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## In-vitro Cholesterol Reducing Property of Human Gut Bacteria from Rourkela Population, Odisha, India.

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### ABSTRACT

Probiotic bacteria are reported to reduce cholesterol by different mechanisms and hence can be utilized to reduce the blood cholesterol level of hypercholesterolemic patients. Probiotic bacteria must be population specific to be used in particular population. Bacteria isolated from faeces of healthy volunteers from Rourkela, Odisha, India were screened for their cholesterol reducing property in MRS broth for 72 hours. Results showed that cholesterol reducing property was increased with time of incubation and there was no increase in cholesterol reduction potential of the gut isolates in presence of 0.05% bile salt in media. Three isolates MS1, MS4 and MS7 were most potent in reducing cholesterol according to our present study. These isolates could emerge as population specific probiotics after further studies.

**Keywords:** Probiotics, Cholesterol, Hypercholesterolaemia, Anti-hypercholesterolaemic, Human gut isolate.

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## INTRODUCTION

Hypercholesterolemia is the major causative agent of coronary diseases. Diseases related to hypercholesterolemia have been predicted to be the number one leading cause of death in the world by 2020 [1]. There is growing interest in the use of probiotics with cholesterol-lowering properties to prevent cardiovascular diseases due to the adverse effects like myopathy and cognitive impairment shown by cholesterol-lowering drugs like statins [2]. Probiotics are live microorganisms administered to provide beneficial effects in human body. They are not only dose and strain specific but also population specific which means that probiotics that work in one population may not work in other population. Hence, it is essential to have probiotics targeted for particular population. Studies in humans indicated that role of fermented milk products as hypocholesterolaemic agents were inconsistent [3, 4] which emphasized the need of probiotics of human origin. Gut microorganisms from healthy individuals of particular population can be utilized as probiotics for that population to alleviate gastrointestinal disorders. Gerard [5] reviewed the mechanisms of metabolism of cholesterol and bile acids by gut microbiota. He concluded that targeting the gut microbiota to modify cholesterol and bile acids metabolisms might be new preventive therapeutic approach in various diseases including cholesterol gallstone disease, colon and liver cancers, inflammatory and metabolic diseases. Efficacy of microbial reduction of cholesterol to coprostanol in human gut is highly variable among population and the mechanisms remain unexplored [6]. In their review on manipulation of gut microbiota, Foxx-Orenstein and Chey [7] concluded by stating that more research is needed to rationally target microbe-directed therapies according to disease state. Hence, the present study has been designed to isolate microorganisms from faeces of healthy individual of Rourkela population, Odisha, India and to test their in-vitro cholesterol reducing property.

## MATERIALS AND METHODS

### Materials

MRS broth, cholesterol, bile, and fecal sample collection container were purchased from HiMedia laboratories, Mumbai, India. All other chemicals and solvents used in the present study were of analytical grade.

### Experimental

#### Collection of Faecal Sample

The protocol of the study was approved by ethical committee of ISPAT General Hospital, Rourkela, Odisha, India. Faecal sample was collected from a healthy volunteer in the age of 24 in HiMedia faecal collection container. The faecal sample was observed for the presence of cysts and parasite and certified for the healthiness by senior pathologist of ISPAT General Hospital, Rourkela, Odisha, India. Absence of cysts and parasite in faecal sample denotes the healthy status of the individual.

#### Isolation and Growth of Microorganisms From Faecal Sample

A loopful of certified faecal sample was enriched by inoculating into MRS broth and incubated at 37°C for 48 h. Then serially diluted sample was spread on MRS agar plates and isolated colonies were separately streaked onto fresh MRS agar plates.

#### Screening For Acid and Bile Tolerance

Isolated microbial colonies were incubated in their respective media for 48 hrs and then pelleted. The pellets were re-suspended and incubated in simulated fasted state Gastric Fluid (Sodium taurocholate(80 µM), Lecithin (20 µM), Pepsin(0.01 g), Lysozyme(0.01 g), Sodium Chloride(34.2 mM),  $\text{KH}_2\text{PO}_4$ (0.06 g), pH 1.6) for 3 hrs and simulated fasted state intestinal fluid (Sodium taurocholate(3mM), Lecithin(0.2mM), Maleic acid(19.12 mM), Sodium hydroxide(34.8 mM), Sodium Chloride (68.62 mM), Pancreatin (200 USP/mg): 0.05 g/100 ml, pH 6.5) for 2 hrs and a small inoculum from each was inoculated on to fresh media to select those which could withstand the hostile gastric and intestinal environments.

