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Research Article

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Biodegradation of calcium oxalate by newly isolated bacterial culture

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ABSTRACT

Kidneys are bean shaped organs having essential regulatory roles. Primary function of the kidney includes maintaining proper balance of water and minerals as well as filtration and excretion of waste products. Kidneys also regulate blood pressure and secrete certain hormones. The major functions of kidney sometime disturbed by formation of kidney stone. About 80% of kidney stones are made up of calcium oxalate. This investigation describes isolation of calcium oxalate degrading facultative bacterial isolate N_4 that can be used as a probiotic tool in the treatment of kidney stone. In this study we isolate bacterial strain N_4 which can degrade 64% of calcium oxalate under in vitro condition.

Keywords: kidney, kidney stone, calcium oxalate, degradation, probiotics

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INTRODUCTION

The kidneys are a pair of vital organs that perform many functions to keep the blood clean and chemically balanced. The main function of kidney is to remove wastes and excess water, by maintaining the chemical balance of the body [1].Kidney function may be disturbed by formation of kidney stone.The most common source of kidney stone includes renal leak hypercalciuria, absorptive hypercalcemia and resorptive hypercalcemia [2].Kidney stone may be formed due to low daily urination; saturation of calcium, oxalate, calcium phosphate, uric acid or cystine in the urine and by means of bacterial infection [3].

Most kidney stones are made up of calcium oxalate or calcium phosphate.Approximately 80% of kidney stones are composed of insoluble calcium oxalate [4]. Oxalates naturally occur in plants,animals and in humans [5, 6].Calcium-oxalate urinary stones can form when oxalate reaches a high concentration in the kidneys[7]. Acute calcium oxalate kidney disease may lead to inflammation and subsequent renal failure [8].

Probiotic microorganisms can be helpful to remove kidney stone. They improve gastrointestinal barrier and increases its function by decreasing absorption of oxalate across the gut [9]. *Oxalobacter formigenes* and freeze dried lactic acid bacteria can be used as probiotic tool to reduce excretion of oxalate in the urine [10].*Oxalobacter formigenes* normally lives in the gut of human and animal [11]. *Oxalobacter* gets its energy only by breakdown of oxalate in colon. *Oxalobacter formigenes* contains two enzymes oxalyl coenzyme decaboxylase and formyl coA transferase. These enzymes degrade oxalate to CO_2 and formate which is further metabolized and excreted through feces [12].

This study focuses to isolate calcium oxalate degrading bacteria which can be used as probiotic tool to treat kidney stone.

EXPERIMENTAL SECTION

Media used

Calcium oxalate ammonium nitrate broth (COAN) containing 0.01% calcium oxalate, 0.15% ammonium nitrate, 0.1% sodium taurocholate with 0.1% glucose at pH 7.5 was used for isolation of calcium oxalate degrading microorganisms. pH of the medium was adjusted by using 0.1 N NaOH and HCL.

Sample used

The common habitat of probiotic organisms is human intestine therefore we use stool samples of different patients suffering from kidney stone.

Harvesting of kidney stone

The kidney stone from patient's urine was harvested as per the method described [13].

Enrichment and isolation of bacterial culture on calcium oxalate under facultative condition

Stool samples were enriched in calcium oxalate ammonium nitrate broth (COAN) broth at pH 7.5 at 28^0C under facultative condition for 14 days. Facultative conditions was created by flushing nitrogen gas inside the flask and sealed with air tight septum. The enriched broth was subjected for the isolation of bacterial strains on COAN agar plates having the same composition as above in addition with 2.5% agar. The bacterial isolates which have the ability to utilize calcium oxalate was subjected for the study of morphological, biochemical, nephlometric titration and UV-Vis spectral analysis.

Nephlometric analysis

The ability of isolates to degrade Ca-Oxalate was detected by estimation of formic acid using $KMnO_4$ solution as per the method described [14].

