Kristu Jayanti Journal of Core and Applied Biology (KJCAB)

Volume 2, Issue 1, 2022, Pages 20-24| Original Research

A Preliminary Study on Oxalate Degradation by *Lactobacillus* Isolated from canine's faeces

Challaraj Emmanuel E.S., Steffi Sebastian & Lydia Mary Thomas

Department of Life Sciences, Kristu Jayanti College (Autonomous), Bengaluru, Karnataka, India-560077

ABSTRACT

This is a preliminary study that reveals the importance of utilizing the potential microorganism for efficient oxalate degradation in the human gut. Hyperoxaluria is considered as the major risk factors related to recurrent ureolithiasis and progressive nephrocalcinosis. The organism isolated was confirmed to strain level by 16SrRNA sequencing and BLAST analysis. *Lactobacillus plantarum,* a gram positive bacterium isolated from the dog faeces was found to show a wide range of oxalate degradation in vitro, especially with sodium and ammonium oxalate. To check the viability of *Lactobacillus plantarum* in gastro- intestine, acid tolerance test was done to confirm whether the organism can survive the gastrointestinal pH of about 1-2.5. *Lactobacillus plantarum* is acid tolerant as well as bile tolerant as it can grow in the pH range of 3-7 and 0.1-3% bile salts. These properties make *Lactobacillus plantarum* a beneficial organism thereby opening a new platform to be used as a therapeutic agent as it is compatible to the human gut environment. Natural kidney stones were studied and the percentage of oxalate degradation by *Lactobacillus plantarum* was analysed in the minimal medium. Previous studies have reported the use of *Lactobacillus* species as probiotic as well as for oxalate degradation from other sources like fermented food, human faeces. But this study aims to screen the potential *Lactobacillus plantarum* from dog faeces which could be used for oxalate degradation for the prophylaxis of hyperoxaluria. This work represents a key milestone in the medical discoveries as an aid to human mankind as a whole.

KEY WORDS: Oxalate*, Lactobacillus,* Kidney stone, degradation, Probiotics

Article History | Received: 15. 09. 2022; Revised: 17. 12. 2022; Accepted: 18. 12. 2022; Published Online: 23. 12. 2022

1. INTRODUCTION:

Oxalate is an organic toxic compound found in high levels in foods such as peanuts, spinach, beets, chocolates, legumes, grains, etc. *(Hodgkinson A, 1978).* Oxalic acid in the body is excreted through urine as minute crystals. These oxalates can form larger kidney stones that obstruct the kidney tubules *(Ratkalkar VN & Kleinman JG, 2011).* Kidney stones present in the human body are of several types and 80% of the estimated kidney stones are formed from calcium oxalate. Nephrolithiasis is the condition of having kidney stones. The presence of stones in the urinary tract is referred to as urolithiasis and those in ureters are referred to as ureterolithiasis. Men of 20-49 years of age are at a greater risk than women to develop urinary tract stones *(Edvardsson VO et. al., 2013).* The bacterial species of genus *Lactobacillus* are non-spore-forming, Gram-positive rods that are microaerophilic. Lactobacilli are recognized as a commensal flora of humans and other animals. They function to inhibit pathogenic bacteria and produce antimicrobial substances like lactic acid, bacteriocins, and hydrogen peroxide. They are found to be normal flora in the oral cavity, the gastrointestinal tract, and also in the female urogenital tract. Food products such as yogurt and cheese are exogenous sources of lactobacilli and hence lactobacilli are vital components of probiotics and the role of *Lactobacillus sp.* in food products and its beneficial effects on human and animal health and longevity have been proved *(Verna EC & Lucak S, 2010).*Probiotics, which often contain members of genera *Lactobacillus* and *Bifidobacterium*, have proven to be successful with beneficial results on a variety of diseases. Lactobacilli have been isolated from dog faecal samples as they play an important role in the canine gastrointestinal tract *(Batt RM et. al., 1991).* *L. mucosae* (*Tzortzis G et. al., 2004*) have been reported previously to be present in the canine faeces. The capacity of Lactobacilli present in canine gut microbiota has been suggested to contribute to the probiotic actions by the inhibition of pathogens *(Elliason DJ & Tatini SR, 1999).* Scientists in 1940 first proved the degradation of oxalic acid by faecal bacteria. Oxalate breakdown by microbes occurs in the rumen of sheep, canines, horses, swine, guinea pigs, and rabbits *(Allison MJ et. al., 1985).* The presence of oxalate metabolizing microbial species in the gastrointestinal tract is inversely correlated to the incidence of calcium oxalate stone formation or hyperoxaluria or both in rodents, humans, and canines *(Sidhu H et. al., 1998)*. It was reported that the oral administration of Lactobacilli and Bifidobacteria were found effective in reducing urinary calcium oxalate levels by Campieri *et al.,* in 2001*(Campieri C et. al., 2001).* Studies have confirmed that oral administration of Lactobacillus species shows an important role in the reduction of luminal oxalate, which also decreased the risk of oxalate excretion in the urine by humans and animals.

