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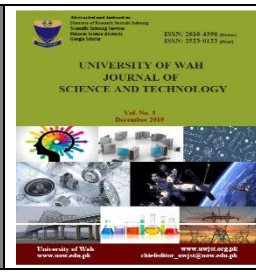

Biodegradation of Calcium Phosphate and Calcium Oxalate by Lactobacillus Strains

Humera Sadaf, Syed Waqas Hassan, Faisal Nawaz, Syed Irfan Raza and Ghulam Jillani

***Abstract*—The purpose of this study is to investigate the effect of locally isolated *Lactobacillus* strains on the degradation of calcium oxalate and calcium phosphate using in vitro analysis. Ten different bacterial strains are isolated from various sources including yogurt, Indian-pickle, rhizosphere and plant root tissue. Results shows that the most efficient strains of *Lactobacillus* are those isolated from the fermented food sources. L-HMY2 proved to be the most effective strain for the biodegradation of calcium salts (PSI =2.88, OSI = 2.65, pH decrease up to 4.32, EC of calcium phosphate broth = 1366 mS cm^{-1} and Ca contents in calcium phosphate broth = 187 mg L^{-1}). Moreover, *Lactobacilli* strains are more effective for the degradation of calcium phosphate than calcium phosphate. This study concludes that these indigenous strains of *Lactobacillus* are potential candidates for probiotic properties for the preclusion of kidney stone formation and this avenue should be further explored.**

Index Terms—*Lactobacillus*, Kidney stones, Calcium oxalate, Biodegradation, Calcium phosphate.

I. INTRODUCTION

Nephrolithiasis is a multifactorial condition marked by the occurrence and formation of salty crystals in kidneys. It is a global problem with an estimation that upto14.8 % population from western world and 9.6% of Asian population is suffering from this disorder [1]. With a high reoccurrence rate of up to 53% within the subsequent 4–11 years after the first episode, it is a public health problem which has significant impact on people's health thus cannot be ignored [2-3]. Numerous epidemiologic variables contribute towards kidney stones formation; these include age, sex, genetics, profession, social class, atmosphere and most importantly the diet [4-7]. Among all these factors, diet is the only one that can be changed easily and it has a noticeable affect on all nephrolithiasis risk factors [8].

Kidney stones mostly result from the accumulation of precipitated salts of calcium phosphate and calcium oxalate [9-10]. Calcium oxalate and calcium phosphate can enter in our body through food. Food and Nutrition Board (FNB), USA recommended a daily intake of 800-1000 mg/day of an adult person, but an increased amount of these salts may lead to stone formation in kidney patients [11]. Stone formation is mainly related with the metabolism of these salts in body as well as with the type of gut microflora. This microflora varies from person to person depending upon many reasons like health, use of antibiotics, diet, lifestyle and environment [12].

There could be several approaches as precautionary or preventive dealing with the disorder of kidney stones including dietary adaptations, forming insoluble calcium oxalate and elimination in feces, excretion in urine, use of drugs and probiotics as such or along with food [13]. Human gastrointestinal passage provides a habitat for many symbiotic nonpathogenic bacteria which are helpful for maintenance of health. This microbiota varies from person to person depending upon many reasons like health, use of antibiotics, diet, lifestyle and environment [12]. The microbes found in the gut plays a critical role for maintenance of health of host body for absorption of nutrition from food, by altering the metabolic pathway and development of resistance [14-15].

Microbial bio-degradation of calcium based salts in the gastro-intestinal tract reduces its circulation in blood, therefore considering a negative risk factor for kidney disorders [16-17]. Oxalate degrading bacteria is found to be effective to reduce chances of kidney stones formation [18]. Due to organic acid production, acidification of nearby environment of the microbial cell solubilizes the calcium compounds through proton substitution for Ca^{2+} [19-20]. As *Lactobacilli* are efficient organic acid producer, so they have been reported with the ability to solubilize calcium oxalate and calcium phosphate containing kidney stones due to their dependence on these compounds to drag energy [21]. They were also considered better candidate for utilizing the mineral nutrients by

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means of enzymes and organic acids [22-23]. Therefore, *Lactobacilli* strains were collected from various ecological niches having different environmental conditions.

