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Induction of Bioactive Compounds Production from some Actinomycetes Genera Using Low Power Electromagnetic Radiation*

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Abstract

Actinomycetes are the source of many secondary metabolites, enzymes, and antimicrobials that have various applications. Microorganism proliferation is aided in practice by mediated mutations utilizing UV and monochromatic laser radiation. This study aimed to evaluate the photostimulatory effect of UV and laser light on the lipase, amylase and antimicrobial productivity of three actinomycetes' strains from genera: *Micromonospora, Pseudonocardia, and Nocardiopsis*. The strains were exposed to UV (300-400 nm) and monochromatic laser (632.8 nm) radiations for 10 and 20 minutes. The influence of radiations on the actinomycetes' growth, pigment production, enzymatic and antimicrobial activity was evaluated after incubation at 28 ±2 °C for 7 days. Two reference bacterial strains Escherichia coli NCMB 11943 and Staphylococcus aureus NCMB 6571, and one clinical yeast strain Candida albicans, were used for antibacterial assessment, compared to five commercial antibiotics. The results showed better radiation response of the selected *actinomycetes* strains to the monochromatic laser. Both *Pseudonocardia* and *Micromonospora* have exhibited significant increase in pigmentation with laser exposure. In contrast, *Nocardiopsis* has shown reduction in pigment production. The UV and Laser radiations have almost doubled the amylase enzyme activity in *Pseudonocardia and Micromonospora* strains, while not affecting lipase production. On the other hand, the crude metabolites from the *Micromonospora* sp. strain exhibited a highly promising inhibitory effect against E. coli (NCMB11943) when compared with five commercially available antibiotics. The laser irradiation for 10 min has almost doubled the inhibitory zone of *Micromonospora* metabolites against S. aureus (from 0.65 to 1.2 cm) and C. albicans (from 0.7 to 1.3 cm). In conclusion, the results of our research recommend using monochromatic laser radiation to enhance the production of amylase and antimicrobial metabolites from *Pseudonocardia and Micromonospora*, respectively.

Keywords: Actinomycetes, Bioactive secondary metabolites, UV, Monochromatic laser 632.8 nm, enzymatic activity, and antimicrobial activity.

1. Introduction:

Actinomycetes are a major source of antimicrobials, many enzymes, and pigments [1]. The generation of their bioactive metabolites can be accelerated by electromagnetic radiation, such as radio waves, microwaves, ultraviolet light, visible light, X-rays, and gamma rays. The UV light as stimulated actinomycetes' strains, isolated from a volcanic cave in Western Canada, to produce novel antimicrobial compounds against six multidrug-resistant pathogens [2]. Also, the low-intensity laser radiation has, shown a promising stimulating effect on actinomycetes. Ouf et al. [3] were able to increase the cholesterol decomposing activity

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of Streptomyces fradiae, isolated from cholesterol-rich materials, by exposure to low-intensity laser radiation.

The stimulating effects of low power magnetic radiation in bacteria have been related to an increase in the energy supply, given by the radiation, to the bacterial respiratory chain [8, 9]. The cytochrome C complex found in bacteria is one of the targets of irradiation. When this protein complex is photoexcited, it increases proton pumping and, as a result, the quantity of accessible cellular ATP. Several investigations have reported that low power magnetic radiation stimulates cell division and protein synthesis in a variety of mesophilic [4], and thermophilic [5] bacteria. While other studies have shown evidences that low-power radiation limit cell activity and decrease survival of bacterial species [6]. The published data imply that the particular physical magnetic radiation and biological characteristics of the bacterial strains are crucial for defining radiation-induced effects on bacteria [7]. Thus, the aim of this study was to assess the effect of UV and laser radiation on selected actinomycetes genera for producing bioactive compounds at different radiation conditions.

