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ORIGINAL ARTICLE

Evaluation of low-intensity laser radiation on stimulating the cholesterol degrading activity: Part I. Microorganisms isolated from cholesterol-rich materials

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Abstract A survey was performed to isolate bacteria and fungi from cholesterol-rich sources including chicken liver, turkey giblets, salmon, lamb, egg yolk, beef brain and shrimps. A total of 34 bacterial and 22 fungal isolates were recovered from the tested sources. The highest count of isolates was recovered from the soil (12 isolates/g), followed by turkey giblets and egg yolk (8 isolates/g, for each). Out of 34 bacterial isolates, five induced the highest level in cholesterol degradation. The most potent bacterial isolate was recovered from turkey giblets and was identified as *Streptomyces fradiae*. In a trial to increase the cholesterol decomposing potentiality of *S. fradiae*, low intensity Nd-YAG laser irradiation was evaluated. The exposure of the chlorophyllin – photosensitized bacterium to 210 mW Nd-YAG laser for 8 min induced significant increase in cholesterol degrading activity reaching 73.8% as compared with 54.2% in the case of non-irradiated, non-photosensitized culture. Under the same conditions but using the reaction mixture containing cholesterol as a substrate and extracellular crude enzyme, the percent decomposition reached 53.7% for the irradiated culture as compared to 28.3% in the case of the control. Our data indicate the importance of the photosensitizer in enhancement of laser radiation to stimulate cholesterol decomposition of *S. fradiae*.

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1. Introduction

Cholesterol as it is now known is a soft waxy compound belonging to steroid family of molecules. Cholesterol is the main sterol found exclusively in animal and human tissues and in plasma lipoproteins either as free cholesterol or combined with long-chain fatty acids as cholesterol esters. Cholesterol and its oxides have been detected in a variety of foods and foodstuffs, especially eggs, milks, meats, seafoods and their processed products (Paniangvait et al., 1995). The major role of cholesterol in pathological process is as a factor in the genesis of atherosclerosis of vital arteries causing

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cerebrovascular, coronary and peripheral vascular diseases (Steinberg, 2006).

Several studies have indicated that serum cholesterol level could be reduced by some bacterial species such as lactic acid bacteria (Smith et al., 1991; Rasic et al., 1992; Tamai et al., 1996; Vesa, 1998; Liong and Shah, 2005), *Bifidobacterium bifidum* (Ghaleb et al., 1998), *Bacillus licheniformis* (Cardona et al., 2003), *Rhodococcus equi* (Watanabe et al., 1986; Chung et al., 1995; Chang et al., 2000).

Drzyzga et al. (2011) reported that the type strain *Gordonia cholesterolivorans*, is able to degrade steroid compounds containing a long carbon side chain such as cholesterol (C_{27}), cholestenone (C_{27}), ergosterol (C_{28}), and stigmasterol (C_{29}). The length of the carbon side chain appears to be of great importance for this bacterium, as the strain is unable to grow using steroids with a shorter or nonaliphatic carbon side chain such as cholic acid (C_{24}), progesterone (C_{21}), testosterone, androsterone, 4-androstene-3,17-dione (all C_{19}), and further steroids. Park and Kim (1994) isolated 120 bacterial strains from foods (pork fat, chicken fat, beef fat, beef liver, butter, egg yolk, Mozzarella cheese and cheddar cheese) using a medium containing cholesterol as the sole carbon source. They tested the cholesterol degradation by 19 isolates using HPLC, and found the isolate C-5, belonged to the genus *Kurthia*, had the highest cholesterol degradation ability. The bacterial strain *Bacillus subtilis* SFF34 producing a high level of extracellular cholesterol oxidase was isolated from Korean traditional fermented flatfish and characterized by Kim et al. (2002).

There is a large body of data about the utilization of laser radiation to inhibit the growth of microorganisms (Keates et al., 1988; Nitsmane and Blitte, 1988; Iwase et al., 1989; Ouf and Abdel-Hady, 1999; Ouf et al., 2003; Maisch et al., 2005; Sharma et al., 2008). However, there are some quantitative studies with organisms of different complexity improving the stimulating action of low-intensity laser radiation. Low intensity laser radiation with wavelength of 400–500 nm and about 600 nm brought about accelerated cell division in various microorganisms and enhanced protein synthesis (Yew et al., 1982; Gamaeva et al., 1983; Boulton and Marchall, 1986).