Current study is undertaken to evaluate the effect of different strains of *Latobacillus* for the degradation of calcium phosphate and calcium oxalate, which represent the major components of the kidney stones. This study is focused to isolate *Lactobacilli* strains from different sources and ecologies to determine the efficiency of various strains for the degradation of oxalate and phosphate compounds.

II. Materials And Methods

A. *Lactobacillus Strains*

Ten different strains of *Lactobacillus* are isolated from various dietary sources. It included L-HMY1, L-HMY2, L-CMY1, L-CMY2, L-PKOP, L-PKP1, L-PKP2, L-MZRS, L-SSD2 and L-SSD3. These strains are isolated on nutrient rich man, rogosa and sharpe (MRS) medium as described for the isolation and counting of *Lactobacillus* from fermented food [24].

B. *Purification of Lactobacillus*

Purification of *Lactobacillus* strains is undertaken on the basis of colony morphology, margins, elevation and color. Bacterial colonies of *Lactobacillus* have a characteristic yellow color. Morphologically distinct colonies of every sample is selected and purified by repeated culturing on the same media through streaking technique and then incubated at 30°C for three days. Purified colonies are maintained at 4 ºC in the refrigerator. The selected isolates are then tested for their efficiency to solubilize calcium oxalate and calcium phosphate. Calcium oxalate solubilizing and calcium phosphate efficiency of the isolates is evaluated on Pikovskaya medium [25].

C. *Analytical Procedures*

1) *Determination of Phosphate Solubilization Index (PSI).*

Analysis for phosphate solubilization is carried out by subjecting all the bacterial isolates for their ability to form the hallow zone/clear zone on Pikovskaya's agar plates [25]. Phosphate solubilization index (PSI) is determined by using the formula as described in equation (1) [26].

$$\mathrm{PSI} = \frac{C_D + H_D}{C_D}, \tag{1}$$

where,
Colony Diameter = C_D
Hallow Zone Diameter = H_D

2) *Determination of Oxalate Solubilization Index (OSI)*

Analysis for oxalate solubilization is carried out by subjecting all the bacterial isolates of *Lactobacillus* for their ability to form the hallow zone/clear zone on media. Oxalate solubilization index (OSI) is determined by using the formula as described in equation (2) [26].

$$\mathrm{OSI} = \frac{C_d + H_D}{C_D}. \tag{2}$$

3) *Measurement of Electrical Conductivity*

Electrical conductivity of all the bacterial broth filtrates after three days of incubation is determined by using a calibrated EC meter [27].

4) *Measurement of Solubilized Calcium Compounds*

The selected isolates are placed in shaking incubator in Pikovskaya's medium broth for three days. After the incubation period, the broths are filtered and calcium contents of the filtrates are determined by using ethylenediamine tetraacetic acid (EDTA) titration method. Control without bacterial strains is also run to compare the effectiveness of selected bacterial strains.

III. Results

A. *Phosphate Solubilization Index (PSI)*

All the bacterial isolates of *Lactobacillus* are subjected to compare their ability of making the hallow zone/clear zone around their colonies on Pikovskaya's agar plates. The fast spreading hallow zones with greater diameter indicate the higher PSI of that particular strain. Statistical analysis of data on ten strains of *Lactobacillus* reflects a statistically significant difference ($p \leq 0.05$) among various isolates as shown in *Fig. 1*.

