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Microbial Degradation Of Calcium Oxalate And Its Potential Application

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Abstract

Calcium oxalate is a naturally occurring compound found in various plants and minerals, and its accumulation in the kidney leads to the formation of kidney stones in humans. One of the main causes of kidney stone formation is a decrease in the number of intestinal microbiota which are capable of degrading calcium oxalate. This study focuses on bacterial degradation of calcium oxalate accumulated in humans. Searching for Calcium oxalate degrading strain with high degrading activity has become one of the priorities from the context of research. The study aimed to isolate and characterize microorganisms which are capable of degrading calcium oxalate. Calcium oxalate degrading microorganisms were isolated from faecal samples by using MRS-calcium oxalate medium. Calcium oxalate degrading bacteria were identified on the basis of colour reduction on calcium oxalate medium plate after the incubation period. The morphological and biochemical characterization of isolates were studied. Quantitative determination of degradation was performed by using an indole assay. The activity of isolates was observed by the degradation of kidney stones. Isolates showed better results for degradation of kidney stones after 2 weeks of the incubation period.

Keywords: Calcium oxalate, kidney stones, Calcium oxalate degrading bacteria, MRS-calcium oxalate medium

1. INTRODUCTION:

Calcium oxalate is a salt of oxalic acid commonly found in nature. calcium oxalate is a compound that can be found in various plants as well as foods and is a major component of kidney stones in humans. Kidney stones are solid concentrations or crystal aggregations of dietary minerals that occur in the kidneys. Calcium is a component of calcium oxalate, the most common kind of human kidney stone. The amount of oxalate accessible for absorption into the bloodstream increases as calcium intake decreases; this oxalate is eliminated in higher amounts into the urine by the kidneys. Oxalate is a highly potent activator of calcium oxalate precipitation in the urine, roughly 15 times greater than calcium. **(Mahalingam, P.U and Rajeshwari, P.,2014)** The majority of kidney stones are formed primarily of calcium oxalate and may contain a variety of different types. Calcium oxalate is most commonly found as calcium oxalate monohydrate (whewellite) or calcium oxalate dihydrate (whedellite). Calcium oxalate monohydrate is present in patients with primary hypercalciuria, but calcium oxalate dihydrate is seen in patients with hypercalciuria. According to epidemiologic studies, calcium oxalate and uric acid calculi have recently become more prevalent. (**Yoshihide Ogawa, M.D., Tomonori Miyazato, M.D., Tadashi Hatano, M.D, 2000)** Idiopathic calcium-oxalate (CaOx)

nephrolithiasis is frequently linked with hypercalciuria, hyperoxaluria, and hypocitraturia in individuals. Because oxalate levels are thought to be the more powerful regulating factor in calcium crystal formation during kidney elimination of water from urine, resulting in CaOx super-saturation, hyperoxaluria has received more attention in CaOx kidney stone disease. **(Ammon B. Peck, Benjamin K. Canales2 and Cuong Q. Nguyen, 2015).** Probiotic bacteria, particularly *Oxalobacter formigenes*, *Lactobacillus spp.*, and *Bifidobacterium spp*.,which can break down dietary oxalate in the gastrointestinal tract and allow it to be absorbed, which may aid in the reduction of urinary oxalate levels. **(Paulina Wigner, Michał Bijak and Joanna Saluk-Bijak, 2022)**

2. MATERIALS AND METHODS:

2.1 Collection and enrichment of faecal samples: Faecal samples were collected from infants aged 3-6 months. Faecal samples were serially diluted and added into sterile MRS (deMan Rogosa Sharpe) broth. Kept it in a desiccator at the microaerophilic condition at 27℃ to 37℃ for 48hrs.

2.2 Isolation of calcium oxalate degrading microorganisms: The loopful enriched culture was streaked on sterile MRS-calcium oxalate plates. Kept it in a desiccator at the microaerophilic condition at 27℃ to 37℃ for 48 hours.

2.3 Identification of calcium oxalate degrading microorganisms: Colonies from MRS-Calcium oxalate media were streaked onto the calcium oxalate medium plates. After the incubation period isolates were maintained in the mineral medium with calcium oxalate and without dextrose.

