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Review

Probiotic Oxalate-Degrading Bacteria: New Insight of Environmental Variables and Expression of The Oxc and Frc Genes on Oxalate Degradation Activity

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ABSTRACT

Oxalate, a compound produced by many edible plants and as a terminal metabolite in the liver of mammals, is a toxin that has a detrimental role to human health. Humans and other mammals do produce the enzymatic machinery to degrade oxalate. However, numerous oxalate-degrading bacteria reside in the mammalian gut and thus provide an important function for hosts. The current review focuses on the environmental factors that influence the efficacy of probiotic oxalate-degrading bacteria, relative to oxalate metabolism. We describe the mechanism of oxalate catabolism and its consumption by obligate and facultative anaerobic oxalate-degrading bacteria, in both in vitro and in vivo environments. We also explore the environmental variables that impact oxalate degradation. Studies on single species degrade oxalate have not shown a strong impact on oxalate metabolism especially in high oxalate conditions such as consumption of foods high in oxalate (such as coffee and chocolate for humans or halogeton in animal feed). Considering effective variables which enhance oxalate degradation could be used in application of effective probiotic as a therapeutic tool in individuals with hyperoxaluria. This study indicates probiotics can be considered a good source of naturally occurring oxalate degrading agent in human colon

Keywords: probiotic bacteria; oxalate-degrading; variables; in vivo; in vitro; oxaluria

1. Introduction

There are several bacteria in the human gut that can degrade significant amounts of oxalate daily [1]. Use of oxalate-degrading bacteria to reduce urinary oxalate has been the focus of numerous studies, with limited success [2]. In the human gastrointestinal tract, there are approximately four hundred different bacterial species with the composition of the gut microbiome exhibiting large, inter-individual variability. Oxalate-degrading bacteria, when present in the gastrointestinal tract are able to decrease urine oxalate up to 40% and significant reduction of oxalate stone formation in the kidneys. Consumption of oxalate rich plant foods and increased digestive absorption of free oxalate can cause kidney stone formation, oxalosis, inflammation, breast cancer, atherosclerosis and cardiovascular diseases

Hypercalciuria (urinary excretion of more than 800 mg of calcium per day) and hyperoxaluria (urinary excretion of more than 400 mg of oxalate per day) are among the most important pathophysiologic causes of kidney stone formation. They are directly related to calcium-oxalate rich diet. In addition, the mentioned complications result in 50%

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increase in calcium and oxalate concentration in urinary tracts, as well as increased level of insoluble precipitates of Ca2+ oxalate or phosphate in the kidney.

Oxalosis leads to kidneys fail. Extra oxlate which cannot been removed from human body will accumulate in blood and organs [3]. Prolonged exposure of breast epithelial cells to oxalate may cause tumor due to expression of proto-oncogene and increase of the proliferation rate of breast cancer cells [4].

The aim of this review study was to evaluate the effective probiotic bacteria (lactic acid bacteria and Oxalobacter formigens) in the breakdown of oxalate to reduce oxalate excreted in the urine. In order to increase and improve the performance of these bacteria, effective variables such as pH, glucose concentration, sucrose concentration, yeast extract, presence of inulin as prebiotic, bacterial age and bacterial inoculation have been identified and studied.

1.1. Chemistry of Oxalate

Oxalate is the anion of a dicarboxylic acid that is commonly found in many plant foods including nuts, fruits, vegetable, grains, and legumes. Different salts of oxalate are found in the plants, such as sodium, potassium or magnesium oxalate, each with unique water solubility characteristics. [5]. Enzymatic synthesis of oxalate occur by hydrolysis of oxaloacetate in fungi e.g. Aspergillus niger, and bacteria e.g. Acetobacter. In mammals, oxalate is produced through the tricarboxylic acid cycle. The chemical structure of the anion is shown in Figure 1. Different form of oxalic acid (H2C2O4, HC2O4-, C2O42 -) may occur depending on the pH of solution. H2C2O4 and C2O4 are the predominant form of oxalate at pH 1.23 and 4.19 (and above), respectively.

