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# ISOLATION AND CHARACTERIZATION OF CHOLESTEROL-OXIDIZING BACTERIA FROM SOIL

Botany

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## ABSTRACT

**BACKGROUND:** Cholesterol and triglyceride are two main lipids in blood that are carried in lipoproteins as high-density lipoproteins (HDL), low-density lipoproteins (LDL) and very low density lipoproteins (VLDL). Cholesterol Oxidase is bifunctional flavoenzymes that catalyze the oxidation of steroid substrates which have a hydroxyl group at 3β position of the steroid ring system. **AIM AND OBJECTIVES:** The present study has been under taken to isolate and characterize the cholesterol oxidizing soil bacteria. **METHODS:** Cholesterol oxidizing bacteria were isolated from soil sample by enriched culture technique. Two types of media were used for this purpose. Medium-I was enriched by adding cholesterol as sole carbon source. Cholesterol Oxidase producing bacterial isolates were identified by staining technique on the growth medium in presence of suitable indicator plates. **RESULTS:** Eight strains of cholesterol oxidizing bacteria were isolated from the soil sample as confirmed by colony staining technique and cholesterol oxidase indicator plates. These included *Arthrobacter* sp., *Brevibacterium* sp., *Bacillus* sp., *Corynebacterium* sp., *Nocardia* sp., *Rhodococcus* sp. and *Pseudomonas* sp. and *Streptomyces*. **CONCLUSIONS:** These bacterial isolates can be considered as cholesterol Oxidase producers because all these isolates changed the colour of the medium to intense brown.

## KEYWORDS

Cholesterol Oxidase, Cholesterol, Triglycerides, Soil bacteria

## INTRODUCTION

Cholesterol and triglyceride are two main lipids in blood that are carried in lipoproteins as high-density lipoproteins (HDL), low-density lipoproteins (LDL) and very low density lipoproteins (VLDL). In fasting serum, most of the cholesterol is carried on LDL particles and is therefore referred to as LDL cholesterol. Most of the triglyceride is found in VLDL particles.

Cholesterol Oxidase (EC 1.1.3.6), also known as 3β-Hydroxysteroid or oxygen oxido-reductase, is bifunctional flavoenzymes that catalyze the oxidation of steroid substrates which have a hydroxyl group at 3β position of the steroid ring system. Cholesterol Oxidases are the flavin adenine dinucleotide (FAD)-containing enzyme that catalyze the first step of cholesterol (cholest-5-en-3 β-ol) catabolism involving a two step process - firstly, the dehydrogenation of C-(3)-OH of a cholestane system to yield the corresponding carbonyl product i.e. the oxidation of the C-OH group of cholesterol and other sterols to give corresponding Δ5-3-ketone (cholest-5-en-3-one); and secondly, the isomerization of Δ5-3-ketone to Δ4 –3-ketone (cholest-4-en-3-one) (Loredano *et al*[1]). Cholesterol Oxidases exist in two forms, one with the FAD cofactor bound non-covalently to the enzyme (Type-I) and other with the cofactor linked covalently to the protein (Type-II) (Sampson *et al*[2]).

Cholesterol Oxidases are produced by a variety of pathogenic and non-pathogenic bacteria (Doukyu *et al*[3], Tahri *et al,*[4] Wantanabe *et al*[5]), actinomycetes (Fukuda *et al*[6] Inoye *et al*[7], Kreit *et al*[8] Lartillot *et al*[9], Richmond[10]) in order to utilize cholesterol as their energy source. Our knowledge regarding the cholesterol oxidizing bacteria is insufficient and hence the present study has been under taken to isolate and characterize the cholesterol oxidizing soil bacteria.

## MATERIALS AND METHODS

Cholesterol oxidizing bacteria were isolated from soil sample by enriched culture technique of Yazdi *et al*[11]. Two types of media were used for this purpose. Medium-I was enriched by adding cholesterol as sole carbon source. This medium consisted of $NH_4NO_3$-1 g, $K_2HPO_4$-250 mg, MgSO4.7$H_2$O-250 mg, NaCl-5 mg, $FeSO_4.7H_2O$-0.5 mg, Cholesterol-1 mg and Distilled water-1000.0 ml. pH of the medium was adjusted to 7.0 by adding 10 %NaOH before autoclaving. The soil sample (1 g) was serially diluted and 1 ml of $10^{-4}$ dilution was poured to this medium. The broth was then plated onto nutrient agar medium (Medium II) consisted of 10 % peptone, 1 % yeast extract, 0.5% NaCl and 2 % agar and pH 7.0. The colonies appeared on the agar plate were picked up and used for morphological and biochemical tests for the identification of bacteria. Cholesterol Oxidase producing bacterial isolates were identified by staining technique on the growth medium in presence of suitable indicator plates following the methods of Lashkarian et al[12]. The results of the present investigation have been presented in Table-1 and 2.