2. Materials & Methods:

2.1. Sources of Strains:

Actinomycetes (three strains, belonging to different genera), previously isolated from desert, and internal tissues of medicinal plants at South Sinai, Egypt, were selected for the current study [8]**.** These were: Micromonospora sp. 1 (strain 32/42), Pseudonocardia sp. 2 (strain 4/14Cn), and Nocardiopsis sp. 3 (strain 9/14Po). All the strains had enzymatic activities, and M. sp. 1 had additional antimicrobial activities. They were obtained from the Actinobacteria Lab., Faculty of Science, Suez Canal University, Ismailia, Egypt, and kept as spore suspension in 20 % v/v glycerol at -15^{o}C for the investigations.

2.2. Irradiation of the strains using UV radiation:

The strains were refreshed on Starch Casein agar plates, in triplicates; then, incubated at 28 ±2 °C for 3-7 days. The plates were allowed to rest in a dark area, at lab. ambient temperature for two hours. The plates were, exposed to UV radiation at -300 - 400 nm for 10 and 20 minutes, after placement 20 cm from the UV lamp. Ultraviolet radiation was performed using Blacklight Blue lamps GE F20T12/BLB 6PK with and power intensity of 5.23 mW.cm^{-2}. The UV directly radiated grown colonies in the plate. To avoid any photo recombination influence, the irradiation was performed in the dark. Then the plates were incubated at 28 ±2 °C for 7 days [9, 10]**.**

2.3. Irradiation of the strains using monochromatic laser radiation 632.8 nm:

The actinomycetes' strains were incubated for 7 days in a static incubator, as described above. All the plates were then placed on the laser devices. This task was performed under dark conditions to prevent photoreactivation [11]. LASER DIOD-2010 was used, with low intensity He-Ne laser wavelength 632.8 nm, a maximum output power of 1.5 mW.cm-2 and a beam diameter of 0.15 cm. The monochromatic laser radiation induction exposure time was 10, and 20 min. Then the plates were incubated at 28 ±2 °C for 7 days.

2.4. Preservation of radiated and unirradiated (wild strain):

All the radiated and wild strains were stored at −20 °C, as spore stocks in 20 % glycerol, for the subsequent investigations.

2.5. Fermentation of the irradiated and unirradiated strains:

The wild and laser-radiated Micromonospora sp. 1 strains were selected for the antimicrobial investigation. Starch Casein (50 ml in 250 ml flasks) were inoculated with 1 ml of 1-3106 CFU/ml spore suspension. The experiment was performed in tripicates for each strain. The strains were incubated in a shaking incubator (100 rpm) for 21 days, at 28 ±2°C. Equal amounts of ethyl acetate was used to extract metabolites three times in a row with vigorous shaking for 20 minutes. After that organic molecule could then be suspended in the less polar solvent. For antimicrobial screens, ethyl acetate

fractions were evaporated under vacuum into preweighed vials, using rotary evaporator HS-2005S-N. Then, redissolved in ethanol or ethyl acetate to give a final concentration of 1 mg/ml.

2.6. Determination of the lipolytic activity of the laser-irradiated strains:

Tributyrin agar plates (Tributyrin (glycerol tributyrate), 10.0 g; Peptone, 5.0 g; Yeast extract, 3 g; Agar, 18.0 g; 1000 ml distilled water) were inoculated with the irradiated strains, compared to their unirradiated wild types, and incubated at 28°C for 7 days. The lipase production was demonstrated by measuring the clear zone diameter around the actinomycetes growth.

2.7. Determination of amylolytic activity of the laser-irradiated strains:

The amylolytic activity was determined by the radial diffusion method. The strains were inoculated in Starch Agar medium plates, consisting of soluble starch, 10.0 g; Yeast extract, 1.0 g; Sodium nitrate, 1.0 g; Potassium chloride, 0.5 g; Magnesium sulfate, 0.5 g; Agar, 18.0 g; 1000 ml distilled water, pH 6.0. The plates were seeded with the irradiated strains, compared to their unirradiated wild types, and incubated for 14 days at 28°C. The cultures were covered by Gram's iodine solution, which allowed the visualization and measuring the diameter of the clear halos around the colonies [12].

