EVALUATION AND SCREENING OF THE CHOLESTEROL-DEGRADING ABILITY OF SOME BACTERIAL ISOLATES

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ABSTRACT

Cholesterol is a waxy, fat-like substance that is made in the body and is found in all cells of the body. The synthesis and utilization of cholesterol must be tightly regulated in order to prevent over accumulation and abnormal deposition within the body. Many bacteria can produce this enzyme. In the present study, a total of 11 cholesterol-degrading bacterial strains were isolated from soil samples. The studies on the morphological and cultural character of cholesterol-degrading bacteria were investigated.

Out of Total 11 different bacterial isolates were studied on the basis of color, elevation, opacity, margin, surface, pigmentation, and character. The studies on evaluating the effect of cholesterol-degrading bacteria on cholesterol concentration were conducted by the CHOD-POD method. The results reveal that all the samples showed a significant decrease in cholesterol concentration, out of which the maximum and minimum decrease levels were 97.20% and 42.88%, respectively.11th isolates and 5th isolates, respectively, as compared to standard cholesterol. From the results, it was observed that all the strains tested showed a significant decrease in the level of cholesterol concentration. Out of 11 isolates, the maximum decrease, i.e., 97.20%, was followed by the eighth isolate, which showed 84.49%, and the fourth isolate, which showed 63.53%, was preliminary taken into consideration as cholesterol-degrading activity, which may be further studied.

(Key words:- Cholesterol and waxy materials, CHOD-POD method, Cholesterol Degrading enzymes.)

I. INTRODUCTION

Cholesterol is a waxy, fat like substance that is made in the body and is found in all cells of body. Cholesterol can also be obtained from the diet .Cholesterol is used in the body to make hormone, vitamin D and substance to aid in digestion. Cholesterol is extremely important biological molecule that has roles in membrane structure as well as being a precursor for the synthesis of the steroid hormones and bile acids. Drzyzga, O. et al (2009)reported that the degradation of cholesterol by a member of the genus Gordonia. In this study the potential of Gordoniacholesterolivorans to use cholesterol as the only carbon and energy source for growth and to degrade other steroid compounds with long carbon side chains (C27). Additionally, show that the pyrosequenced genome of G. cholesterolivorans contains two putative genes that code for conventional intracellular acting cholesterol oxidases, both genes located in unique genetic organizations when compared with the genomes of other cholesterol-degrading bacteria.

FAO/WHO (2001) suggested that people affected with hypercholesterolemia may avert the use of cholesterol-lowering drugs by practicing dietary control or supplementation of probiotics and/or prebiotics.Fernandez de lasHeras L., et al (2011) described that extracellular cholesterol oxidase activity with whole bacterial cells is indicated by the production of brown color in the agar and minimal medium around the colonies. Kim et al. (2001), investigated that the role of cholesterol-degrading bacteria in the fermentation of Korean traditional fermented seafood as well as isolated and characterized a bacterial strain, *B. subtilis* SFF34, producing a

high level of extracellular cholesterol oxidase from Korean traditional fermented flatfish.

Cholesterol oxidase (CHO) is an enzyme which catalyse the oxidation of cholesterol and converts 5-cholesten-3β-ol into 4-cholesten-3-one.Many bacteria can produce this enzyme including members of the genera *.Arthobacter, Brevibacterium, Pseudomonas, Nocardia, Rhodococcus ,Treptomyces, Cornebacterium*and *Schizophyllum*.This enzyme can be produced from a bacterium in 3 forms: intracellular, extracellular and membrane bound. Due to the wide spectrum application of (CHO), screening and isolation of bacterial strains producing extracellular form of (CHO), screening and isolation of bacterial strains producing extracellular form of (CHO) are of great importance.

