after laser irradiation, the presence of thiophene was investigated (Table 2).

Thiophene, also known as acetylenic thiophene, is biosynthetically derived from fatty acids or polyacetylenes through acetylene intermediates. Thiophenes are a class of sulfur-containing molecules usually composed of one to five thiophene units and various alkyl groups on the α-carbon of the terminal ring[31].

Therefore, fatty acids and polyacetylenes are derived from oxidative stress upon laser irradiation and cause direct DNA damage through the formation of photoproducts, including cyclobutane-pyrimidine dimers and pyrimidine-(6-4)-pyrimidone[32–35]. These dimers eventually form the fatty acid polyacetylene, which is a precursor for thiophene. This hypothesis was created on the basis of the abundance of cyclobutane compounds in the supernatants of the irradiated samples (Table 2).

Conclusion

Laser light can induce certain natural isolates of *Strept.* to synthesize thiophenes, which, due to their bioactivities, have garnered significant interest from researchers for further exploration of their mechanisms, efficacy, and safety. The structural diversity of thiophenes may hold significant synthetic value as innovative chemical entities for drug discovery. This study may stimulate additional research on the appropriate dosage and wavelength of laser irradiation for related investigations.

Data availability

No datasets were generated or analysed during the current study.

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N.J planned and wrote the paperT.A isolate and characterized the Streptomycetes Both authors reviewed the paper.

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Declarations

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