2.8. Antimicrobial activity of metabolic extracts from Micromonospora sp 1 strains:

The metabolic organic extracts, from the fermentation process, were evaluated against a representative panel of human pathogenic microorganisms using the disc diffusion method, using 0.1 mg per disc of the dried metabolic extract. All the tests were performed in triplicates. Two bacterial reference strains, Escherichia coli NCMB 11943 and Staphylococcus aureus NCMB 6571, and one clinical yeast strain Candida albicans, were used in the study. The bacterial strains were grown on a nutrient agar medium, while C. albicans was grown on potato dextrose agar. Negative controls were included as ethanol/ethyl acetate-loaded discs in the experiment, which was done in triplicate. After one day of incubation at 35 °C, the clear zone diameter was measured to determine inhibitory action.

For comparison, the tested bacteria Escherichia coli, Staphylococcus aureus, and Candida albicans were evaluated using the disc diffusion technique on nutritional agar medium plates against 5 commercially available antibiotics (Oxoid susceptibility discs) [13]. After one day of incubation at 35 °C, the inhibition zones were measured. The tested antibiotics were: Chloramphenicol (30 μg), Streptomycin (10 μg), Rifampicin (5 μg), Novobiocin (30 μg), and Neomycin (10 μg) were the antibiotics tested.

2.9. Statistical analyses:

Data were collected and handled using Microsoft Excel. Normality was checked using the Shapiro-Wilk test to assess whether data are parametric or nonparametric. Differences between treatment groups were performed using one-way analysis of variance (ANOVA) for parametric data and the Kruskal-Wallis test for nonparametric data at a 0.05 level. Statistical analyses were performed using SPSS version 28.0 for Mac OS (add a reference for SPSS).

3. Results:

The three actinomycetes strains, *Micromonospora sp. 1*, *Micromonospora sp. 2*, and *Nocadiopsis sp. 3*, were selected for this study according to their ability to produce enzymes and antimicrobials. Our results have indicated that the strains are significantly affected by the electromagnetic radiation in the UV ~280–400 nm range and He-Ne monochromatic laser 632.8 nm, with only one exception for *Nocardiopsis. sp 3.* Schematic preview of the experiment is shown in Figure 1. In the following sections, we will show the influences of both radiation types on the three strains.

3.1. Macromorphological changes in colony size of the strains after exposure to UV radiation:

All the three strains were irradiated using ultraviolet radiation at 300 - 400 nm (Figure 2), then incubated at 28 ±2 °C for 7 days. Based on the results (Table 1), after 20 minutes of UV radiation induction the colony size has significantly increased

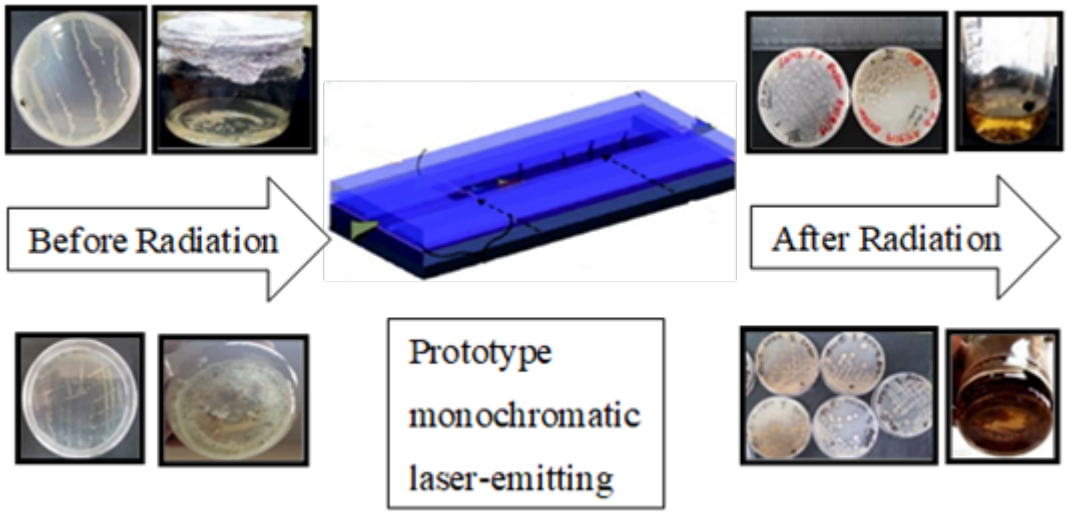

Figure 1: Schematic preview for isolates before and after exposure of radiations.