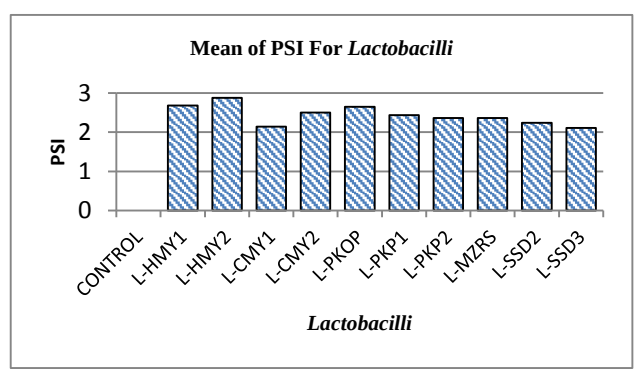

Fig. 1. Phosphate Solubilization Index (PSI) of different Lactobacillus isolates cultured on Pikovskaya's medium.

B. *Oxalate Solubilization Index (OSI).*

All the bacterial isolates of *Lactobacillus* are subjected to compare their ability of making the hallow zone/clear zone around their colonies on Pikovskaya's agar plates. The fast spreading hallow zones with greater diameter indicate the higher OSI of that particular strain. Data analysis revealed a statistically significant difference ($p \leq 0.05$) among various isolates as shown in *Fig. 2*. L-HMY2 shows the highest solubilization of calcium oxalate with a significant difference ($p \leq 0.05$) from all other isolates. Samples L-CMY1, L-CMY2, L-PKP1 and L-PKP2 gave intermediate response for the solubilization of oxalate phosphate in terms of OSI. The *Lactobacillus* L-MZRS, L-

SSD2 and L-SSD3 rendered the lowest OSI values. Microbial bio-degradation of oxalate in the gastro-intestinal tract reduces its circulation in blood, so considered a negative risk factor for kidney disorders [16]. Due to organic acid production, acidification of nearby environment of the microbial cell solubilizes the calcium compounds through proton substitution for Ca^{2+} [19-20].

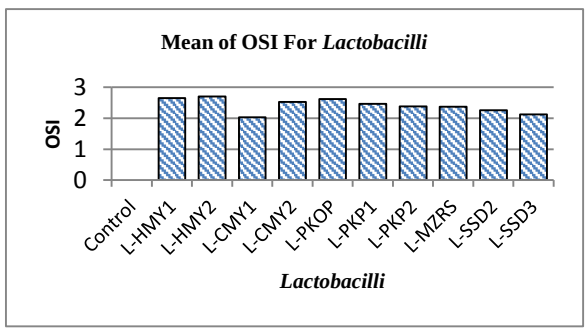

Fig. 2. Oxalate Solubilization Index (OSI) of different Lactobacillus isolates cultured on Pikovskaya's medium.

C. *Acidification of Phosphate and Oxalate Broth.*

Isolates of *Lactobacillus* are tested for their efficiency to acidify the medium over a period of 10 days with an interval of 2 days. Statistical analysis of data on the pH in *Fig. 3(b)*. With increase in time duration of incubation, pH values decreased significantly by all the bacterial strains over that in control treatment. Here L-HMY2 also shows the lowest pH with a significant difference from all other isolates followed by L-HMY1 and L-PKOP respectively. The strains L-CMY1, L-MZRS, L-SSD2 and L-SSD3 shows higher pH values. Generally, the isolates sampled from fermented food products, gave better response for the solubilization of calcium phosphate in terms of pH reduction.

D. *Electrical Conductivity (EC) of Phosphate and Oxalate Broths.*

Increased values of electrical conductivity (EC) in a liquid medium indicate enhanced solubilization of precipitated salts rendering greater amount of soluble salts. All the collected bacterial isolates of *Lactobacillus* are subjected to compare their ability of solubilizing the calcium phosphate in Pikovskaya's broth medium. The fast growing bacterial populations with more acid production capacity showed higher phosphate solubilization. This is reflected by greater EC values by that particular strain.