In this work a survey was performed to isolate bacteria and fungi from cholesterol-rich sources and the recovered isolates were evaluated as cholesterol decomposers. The most potent isolate exerting the highest potentiality in cholesterol decomposition was evaluated under different doses of low intensity Nd-YAG laser radiation in increasing the cholesterol degrading activity.

2. Materials and methods

2.1. Isolation and growth media

2.1.1. Sources of isolation

Eight sources rich in cholesterol were used for isolation of bacteria and fungi. The sources were chicken liver, turkey giblets, salmon, lamb, egg yolk, beef brain, shrimps as well as soil.

2.1.2. Isolation from soil

One gram of finely crystalline cholesterol was mixed with 100 g clay soil, from the garden of Faculty of Science, Taibah University. The mixture was divided into two pots and buried below the ground surface in the soil from which the sample was taken. After one month, the experimental soil was removed from the ground and 10 g soil was suspended in 100 ml sterilized distilled water and shaken for 30 min at 200 rpm. Ten milliliter suspension was transferred to 10 plates where each plate contains 1 ml suspension. Fifteen ml of each of the media described before was poured into each plate, shaken gently and incubated for 15 days at 30 °C.

2.1.3. Isolation from the other sources

One hundred grams of the tested source was exposed to air for 2 weeks and then macerated to smaller pieces. The pieces were suspended in 250 ml sterilized distilled water and shaken for 30 min at 200 rpm. Ten milliliter suspension from each tissue was transferred into 10 plates, where each plate received 1 ml. Fifteen ml of each of cholesterol media described before was poured in each plate and shaken gently and incubated for 15 days at 30 °C. The developing colonies were picked up on agar slant of the same medium and purified by repeated serial platings.

2.1.4. Media used for bacterial and fungal isolation

The cholesterol-degrading bacteria were isolated from different sources using the medium described by Nishiya et al. (1997). It consists of (g/L): NH_4NO_3, 1.0; K_2HPO_4, 0.25; $MgSO_4{\cdot}7H_2O$, 0.25; yeast extract, 5; $FeSO_4.7H_2O$, 0.001; cholesterol, 1.0; agar-agar, 15. The mineral salts basal medium of Zanin (1968) was used for isolation of fungi. It consists of (g/L): NH_4NO_3, 1.0; K_2HPO_4, 0.25; $MgSO_4{\cdot}7H_2O$, 0.25; NaCl, 0.05; $FeSO_4{\cdot}7H_2O$, 0.001; cholesterol, 1.5; agar-agar, 15. The medium with agar but without carbon source (cholesterol) was sterilized separately. The recovered colonies were purified by subculturing on the same cholesterol medium and incubated at 28 °C for 5 and 7 days, in the case of bacterial and fungal isolates, respectively. The purified colonies were subcultured on slants and then kept in the refrigerator.

2.2. Preliminary test for cholesterol degrading ability of the recovered bacteria and fungi

The ability of the isolated bacteria and fungi to decompose cholesterol was evaluated by measuring the zone of translucency around colonies on agarized medium containing cholesterol as a sole carbon source (Zanin, 1968).

2.3. Identification of bacterial isolate

The most potent bacterial isolate in decomposing cholesterol (isolate No. 16) was identified according to Biolog GP2 MicroPlate, which performs 95 discrete tests simultaneously and gives a characteristic reaction pattern called a ''metabolic fingerprint''. These fingerprint reaction patterns provide a vast amount of information conveniently contained in a single Biolog MicroPlate. The metabolic fingerprint patterns are compared and identified using the MicroLog™ database software. The results showed that *Streptomyces fradiae* was the most potent microorganism in decomposing cholesterol, so, it was employed as test organism.

2.4. Quantitative determination of cholesterol decomposition by S. fradiae

2.4.1. Extraction of total cholesterol from different materials

One gram of each sample was homogenized with 2:1 chloroform–methanol (V/V) (Folch et al., 1956) for one hour, and the homogenate was filtered. The extract of each sample was placed in a 15 ml centrifuge tube and 2 ml of water was added, mixed with a stirring rod and centrifuged at 2400 rpm for about 20 min until complete separation of the system into 2 phases. The upper phase is removed as completely as possible with a pipette; while the lower phase (lipid extract) was concentrated to dryness by vacuum distillation of the solvents and the concentration of cholesterol in the lipid extracts was measured enzymatically by using a kit (Asan Pharmaceutical, Seoul, Korea).