in Nocardiopsis sp. 3 from 1.54 mm to 2.1 mm, Pseudonocardia sp. 2 from 2.7 mm to 3.55 mm, and Micromonospora sp. 1 from 1.58 mm to 7.12 mm. In all strains, the exposure for 20 min has influenced the colony size more than the 10 min exposure.

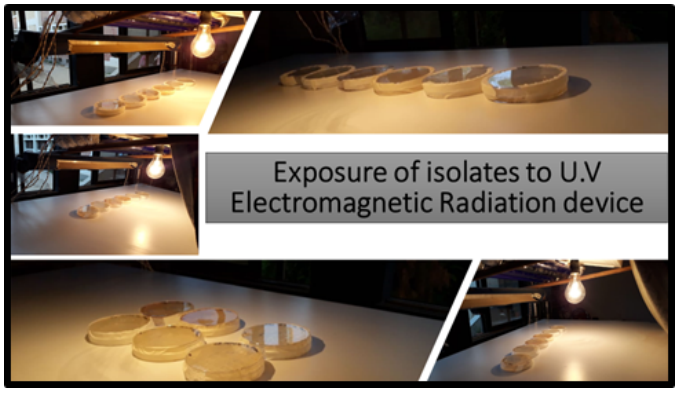

Figure 2: Schematic preview for the exposure of isolates to ultraviolet radiation

3.2. *Colour changes in the fermentation products after exposure to UV radiation:*

It was observed that exposure of the strains to 10 min, or 20 min of UV radiation has significantly affected the colour of the metabolites (Table 2). *Pseudonocardia sp. 2* after receiving a 10 min radiation dosage, has shown colour changes from pale yellow to brown, exhibiting the strongest sensitivity to irradiation. The UV exposure for 20 minutes has caused Micromonospora sp. 1 to change its metabolite colour from deep yellow to pale brown (Table 2). The least UV effects on the colour of fermentation products was that recorded with Nocardiopsis sp. 3.

3.3. *Macromorphological changes in colony size of the strains after exposure to monochromatic laser radiation:*

All the three strains were irradiated using monochromatic laser radiation at 632.8 nm (Figure 3), then incubated at 28 ±2 °C for 7 days. After exposure for 10 and 20 minutes, Nocardiopsis sp. 3 and Micromonospora sp. 1 has significantly increased in colony size. However, Pseudonocardia sp. 2 didn't exhibit significant changes in colony size in response to the laser radiation (Table 3).

3.4. *Colour changes in the fermentation products after exposure to monochromatic laser radiation:*

Exposure of all strains to either10 min, or 20 min laser radiation has significantly affected Both Pseudonocardia sp. 2 and Micromonospora sp. 1 have exhibited significant increase in colour intensity with laser exposure. In contrast, Nocardiopsis sp. 3 have shown reduction in the colour intensity after exposure (Table 4).

3.5. *Lipase production before and after laser radiation:*

Data shown in (Table 5) indicate that laser radiation has an inhibotory effect on the lipolytic acitiv-

Table 1: Colony size responses to different radiation doses (control, UV-10 min, UV-20 min) grown on SC agar medium.