A set of discrete genes in *Lactobacillus acidophilus* was confirmed and was believed to be involved in oxalate degradation. The *Lactobacillus acidophilus* cells survived with low levels of ammonium oxalate (3.5 mM) and to a maximum of 32 mM ammonium oxalate in MRS media. The *in vitro* oxalate degradation ability of *Lactobacillus gasseri* in MRS-Ox was generally comparable to 61% reported for oxalate degrading probiotic bacteria *B. lactis* DSM 10140 which were grown under identical conditions *(Federica F et. al., 2004).* Feeding the mixture of freeze-dried Lactobacilli led to a significant reduction of the excretion of oxalate in urine among a group of patients suffering from calcium oxalate urolithiasis and also mild hyperoxaluria *(John CL et. al., 2004).* Lactobacilli were also shown to have good colonization and survival properties in

*Author for correspondence: Challaraj Emmanuel ES | Email: challaraj.e@kristujayanti.com

laboratory conditions as well as in human trials elsewhere *(Hamilton-Miller JMT, 2003).* However, the number of identified bacterial species with oxalate degradation ability is limited and there is no report regarding the oxalate degrading ability of Lactobacilli from dog (canine) gut microbiota. Hence, this study is aimed to screen and identify an efficient oxalate degrading Lactobacillus spp. from a healthy dog's fecal sample and to evaluate its potential oxalate degrading characteristics in vitro.

2. MATERIALS AND METHODS

2.1. Total viable bacterial count:

A fresh fecal sample was collected from a healthy dog in an aseptic condition, stored in a sterile container, and brought to the laboratory for further analysis. The samples collected were serially diluted using 9 mL sterile saline. Total viable bacterial counts were enumerated by pour plate method, using MRS (de Man Rogosa Sharpe) agar plates (Hi-Media, Mumbai, India). One mL aliquot of appropriate dilution was pipetted out into the sterile Petri plates and 20 mL of MRS agar was added into each Petri plate. Then the inoculated plates were incubated at 28°C, duplicate plates were also maintained. Petri plates with 30-300 colonies were selected after 24 - 48 h incubation and the total viable bacterial counts were enumerated. The bacterial population was expressed as the number of colony-forming units CFU/mg of a sample *(Trias R et. al., 2008).* Bacterial colonies from primary isolation were sub-cultured onto fresh MRS plates and incubated at 37°C for 24 - 48 hours. 10 – 15 colonies were randomly selected and purified by streaking on MRS agar plates. The pure culture was then stored at 4°C in the refrigerator.

2.2. Biochemical and Molecular Characterization of the isolate:

The isolate was microscopically examined and the bacterial strains isolated from all the samples were identified up to a generic level *(Holt JG et. al., 1994).* The bacterial genomic DNA was extracted and amplified by PCR (Polymerase Chain Reaction) technique. The amplified PCR product was purified and sequenced commercially. The 16S rDNA sequences were compared with the sequence in the GenBank Public database using BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) *(Altschul SF et. al., 1997)*