2.4.2. Cholesterol decomposition by S. fradiae

The promising bacterial isolate in cholesterol decomposition (isolates number 20 recovered from salmon) was assayed for their cholesterol degrading activity. One ml bacterial suspension (10^7–10^8 bacterial cells/ml) was incubated with 150 ml of the corresponding cholesterol medium described before for 7 days at 28 °C under stationary condition. At the end of the incubation time, the percentage of cholesterol reduction was measured according to the calorimetric method of acetic anhydride/sulfuric acid (Ratilff and Hall, 1973; Tietz, 1987) using the following general formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard (200mg/100ml)}$$

2.5. Preparation of crude enzymes

2.5.1. Preparation of extracellular crude enzyme

Solid ammonium sulfate was added in small amounts to the clear filtrate to bring salt concentration to 80%. The mixture was allowed to stand in cold (5 °C) for 48 h. and the precipitate was collected by centrifugation at 5000 rpm for 20 min. The precipitate was dissolved in 0.05 M potassium phosphate buffer (pH 7.0) and stirred for 30 min at room temperature. Any insolubles were then removed by centrifugation (Somkuti and Babel, 1968). Elimination of excess salt was carried out by dialyzing the enzyme preparation overnight at 5 °C using 0.05 M potassium phosphate buffer (pH 7.0).

2.5.2. Preparation of intracellular crude enzyme (cell free extract)

The bacterial cells from liquid cultures were harvested by centrifugation at 5000 rpm for 10 min at 5 °C and then washed twice with 0.05 M potassium phosphate buffer (pH 7.0). The cell crop was suspended in a volume of 0.1 M Tris–HCl buffer (pH 6.5) corresponding to 1% of the original culture volume and then broken up in a disintegrator under the pressure of 3000 kg force/cm^2. The cell debris was removed by centrifugation at 7000 rpm for 10 min and the supernatant was the source of enzyme (Beesley et al., 1967).

2.5.3. Determination of extracellular and intracellular cholesterol enzyme preparation: cholesterol oxidase

This experiment was carried according to Johnson and Somkuti (1990). The reaction mixture used for this purpose contained: 200 μl of enzyme preparation in 1.5 ml of potassium phosphate buffer (pH 7.0) containing 200 μl of cholesterol (2 mg/ml) in *n*-propanol. The reactions were carried out for 60 min. at 37 °C and stopped by extraction with 4 ml ethyl acetate. Aliquots (1 ml) of the ethyl acetate layer were assayed for cholesterol concentration calorimetrically. Cholesterol degrading activity was calculated as μg cholesterol degraded per minute per mg protein.

2.6. Irradiation of the test bacterium using Nd-YAG laser radiation

2.6.1. Source of radiation

The source of irradiation was located at the National Institute of Laser Enhanced Science (NILES), Cairo University (laser model BL-106C; Spectra-Physics Lasers, Inc., Mountain View, CA). Samples were irradiated by 210 mW, pulse duration 8–9 ns Nd-YAG at a wavelength of 1064 nm.

2.6.2. Photosensitizers

Three photosensitizers were used in this research which were chlorophyllin, phthalocyanine (*Sigma* Chemical Co., St. Louis, MO) and hematoporphyrin (HP, Porphyin products-Logan, Utah). Each of photosensitizers was used at a concentration of 0.5 mg/ml and was incubated with the test bacterium for 5 min before irradiation.

2.6.3. Bacterium treatment

The test bacterium was grown on plates containing the cholesterol medium and incubated at 28 °C for 5 days. Bacterial suspension (10^7–10^8 cells/ml) was prepared and incubated with the photosensitizers for 5 min at room temperature before irradiation. A photosensitizer-free sample was used for comparison. The samples were then irradiated with Nd-YAG laser for 2, 4, 8, 16 and 32 min. The irradiated bacterial suspension was inoculated into 100 ml of the cholesterol medium (Nishiya et al., 1997). After 7 days of incubation at 28 °C, the bacterial cells were separated by centrifugation at 3000 rpm. The bacterial cells were washed three times with sterile distilled water, dried at 70 °C and then weighed. The decomposition of cholesterol (mg/100 ml) was determined in the medium as stated before.