Strain	The average size of the colony mm						ANOVA (p-value)
	Control		UV-10 min		UV-20 min		
	Mean	S. D	Mean	S. D	Mean	S. D	
Micromonospora sp.1	1.584^{F}	0.055	6.817^{B}	0.408	7.123^{A}	0.132	<0.001***
Pseudonocardia sp.2	2.698^{D}	0.159	3.328^{C}	0.139	3.554^{C}	0.053	<0.001***
Nocardiopsis sp.3	1.541^{F}	0.116	1.685^{F}	0.069	2.109^{E}	0.057	<0.001***

Differences were assessed by a one-way ANOVA test. ***, significant at p <0.001.
^{A,B,C,D,E,F} Letters refereeing to different groups, arranged between different exposure times and different strains.

Table 2: Color changes in the fermentation products of the actinomycetes' strains after UV radiation and incubation at 28 ±2 °C for 7 days

Strain	Average colour score of the fermentation product^{A}			Kruskal-Wallis (p-value)
	Control	UV-10 min	UV-20 min	
Micromonospora sp.1	3	3	4	0.011*
Pseudonocardia sp.2	1	5	2	0.001***
Nocardiopsis sp.3	6	5	6	0.011*
Kruskal-Wallis significance (p-value)	0.001 ***	.011*	0.001 ***	0.000***

Differences were assessed by a one-way ANOVA test. *,**, ***, significant at p<0.05, 0.01, 0.001.
^{A}Color code for fermentation products: (1) pale yellow, (2) yellow, (3) deep yellow,(4) pale brown, (5) Brown, (6) deep brown.

Table 3: Colony size responses to different laser radiation doses (control, L-10 min, L-20 min) grown on SC agar medium.

Strain	The average size of the colony mm						ANOVA (p-value)
	Control		L-10 min		L-20 min		
	Mean	S. D	Mean	S. D	Mean	S. D	
Micromonospora sp.1	1.850^{E}	0.143	8.467^{A}	0.567	5.775^{B}	0.001	<0.001***
Pseudonocardia sp.2	2.549^{D}	0.603	3.203^{C}	0.309	3.570^{C}	0.105	>0.051 NS
Nocardiopsis sp.3	1.901^{E}	0.067	5.323^{B}	0.359	3.376^{C}	0.262	<0.001***

Differences were assessed by a one-way ANOVA test. NS,non-significant at p>0.05. *, **, ***, significant at p<0.05, 0.01,0.001.
^{A,B,C,D,E} Letters refereeing to different groups, arranged between different exposure times and different strains.

Table 4: Color changes in the fermentation products of the strains after laser radiation and incubation at 28 ±2 °C for 7 days.

Strain	The average colour score of the fermentation product ^{A}			Kruskal-Wallis (p-value)
	Control	L-10 min	L-20 min	
Micromonospora sp. 1	2	3	3	0.011*
Pseudonocardia sp. 2	1	2	2	0.011*
Nocardiopsis sp. 3	6	4	5	0.001***
Kruskal-Wallis significance (p-value)	0.001***	0.001***	0.001***	0.003*

Differences were assessed by a one-way ANOVA test. *,**, ***, significant at p<0.05, 0.01, 0.001
^{A}Color code for fermentation products: (1) pale yellow, (2) yellow, (3) deep yellow,(4) pale brown, (5) Brown, (6) deep brown.

