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"COMPARATIVE STUDIED ON KIDNEY STONE DEGRADATION ACTIVITY OF LACTOBACILLUS"

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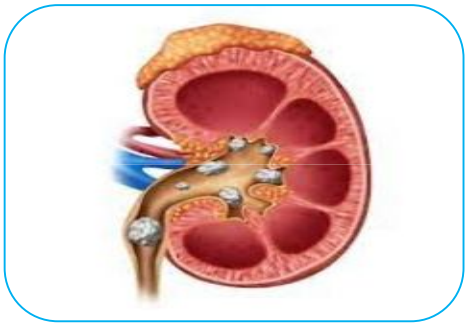
ABSTRACT:

Kidney stone disease is a common, worldwide problem that affects approximately 20 per cent of the general population. However, it should be noted that, super saturation is not the only contributing factor to the formation of kidney stones, as there are other factors involved in stone formation. In current report the present investigations were undertaken to isolated and screen the lactobacillus from different environmental sources.in present study perceived that the result for percentage of kidney stone degradation by Lactobacillus were observed. Hence, the researches focus on isolate the urine stone degrading lactobacillus strain from different environmental sources.



KEYWORDS: Kidney stone,Lactobacillus, calcium oxalate,LAB species,Probiotics,

INTRODUCTION

Kidney stone disease is a common, worldwide problem that affects approximately20 per cent of the general population. The treatment is costly and is not affordable to poor community. It is estimated that kidney stones more frequent and common in males than female. One of the contributing factors is likely to be the super saturation of urine with the respect to its constituents such as calcium, oxalate and uric acid etc. This super saturation may lead to the formation of crystals, which attach to the inner surface of the kidney and build up into kidney stones.

However, it should be noted that, super saturation is not the only contributing factor to the formation of kidney stones, as there are other factors involved in stone formation. 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. (Baetzet al., 1992)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.However, the increased absorption of oxalate-rich food in the gut could be dangerous, as this may lead to the formation of calcium oxalate stones (Argenzioet al., 1988).

Approximately 80 per cent of stones are made up of calcium oxalate and, less often, calcium phosphate. Of the remaining, 20 per cent are composed of mixture of magnesium, ammonia, phosphate and uric acid etc. Several types of stones can be formed and these are categorized based on their chemical composition.(Abe et al.,1996) Approximately 80% of stones are made up of calcium oxalate and, less often, calcium phosphate. Of the remaining, 10% are composed of struvite (which forms in the

presence of urine infection and is made up of a mixture of magnesium, ammonia and phosphate), 9% contain uric acid (which occurs when the acidity level in urine is high), and only 1% contain cystine. The latter is a rare phenomenon and occurs mainly in an inherited condition called cystinuria (Chandrajithet al., 2006).

Treatment with oxalate degrading bacteria could be a new therapeutic choice for the treatment of kidney stone disease. In India the use of curd and butter milk is the traditional dietary practice for maintaining the good health, on the other hand 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. (Holzapfelet al., 2001)

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. Probiotics are defined as live microorganisms, that when ingested, provide a health benefit on the host (Reid et al., 2003) and hence they promote intestinal microbial balance. LAB species are frequently used as probiotics,(du Toitet al., 1998) especially Lactobacillus species due to its beneficial properties and the important role it plays in maintaining the intestinal environment and in stimulating the immune system of the host.(Reid, 1999,Campieriet al 2001).Hence, with this view the pilot experiment has been carried out to isolate the urine stone degrading lactobacillus strain from different environmental sources.

Materials and Method

Collection of sample

For presentation investigation, the Raw milk, Curd, Cabbage, Soil and Sewage samples were collected from different environmental sources. All the samples were transported to microbiology Research laboratory for isolation of lactic acid bacteria. Kidney stone degrading lactic acid bacteria was screened by adopting method suggested by Mahalingam et.al. (2014) with slight modifications.