2.7. Statistical analyses

All data were subjected to statistical analyses of variance (*F*-test) ''one way ANOVA'' (Armitage, 1971).

3. Results

3.1. Quantitative determination of cholesterol content in the isolation materials

The highest source of cholesterol content was beef brain followed by lamb and egg yolk (Table 1). Chicken liver, turkey giblets, shrimps, and salmon came next according their cholesterol content. The cholesterol content of the soil was the lowest.

Table 1 Cholesterol content (mg/g) in the different tested materials.

Tested materials	Cholesterol content (mg/g)
Soil	0.03
Chicken liver	6.41
Turkey giblets	5.66
Salmon	1.93
Lamb	16.32
Egg yolk	13.71
Beef brain	30.03
Shrimps	4.88
L.S.D.	
At 1%	0.80
At 5%	0.96

Values for significant difference were determined according to Fisher's LSD test, $P = 0{:}05$.

3.2. Isolation of bacteria and fungi and their assay for cholesterol degrading enzymes

A total of 34 bacterial isolates and 22 fungal isolates were recovered from the tested sources (Tables 2 and 3). The highest counts of bacterial isolates were recovered from soil (7 isolates/g) followed by turkey giblets (6 isolates/g) representing 20.59% and 17.65%, respectively. Chicken liver, salmon, lamb, egg yolk and beef brain, each recorded 4 isolates/g. The lowest count of bacteria (1 isolate/g) was recovered from shrimp constituting 2.94% of the total population. On the other hand, the highest count of fungi was recovered from soil (5 isolates/g), followed by egg yolk (4 isolates/g) and both of chicken liver and salmon (3 isolates/g for each).

Data in Table 4 revealed that out of 34 bacterial isolates, 5 induced the highest level in cholesterol degradation as indicated by the very clear translucency in agarized cholesterol medium (+4). One of these isolates was recovered from the chicken liver (isolate no. 9), one from turkey giblets (isolate no. 13), one from salmon (isolate no. 20), one from lamb (isolate no. 25) one from egg yolk (isolate no. 29). None of the fungal isolates induced +4 translucency in the test medium (Table 5). Therefore the five bacterial isolates were the target of the experiments ahead.

Bacterial isolate no. 13, as indicated in Table 6 exerted the highest percentage of cholesterol decomposition after 7 days (55.41 mg/100 ml) and the highest dry weight gain (234.5 mg/100 ml). The percentage of cholesterol decomposition of this isolate was followed by that of isolate number 20 (46.11%) and isolate number 29 (38.52%).

The data in Table 7 indicate that isolates number 13 and 29 were able to produce extracellular and intracellular cholesterol degrading enzymes, while isolate 9 produced only intracellular enzyme. The other tested isolates (nos. 20 and 25) produced only extracellular enzyme. Isolate number 13 recorded the highest production of the extracellular cholesterol degrading enzyme which decomposed cholesterol in the reaction mixture by 36.6%. The other isolates can be arranged in descending order according to their cholesterol degrading ability of extracellular enzyme as follows: isolate 25 (cholesterol degradation 29.1%) > isolate 20 (16.8%) > isolate 29 (11.3%).

The bacterial isolate number 13 was the most potent isolate in cholesterol degrading ability as indicated by the production of the extracellular degrading enzyme. Thus, this bacterium was selected to continue this work. This isolate was identified according to Biolog GP2 MicroPlate, as *S. fradiae*.

3.3. Effect of Nd-YAG laser on dry weight gain and cholesterol degrading activity of S. fradiae

Fig. 1 reveals the effect of different doses of Nd-YAG laser irradiation emitted at power output of 210 mW, in absence and presence of three photosensitizers on the dry weight gain of *S. fradiae*. The photosensitizers, namely chlorophyllin, hematoporphyrin, and phthalocyanine, each was incubated with the test bacterium for 5 min before irradiation.

In absence of the photosensitizer, there was an increase in the dry weight of *S. fradiae* when exposed to the irradiation for 2, 4 and 8 min, reaching 216, 230 and 246 mg/100 ml, respectively. The longer duration of exposure induced a decrease in dry weight gain reaching 136 mg/100 ml at 32 min exposure time.

On the other hand, the irradiation of photosensitized cells up to 8 min induced gradual significant increase in dry weight

Table 2 Count of bacterial isolates recovered from different cholesterol-rich sources.