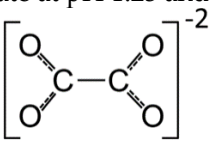

Figure 1. Chemical structure of oxalate anion

2. Oxalate Sources in The Body

The oxalate in the body has two sources: from dietary sources or from endogenous synthesis [6]. The endogenous synthesis takes place mainly within the liver, from different dietary precursors, such as glyoxalase, ascorbic acid and some amino acids [7]. Oxalate synthesis in the body has essential impact on the rate of oxalate content in the urine and formation of calcium oxalate stone in kidney. Glyoxylate is the major precursor to oxalate production. The main sources of in vivo glyoxylate metabolism are phenylalanine, glycine, hydroxyproline, tryptophan, pentose sugars, glucose, fructose, ethanolamine and glycolate. Metabolism of oxalate formation from the glyoxal precursor is performed according to the cycle shown in Figure 2 [8].

All these dietary precursors are metabolized to oxalate in order to produce NADH [9]. The human body lacks any enzyme to degrade oxalate and kidneys are the main routes for eliminating oxalate in the body [10]. Recently, it has been shown that different segments of the mammalian intestine have the ability to secrete oxalate in some condition. This extra-renal route of oxalate elimination may have a significant impact on urinary oxalate [11].

3. Degradation Of Oxalate By Obligate And Facultative Anaerobic Gut Bacteria

There is considerable inter-individual variability in the composition of the gut microbiota, but generally remains stable within individuals. Gut microbiota composition can, however, differ over time in individuals with varying diets and other factors such as antibiotic use. Oxalate-degrading bacteria in the gut are able to decrease oxalate (as a source of carbon and energy) by 40% and reduce oxalate stone formation in kidney. The absence of oxalate degrading bacteria in the GIT had shown to be a risk factor for the hyperoxaluria and urolithiasis [12].

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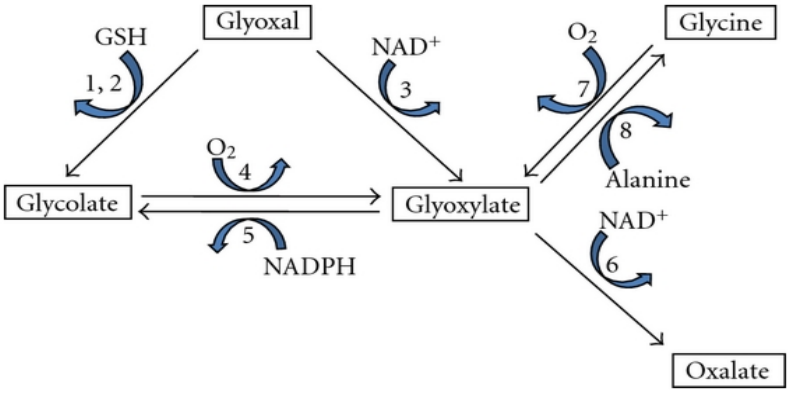

Figure 2. Oxalate synthesis pathway from glyoxal. In this mechanism, enzymes: alanine: glyoxylate and aminotransferase are active.

For the first time, Alison et al. isolated Oxalobacter formigenes as an obligate anaerobic bacterium in the human digestive system and other vertebrates with ability of consumption of oxalate as a source of carbon and energy [13]. *O. formigenes* are very sensitive to common antibiotics. Reproduction of *O. formigenes* normally It is higher in healthy people than in patients with kidney stones. The relationship between *O. formigenes* proliferation and urinary oxalate secretion is unclear [14-17]. Patients with primary hyperoxaluria may not respond to the probiotic *O. formigenes* [2]. It has been shown that in individual *O. formigenes* were lost after therapeutic use of antibiotics and other drugs as well as in patient with cystic fibrosis [1,18]. *O.formigenes* with anaerobic oxalate degrading activity can degrade oxalic acid with three enzyme (Figure 3).