2.3. Oxalate degradation:

Minimal media with different concentrations of oxalates like ammonium oxalate and sodium oxalate are used separately for oxalate degradation studies. Minimal media with different concentrations (1%, 2%, 3%… up to 8% and 9%) of each oxalate were taken in different sterilized conical flasks. An isolated test organism was inoculated in each of the flasks and incubated at 37°C for 24 hours. Growth of the organism was observed in the minimal media of different concentrations of oxalate derivatives until there was complete inhibition of growth. The only carbon source provided in minimal media was glucose. The isolated organism was standardized by using 0.5 Mc Farland's standards. This was used as a reference. 0.5 Mc Farland's gives an approximate cell density of 1.5 x 108 CFU/ml, having an absorbance of 0.132 at a wavelength of 600 nm. The microbial suspensions were prepared in their respective sterile Minimal broth with Oxalate sources and are compared with that of the standard. The isolated LAB was screened for oxalate utilization using the agar well diffusion method in Sodium oxalate. 6 mm diameter wells were prepared in sodium oxalate plate and each well was inoculated with 0.1 mL of overnight culture and incubated at 28°C for 12 hours (*(Allison MJ et. al., 1985).*

2.4. Precipitation and Quantification of oxalate:

The amount of oxalate in the sample was determined by using the concepts of redox titration in permangimetry. Redox titration in this experiment included the use of permanganate, which oxidized oxalate, in an unknown sample. Oxalate precipitated filtrate was measured into beakers and four drops of methyl red indicator were added to it. Ammonium hydroxide solution was added until the test solution changed from pink to a faint yellow colour (pH 4.0 – 4.5). Then the sample was heated to around 90ºC, cooled, and then filtered to remove the precipitate containing ferrous ions. The solution was then heated and left overnight at 25ºC; it was centrifuged at 2000 rpm for 5 minutes the supernatant was decanted and the precipitate was completely dissolved in 20 ml of 25% (v/v) H_2SO_4 solution. At this point, the total filtrate resulting from the digestion of 2g of sample was made up to a volume of 100 ml. aliquots of 10 ml of the filtrate was heated until near boiling and then titrated against 0.02M standardized $KMnO_4$ solution to a faint pink colour which persisted for 30 seconds *(Kingsley OI, 2019).*

Sodium oxalate content was calculated using the formula:

N x Eq.Wt x Total Volume /1000

= 'X'g of oxalate in the sample.

2.5. Acid and Bile tolerance test:

The overnight grown culture of *Lactobacillus plantarum* in MRS broth was used. 1ml of culture was inoculated into different tubes of MRS broth (20 ml each tube) which was acidified to a pH of 1 - 6 with 0.1 N HCl. Tubes lacking acid were taken as control. Growth of *Lactobacillus plantarum* was observed at different pH concentrations and optimal bile salt concentrations were observed based on the turbidometric method *(Wang CY et. al, 2010)*. Similarly, 1 ml of the culture was inoculated into different test tubes of MRS broth (20 ml each tube) with 0.3 - 1.5% bile salts (sodium cholate), along with one tube without bile salts acting as a control. After 24 h incubation, viable cell count was determined on MRS agar. These plates were then incubated at 37 °C in an anaerobic atmosphere for 48 hours *(Hyronimus, B, 2000).* The percentage of growth was calculated as follows:

Percentage growth of the test organism can be analysed by the following formula:

% growth = No. of CFU in MRS with bile x 100/

No. of CFU in MRS without bile

2.6. Kidney stone degradation:

2.6.1. Physical analysis:

The physical characteristics of collected kidney stones from patient (M, 54) were analysed *viz.,* weight, solubility, diameter, length, colour, physical appearance etc.

2.6.2. Confirmation of presence of oxalate:

The kidney stone was powdered added to test tube containing

sterile distilled water with a neutral pH (pH 7). Uric acid stones are usually similar to kidney stones in appearance, to detect the absence of uric acid in the stone or to confirm the presence of oxalate in the stone; this experiment is of great significance. The presence of uric acid in the stone shows an acidic pH of the distilled water after the addition of powdered stone whereas oxalate stones show completely neutral pH in the distilled water. Uric acid stones form when the levels of uric acid in the urine are too high, and/or the urine is too acidic (pH level below 5.5) regularly. The presence of oxalate in the stones was confirmed by the decolourization of Potassium permanganate in the presence of diluted sulphuric acid *(McBride RS, 1912).*