**In-Vitro Cholesterol Reducing Property**

A starter culture was prepared by inoculating acid and bile tolerant isolates into 10 ml of MRS broth. After 24 h incubation at 37°C the cultures were enumerated and 1ml of each culture was used to inoculate the freshly prepared MRS broth supplemented with 0.517 mM of cholesterol and 0.05% bile salt. Comparative study of reduction in cholesterol percentage was done by using media with and without bile salt. Zak’s method [8] was used to determine the amount of total cholesterol in spent broth and uninoculated sterile broth.

**Statistics**

All the data represented were mean of three different experiments.

**RESULTS AND DISCUSSION**

**Table 1: Morphological characterization, Gram reaction, acid and bile tolerance of the human gut isolates**

Isolate	Morphology	Gram reaction	Acid tolerance	Bile tolerance
MS1	Rod	Positive	Yes	Yes
MS2	Rod	Positive	Yes	Yes
MS3	Rod	Positive	Yes	Yes
MS4	Rod	Positive	Yes	Yes
MS5	Rod	Positive	Yes	Yes
MS6	Spherical	Positive	Yes	Yes
MS7	Oval	Positive	Yes	Yes
MS8	Oval	Positive	No	No
MS9	Oval	Positive	No	No
MS10	Oval	Positive	No	No
MS11	Spherical	Positive	No	No
MS12	Spherical	Positive	No	No

**Table 2: Inoculum level taken for the study of cholesterol reducing property**

Isolate	CFU/mL
MS1	$3.04 \times 10^9$
MS2	$0.57 \times 10^9$
MS3	$1.50 \times 10^9$
MS4	$1.84 \times 10^9$
MS5	$0.94 \times 10^9$
MS6	$4.96 \times 10^9$
MS7	$1.00 \times 10^9$

A total of 12 microorganisms were isolated from the fresh faecal sample and were designated with the strain numbers MS1 to MS12. Pure cultures of the isolates were maintained in MRS agar. Their cell morphology and Gram reaction were determined by Gram staining. Amongst the 12 isolates 4 were found to be yeasts, 3 were spherical and rest 5 were rods. All of them were gram positive (Table 1). Out of the 12 isolated organisms only 7 could survive the harsh gastric and intestinal conditions (Table 1). Cholesterol content in spent broth of individual isolates was studied after 24h, 48h and 72h of incubation at 37°C. Then the cholesterol reduction percentage was calculated in comparison to uninoculated broth. Cholesterol reduction by individual isolates was also studied comparatively in presence and absence of bile salt (0.05%). Table 2 shows the amount of inoculum of gut isolates taken for the study of cholesterol reducing ability. Cholesterol reduction property of gut isolates in the absence of bile is shown in figure 1 and figure 2 shows the same in the presence of bile. As clearly seen in figure 1 and figure 2, the cholesterol reducing property of human gut isolates are increased with increased in time irrespective of the presence of bile. Among seven isolates, cholesterol reduction capacity of MS4 isolate increased from 39% after 24 hours to 61% after 72 hours of incubation in the absence of bile. Similarly, MS7 isolate’s capacity also increased from 29% to 60% (Figure 1). When compared to other isolates, MS1 and MS7 performed better in the presence of bile. From figure 2, it is evident that MS1 and MS7 isolate’s ability to reduce cholesterol increased from 44% to 50% and 16% to 49.5%, respectively after 72 hours of incubation in the presence of bile. They were followed by MS4 with increase of

cholesterol reduction from 14.2% to 48% after 72 hours of incubation. In the present study, it was also observed that the presence of 0.05% bile in the media did not increase the cholesterol reducing effect of human gut isolates except MS1.