Microbial cholesterol oxidase is an enzyme of great commercial value, widely employed by laboratories routinely devoted to the determination of cholesterol concentration in serum, other clinical sample and food. In addition, the enzyme has potential application as a biocatalyst which can be used as an insecticide and for the bioconversion of a number of sterol and non-steroidal alcohols. The enzyme has several biological roles, which are implicated in the cholesterol metabolism, the bacterial pathogenesis and the biosynthesis of macrolide antifungal antibiotics. Cholesterol oxidase has been reported from a variety of microorganism. Recently reported cholesterol oxidises from gram negative bacteria such as *Burkholderia*and *Chromobacterium.*

Arenskotter, (2004) and Drzyzga, O.,et al(2009) proved that cholesterol degradation by Gordoniae appear to be widely distributed in nature, and strains have been isolated from environments such as soil, wastewater, estuary sand, mangrove rhizosphere, oil-producing wells, sewage sludge, and activated sludge foam as well as from clinical samples (Arenskotter, (2004)and Blaschke, A. J., et al. (2007). According to Maris, A. E., et al (2005.), the long leader sequence (232 bp) of the mRNA of cholesterol degradative G. cholesterolivorans contains a putative binding site for the OmpR regulator, a two domain response regulator frequently found in Gram-negative bacteria such as *Escherichia coli.*

Doukyu, N. (2009), Fernandez de lasHeras, et al (2009), Kreit, Jer al(2009), Pollegioni, L., et al (2009), Van der Geize, R., et al. (2007), Vrielink, A., and S. Ghisla(2009) showed that richness of metabolic activities of gordoniae and widen our view about the possible environmental and industrial application of these bacteria. The ability to degrade steroid compounds such as cholesterol by members of the genera *Rhodococcus, Mycobacterium, Streptomyces, Brevibacterium,* and some further Gram-positive genera as well as some Gram-negative genera such as *Pseudomonas, Comamonas, Burkholderia, and Chromobacterium* is well documented.

Fernandez de lasHeras L., et al (2011) described that extracellular cholesterol oxidase activity with whole bacterial cells is indicated by the production of brown color in the agar and minimal medium around the colonies.

The WHO (2009) predicted that, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the World.Experimentally proved that many bacteria can produce cholesterol oxidase (CHO) enzyme including members of the genera *Arthrobacter, Brevibacterium, Pseudomonas, Nocardia, Rhodococcus, Streptomyces, Corynebacterium and Shizophyllum.*

II. MATERIALS AND METHODS

In order to screen microorganisms with special interest to study the cholesterol degrading soil originating bacteria present work has been undertaken.

A. ISOLATION OF CHOLESTEROL DEGRADING BACTERIA

The soil samples approximately 100gm were collected from local farm, petrol pump and urban composts. All the samples were transported to research laboratory and mix together so as to produced composite soil sample and further subjected for dilutions.

To create a homogenate mixture, a 1 gram sample of composite soil was dissolved in 100 milliliters of distilled water that had been sterilized and blended on a cyclomixer. A 30-

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minute centrifugation at 1000 rpm was performed on the homogenate. Studies on isolation were conducted using the resulting supernatant.

The spread plate method was used to prepare the cholesterol medium (A) plates, which were then inoculated with 0.1 ml of supernatant and incubated for 12 days at 300 C.

The colonies grow on cholesterol medium (A), which was then re-injected into cholesterol medium (B) and infected again for a whole day at 300 degrees Celsius. After being subcultured on a cholesterol medium (B) slant, each colony on cholesterol medium B was incubated for 24 hours at 300 C. Next, the colonies grown on the cholesterol medium (B) slant were analyzed to look for cultural traits.

B. ENRICHMENT AND EXTRACTION OF CRUDE ENZYME

Pure sub cultured colonies further enriched in cholesterol medium broth (B) and incubated at 30 ^{0} C for 24 hour. The enriched culture was homogenizes to obtain cell free extract. The cell free extract contain crude enzyme was ultra-centrifuged at -5^{0} C for 1000 rpm (revolution per minute) for 3 mins. The supernatant thus obtain is taken for the evaluation decreasing total cholesterol concentration. Evaluation of decreasing total cholesterol concentration: - The cholesterol degradation was analyzed by CHOD - POD Enzymatic Method (D.V.Plummer,(2006).