2.4 Characterization and screening of isolates: The colonies were selected based on their morphological characteristics, followed by biochemical tests were done for isolates.

2.5 Quantitative determination of calcium oxalate degradation: Quantitative determination of calcium oxalate was determined by using an Indole reagent. Absorbance was measured at 525 nm on a spectrophotometer.

2.6 Application: Kidney stone degradation was checked by inoculating colonies into the mineral medium with kidney stones. Incubate it for 21 days at microaerophilic conditions at 27℃ to 37℃.

3. RESULTS AND DISCUSSION:

3.1 Isolation of Calcium Oxalate degrading microorganisms: Bacterial colonies with a zone of calcium oxalate degradation were obtained on MRS-Calcium oxalate media plates.

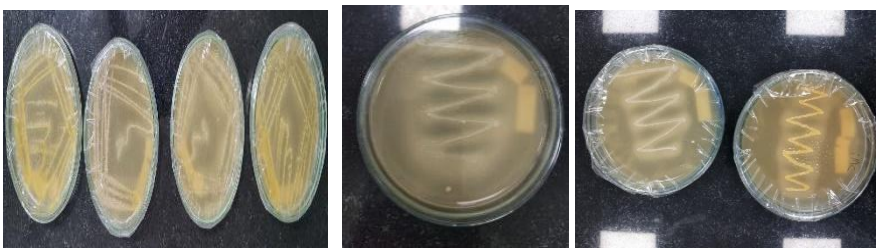

Figure 1: Isolation of Calcium Oxalate Degrading Bacteria on MRS-Calcium Oxalate Media Plates

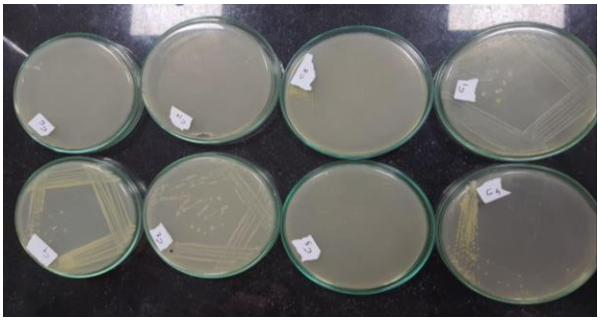

Figure 2: Identification of Ca-Oxalate Degrading Bacteria

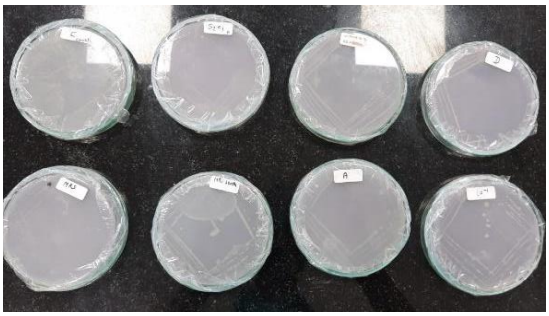

Figure 3: Maintenance of Isolates

3.2 Identification of calcium oxalate degrading microorganisms: The colour of resazurin dye changes from purple to colourless which is present in media plates after colony formation.Isolates were maintained on a mineral medium with calcium oxalate.

3.3 Morphological characterization and biochemical tests of isolates: The 10 morphologically different colonies were selected and biochemical tests were done for the isolates.

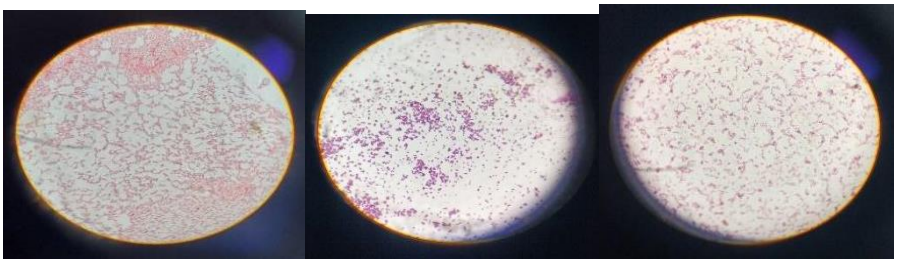

Figure 4: Gram Staining of Isolates

3.4 Quantitative determination of calcium oxalate degradation: Pink coloured compound was formed in the reaction between indole and oxalic acid which indicates the degradation of calcium oxalate. Absorbance was measured at 525 nm**.**

Figure 5: Quantitative Determination of Oxalate

3.5 Application: After one month incubation of kidney stones with inoculated mineral medium. The weight and length of kidney stones were decreased. Out of 10, five isolates were found which have a potency to degrade the kidney stones at microaerophilic conditions at 27℃ to 37℃.