Source of isolation	Bacterial isolates		
	Count (isolate/g)	By number	% of total population
Soil	7	1–7	20.59
Chicken liver	4	8–11	11.76
Turkey giblets	6	12–17	17.65
Salmon	4	18–21	11.76
Lamb	4	22–25	11.76
Egg yolk	4	26–29	11.76
Beef brain	4	30–33	11.76
Shrimps	1	34	2.94
Total count	34	34	100
L.S.D.			
$P = 0.01$	2		
$P = 0.05$	1		

Values for significant difference were determined according to Fisher's LSD test, $P = 0{:}05$.

Table 3 Count of fungal isolates recovered from different cholesterol-rich sources.

Source of isolation	Fungal isolates		
	Count (isolate/g)	By number	% of total population
Soil	5	1–5	22.73
Chicken liver	3	6–8	13.64
Turkey giblets	2	9–10	9.10
Salmon	3	11–13	13.64
Lamb	2	14–15	9.10
Egg yolk	4	16–19	18.19
Beef brain	2	20–21	9.10
Shrimps	1	22	4.55
Total count	22	22	100
L.S.D.			
$P = 0.01$	2		
$P = 0.05$	1		

Values for significant difference were determined according to Fisher's LSD test, $P = 0{:}05$.

Table 4 Preliminary test for cholesterol decomposition by bacterial isolates recovered from cholesterol rich materials after 5 days growth on medium containing cholesterol as the sole carbon source.

Isolate number	Cholesterol decomposition	Isolate number	Cholesterol decomposition
1	+	18	+ +
2	+ +	19	+
3	+	20	+ + + +
4	+	21	+ +
5	+	22	+ + +
6	+ +	23	+
7	+ +	24	+
8	+ +	25	+ + + +
9	+ + + +	26	+
10	+	27	+ +
11	+ +	28	+
12	+ +	29	+ + + +
13	+ + + +	30	+
14	+ +	31	+
15	+	32	+ +
16	+	33	+ +
17	+ + +	34	+ + +

+ = weak translucence; + + = moderate translucence; + + + = clear translucence; + + + + = very clear translucence.
Values for significant difference were determined according to Fisher's LSD test, $P = 0{:}05$.

gain of *S. fradiae* reaching 288 and 277 mg/1 ml after 4 min in the case of chlorophyllin and phthalocyanine. The maximum dry weight gain was obtained on irradiation for 8 min in the case of chlorophyllin (311 mg/100 ml). There was a clear reduction in dry weight at longer exposure times (16 and 32 min) under different treatments of photosensitized bacterium.

Fig. 2 includes the percentage cholesterol decomposition of the irradiated *S. fradiae* under different photosensitizers. In absence of photosensitizer, the cholesterol degrading activity of test bacterium significantly increased with extension of exposure time up to 32 min reaching 65.3% as compared to 54.2% in the case of non-irradiated culture.

For photosensitized bacterial cells, the maximum cholesterol degrading activity was reached after 8 min irradiation amounting 73.8%, 65.2% and 66.3%, in the case of chlorophyllin, hematoporphyrin and phthalocyanine, respectively. The cholesterol degrading activity of the irradiated photosensitized bacterial cultures was significantly reduced as compared with the corresponding photosensitized non-irradiated cells, on exposure for 32 min.

Assay of cholesterol degrading enzyme activity in reaction mixture containing cholesterol as a substrate and extracellular crude enzyme of non-irradiated and 210 mW Nd-YAG laser irradiated *S. fradiae* in presence or absence of the photosensitizers is shown in Table 8.

The results show a similar pattern to that recorded for cholesterol degrading activity in the culture medium. The maximum amount of decomposed cholesterol, in absence of photosensitizers was achieved with *S. fradiae* exposed to Nd-YAG laser for 16 min. The gradual extension of laser irradiation of the photosensitized bacterial cells induced a significant increase in the cholesterolytic activity in the reaction mixture reaching maximum values in the case of the bacterial suspension exposed to 8 min (53.7%, 46.0%, and 44.6%, in the case of chlorophyllin, hematoporphyrin and phthalocyanine,

Table 5 Preliminary test for cholesterol decomposition by fungal isolates recovered from cholesterol rich materials after 8 days growth on medium containing cholesterol as the sole carbon source.