$$2KMnO_4+3H_2SO_4+5H_2C_2O_4 \longrightarrow K_2SO_4+2MnSO_4+10CO_2+8H_2O$$

2.6.3. Weight loss study:

The kidney stone was added to a test tube containing minimal media with an overnight grown culture of isolated strain. This tube was incubated at 37ºC for 6 – 7 days. The physical changes and weight loss of the kidney stone were assessed after 7 days of incubation. A difference between the initial and final weight of the stone was detected to determine the degradation of oxalate by the bacterial strain in a minimal medium. This method is applied for metal deterioration and to find out the corrosion rate of metal *(Chen XH, 2005)*. In this study, the weight loss of the stone for 1 year by the utilization of oxalate by the action of isolated *Lactobacillus* strain can be determined by using the following formula

The dried weight of the stone is calculated by the formula:

Initial weight = (X); Final weight = (Y)

So weight loss for 30 days is X-Y = (A)

Therefore the weight loss determination for 1 year =

$$\frac{A \times 365}{30} = (Z)$$

3. RESULTS AND DISCUSSION

The total number of viable cells obtained after the incubation period of the serially diluted sample was found to be 112 ± 2 x 10^5 CFU/gm. Screening for potential bacterial species in dog fecal samples demonstrated the presence of significant *Lactobacillus spp*. in the dog intestine after serial dilution

Table 1: Molecular identification of *Lactobacillus* isolate

S. No.	Sequence length	Accession Number	BLAST results
1.	1139 bp	MT043815	*Lactobacillus plantarum*

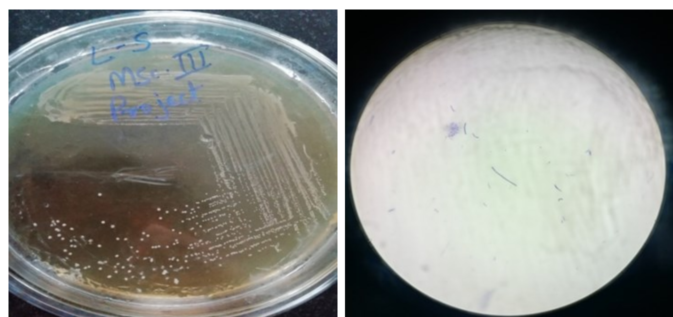

Figure 1: (a) Isolated pure colonies of *Lactobacillus sp*. on MRS agar plates, and (b) Microscopic view of isolated culture showing gram positive rods - *Lactobacillus*

of the sample and growth of the culture on MRS agar plate. The culture was identified as a Gram-positive rod-shaped organism (Figure 1). The selected bacterial strain was identified based on cultural, morphology, and biochemical characteristics. Results were compared with Bergey's manual of systemic bacteriology classification. The strain was identified as *Lactobacillus plantarum* further using 16S rRNA sequencing (Table 1, Figure 2).

The isolated strain of *Lactobacillus plantarum*, showed potential growth in minimal medium with ammonium oxalate and minimal medium with sodium oxalate *in vitro L. plantarum* was able to grow in the presence of 8% ammonium oxalate