Statistical analysis of data on ten strains of *Lactobacillus* reflected a statistically significant difference ($p \leq 0.05$) among various isolates on electrical conductivity of the broth medium as shown in *Fig. 4(a)*. Among them, L-

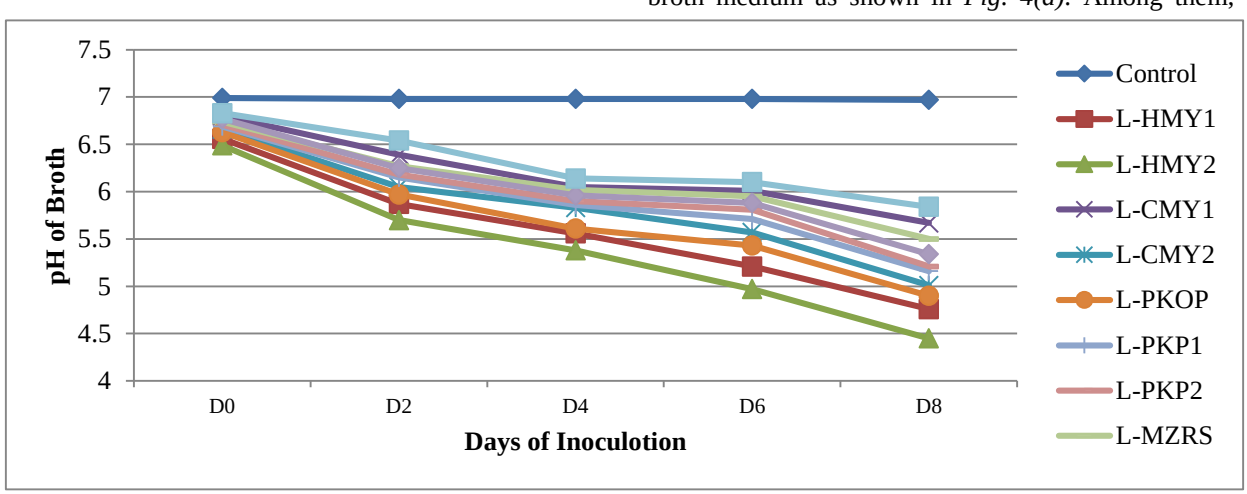

Fig. 3(a). The pH dynamics in culture medium containing calcium phosphate by Lactobacillus isolates.

changes in the broth containing calcium phosphate as influenced by all the collected isolates of *Lactobacillus*, exhibited significant difference ($p \leq 0.05$) among them as well as among the time intervals as shown in *Fig. 3(a)*. With the increase in time lapse during incubation, the values of pH are decreased significantly by all the bacterial strains over that in control treatment. L-HMY2 shows the lowest pH indicating for the highest solubilization of calcium phosphate with a significant difference ($p \leq 0.05$) from all other isolates and is followed by L-HMY1. Reduction in pH by all the isolates continued till 10^{th} day of incubation to attain values between 4 and 5, while in control sample of broth the pH remained at the original level around 7.00 throughout the study period of ten days.

Similarly for the acidification of calcium oxalate broth all isolates of *Lactobacillus* exhibited significant difference ($p \leq 0.05$) among them and among time intervals as shown

HMY2 shows the highest solubilization of calcium phosphate with a significant difference ($p \leq 0.05$) from all other isolates. It is followed by L-HMY1 and L-PKOP with statistically non-significant difference for both these isolates. The Oxalate broth L-HMY2 shows the highest solubilization of calcium phosphate with a significant difference ($p \leq 0.05$) from all other isolates. The strains L-MZRS, shows the lowest EC values. Nonetheless, the isolates L-CMY1, L-CMY2, L-PKP1 and L-PKP2 exhibited intermediate response in terms of EC as an indicator for the solubilization of calcium oxalate as shown in *Fig. 4(b)*.