Isolate number	Cholesterol decomposition	Isolate number	Cholesterol decomposition
1	+ +	12	+ +
2	+ + +	13	+ + +
3	+	14	+ +
4	+	15	+ +
5	+	16	+ +
6	+ +	17	+
7	+ +	18	+
8	+	19	+
9	+ +	20	+
10	+	21	+ + +
11	+ + +	22	+

+ = weak translucence; + + = moderate translucence; + + + = clear translucence; + + + + = very clear translucence.
Values for significant difference were determined according to Fisher's LSD test, $P = 0{:}05$.

Table 6 Average values of percentage residual and decomposed cholesterol and dry biomass (mg/100 ml) of the most cholesterol-decomposing bacterial isolates after 5 days growth on cholesterol medium containing 100 mg cholesterol/100 ml.

Isolate No.	Source of isolation	Residual cholesterol (mg/100 ml)	Decomposed cholesterol (mg/100 ml)	Dry weight (mg/100 ml)
9	Chicken liver	72.77	27.23	143.3
13	Turkey giblets	44.59	55.41	234.5
20	Salmon	65.81	34.19	166.7
25	Lamb	53.89	46.11	197.1
29	Egg yolk	61.48	38.52	174.8
L.S.D.				
$P = 0.01$		4.6	4.4	10.3
$P = 0.05$		6.7	6.8	13.7

Values for significant difference were determined according to Fisher's LSD test, $P = 0{:}05$.

respectively). The exposure of the bacterial cells to 16 min caused a drop in cholesterol decomposing activity as compared to the activity exerted at 8 min. The cholesterol decomposition in the reaction mixture was significantly decreased in the case of the culture medium developed from *S. fradiae* exposed to Nd-YAG irradiation for 32 min.

4. Discussion

A high level of serum cholesterol in human is generally considered to be a risk factor and increases the risk of the other conditions, depending on which blood vessels are narrowed or blocked (Pekkanen et al., 1990). Some of the diseases linked to high level of cholesterol include coronary heart disease, stroke, peripheral vascular disease, diabetes and high blood pressure.

Several studies have indicated that serum cholesterol level could be reduced by some bacterial species including lactic acid bacteria (Smith et al., 1991; Rasic et al., 1992; Tamai et al., 1996; Vesa, 1998), *Bifidobacterium bifidum* (Ghaleb et al., 1998), *Bacillus licheniformis* (Cardona et al., 2003), *Rhodococcus equi* (Chung et al., 1995), Kimoto et al. (2002) reported that seven strains of the genus *lactococcus* were able to remove cholesterol from laboratory media during growth without degrading cholesterol. The amount of cholesterol removed was strain specific. Among the tested strains was *Lc. Lactis* subsp *lactis* biovar *diacetylactis* N7 which could remove as much cholesterol as *L. acidophilus* ATCC 43121.

Table 7 Assay of cholesterol degrading enzyme activity in reaction mixture containing extracellular and/or intracellular enzyme of the most cholesterolytic bacterial isolates and cholesterol as a substrate.

Isolate No.	Cholesterol decomposed (%)	
	Extracellular enzyme	Intracellular enzyme
9	0.0	17.9
13	36.6	3.8
20	16.8	0.0
25	29.1	0.0
29	11.3	2.2
L.S.D.		
$P = 0.01$	3.2	2.1
$P = 0.05$	5.8	3.2

Values for significant difference were determined according to Fisher's LSD test, $P = 0{:}05$.

In the present investigation, the isolation of bacteria, which have the ability to utilize cholesterol as carbon source was carried out from different sources. Thirty-four bacterial isolates and twenty-two fungal isolates were recovered from the tested sources. The highest population of bacterial isolates were

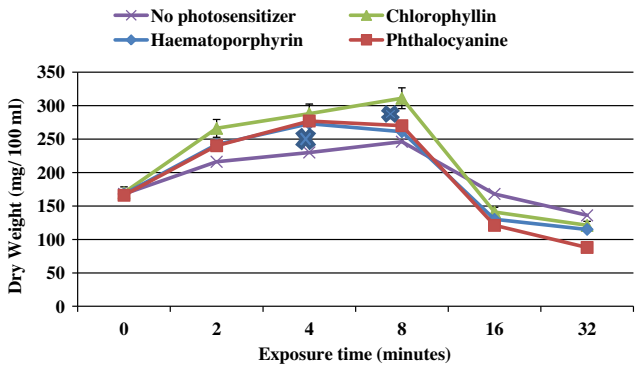

Figure 1 Effect of different doses of Nd-YAG laser emitted at power output of 210 mW, in absence and presence of photosensitizers, on dry weight gain (mg/100 ml) of *Streptomyces fradiae*. Treatments above an X are significant different from treatments below an X. Treatments that appear together with no X are not different.