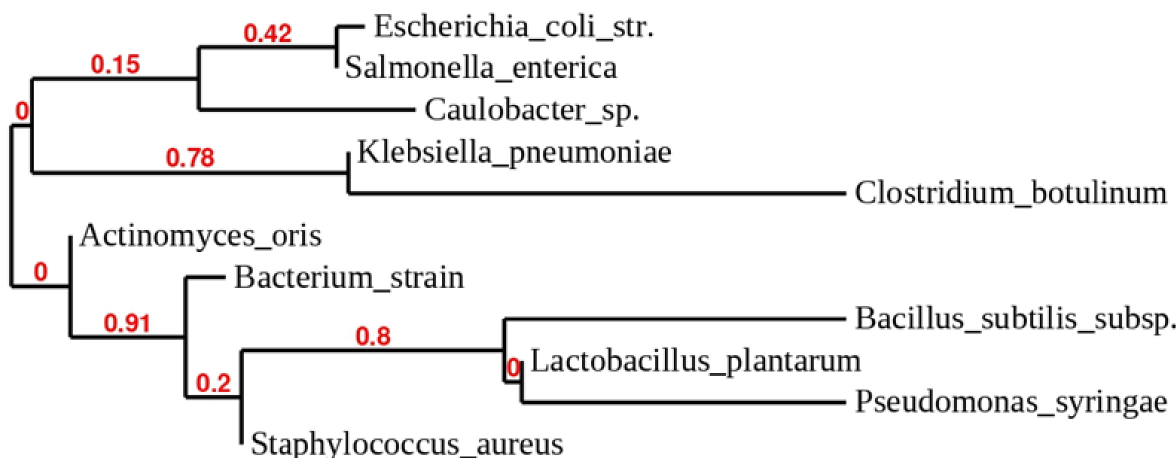

Figure- 1: Phylogenetic tree and systematic position of *Lactobacillus plantarum*

and 6% sodium oxalate. Growth beyond the mentioned oxalate concentrations was inhibited and *L. plantarum* could not survive in higher concentrations of oxalates. According to Mc Farland's standard gives an approximate cell density of 1.5 x 10^8 CFU/ml, having absorbance of >0.1 OD at a wavelength of 600 nm (Table 2). The isolate was found to be oxalate utilizing the formation of clear zones around the well when 0.1 ml of culture was inoculated onto minimal media containing 6% of Sodium oxalate at 28°C for 12 hours. The canine intestine showed a high degree of variability in the degradation of oxalates. Turroni *et al.,* in 2007 *(Turroni S et al., 2007)* found that *Lactobacillus gasseri* and *Lactobacillus acidophilus* showed significant oxalate degradation whereas other strains showed comparatively less oxalate utilization especially; *Lactobacillus salivarius,* which showed only 20%, oxalate degrading ability.

The initial and final amount of oxalate present in the medium, when treated with the isolate, was determined by the end of the experiment using the concepts of redox titration in permangimetry. After titration, it was found out that the oxalate minimal broth with 6% sodium oxalate has around 66.6% of oxalate content in it. In addition, after titration, the oxalate minimal broth with 8% ammonium oxalate has around 75% of oxalate content in it. This means that the significant oxalate utilization by the bacterial isolate (*L. plantarum*) was found to be around 33.4% after 72 hours of incubation.

To standardize the isolated *Lactobacillus plantarum* with a high degree of oxalate degradation, isolated *Lactobacillus plantarum* was assessed for acid tolerance. The isolate was viable at pH 3 indicating that it was acid tolerant. When exposed to pH 2, the survival rate was strongly reduced, and eventually there was no viability observed which suggested that this strain was highly sensitive to high acidic pH. Growth above pH 7 was also inhibited. Hence, *L. plantarum* is acid tolerant and can grow in the pH range of 3-7.

The isolate was found viable up to 1.3% of bile salts indicating that it was bile tolerant. At 1.5% concentration of bile salts, the survival rate was strongly inhibited, and eventually there was no viability observed which suggested that this strain was sensitive to higher concentrations of bile. Hence, *L. plantarum* is bile tolerant and can grow up to a concentration of 0-1.3% of bile salts. *L. plantarum* is also acid-tolerant and highly tolerant to bile salts and hence it can survive in a human acidic gut environment. Such capacity of Lactobacilli has got the probiotic actions by the inhibition of pathogens present in canine gut microbiota *(Elliason DJ & Tatini SR, 1999).* .

Previous studies of Allison *et al.,* in 1985 *(Allison MJ et. al., 1985)* indicate that the normal human gut harbors a significant population of oxalate degrading microorganisms and that the people who have undergone surgeries. *Lactobacillus* genera in particular are known to metabolize the oxalates in canines efficiently *(Gnanandarajah JS et. al., 2012).* In this study, an isolated strain, *Lactobacillus plantarum*, showed potential growth in minimal medium with 8% ammonium oxalate and minimal medium with 6% sodium oxalate *in vitro*. Oxalic acid in the body combines with divalent metallic cations like calcium (Ca^{2+}) and iron (II) (Fe^{2+}) to form corresponding oxalate crystals, which are excreted through urine as minute crystals. The significant oxalate utilization by the bacterial isolate (*L. plantarum*) was found to be around 33.4% after 72 hours of incubation. In the present study, isolated *L. plantarum* showed potential degradation of oxalate crystals in the medium and hence can be further used for several clinical applications. After incubation in minimal medium and weight loss of 0.02 g of the stone was also attained within 7 days. The oral administration of *Lactobacillus* species was proved to be an important role in the reduction of luminal oxalate, which reduced the oxalate excretion in the urine by humans and animals *(Joseph Okombo & Michael Liebman, 2010).*