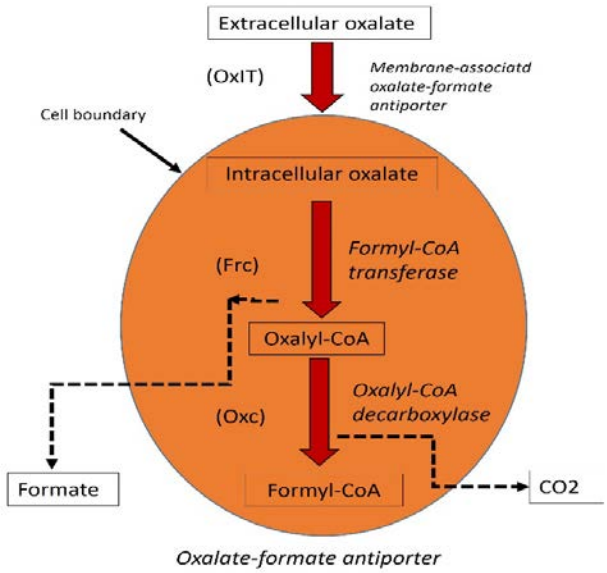

Figure 3. Oxalate degradation pathway in *O. formigenes*. Enzyme names are italicized with their protein abbreviations in brackets; dotted lines show secreted product

Furthermore, different investigations showed that probiotic bacteria, especially Bifidobacterium (B.) spp. and Lactobacillus (L.) spp. having the ability of degradation oxalate into carbon dioxide and formate. Lactobacillus and Bifidobacteria are gram-positive, non-spores and rod-shaped species and are found in large numbers in the human intestine. Bifidobacteria are anaerobic, while Lactobacillus species are often highly tolerant to air. Since these bacteria are in the safe group for human consumption, different species of these two bacteria are widely used as probiotic bacteria to improve human health [1]. It should be noted that the breakdown of oxalate in Lactobacilli and Bifidobacteria is specific

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to the genus and species of certain bacteria. In 2202, Jiang et al. studied the decomposition of oxalate in a wide range of human microbiota especially Bifidobacterium species [19].

4. Variables Affecting the Activity of Oxalate Degrading Bacteria

4.1. pH

Azcarat Peril et al. (2006) reported transcription of genes (oxc and frc) in *Lactobacillus acidophilus* which was stimulated at pH 5.5 and inhibited at pH 6.8 in the presence or absence of oxalate [20]. Lewanica and colleagues showed that *Lactobacillus gasseri* at pH 5.5 can decompose 74% of oxalate. Also in the simulated colon medium, Lactobacillus reduces the fraction of 40% of oxalate in the culture medium (at 60 hours and pH 5.5) (21). Karamad et al. (2019) showed that in L. acidophilus at pH 5.5 and with increasing sodium oxalate concentration from 5 mmolL-1 to 22.7 mmolL-1 sodium oxalate decomposition shows an increase. At this pH, the bacterium has the highest expression of the oxalate degradation gene and due to the pre-adaptation of the bacterium to high oxalate content the oxalate degradation ability of bacterium significantly increased. They also showed that in *O. formigenes* ammonium oxalate degradation rate increases with increasing pH from 5.5 to 6. In pH less than 6 oxalate degradation drops sharply [21].

4.2. Glucose and sucrose concentration

Tarruni et al. (2007) showed that Bifidobacteria were unable to grow and degrade oxalate, and found this by completely recovering oxalate from the growth medium after 5 days in the incubator. Growth retardation has also occurred due to the lack of oxalate and sucrose in the bacterial growth medium [22]. The researchers also showed that rapidly proliferating cells consumed carbon sources (30% decomposition, 0.25 gL-1 per hour) during the first 24 hours. From 24 hours to 120 hours of incubation, after logarithmic growth, the residual sucrose is slowly reduced to half the initial concentration (0.07 gl-1h-1). In in vivo mouse study Miller et al. showed that that both antibiotics and high fat, high sugar diet lowers microbial oxalate metabolism [23].

4.3. Yeast extract

Dawson (1980) showed that removing yeast extract from the growth media reduced the relative growth rate of *O. formigenesis* by 80% in 4 days. In this experiment, it was finally shown that the only energy source needed - other than oxalate - for the growth of *O.* formigens is yeast extract, and the best amount of yeast added to the growth medium is 0.1%. So that if this rate increases to 0.2%, it will not have an effect on increasing bacterial growth [24].

4.4. Bacterial age

Gholami and Khosravi Darani (2014) showed that the age of inoculation is 36 hours and the inoculation rate of 0.8% leads to higher production of dual linoleic acid. Biohydrogenation is a process that requires energy, so it does not often occur in older cells [25].

