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Degradation of Cholesterol by Probiotic Bacteria Grown in Presence of Polyphenol Tannic Acid Extracted from Cocoa Beans.

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Abstract: ***Polyphenols are a structural class of mainly Natural, but also synthetic or semi synthetic, organic chemicals characterized by the presence of large multiples of phenol structure units. Cholesterol is a type of lipid molecule, and is biosynthesized by all animal cells. Tannic acid was extracted from cocoa beans by using organic solvent methanol.Polyphenol - tannic acid supports the growth of probiotic bacteria when grown on Rogosa Agar medium containing Tannic Acid (10mg/ml). Two types of colonies from Rogosa agar plates grown individually on Rogosa medium slant and then inoculated in MRS broth containing Cholesterol (200mcg/ml). After incubation checked the decrease in Concentration of Cholesterol by using Wybenga& Pileggi Reagent and take O.D at 530nm.***

Keywords: Polyphenol, Tannic acid, Probiotic bacteria, Cholesterol, Degradation, Wybenga& Pileggi method

1. Introduction

Cholesterol is a type of lipid molecule, and is biosynthesized by all animal cells, because it is an essential structural component of all animal cells membrane and it is essential to maintain both structural integrity and fluidity. But high concentration of cholesterol anabolism leads many health illnesses. High Cholesterol can cause atherosclerosis, a dangerous accumulation of cholesterol and other deposits on the walls of arteries, this deposit can reduce blood flow through arteries which can result in chest pain. Cardiac arrest etc.In addition, cholesterol serves as a crucial precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D. The World Health Organization (WHO) reported that Cardiovascular Diseases (CVDs) were responsible for 30% of deaths worldwide and predicted that CVDs will remain the leading causes of death. Cholesterol-lowering ability of certain pharmacological agents, unwanted side effects can occur in some cases, such as gastro intestinal discomfort. Many *Mycobacterium*species known to degrade the Cholesterol, but due to their pathogenic nature, they cannot use for preventive measure of excess production of Cholesterol. This study was carried out to check the degradation of Cholesterol by Probiotic bacteria isolated from probiotic capsule grown in presence of Polyphenol – Tannic Acid, as polyphenols support the growth of probiotic bacteria and probiotic bacteria shows many metabolic pathways for degradation of many organic compounds.

2. Materials and Methods

A] **Preparation of suspension of probiotic bacteria:**
For preparation suspension probiotic capsule "Vibact" was used. Entire capsule was dissolved in 10 ml sterile saline. Vortex the suspension to mix the content properly.

B] **Isolation of bacteria in presence of polyphenol-tannic acid**
Loopful of suspension was streaked on Sterile Rogosa agar plate containing 10mg/ml of Tannic acid. Incubate plates at Room temperature under anaerobic condition for 48 hours. After the isolation pass the pure colonies from same plate on Sterile Rogosa agar slants individually incubate at Room temperature under anaerobic condition for 48 hours.

C] **Estimation of cholesterol in medium after utilization by probiotic bacteria**
Prepared the suspension of isolates passed on Rogosa agar slants individually in different tubes by using sterile saline adjusted O.D to 0.5, Inoculated 1ml of O.D adjusted culture in Sterile Rogosa Broth medium containing 200mcg/ml of Cholesterol. Incubated for 48 hours at Room temperature. After the incubation the broth medium is transferred to Centrifuge tubes for centrifugation at 5000rpm for 15 minutes to separate the cells from medium and supernatant is used as test sample for estimation of Cholesterol by using Wybenga& Pileggi reagent. Total Cholesterol content can be calculated by using following formula-

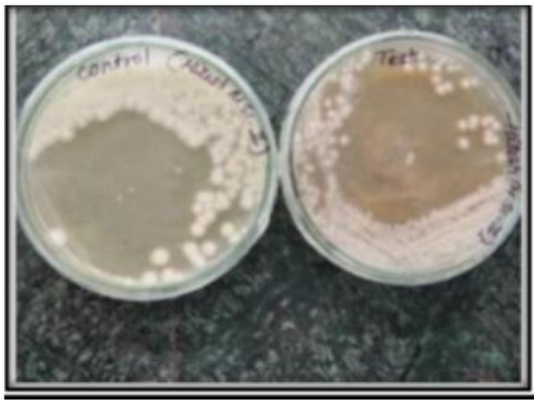
Cholesterol content=(Absorbance of Test ÷ Absorbance of Standard.)×Conc. of Standard

Content	Blank	Standard Cholestrol	Test sample
Distilled Water	0.1ml	-	-
Sample	-	-	0.1 ml
Std cholesterol	-	0.1 ml	-
W-P Reagent	2.0 ml	2.0 ml	2.0 ml
O.D at 530 nm	-	-	-

Note – After adding reagent all tubes keep in Boiling water Bath for 90 Seconds. Cool it and take O.D

3. Results

A] Isolation of probiotic organisms
From suspension prepared by using Vibact probiotic capsule in Sterile saline and streaked on Sterile Rogosa medium agar plate with and without Tannic acid. Same growth pattern is observed, indicated that polyphenol Tannic acid extracted from cocoa beans supported the growth of probiotic organisms.



B] Estimation of cholesterol in medium after utilizing by probiotic bacteria

Particulars	O.D at 530 nm
Blank	0.0
Standard	0.62
Test sample 1	0.45
Test Sample 2	0.36

Calculation:

1) Test Sample 1 -
Cholesterol content = (0.45÷ 0.62) × 200
= 144 mcg/ml

2) Test Sample 2–
Cholesterol content = (0.36÷0.62) × 200
=116 mcg/ml

4. Conclusion

Polyphenols are the compound known to boost up the immunity and support the growth of probiotic bacteria which have diverse functions and plays an important roles in metabolism of many organic compounds. Cholesterol (200 mcg/ml) was incorporated in medium which was metabolised by inoculated probiotic bacteria which was detected by Wybenga& Pileggi's Method. The cholesterol content in two mediums after metabolising by probiotic bacteria was 144mcg/ml and 116 mcg/ml in sample 1&2 respectively. Hence this experiment concluded that the Probiotic Bacteria grown in presence of polyphenols degrade the Cholesterol. To reduces the Cholesterol level and to maintain proper metabolism one should use probiotic food along with proper polyphenolic fruits, vegetables etc. in diet.

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