Enrichment

The collected samples were further enriched @ 10 percent level separately in 10ml nutrient broth supplemented with 1 percent kidney stone powdered substrate in boiling tube and incubated anerobically at 370C for 48 hrs. The PH of the media was adjusted to 6.8 using 1N. NaOH.

Secondary enrichment

Further by the enrichment culture 1ml of enriched samples were re- enriched separately in 9ml (MRS) broth (Biolab) with 50% glycerol, supplemented with1 percent powdered substrate of kidney stone. Followed by the incubation the enriched tubes were observed for the presence of turbidity by comparing it with the un-inoculated control at 600 nm.

Final isolation:

The enriched cultures were separately streak on MRS agar plates supplemented with1 percent powdered substrate of kidney stone.All the plates were anaerobically incubated at 370C for 48 hrs. As per the colony diversity well isolated colony were evaluated for its morphological& biochemical characterization by adopting conventional method.Followed by the characterization isolates sub-culturing were inoculated separately in 10ml (MRS) broth (Biolab) with 50% glycerol, supplemented with 1 percent powdered substrate of kidney stone..The growth performance of lactobacillus in kidney stone supplemented medium was observed spectroscopically at 600 nm.

Studies On Kidney Stone Degradation Efficacy Of The Isolated Lactic Acid Bacterial Cultures:

The set of seven screw cap bottle vials were prepared. The weight of all collected kidney stones were taken and added to all seven screw cap bottle and dried in hot air oven at 40o c for 1 hour till the constant weight is achieved and considered as (W1).Thereafter 5ml of MRS medium and 5ml of dextrose solution were added to all above mentioned bottles. Then 2 per cent v/v of broth cultures of

isolated lactobacillus species from different samples. All the vials were incubated anaerobically at 370C for 1 week. After incubation kidney stones from all the bottles were removed and dried in hot air oven at 40o c for 1 hour till the constant weight is achieved and considered as (W2). The final weight of kidney stones was measured. The reduction in weight of stone accompanied to un- inoculated control was evaluated and express in terms of degradation efficacy.

Result& Discussion

"Comparative studies on kidney stone degrading activity of lactobacillus isolated from different environmental samples viz, raw milk, curd, cabbage, soil, sewage and commercial available strain" have been under taken. The experiment conducted in two phages viz, Isolation of lactobacillus from different environmental sources and Lactobacillus analyzed for kidney stone degradation. The observation obtained in present study has been presented as follows

The result on isolation and identification of lactobacillus isolated from different sources viz, Raw milk ,curd , cabbage, soil, sewage and commercial strain were presented in Table 1.The lactobacillus isolated from all above mentioned sources was confirmed morphologically and biochemically by comparing with standard literature. The organism was found Gram positive .motile ,rod ,circular in shape ,white color, convex, opaque, size 0.5-0.8 and also observed biochemical tests viz, IMViC and sugar fermentation as shown in Table no 1.

The growth performance of lactobacillus on kidney stone supplemented medium was observed spectroscopically at 600 nm as shown in Table 2.Isolate SEI-LAB were showed the maximum growth (1.60 OD at 600 nm) on 7th day as show in figure no 1

The result for percentage of kidney stone degradation by Lactobacillus were observed and recorded as shown in Table 3. Lactobacillus species play a protective role in the gut by hindering the growth of pathogenic bacteriathrough the production of a variety of compounds with inhibitory activity (Piard and Desmazeaud, 1992). The maximum percentage (75.47%) of kidney stone degradation by Lactobacillus isolated from sewage as shown in figure no 2.and followed by raw milk (38.46%),curd(53.57),cabbage(18.51),soil(39.21),commercial strain(72.72).

Hence proved SEI- LAB showed better degradation of kidney stone. The present work is in accordance with the experimental findings of Maralinga in 2014 they reported the degradation activity of lactobacillus sp against kidney stone.