**Table-1: Biochemical Characterization Of Cholesterol Oxidase Producing Bacteria**

| Isolates | Gram staining | Motility | Indole | Methyl red | Voges-Proskauer | Citrate | Catalase | Oxidase | Nitrate | Urease | Starch hydrolysis | Hemolysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Arthrobacter* | + | + | - | + | - | - | + | + | + | - | - | - |
| *Brevibacterium* | + | + | - | + | - | - | + | + | + | - | + | - |
| *Bacillus* | + | + | - | + | - | - | + | + | + | - | + | - |
| *Corynebacterium* | + | + | - | + | - | - | + | + | + | - | - | - |
| *Nocardia* | + | + | - | + | - | - | + | + | + | - | - | |
| *Pseudomonas* | - | + | - | - | - | + | + | + | + | - | - | + |
| *Rhodococcus* | + | + | - | + | - | - | + | + | + | - | - | - |
| *Streptomyces* | + | - | - | + | - | + | + | - | - | - | - | - |

**Table-2: Population Density Of Cholesterol Oxidizing Bacteria**

| Cholesterol oxidizing bacteria | Population density in CFU/g of soil |
|---|---|
| *Arthrobacter* | 4.7 X $10^8$ |
| *Brevibacterium* | 3.8 X $10^8$ |
| *Bacillus* | 4.5 X $10^8$ |
| *Corynebacterium* | 4.8 X $10^8$ |
| *Nocardia* | 4.6 X $10^8$ |
| *Pseudomonas* | 5.5 X $10^8$ |
| *Rhodococcus* | 3.7 X $10^8$ |
| *Streptomyces* | 6.5 X $10^8$ |

## RESULTS AND DISCUSSION

Eight strains of cholesterol oxidizing bacteria were isolated from the soil sample as confirmed by colony staining technique and cholesterol oxidase indicator plates. These included *Arthrobacter* sp., *Brevibacterium* sp., *Bacillus* sp., *Corynebacterium* sp., *Nocardia* sp., *Rhodococcus* sp. and *Pseudomonas* sp. and *Streptomyces*. The morphological and biochemical characteristics of these isolates have been presented in Table-1, and can be considered as cholesterol Oxidase producers because all these isolates changed the colour of the medium to intense brown. The population of cholesterol producing

bacteria is illustrated in Table-2.

Except *Streptomyces* all bacterial isolates were motile, and all the eight strains exhibited indole negative test. All the isolates except *Pseudomonas* exhibited negative methyl red test. All the eight isolates exhibited negative Voges Proskauer and urease tests. Only *Pseudomonas* and *Streptomyces* showed citrate positive. Catalase test was found to be positive for all the eight bacterial isolates. All showed positive Oxidase and nitrate tests except *Streptomyces*. The starch hydrolysis test was positive only for *Brevibacterium* and *Bacillus.* Except *Pseudomonas* all the isolates exhibited negative test for hemolysis (Table-1).

The population density of *Streptomyces* was maximum ($6.5 \times 10^8$), followed by *Pseudomonas* ($5.5 \times 10^8$), *Corynebacterium* ($4.8 \times 10^8$), *Nocardia* and *Corynebacterium* ($4.8 \times 10^8$ and $4.6 \times 10^8$ respectively) and minimum for *Brevibacterium* and *Rhodococcus* ($3.8 \times 10^8$ and $3.7 \times 10^8$ respectively) (Table-2).

In the isolation media cholesterol Oxidase indicator plates were used to identify the cholesterol oxidizing soil bacteria. The present investigation gains support from the work of Saranya et al[13], Hamed Esmaeil Lashgarian *et al*[14], Lata Kumari *et al*[15] etc.

## CONCLUSIONS:

From the present investigation, it can be concluded that the eight bacterial isolates viz. *Arthrobacter* sp., *Brevibacterium* sp., *Bacillus* sp., *Corynebacterium* sp., *Nocardia* sp., *Rhodococcus* sp. and *Pseudomonas* sp. and *Streptomyces* sp. showed cholesterol Oxidase activity.

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## Conflict Of Interest:

Authors declare no conflict of interest directly or indirectly.

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