4.5. Inulin

Previous research by Balthazar et al. has shown that among prebiotics, inulin may cause increase survival and activity of lactic acid bacteria during shelf life [26]. Stepanova et al. showed that impact of prebiotics on increased degradation of oxalate is due to the increased growth of bacteria resulted from the presence of short chain free fatty acids [27]. Another study in 2018 by Darilmaz et al. Showed that prebiotics could increase the degradation of oxalate by lactobacilli in vitro. Inulin also plays a key role in the anti-E coli activity, which can be increased by the use of *Lactobacillus fermentum* IP5 [28]. Karamad et al. (2019) resulted from their researches on L. acidophilus that by increasing the amount of inulin from 0.5 gL-1 to 0.97 gL-1 at pH: 5.5, the highest rate of oxalate decomposition has been performed (about 90% of 20 mmolL-1 sodium oxalate concentration). They also showed that in *O. formigenes* as the concentration of inulin increases, the rate of oxalate

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degradation increases and its optimal value was 1.35 gL-1. In fact, this study for the first time investigated the effect of inulin on the oxalate degradation activity of *O. formigens*.

4.6. Antibiotics

The correlation between antibiotic therapy and kidney stone disease has been approved [29]. Although specific mechanisms has not been identified but it is clear that *O. formigenes* is antibiotic sensitive. Absence of intestinal *O. formigenes* could represent a pathogenic factor in calcium oxalate urolithiasis when antibiotics are prescribed generously [8].

5. Analysis of Transcription and Function of The Oxc and Frc Genes in Lactic Acid Bacteria and *O. formigenes*

The genes for oxalyl-CoA decarboxylase (oxc) and formyl-CoA transferase (frc) play a key role in oxalate metabolism in *O. formigenes* and lactic acid bacteria were isolated by Baetz and Allison in 1989 and 1990. By utilizing oxc and frc as catalysts in a two-step enzymatic reaction, oxalate can be metabolized into CO2 and formate. [30].

pH and oxalate exposure may interact directly to affect oxalate degradation, but may also have wider effects on microbial community dynamics and function. In L. acidophilus, 315 genes are down-regulated with exposure to 1% oxalate at pH 6.8, and 16 genes are up-regulated with exposure to 1% oxalate at pH 6.8 [20]. Under these conditions, oxc and frc, which degrade oxalate, are down-regulated. The flow of oxalate between gut regions with varying pH can affect gene expression in whole microbial communities using next-generation metagenomic strategies. Using this technique, oxalate-induced shifts in microbiota function and community composition could be predicted more accurately. Several efforts have been made in this field, including the sequencing of *O. formigenes* as part of the human microbiome project (Broad Institute). Oxalate degradation is particularly sensitive to pH, and the cyclic fatty acid configuration of O. formatigenes indicates a degree of acid tolerance in this species [31].

The oxalyl-CoA decarboxylase function was attributed to the product of the open reading frame (ORF) based on amino acid similarity with proteins of known function. The oxalyl-CoA decarboxylase of *O. formigenes* (accession no. M77128) presented the highest nucleotide homology (56%) and amino acid similarity (identities, 47%; positives, 64%). Furthermore, most of the decarboxylase enzymes described to date, including the oxalyl-CoA decarboxylase of *O. formigenes*, present a conserved thiamine pyrophosphate (TPP)-binding region [19].

The current understanding of the phylogenetic relatedness of *O. formigenes* with L. acidophilus and B. lactis is summarized in Fig. 1.