E. *Calcium Concentration in Broth*

Lactobacillus strains isolated during this study are compared for their ability of solubilizing the calcium compounds in the broth culture medium. Statistical

analysis of data on these strains reflected a statistically significant difference ($p \leq 0.05$) among various isolates grown in both calcium phosphate and calcium oxalate containing media. Among all the isolates, L-HMY2 showed the highest solubilization of calcium phosphate as well as calcium oxalate with a significant difference ($p \leq 0.05$) from all other isolates as shown in *Fig. 5*. The strain L-SSD3 exhibits the lowest Ca contents.

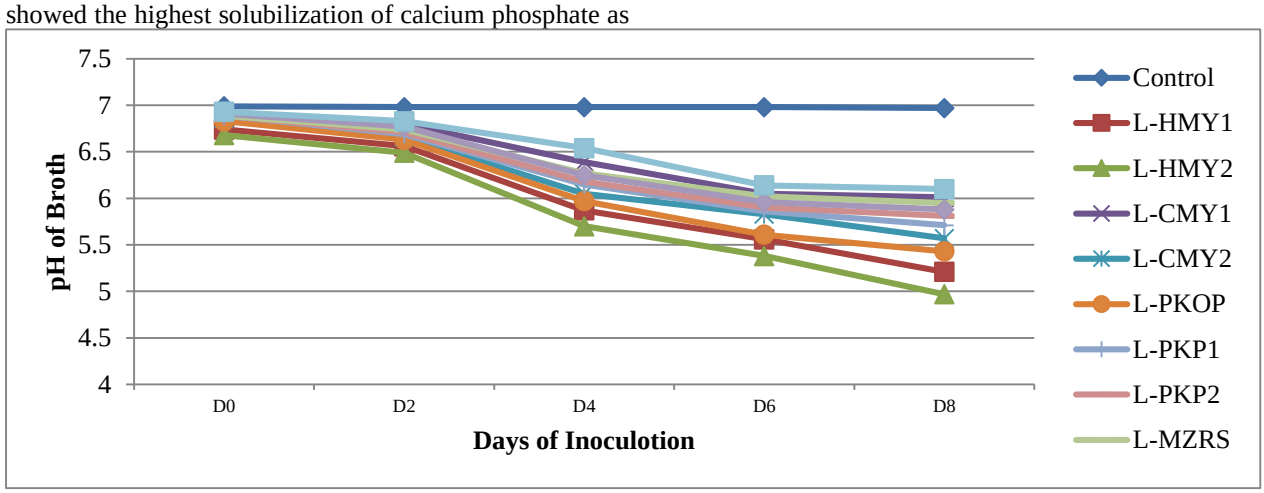

Fig. 3(b). The pH dynamics in culture medium containing calcium oxalate by Lactobacillus isolates.