III. RESULT AND DISCUSSION

The study on screening of the cholesterol degrading bacteria has been undertaken. The observation obtained in present study with its discussion of literature has been presented as follows:-

Sr. No.	**Shape**	**Colour**	**Elevation**	**Opacity**	**Margin**	**Surface**	**Pigmentation**	**Gram character**
1	Circular	White	Convex	Opaque	Entire	Smooth	Slight yellow	Gm(+)ve, cocci, purple
2	Circular	Milky white	Convex	Opaque	Entire	Smooth	No	Gm (-)ve,cocci,pink
3	Circular	White	Convex	Opaque	Entire	Smooth	Dark yellow	Gm(+)ve,cocci,purple
4	Circular	White	Convex	Opaque	Entire	Smooth	No	Gm(-)ve,cocci,pink
5	Circular	White	Submerged	Opaque	Entire	Smooth	No	Gm(-)ve,cocci,pink
6	Circular	White	Convex	Opaque	Entire	Smooth	No	Gm(-)ve,cocci,pink
7	Circular	Slightly Yellow	Submerged	Opaque	Entire	Smooth	Greenish yellow	Gm(+)ve,cocci,purple
8	Circular	White	Submerged	Opaque and transparent	Entire	Smooth	Slight green	Gm(+)ve,cocci,purple
9	Circular	White & yellow	Submerged	Opaque	Entire	Smooth	Slight green with yellow tinch	Gm(+)ve,cocci,purple
10	Circular	White	Convex	Opaque	Entire	Smooth	yellow	Gm(-)ve,cocci,pink
11	Circular	White	Convex & submerged	Opaque	Entire	Smooth	yellow	Gm(-)ve,cocci,pink

Table (1):- colony characteristics of cholesterol degrading bacteria isolates.

Numerous bacterial species have been implicated in the biodegradation of cholesterol through the action of functional cholesterol oxidase, which contains flavin adenine dinucleotide and oxidizes cholesterol to 4-

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cholesten-3-one while reducing oxygen to hydrogen peroxide.

Cholesterol oxidase has drawn a lot of attention since it is more widely used to detect cholesterol in food and blood samples, which have a direct, impact on lipid disorders such atherosclerosis and coronary heart disease. Furthermore, the synthesis of steroids involves the usage of cholesterol oxidase. While cholesterol is an essential component in the human body, as people age,

Solution	Optical density (505nm)	Total cholesterol (mg/dl)	Percent decrease in cholesterol concentration
Blank	0.162		
Standard	0.3148		
Test			
1	0.155	98.47	49.23
2	0.160	101.65	50.82
3	0.186	118.17	59.08
4	0.200	127.06	63.53
5	0.135	85.76	42.88
6	0.189	120.07	60.0035
7	0.160	101.658	50.829
8	0.266	168.99	84.49
9	0.162	102.92	51.46
10	0.170	108.00	54.00
11	0.179	194.40	97.20

Table (2): Effect of cholesterol degrading bacteria isolates on cholesterol concentration

In the present study total 11 cholesterol degrading bacterial strains were isolated form soil samplesthe studies on morphological cultural character of cholesterol degrading bacteria represented (Table 1).A total of 11 different bacterial isolates were studied on the basis of color elevation opacity margine surface pigmentation and grams characters as shown in table No.1.

Studies on evaluating effect of cholesterol degrading bacteria on cholesterol concentration were conducted by CHOD-POD Method. Result revels that all the samples showed significant decrease in cholesterol concentration. Out of which maximum and minimum decrease level were 97.20 % and 42.88 % by eleventh isolates and 5 th isolates respectively. As compare to standard cholesterol. (Fig.No. 1). As per results it was observed that all the strains tested showed significant decrease in the level of cholesterol concentration out of eleven isolates maximum decrease 97.20 % followed by 18 th isolates showed 84.49% 4 th isolates showed 63.53% was preliminary taken in to consideration as cholesterol degrading activity which may be further study. The result from present studies enlighten that cholesterol degrading bacteria were screen from the soil samples.

The cholesterol degradation was calculated using the enzymatic colorimetric cholesterol oxidase peroxidase technique. Using a spectrophotometer, growth was observed at 600 nm. The cholesterol assay was carried out using the Merck cholesterol estimation kit. Every reagent was thoroughly combined in accordance with the manufacturer's instructions. 10 µL of cell free supernatant (CFS) was added to the reaction mixture, mixed by inversion, and incubated for 10 minutes at 37°C.