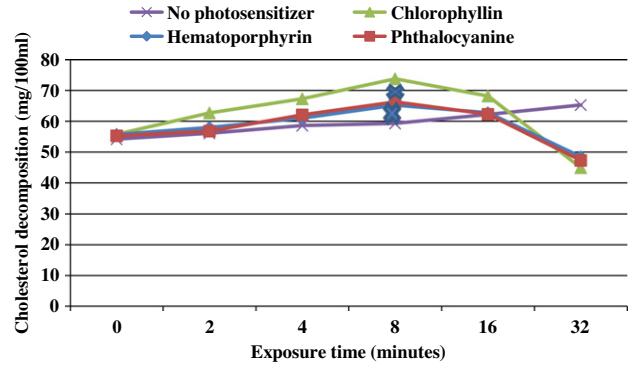

Figure 2 Effect of different doses of Nd-YAG laser emitted at power output of 210 mW, in absence and presence of photosensitizers, on decomposition of cholesterol (mg/100 ml) after 5 days growth of *Streptomyces fradiae* on cholesterol medium containing 100 mg cholesterol/100 ml. Treatments above an X are significant different from treatments below an X. Treatments that appear together with no X are not different.

estimated from soil (7 isolates/g) followed by turkey giblets (6 isolates/g), chicken liver, salmon, lamb egg yolk and beef brain (each of 4 isolates/g). It seems that there was no certain relation between the number of bacterial population recovered from each source and its cholesterol content. The extremely low number of isolates per gram recovered from the different cholesterol-richer sources indicates the difficulty of most bacterial isolates to metabolize the higher concentration of cholesterol or indicates the failure of bacteria to degrade cholesterol to intermediate products suitable for metabolism.

In similar previous studies, Watanabe et al. (1986) isolated cholesterol-degrading organisms from food of animal origin such as butter, bacon, pork and chicken fat. Most of the isolated strains were identified as belonging to the genus *Rhodococcus*. In view of other investigators, Paniangvait et al. (1995) detected cholesterol and its oxides in a variety of foods and foodstuffs, especially eggs, milks, meats, sea foods and their processed products.

In a preliminary screening of cholesterol decomposition by the isolated bacteria, it was found that 5 bacterial isolates induced the highest levels as cholesterol decomposers. The fungal isolates were less potent in decomposing cholesterol. Abo-El-Khair (1989) isolated 11 bacterial isolates together with 12 fungal species from 4 different Egyptian soils using cholesterol as sole carbon source. He reported that the bacterial isolates were more active in cholesterol decomposition than fungal isolates. The highest cholesterol degradative activity and bacterial biomass were achieved by *Mycobacterium fortuitum*.

In a confirmation of the preliminary screening, the 5 bacterial isolates were screened for their ability to decompose cholesterol in liquid cultures. The cholesterol decomposition ranged from 55.41% (by isolate no. 13) to 27.23% (by isolate no. 9). The high decomposing activity of the 5 isolates was mostly due to production of extracellular enzyme.

Cholesterol oxidase (cholesterol/oxygen oxidoreductase) is the first enzyme of the cholesterol degradation pathway found in many microorganisms. It catalyzes the oxidation of cholesterol (5-cholesten-3β-ol) to 4-cholesten-3-one. At the same time molecular oxygen is reduced to hydrogen peroxide. Since it was first described in a soil bacterium, the enzyme has been identified and purified from various microbial sources, including members of the genera *Nocardia*, *Streptomyces*, *Mycobacteria*, *Brevibacterium*, *Schizophyllum* and *Pseudomonas fluorescens* (Warnick 1986; Lolekha and Jantaveesirirat, 1992; Chen et al. 2006; Wang et al., 2008; Doukyu, 2009).

