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A Study on Kidney Stone Degradation by Lactobacillus

KEYWORDS: Oxalate, kidney stone, probiotics, Lactobacillus

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ABSTRACT *In this study, an attempt was made to screen to isolate and characterize probiotic organisms for kidney stone degradation. Oxalate degrading probiotic Lactobacillus was isolated from curd sample using MRS medium and identified based in the morphological and biochemical characteristics. Then the strain was further subjected for kidney stone degradation. Lactobacillus isolate showed better results for degradation of kidney stone of on 7th day*

Introduction

Kidney stones are solid concentrations or crystal aggregations formed in the kidneys from dietary minerals. Calcium is one component of the most common type of human kidney stones, calcium oxalate. As the amount of calcium intake decreases, the amount of oxalate available for absorption into the bloodstream increases; this oxalate is the excreted in greater amounts into the urine by the kidneys. In the urine, oxalate is a very strong promoter of calcium oxalate precipitation, about 15 times stronger than calcium (Holmes and Assimos, 1998). Probiotics are dietary supplements containing the microorganisms which have the potential to confer health benefits beyond inherent general nutrition to the host lactic acid bacteria are the most commonly used group of probiotic microorganisms (Ouwehand et al., 2002). Lactic acid bacteria are important inhabitants of the human gastrointestinal tract and have been traditionally used as probiotics due to their reported health promoting benefits (Gilliland, 1990). Some lactic acid bacteria have been reported to express the genes encoding for Oxalyl coenzyme A transferase, which includes strains such as Lactobacillus acidophilus, Lactobacillus gasseri and Bifidobacterium lactis (Lewanika, 2007; Fedrick, 2004).

Materials and Methods

Isolation of Lactobacillus **(Murphy** et al. **(2009)**

Lactobacillus was isolated from curd sample by using a selective medium (i.e., MRS medium contains Peptone from casein – 10g; Yeast extract – 8g; D (+) glucose – 20g; Di-potassium hydrogen phosphate – 2g; Tween 80 – 1ml; Di-ammonium hydrogen citrate – 2g; Sodium acetate – 5g; Magnesium sulphate – 0.2g; Manganese sulphate – 0.04g; Distilled water – 1000ml; pH - 5.7 ± 0.2).

Identification of Lactobacillus

Morphological characteristic of the bacteria were observed under microscope. Microscopic observations of the bacterial isolates were studied using Gram Staining and motility tests (Hanging drop Technique). Various biochemical tests such as Methyl red, Voges proskauer, Catalase activity, gelatin liquefaction, starch hydrolysis, urease production, and carbohydrate fermentation were carried out for the identification of Lactobacillus.

Kidney Stone degrading activity of Lactobacillus

The kidney stones were taken in the crucible vials and dried in hot air oven at 100. Initially, the weight of the crucible was taken. Then the weight of crucible containing the kidney stone was taken. Likewise, the weight of each stone and crucibles are taken. Instead of the Potassium Oxalate, kidney stone was added to each glass vials. 5ml of base media(Protease peptone – 20g; Yeast extract – 10g; Tween 80 – 2ml; Potassium di-hydrogen phosphate – 4g; Sodium acetate – 10g; Di-ammonium hydrogen citrate – 4g; Magnesium sulphate – 0.1g; Manganese sulphate – 0.1g; Distilled water – 1000ml) and 5ml of Dextrose solution(Dextrose – 0.1g; Distilled water – 1000ml) were added to the glass vials. Pre-weighed kidney stone was added to each glass vial. 2% of broth culture Lactobacillus was inoculated to each glass vials and incubated anaerobically at 37 for one week. The Kidney stone was removed from treated vials and dried in Hot air oven at 100 for one hour. The final weight of the kidney stone was taken. The optical density of culture broth was also measured.

Results and Discussion

Kidney stones are crystal aggregations formed in the kidneys from dietary minerals in the urine. Oxalate is formed in the liver by amino acid catabolism (Holmes and Assimos, 1998). It is also present in a wide range of food and drinks, including tea, coffee, chocolate, fruits and vegetables (Holmes and Kennedy, 2000). It is an end product of endogenous metabolism of ascorbate, glyoxylate and glycine (Noonan and Savage, 1999).

In this study, an attempt was made to screen to isolate and characterize probiotic organisms for kidney stone degradation. The third probiotic organism Lactobacillus was isolated from curd sample. The results of various biochemical tests and morphological analysis such as Methyl red, Voges Proskauer, Catalase activity, gelatin liquefaction, starch hydrolysis, urease production, and carbohydrate fermentation were tabulated in Table 1. This similar work was already done by Murphy et al, 2009.

Table 1: Morphological and biochemical characterization of Lactobacillus

Biochemical tests	Lactobacillus
Gram's staining	+
Shape	Rod
Motility	-
Methyl red	+
Voges proskauer	-
Urease production	-
Catalase activity	-

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Starch hydrolysis	-
Gelatin hydrolysis	-
Carbohydrate fermentation	
Glucose	+
Fructose	+
Sucrose	+
Lactose	+

The growth performance of Lactobacillus in the medium containing kidney stone was observed spectroscopically at 600nm (Table 2). T_{1-c} replicate showed the maximum growth (1.81 OD at 600nm) on 7th day. The results for percentage of kidney stone degradation by Lactobacillus were observed and recorded (Table 5). T_{1-c} showed the maximum percentage (28.6%) of kidney stone degradation by Lactobacillus Lactobacillus showed better results for degradation of kidney stone.

Table 2: Growth of Lactobacillus on kidney stone degradation at 7 days.

Treatments	Optical Density at 600 nm
T_0	0
T_{1-a}	1.55
T_{1-b}	1.55
T_{1-c}	1.81

T_0 – Control; T_{1-a}, T_{1-b}, T_{1-c} – Replicates treated with Lactobacillus.

Table 3: Percentage of kidney stone degradation by Lactobacillus at 7 days.

Treatment	Initial Weight of Kidney stone (g)	Final Weight of Kidney stone (g)	Percentage of Kidney Stone Degradation (%)
T_0	0.054	0.054	0
T_{1-a}	0.026	0.020	23
T_{1-b}	0.026	0.023	19.2
T_{1-c}	0.021	0.015	28.6

T_0 – Control; T_{1-a}, T_{1-b}, T_{1-c} – Replicates treated with Lactobacillus.

Conclusion:

Study on isolation and characterization of probiotic Lactobacillus organisms for kidney stone degradation clearly indicates its ability to degrade kidney stone under in vitro conditions. Hence it was suggested that these probiotics could be a better option for in-vivo treatment of patient with kidney stone complications provided these organism has to be screened through bioassay with animal cell lines or animal models.

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