Table 1(a): Morphological and biochemical characterization of isolated lactobacillus strains

Isolates	RMI-LAB	CBI-LAB	SI-LAB	SEI-LAB	CS-LAB
1.Gram nature	+ Rod	+ Rod	+ Rod	+ Rod	+ Rod
2.Motility	Motile	Motile	Motile	Motile	Motile
3.Colonycharacters • Size • Shape • Color • Margin • Elevation • Opacity	0.5-0.8 Circular White Swarming Convex Opaque	0.5-0.8 Circular White Swarming Convex Opaque	0.5-0.8 Circular White Swarming Convex Opaque	0.5-0.8 Circular White Swarming Convex Opaque	0.5-0.8 Circular White Swarming Convex Opaque
4.Sugar fermentation • Glucose • Lactose • Sucrose	Positive PositivePositive	Positive PositivePositive	Positive PositivePositive	Positive PositivePositive	Positive PositivePositive

5.Indole	Positive	Positive	Positive	Positive	Positive
6.MR	Positive	Positive	Positive	Positive	Positive
7.VP	Negative	Negative	Negative	Negative	Negative
8.Citrate	Negative	Negative	Negative	Negative	Negative
Possible species	Lactobacillus	Lactobacillus	Lactobacillus	Lactobacillus	Lactobacillus

Table 2: Growth of lactobacillus on kidney stone supplemented medium

Isolates	Optical Density at 600 nm
Control	0
RMI-LAB	1.44
CRI-LAB	1.52
CBI-LAB	1.00
SI-LAB	1.40
SEI-LAB	1.60
CS-LAB	1.56

Figure1: Growth of lactobacillus on kidney stone supplemented medium

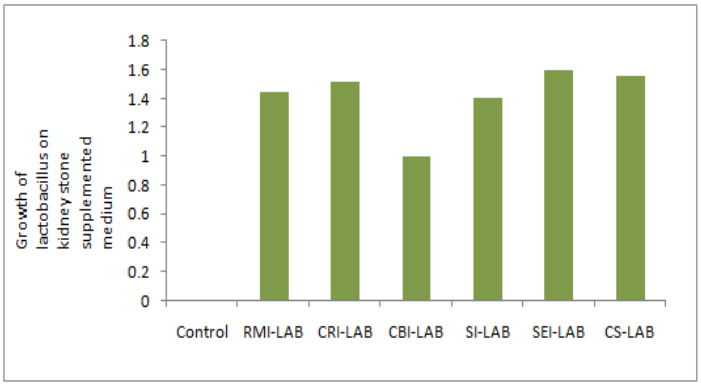
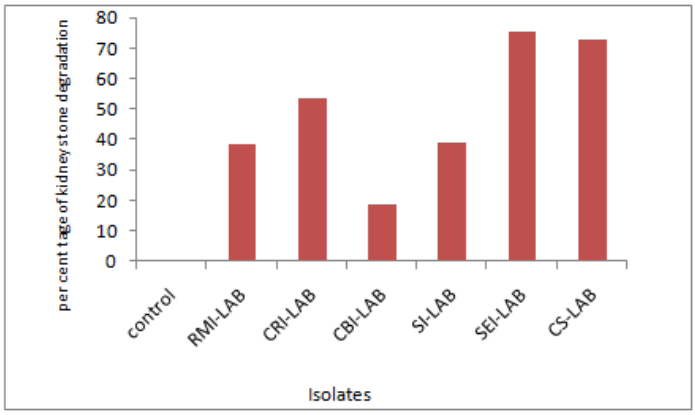


Table 3: Percentage of kidney stone degradation by LAB at 7days

Sr. No.	Isolated LAB	Mean Initial weight of kidney stone (g)	Mean Final weight of kidney stone (g)	Amount of weight reduced	Percentage of kidney stone degradation (%)
1	Control	0.051	0.051	00	00
2	RMI-LAB	0.052	0.050	0.02	38.46
3	CRI-LAB	0.056	0.053	0.03	53.57
4	CBI-LAB	0.054	0.053	0.01	18.51
5	SI-LAB	0.051	0.049	0.02	39.21
6	SEI-LAB	0.053	0.049	0.04	75.47
7	CS-LAB	0.055	0.051	0.04	72.72

Figure2: Percentage of kidney stone degradation by LAB at 7days



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