Several investigators demonstrated the ability of microorganisms (bacteria, actinomycetes and fungi) to utilize cholesterol as sole source of carbon (Watanabe et al., 1986, 1987; Zhang and Luo, 1998; Sojo et al., 2002; Sarkar, 2003; Gupta and Prabhu, 2004; Brzostek et al., 2009). Arima et al. (1978) reported that *Arthrobacter simplex*, *Bacillus roseus, Brevibacterium lypolyticum, Corynebacterium equi, C. sepedonicum, Microbacterium lacticum, M. avium, M. phlei, M. smegmatis*, *Nocardia erythropolis, N. gardneri, N. restrictus, Serratis marcescens* were able to decompose cholesterol. Several investigators reported that *Pseudomonas fluorescens*, *P. pyocyane*, *Mycobacterium fortuitum* and *Rhodococcus* sp. have the ability to degrade and utilize cholesterol as the sole source of carbon (Owen et al., 1983, 1978; and Watanabe et al. 1986, 1987, 1989).

The most potent cholesterol degrading bacterium (isolate 13) was identified as *S. fradiae*. It appears that the identified isolate was recovered from turkey giblets and not from the other investigated cholesterol richer materials. This indicates the adaptability of this isolate to produce sufficient enzyme (s) under different cholesterol concentrations. Cholesterol oxidase is derived from the several bacterium sources, including *Streptomyces*, (Srisawasdi et al., 2006).

In a trial to increase the cholesterol-degrading potentiality of the tested *S. fradiae*, the isolate was exposed to a low intensity laser radiation in presence of three photosensitizers, namely, chlorophyllin, haematoporphyrin phthalocyanine. The exposure of the chlorophyllin photosensitized bacterium to 210 mW Nd-YAG laser for 8 min induced significant increase in cholesterol degrading activity reaching 73.8% as compared with 59.3% in the case of non-irradiated, non-photosensitized *S. fradiae*. However, the prolongation in duration of exposure to 32 min led to a decrease in cholesterol-degrading activity in the case of the photosensitized cells.

Table 8 Assay of cholesterol degrading enzyme activity (calculated as % cholesterol decomposition) in reaction mixture containing cholesterol as a substrate and extracellular crude enzyme of non-irradiated or 210 mW Nd-YAG laser irradiated *Streptomyces fradiae* in presence or absence of some photosensitizers.

Exposure time (min)	No photosensitizer	Photosensitizers			L.S.D.	
		Chlorophyllin	Hematoporphyrin	Phthalocyanine	$P = 0.05$	$p = 0.01$
0	28.3	29.6	29.7	28.8	3.0	5.1
2	31.7	34.4	31.3	30.2	4.0	5.3
4	35.6	41.8	38.6	37.1	3.3	4.7
8	40.7	53.7	46.0	44.6	2.1	4.2
16	45.8	39.9	40.0	41.0	3.6	4.9
32	48.9	30.8	34.8	36.9	3.0	4.3
L.S.D						
$P = 0.05$	3.8	2.5	2.9	3.6		
$P = 0.01$	4.8	4.6	4.2	5.0		

Values for significant difference were determined according to Fisher's LSD test, $P = 0{:}05$.

Several types of low-intensity laser radiation, including helium neon laser (He–Ne), the gallium aluminum laser (Ga–Al), the helium neon arsenate laser (He–Ne–As), the gallium arsenate laser (Ga–As) and the diode laser, were tried against different microorganisms to stimulate the growth (Karu et al., 1984). Geweely et al. (2006) indicated that a dose of 7.3 mW He–Ne laser irradiation of *Aspergillus terreus* in the presence of photosensitizer, induced an increase in the production of xylanase. Jiang et al. (2007) reported an increased growth and phenol biodegradation rates on irradiation of *Candida tropicalis* by using a He–Ne laser probably due to promotion of phenol hydoxylase. They also found that four amino acids in phenol hydrolyase gene were mutated by irradiation and the mutant strain possessed a higher capacity to resist phenol toxicity than wild strain. The use of low-power laser irradiation technology to mutate the biological strains has been attracting attention. Kohli et al. (2001) have reported that irradiation with a He–Ne laser (632.8 nm) could stimulate *Escherichia coli* strain KY706/pPL-1, which led to the induction of *phr* gene expression. The optimal irradiation parameters were also obtained. Karu et al. (1994) determined the mechanisms of the effects of irradiation on *E. coli* with a He–Ne laser, and the quantity of viable cells changed in the irradiated culture.

In conclusion, The Nd-YAG laser-induced technique can be successfully applied in microorganism to stimulate its capacity of degradation.

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