The Physical Characteristics of the kidney stone were analyzed and were found to be an irregular amorphous mass collected and dried from a patient (M, 54) from the ureter. There was no change in pH when the stone was mechanically crushed into powder and mixed with sterile distilled water with neutral pH. The amorphous powder obtained after crushing the stone was found to be insoluble. These properties indicate that the stone was not a uric acid crystal. The presence of oxalate in the powdered stone was confirmed by the decolourization of Potassium permanganate in the presence of Sulphuric acid. With these observations, it was evident that the stone received had no evidence of uric acid and confirmed the presence of oxalate in it. Studies have identified clinical applications in which the probiotics with Lactobacilli are reported in being effective for urogenital infections *(Sarah C et. al., 2009)*. *Lactobacillus plantarum* was never evaluated before for oxalate degradation and an interesting potential factor for the degradation of oxalates proved based on the outcomes obtained in the present study. Since *L. plantarum* is well known for its ability to survive and tolerate adverse conditions in dog fecal samples, new perspectives may be hypothesized for the production of clinically significant products or probiotic food, with reduced oxalate content and endowed with *L. plantarum* thus likely to promote the reduction of oxalates in the human biological system. Additionally, this strain can survive well during gastrointestinal transit and all Lactobacilli reduce the pathogenic coliform counts in feces with a good fecal recovery rate according to the previous studies. Hence these interesting properties make *Lactobacillus plantarum* a potentially good candidate that could be utilized for the prophylaxis of CaOx (calcium oxalate) urolithiasis and hyperoxaluria in humans.

Table 2: Growth of *L. plantarum* in minimal media with different oxalate concentrations

S. No.	Sodium Oxalate (6%)	Ammonium Oxalate (8 %)
1.	+++	+++

Table 3: Weight Loss study of kidney stone with *L. plantarum*

Initial weight	Final weight	Weight loss in 7 days	Weight loss in a year
0.08g	0.06g	0.02g	0.243g

This method was adopted to find out the material deterioration and corrosion. On a trial and error basis, this method is applied for the stone degradation in the presence of the isolate *L. plantarum*. The difference in weight of the stone was measured after seven days of incubation at 28°C. This indirectly reflects the utilization and degradation of oxalate by the bacterial strain in a minimal medium. Based on the weight variation before and after inoculation with *L. plantarum* the measurement was done and the results were tabulated (Table 3).

REFERENCES

Allison MJ, Dawson KA, Mayberry WR, Foss J.G. (1985), *Oxalobacter formigenes : oxalate-degrading anaerobes that inhabit the gastrointestinal tract*, Archives of Microbiology. 141 (1): 1–7.

Altschul SF, Thomas L, Madden A, Alejandro S, Jinghui Z, Zheng Z, Webb MD, Lipman J (1997). *Gapped BLAST and PSI -BLAST A new generation of protein database search programs*, Nucleic Acids Res. 25: 3389-3402.

Batt RM, Hall EJ, McLean L, Simpson KW (1991). *Small intestinal bacterial overgrowth and enhanced intestinal permeability in healthy beagles*. Int. J. Vet. Res. 53: 1935– 1937.

Campieri CM, Campieri VB (2001) *Reduction of Oxaluria After an Oral Course of Lactic acid Bacteria at High Concentration,* Kidney International 60 (3): 1097-1105

Chen XH, Chen CS, Xiao HN, Cheng FQ, Zhang G, Yi GJ, (2005). *Corrosion behavior of carbon nanotubes–Ni composite coating, Surface and Coatings Technology*. 191(2-3): 351-356.

Edvardsson VO, Goldfarb DS, Lieske JC, Beara-Lasic L, Anglani F, Milliner DS, Palsson R (2013). *Hereditary causes of kidney stones and chronic kidney disease*, Pediatr. Nephrol. 28 (10): 1923-1942.