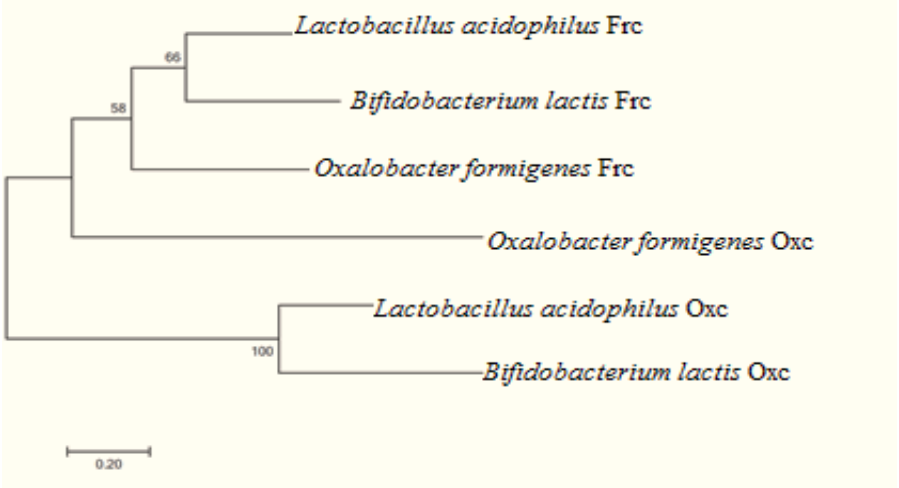

Figure 4. Molecular phylogenetic analysis by maximum likelihood method

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The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [33]. The tree with the highest log likelihood (-4566.2828) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 634 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [34]. In this analysis we use bootstrap method and the number of bootstrap application is 1000 [35].

Consumption of probiotic bacteria can be a suitable treatment method for people with kidney stone disease and individuals with high risk of infection. *Lactobacillus acidophilus* and *O. formigenes* have shown good results in these studies. However, more research is needed on the appropriate amount and conditions of use of these dietary supplements to achieve the highest rate of oxalate degradation, especially when consuming high oxalate foods. In our studies, it has been shown that the simultaneous use of variables affecting the decomposition of oxalate and can greatly increase oxalate degradation by probiotic bacteria in high oxalate content.

In the postnatal period, colonic anaerobes play an important role in the development and functioning of the organism. If we are able to recognize the functions performed by colonic bacteria, we should be able to develop therapies under medical supervision that can be administered to individuals lacking key bacteria in the future. The degradation of toxic compounds in the intestine provides ecological niches for anaerobic bacteria in the gut. Some examples other than *O. formigenes* include Synergistes jonesii and bacteria that degrade nitropropanol [24]. Both the human colon and the animal gut are thought to offer an ecological niche for oxalate degradation. The potential for replacement therapy with probiotic preparations of *O. formigenes* should be excellent in such a niche [36]. In these investigations, molecular quantitative methods can be used since Oxalobacter's loss correlates with other diseases, in addition to the potential link between antibiotic use and its loss [37] will be helpful. Other than lactic acid bacteria and *O. formigenes*, it seems unclear what bacteria are responsible for oxalate degradation in the gut [6,38]. In studying microbial oxalate degradation in the intestinal tract, one of the main goals is to reduce the incidence of recurrent renal colic, an economically damaging condition.

There are four key enzymes that degrade oxalate: oxidase, decarboxylase, FCR, and OXC. The oxalate decarboxylase and oxalate oxidase belong to the cupin superfamily of proteins, which show strong similarities at the amino acid level. There were significantly more genes encoding Frc and Oxc in the gut than genes encoding oxalate oxidase and decarboxylase.

According to the analysis of 660 subjects, the four genes encoding the enzymes were widely present in the healthy gut microbiome [39]. In the metagenomes of 660 subjects, Oxc can be detected in 554 (84%) and Frc in 581 (88%).

Gut microbiota play an important role in gut-kidney physiopathology. Inhibiting urinary stone disease by maintaining healthy oxalate homeostasis could be achieved by a multi-species bacterial network [23]. In addition to research on isolated species of oxalate-degrading bacteria, particularly those that require oxalate to function, recent studies indicate that microbiota play broader roles in oxalate metabolism and in inhibiting urinary stone formation.

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7. Conclusion

Consumption of probiotic bacteria can be a suitable treatment method for people with kidney stone disease and individuals with a high risk of infection. Review of all reports showed that L. acidophilus and *O. formigenes* have shown promising results in these studies. However, more research is needed on the appropriate amount and conditions of using these dietary supplements to achieve the highest rate of oxalate degradation, especially when consuming high oxalate foods. Studies indicate that the simultaneous use of variables affecting the decomposition of oxalate can significantly increase oxalate degradation by probiotic bacteria in high oxalate content.

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