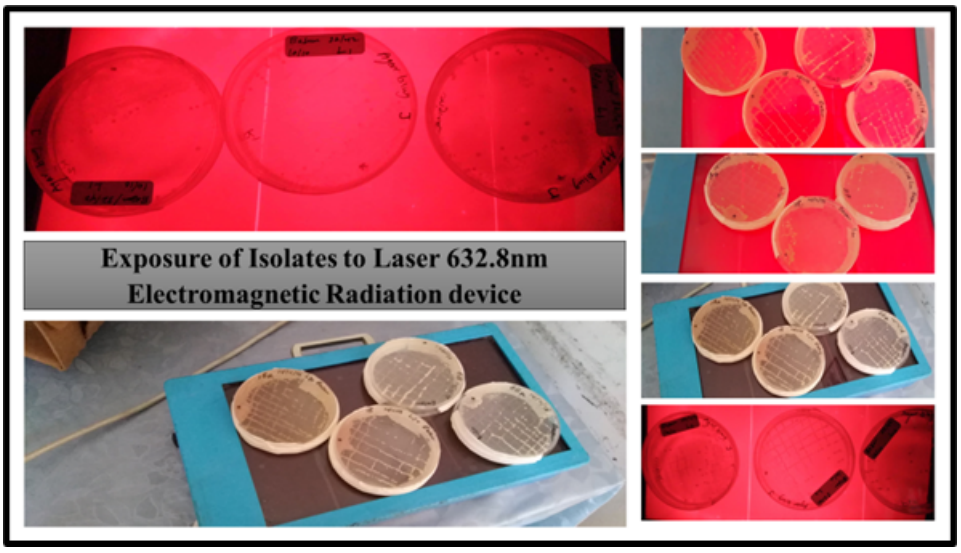

Figure 3: Schematic preview for the exposure of isolates to monochromatic laser radiation

ities. . The control group was significanlty higher in two strains, Nocardiopsis and Pseudonocardia (2.13 and 1.85 mm, respectively), compared to L-10 and L-20 minutes regarding the production of lipases.

3.6. Amylolytic enzymes production before and after laser radiation:

Initial screening results for amylase production by the three strains has shown that all the wild strains were amylase producers, and the produciton activity was significantly affecte dy laser radiation. The activity of Nocardiopsis sp. 3 was negativlely affected by laser radiation. While, Pseudonocardia sp. 2 have shown almost douple activity (from 1.21 to 2.09 mm index) after exposure to laser light for 20 minutes. Similarly, Micromonospora sp. 1 activity have been almost doupled (from 1.16 to 2.24 mm index) after exposure to laser light for 10 minutes (Table 6).

3.7. Antimicrobial activity of metabolic extract from Micromonospora sp1:

Table 7 shows the antimicrobial activity of the metabolic extracts from both wild and laser-irradiated Micromonospora sp. 1 strains, compared to five commercial antibiotics for guidance. The results have indicated positive effect of laser irradiation for 10 min, where the antimicrobial activity has almost doubled against S. aureus and C. albicans. While antimicrobial activity against E. coli did not show significant changes.

4. Discussion

Actinomycetes are among the largest producers of enzymes and antimicrobials [14]. Mediated genetic changes using electromagnetic radiation such as UV and lasers are practiced for enhancing the microbial activity [15]. In our microbiological laboratory, enormous efforts are made to maximize the production of these natural products because of their significance. Utilizing various low-power, high-wavelength radiation energy, such as laser and ultraviolet radiation, for "physical induction" was investigated on three strains representing different genera of actinomycetes. This study demonstrates that Micromonospora, Pseudonocardia, and Nocardiopsis irradiated strains can change their pigmentation colours according to exposure dose and the type of radiation exposure. These results, also validate the effects of UV and monochromatic laser radiation in enhancing bioactive secondary metabolites. Likewise, according to a prior study, Streptomyces coelicolor, which generates blue pigment, has been genetically modified to create bright yellow (kalafungin), orange, or yellow-red pigments (anthraquinones) [16].

Our results showed that the better radiation choice for growing the selected actinomycetes

Table 5: Lipase production before and after exposure of laser 632.8nm, differences were assessed by One Way ANOVA

Groups	Lipolytic activity		
	Nocardiopsis sp. 3	Pseudonocardia sp. 2	Micromonospora sp. 1
Control	2.13±0.07	1.85±0.04	NS
L-10 min	0.84±0.04	1.10±0.04	NS
L-20 min	1.83±0.05	1.58±0.06	NS
ANOVA	<0.001***	<0.001***	NS

NS, non-significant at p>0.05. ***, significant at p<0.001.
The highest lipase production is highlighted in grey for each strain.