Elliason DJ, Tatini SR (1999) *Enhanced inactivation of Salmonella typhimurium and verotoxigenic Escherichia coli by nisin at 65°C.* Food Microbiol. 16: 257– 267.

Federica F, Beatrice V, Roberto G, Maria RP, Silvia G(2004) . *Characterization and Heterologous Expression of the Oxalyl Coenzyme A Decarboxylase Gene from Bifidobacterium lactis.* Applied And Environmental Microbiology, 70(9): 5066–5073

Gnanandarajah JS, Johnson TJ, Kim HB, Abrahante JE, Lulich JP (2012). *Comparative Faecal microbiota of dogs with and without calcium oxalate stones*. Society for Applied Microbiology, 113 (4): 745-56

Hamilton-Miller J.M.T. (2003). *The role of probiotics in the treatment and prevention of Helicobacter pylori infection.* International Journal of Antimicrobial Agents, 22 (4): 360-366

Hodgkinson A. (1978), *Evidence of increased oxalate absorption in patients with Calcium-containing renal stones.* Clinical Science and Molecular Medicine, 54 (3): 291-294.

Holt JG, NR Kreig, PHA. Sneath, JT Staley, ST Williams. (1994). *Bergey's manual of determinative bacteriology 9th Edn*. Williams and Wilkins, Maryland, USA.

Hyronimus B, Le Marrec C, Sassi AH, Deschamps A (2000), *Acid and bile tolerance of spore-forming lactic acid bacteria*, Int. J. Food Microbiol. 61: 193–197.

John CL, David SG, Claudio D, Cynthia R (2005). *Use of a Probiotic to Decrease Enteric Hyperoxaluria*, Kidney Int. 68 (3): 1244-9.

Joseph O and Michael L (2010). *Probiotic-induced Reduction of Gastrointestinal Oxalate Absorption in Healthy subjects*. Urol Res. 38 (3): 169-78.

Kingsley OI (2019) *Qualitative and Quantitative Determination of Anti-Nutritional Factors of Five Wine Samples.* Advanced Journal of Chemistry-Section A, 2(2), 136-146.

McBride RS (1912). *The Standardization of Potassium Permanganate Solution by Sodium Oxalate.* Journal of the American Chemical Society, 1912 34 (4), 393-416

Ratkalkar VN and Kleinman JG (2011) *Mechanisms of stone formation, Clinical Reviews in Bone and Mineral Metabolism* vol. 9, no. 3-4, pp. 187–197.

Sarah Cribby, Michelle Taylor, and Gregor Reid (2008). *Vaginal Microbiota and the Use of Probiotics.* Interdisciplinary Perspectives on Infectious Diseases. Volume 2008, Article ID 256490, 1-9

Sidhu H, Hoppe B, Hesse A, Tenbrock K (1998). *Absence of Oxalobacter formigenes in Cystic Fibrosis Patients: A Risk Factor for Hyperoxaluria*. National Library of Medicine, 352 (9133):1026-9.

Trias R, Baneras L, Badosa E. and Montesinos E (2008). *Bioprotection of Golden delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria*, Int J Food Microbiol., 123:50-60.

Turroni S, Vitali B, Bendazzoli C, Candela M, Gotti R, Federici F, Pirovano F, Brigidi P (2007). *Oxalate consumption by Lactobacilli: evaluation of oxalyl-CoA decarboxylase and formyl-CoA transferase activity in Lactobacillus acidophilus,* Journal of Applied Microbiology Vol. 103(5), 1600-9

Tzortzis G, Baillon LA, Gibson GR, Rastall RA (2004). *Modulation of anti-pathogenic activity in canine-derived Lactobacillus species by carbohydrate growth substrate*, Journal of Applied Microbiology 96 (3), 552-9

Verna EC, and Lucak S (2010). *Use of probiotics in gastrointestinal disorders: what to recommend?* Therap. Adv. Gastroenterol. 3, 307–319.

Wang CY, Lin PR, Ng CC, Shyu YT (2010). *Probiotic properties of Lactobacillus strains isolated from the feces of breast-fed infants and Taiwanese pickled cabbage*, Anaerobe. 16 (6): 578–585.