UV-Vis Spectral analysis

The in vitro degradation of calcium oxalate was studied by taking 4ml of COAN enriched microbial broth, centrifuged at 12000 x g for 10min. The obtained supernatant was subjected for UV-Vis spectrophotometer analysis (Cyberlab UV 100). The percent degradation of calcium oxalate was studied by using the formula [14], Percent degradation = Ab – Aa /Ab X 100, where Ab is absorbance of compound before degradation and Aa is absorbance at same wavelength after degradation.

RESULTS AND DISCUSSION

Morphological and biochemical study of N_4 isolate

On agar plate different isolates were obtained and are serially numbered as N_1, N_2, N_3, N_4 and N_5. On agar surface N_4 isolate shows circular, colorless, sticky, smooth, transparent, moist colony. Microscopic examination of N_4 isolate shows Gram negative, non motile short rods arranged singly. The isolate N_4 shows only acid production with utilization of glucose, sucrose, maltose, lactose and arabinose.

Enrichment and isolation of bacterial culture on calcium oxalate under facultative condition

The isolated strain N_4 shows optimum degradation of Ca-Oxalate at 37^0C after 6 days of incubation, which was measured by estimating the amount of formic acid formed (Table1).

Table 1 Percent degradation of calcium oxalate at every 4days incubation up to 14 days

Degradation	After 4 days of incubation	After 8 days of incubation	After 12 days of incubation	After 14 days of incubation
Percent degradation	25±0.033	32±0.033	55±0.033	64±0.033

- *Values are ±SEM of three experiment*

Nephlometric analysis

As far as the common way for calcium oxalate degradation in kidney is concern it was found to be converted into formic acid [15]. Thus, we focused on measurement of formic acid, which was observed by change in color of broth from purple-pink to colorless. The formic acid formed was estimated by addition of 0.1 N $KMnO_4$ solution in 1:1 proportion in broth. Addition of $KMnO_4$ in broth shows decolourization from pink-purple to colorless.

UV-Vis spectral analysis

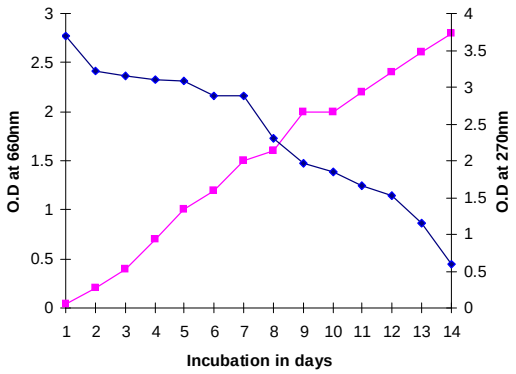

Fig.1

-Growth of N_4 isolate measured at 660nm and degradation of calcium oxalate at 270nm

The wavelength maxima (λ_{max}) of calcium oxalate was found and studied at 270 nm whereas growth of bacteria was studied at 660nm (Fig.1)

Oxalate degradation by the anaerobic bacterium *Oxalobacter formigenes* was previously reported where it was concluded that it is important for human health as it helps to prevent hyperoxaluria and disorders such as the development of kidney stones [16]. Bacteria such as *Eubacterium lentum* WYH-1 and *Enterococcus faecalis* were also found to degrade oxalate [17, 18]. As far as the human anatomy and metabolism is concerned gastric acidity and action of bile salt are the barriers for the survival of ingested bacteria in the gut[19]. There are number of bacteria yet to be studied which are responsible for oxalate degradation in the gut. Therefore it is possible to isolate and select the functional oxalate degrading bacteria by batch or continuous culture.

CONCLUSION

As far as the study of *Oxalobacter formigenes*is concerned anaerobic condition are needed for the growth of *Oxalobacter formigenes* however in this study we reported oxalate degradation by new bacterial isolate N_4 which can degrade calcium oxalate under facultative condition and converts it to formic acid. There is scope for future research by means of isolate N_4 as a probiotic tool in the in vivo treatment of kidney stone.

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