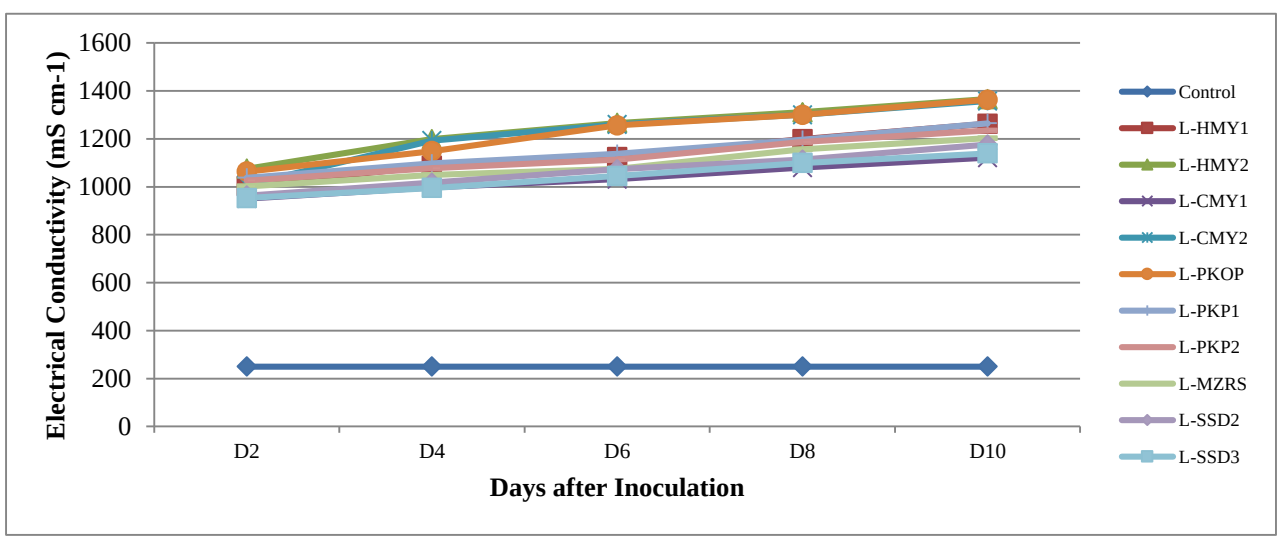

Fig. 4(a). Electrical conductivity (mS cm^{-1}) of Pikovskaya's broth medium containing calcium phosphate as affected by different bacterial isolates of Lactobacillus.

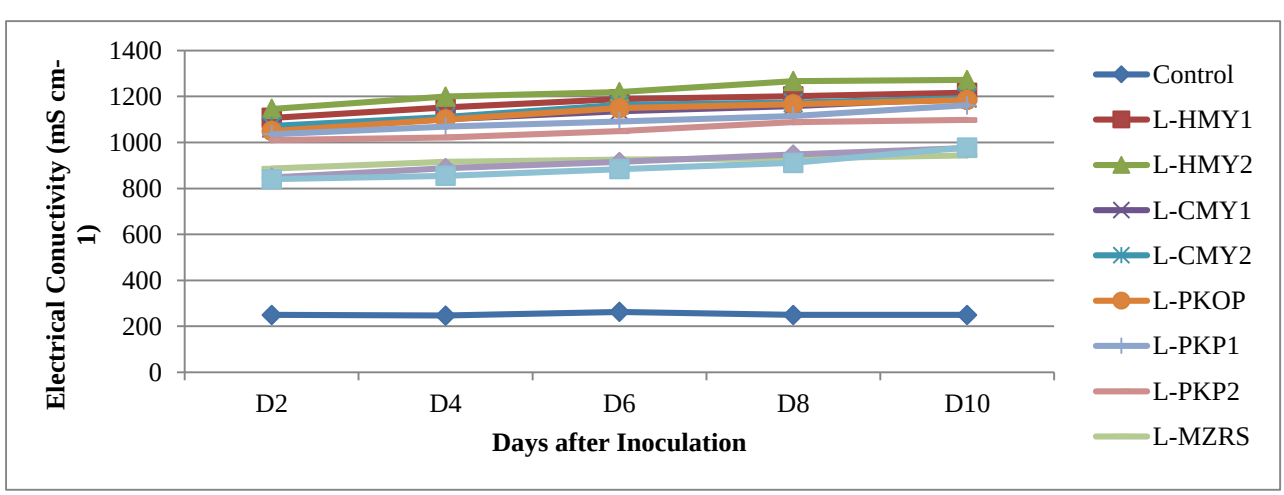

Fig. 4(b). Electrical conductivity (mS cm^{-1}) of Pikovskaya's broth medium containing calcium oxalate as affected by different bacterial isolates of Lactobacillus.