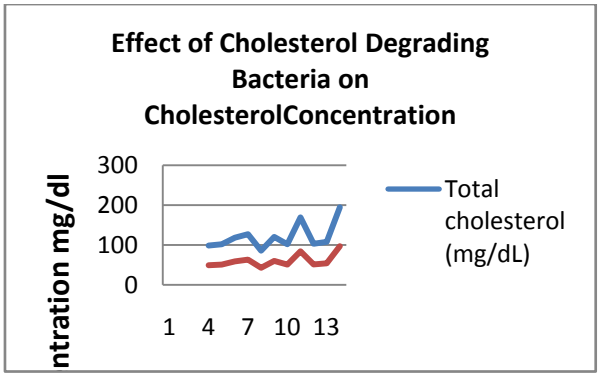

Fig. No. 1:- Effect of Cholesterol Degrading Bacteria on Cholesterol concentration

The results showed that sample number 6,7, 11 and 14howed the significant degradation of cholesterol by bacterial species. Kim et al. (2001) investigated that the role of cholesterol-degrading bacteria in the fermentation of Korean traditional fermented seafood as well as isolated and characterized a bacterial strain, *B. subtilis* SFF34, producing a high level of extracellular cholesterol oxidase from Korean traditional fermented flatfish.Kimoto et al(2002) examined the removal of cholesterol by several strains of *lactococci* from media.

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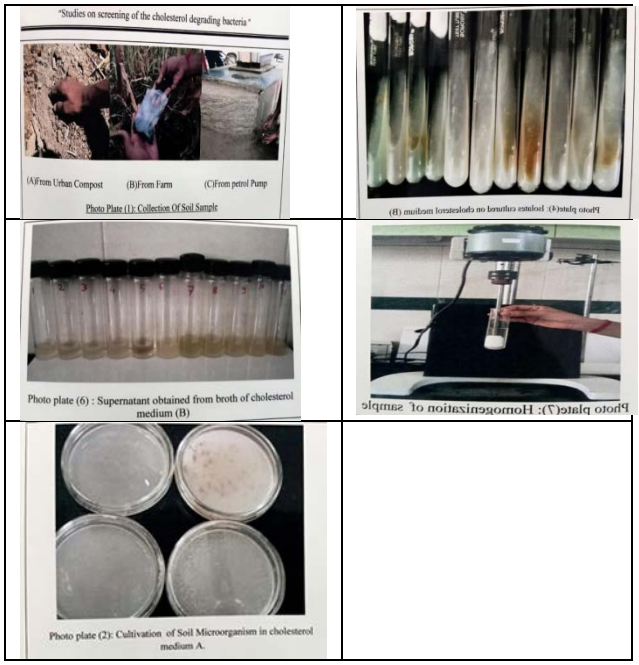

KirtiPawar et al (2011) experimenatally proved that many microorganisms were found to utilize cholesterol as sole source of carbon and energy.A pharmacologically important 17-keto steroid accumulated during side chain cleavage of cholesterol by *Pseudomonas putida* MTCC 1259 when n-propanol was used as an inhibitor of ring cleavage.

Lambert, JM et al (2008), Pereira, D.I.A. et al (2002), Liong. M.T. (2006), Liong, M.T. (2005), Lye, H. S. (2010), De Preter, V. (2007) studied that efficacy of probioties in reducing cholesterol often do not sufficiently address the mechanisms by which probiotics modulate hypocholesterolemic effects and the optimum dose, frequency, and duration of treatment for different probiotic strains Several mechanisms have been hypothesized, which include enzymatic de-conjugation of bile acids by bile-salt hydrolase of probiotics, assimilation of cholesterol by probiotics, co-precipitation of cholesterol with deconjugated bile, cholesterol binding to cell walls of probiotics, incorporation of cholesterol into the cellular membranes of probiotics during growth, conversion of cholesterol into co-prostanol and production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics.

IV. CONCLUSION

Soil microorganism may utilize cholesterol as there carbon source. Remarkable decreased in cholesterol may enlighten the cholesterol degrading bacteria to be manifested for the development of pro/pre-biotic specially to prevent the cardio artery diseases.

V. RECOMMEDDATION

Cholesterol degrading bacteria may be provided as pre/pro-biotic nutrient supplement. However, in vivo studies in healthcare environment. Cholesterol degrading enzyme can also been isolated and characterized with respective to the development of management of coronary heart diseases.

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