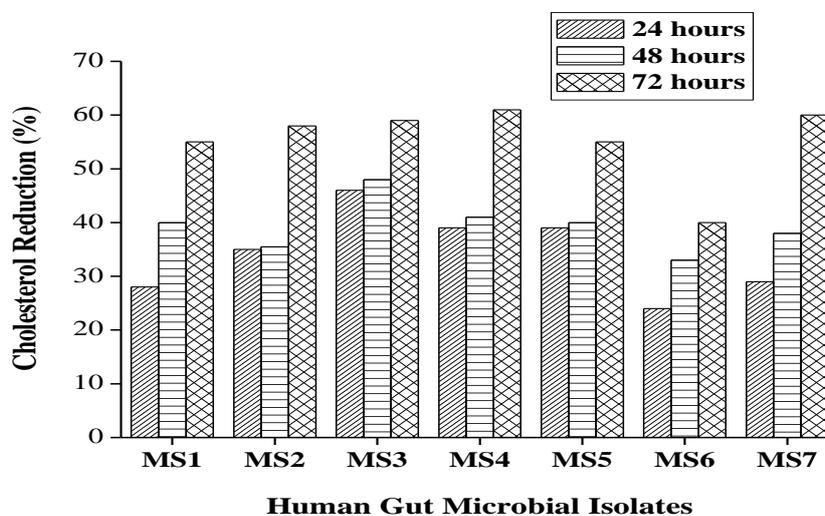


Figure 1: Cholesterol reduction percentage of gut isolates in absence of bile

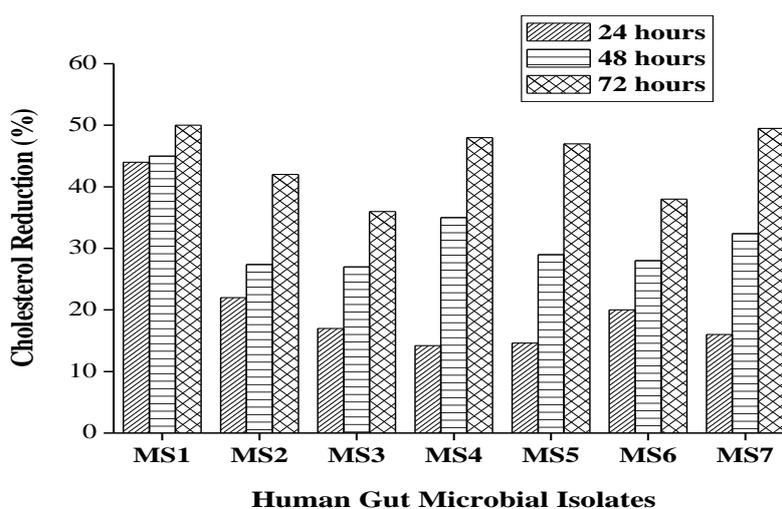


Figure 2: Cholesterol reduction percentage of gut isolates in presence of 0.05% bile salt

Gerard et al. [6] reported that *Bacteroidesdorei* isolated from human feces had the capacity to reduce cholesterol. Yue et al. [9] stated that the use of cocktail comprising *Lactobacillus plantarum*, *L. acidophilus* and *L. casei* regulates gut flora and prevent lipid accumulation in mice model. Our results were in accordance to Tahri et.al [10] who stated that very less amount of cholesterol is removed from the broth until more than 0.1% bile is present in the medium. This may be because the bile salt hydrolase activity in the test organisms is induced beyond bile concentration of 0.1%.It is important to study the relationship between number of microbial cells and cholesterol reducing property of microbial isolate. However, it is not possible to study this relationship in the present study as only the initial load of microbial isolates was studied.

Probable strain dependent mechanisms underlying cholesterol-reducing capability of probiotics involve assimilation of cholesterol, cholesterol adherence or incorporation into bacterial cell wall, destabilization and co-precipitation of the micelles of cholesterol, bile salt hydrolase or cholesterol oxidase activity, physiological action of fermentation end products of short chain fatty acids, and production of

functional peptides [11]. Cholesterol is attached to bacterial cells through a physical phenomenon, effected by the chemical and structural properties of cell wall peptidoglycans, which contain amino acids capable of binding cholesterol [12]. Cholesterol adheres to the surface of lactobacilli cells upon fermentation and the attached cholesterol is removed along with the cells upon centrifugation [13]. The microbial community in the human colon contains bacteria that reduce cholesterol to coprostanol by producing cholesterol reductase. This bacterial metabolism for cholesterol-to-coprostanol conversion proceeds according to two pathways, one through formation of intermediates like 4-cholesten-3-one and coprostanone and the other by direct conversion of cholesterol into coprostanol through reduction of the 5-6 double bond [6]. Coprostanol, unlike cholesterol, is poorly absorbed by the human intestine, and the serum cholesterol levels and the coprostanol/cholesterol ratio in faeces are inversely related, thus the conversion of cholesterol to coprostanol has been considered to be a natural means for lowering serum cholesterol in humans [14]. Higher cholesterol removal is reported when cells are actively growing compared with resting and dead forms [13]. An increase in cholesterol binding onto bacterial surface inhibits the intestinal formation of cholesterol micelle which is essential for optimal absorption of fat catabolic products by solubilizing and transporting dietary lipids to the enterocytes. Therefore, the inhibition of cholesterol micelle formation reduces cholesterol absorption in our intestine [15]. As bile salts, phospholipids and cholesterol molecules are needed to form micelles, binding of cholesterol onto cellular surface of bacteria leads to their decreased availability for the formation of micelle [13].

### CONCLUSION

Probiotics are gaining popularity for their beneficial effects on human health especially in the prevention of disease. In specific, role of probiotics as anti-hypercholesterolaemic agent has been studied extensively. The present study suggests that the human gut isolates of Rourkela population, MS1, MS4 and MS7 isolates were capable of reducing higher amount of cholesterol in laboratory model after 72 hours of incubation than the other gut isolates tested. Further studies have to be carried out in order to prove their in-vivo efficacy and other probiotic characteristics. These human gut isolates could emerge as population specific cholesterol reducing probiotic bacteria among Odisha people.

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