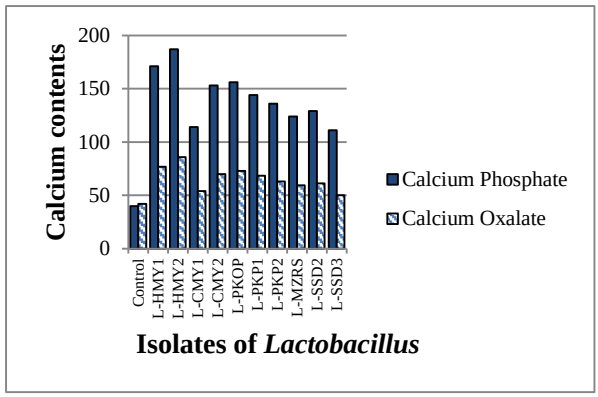

Fig. 5. Calcium contents (mg L^{-1}) in Pikovskaya's broth medium containing calcium phosphate or calcium oxalate as solubilized by Lactobacillus isolates.

IV. Discussion

The results obtained through this study indicate that bacterial ecologies play an important role for their efficiency towards solubilization of mineral compounds. Generally, microbes have variable tolerance to the outer environmental conditions (ecological niche) for their living [28]. Bacteria in the harsh conditions are more tolerant and have better efficiency for utilizing the fixed or insoluble mineral nutrients by releasing enzymes and organic acids [22-23]. Understanding of microbial association with nearby environment may help to plan the technology of by means of potential strains for bio-degradation [29].

Statistical analysis of data on ten strains of *Lactobacillus* reflected a statistically significant difference ($p \leq 0.05$) among various isolates as shown in *Fig. 1*. Among *Lactobacillus* isolates, L-HMY2 shows the highest solubilization of calcium phosphate with a significant difference ($p \leq 0.05$) from all other isolates. It is followed by L-HMY1 and L-PKOP with statistically non-significant difference for both these isolates. The *Lactobacillus* strains isolated from the L-MZRS, L-SSD2 and L-SSD3 rendered the lowest PSI values. *Lactobacilli* have been known to produce lactic acid, which solubilize the phosphate compounds as that found in kidney stones or reduce the chance of their formation in the body [18].

Isolates of *Lactobacillus* are tested for their efficiency to acidify the medium over a period of 10 days with an interval of 2 days. Due to organic acid production, acidification of surrounding environment of the microbial cell solubilizes the calcium compounds through proton substitution for calcium [20]. As *Lactobacilli* are efficient organic acid producer, so they have the ability to solubilize calcium oxalate and calcium phosphate containing kidney stones due to their dependence on these compounds to drag energy [21]. As L-HMY2 and L-PKOP both strains originated from fermented products hence their ability to reduce pH is more efficient then strains isolated from non fermented sources.

Lactobacillus strains isolated during this study is compared for their ability of solubilizing the calcium compounds in the broth culture medium. The ability of *Lactobacillus* to dissolve more calcium phosphate is due to production of lactic acid [29] as well as specific enzymes for biodegradation of Calcium phosphate [30]. The management of *Lactobacillus* component of the gut microflora may decrease the intestinal absorption of oxalate, and ultimately the intestinal oxalate absorption and renal excretion, so potentially reducing oxalate urolithiasis [31].

Acidification of nearby environment of the microbial cell because of organic acid production is known to solubilize the calcium compounds through proton substitution for calcium [20]. As *Lactobacilli* are efficient organic acid producer, therefore they have the ability to solubilize calcium oxalate and calcium phosphate containing kidney stones due to their dependence on these compounds to drag energy [21]. As L-HMY2 and L-PKOP both strains originated from fermented products hence their ability to reduce pH is more efficient then strains isolated from non-fermented sources.

V. Conclusion

The results of this study shows that gut microbiota plays an important role towards solubilization of mineral compounds. Data suggests that *Lactobacilli* strains can be an effective component to be used as probiotics against Nephrolithiasis. The combined use of probiotics along with fermented food products especially homemade yogurt (Dahi) can enhance the degradation of kidney stones.

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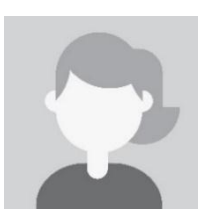

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