Figure 6: Checking for Kidney Stone Degradation

Colony	Weight (gm) Before	Weight (gm) After	Diameter (length)	Diameter (length)
C1	0.123	0.114	5mm	5mm
C2	0.160	0.145	1cm	8mm
C3	0.075	0.071	5mm	5mm
C4	0.140	0.126	5mm	5mm
C5	0.056	0.050	6mm	6mm
C6	0.240	0.217	9mm	7mm
C7	0.110	0.095	6mm	6mm
C8	0.175	0.163	8mm	8mm
C9	0.152	0.138	6mm	5mm
C10	0.089	0.075	5mm	5mm
Control	0.137	0.137	8mm	8mm

Table 1: Kidney stone measurement

	Weight (Before)	Weight (After)	Length (Before)	Length (After)
Mean	173.4	157.8	7.6	6.6
Variance	1547.8	1274.7	4.3	2.3
Observations	5	5	5	5
Pearson Correlation	0.998200022		0.890348201	
Hypothesized Mean Difference	0		0	
df	4		4	
t Stat	8.154250626		2.236067977	
P(T<=t) one-tail	0.00061553		0.044504671	
t Critical one-tail	2.131846786		2.131846786	
P(T<=t) two-tail	0.00123106		0.089009343	
t Critical two-tail	2.776445105		2.776445105	

Table 2: t-Test Paired Two Sample for Mean

4. DISCUSSION:

Isolation of bacterial strains from faecal samples to determine their activity of calcium oxalate degradation. Iryna Akulenko, Marharyta Skovorodka, Tetiana Serhiichuk, and Ganna Tolstanova has isolated 7 bacterial strains from food samples on media containing Oxalate which compared to this work in which 10 isolates were found from faecal sample on MRS media containing calcium oxalate. The isolates were further identified by streaking isolates on a Calcium Oxalate medium. The potential isolates were identified by checking the colour change of resazurin from purple to colourless. The selected isolates were maintained on a mineral medium with Calcium Oxalate without dextrose which is compared to work done by Vaibhavi Mandanka and Brijesh Shukla in which they maintained their isolates on modified Barber's medium. Morphological characterization and biochemical tests of isolates were studied. Vaibhavi Mandanka and Brijesh Shukla have studied the Quantitative determination of oxalate degradation by $KMnO_4$ assay which is compared to this work in which indole reagent is used to determine the Oxalate degradation. Challaraj Emmanuel E.S., Steffi Sebastian & Lydia Mary Thomas studied kidney stone degradation with *L. plantarum* in which they found 0.06 g of weight loss in 7 days which compared to this work in which 0.015 g of weight loss was found with one isolate after 21 days of incubation.

5. CONCLUSION:

A study on isolation, identification and characterization of Calcium oxalate degrading microorganisms indicates it can degrade kidney stones under in vitro conditions.Faecal samples were collected and enriched in MRS broth after 48h of incubation in a desiccator. Isolation of calcium oxalate degrading microorganisms shows the colonies with a zone of calcium oxalate degradation on MRS-Calcium oxalate media plates. Identification of calcium oxalate degrading microorganisms shows the colour change of resazurin dye from purple to colourless on calcium oxalate media plates after colony formation. Isolates were maintained on mineral media with calcium oxalate and without dextrose. All 10 isolates were characterized and screened on their morphological characterisation, gram staining and biochemical characterisation. Quantitative determination of calcium oxalate degrading microorganisms shows oxalic acid decreases pink coloured compounds formed in the reaction between indole and oxalic acid by indole assay. Out of 10 isolates, 5 isolates have a higher potency to degrade kidney stones.

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