Table 6: Amylase production before and after exposure to laser 632.8 nm, differences were assessed by One Way ANOVA

Groups	Amylolytic activity		
	Nocardiopsis sp.3	Pseudonocardia sp.2	Micromonospora sp.1
Control	1.82±0.03	1.21±0.03	1.16±0.03
L-10 min	1.38±0.03	1.92±0.20	2.24±0.02
L-20 min	1.10±0.03	2.09±0.05	1.47±0.05
ANOVA	<0.001***	<0.001***	<0.001***

NS, non-significant atp>0.05. ***, significant atp<0.001.
The highest amylase production is highlighted in grey for each strain.

Table 7: Antimicrobial activity of the metabolic extracts from wild and laser-irradiated Micromonospora sp. 1 strains

Strain / Antibiotics	Antimicrobial activity (cm)		
	E. coli (NCMB11943)	S. aureus (NCMB6571)	C. albicans (Clinical culture)
Micromonospora sp. 1 (100 μg)			
M. sp. 1 Wild	0.8 ±0.03	0.65 ±0.04	0.7 ±0.05
M. sp. 1 L-10	0.9 ±0.05	1.2 ±0.09	1.3 ±0.03
Commercial antibiotics (conc., μg)			
Chloramphenicol (30 μg)	0.9	0.7	2
Streptomycin (10 μg)	0.7	0.6	1.2
Rifampicin (5 μg)	0.9	0.6	1.2
Novobiocin (30 μg)	0.5	Nil	2
Neomycin (10 μg)	1	1.2	1.1

strains is monochromatic laser. It has stimulation activity for Pseudonocardia sp. 2 and Micromonospora sp. 1, rather than Nocardiopsis sp. 3, in the production of pigments in SC broth medium. These results agreed with previous studies, where monochromatic laser radiation exposure enhances the activity of various bacteria, [17, 18].

Lipases are enzymes that catalyze the hydrolysis or production of lipids. They're employed in various industries, including detergents, oils and fats, baking, organic synthesis, hard surface cleaning, the leather business, and the paper industry [19]. Our study showed that the production of lipase enzyme differs according to the strain selected, where only Nocardiopsis sp. 3 and Pseudonocardia sp. 2 could produce lipase enzyme.

Previous studies agreed with our study that the

decrease in lipase production following laser induction could be due to extracellular protease inactivation, as seen in other lipase-producing bacteria. Lipases are generally inducible enzymes that require inducers like oils to produce them [20]. Lipase induction in actinomycetes has also been documented in lipid-based carbon sources [21] .

Our initial screening results for amylase production by actinomycetes on the agar plates containing starch medium showed that all isolates were amylase producers. A comparison between amylase production among the three groups of strains (before and after exposure to laser 632.8 nm) revealed that monochromatic laser radiation have greatly enhanced Micromonospora, and Pseudonocardia abilities when exposed for 10 and 20 min, respectively. While the laser radiation did not affect Nocardiopsis ability for amylase production.

As reported in the previous studies, actinomycetes have been recognized as an essential source of antimicrobial, and antitumor compounds [22]. Another study revealed that high yield antibiotic producing mutants of actinomycetes, such as Streptomyces erythreus, can be induced by low energy electromagnetic radiation [15] . Similarly, our study showed that Micromonospora strain can double its antibacterial activity against the reference cultures Staphylococcus aureus NCMB 6571, and Candida albicans NCMB 6571 when exposed to laser monochromatic radiation for10 min. It is worth noting that, the crude metabolites from the irradiated Micromonospora sp. 1 strain exhibited a highly promising inhibitory effect against the reference culture S. aureus and C. albicans, compared with five commercially available antibiotics.

5. Conclusion:

Based on the protocol used in the present study, it is possible to conclude that, UV and monochromatic laser radiation has enhanced cell proliferation and pigmentation production of actinomycetes' strains. Also, these radiations have increased the productivity of lipase and amylase enzymes. On the other hand, the crude metabolites from Micromonospora sp. 1 strain exhibited a highly promising inhibitory effect against the reference cultures, compared with five commercially available antibiotics. Utilizing various low-power, high-wavelength radiation energy, such as laser and ultraviolet radiation, for bioactivity induction, and metabolomic analyses of secondary metabolites generated by Micromonospora sp. 1 will be investigated.

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