Enrichment of Anaerobic Glyoxylate-Degrading Bacteria from the Human Gut

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Introduction

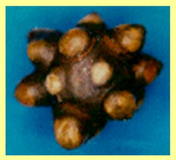
Kidney stones are a painful burden that many people have to deal with. It is known that inasmuch as 70% of all such stones in humans contain calcium oxalate (Figure 1)(3). Oxalate passes into the urinary tract where it combines with calcium to form calcium oxalate. Urinary oxalate in humans originates from the absorption of dietary oxalate (3-11%) and endogenous synthesis from several precursors (Figure 2)(5). Among the various oxalate precursors, glycolate and glyoxylate appear to be the most effective substances for oxalogenesis (4).

Several species of anaerobic oxalate-degrading bacteria have been isolated from the human gastrointestinal tract. One of these isolates is *Oxalobacter formigenes*. This bacterium has been isolated from the rumen of cattle and sheep; from the large bowel contents of pigs, humans, and rats; and from anoxic sediments (2). It is believed that these bacteria may limit absorption of oxalate by degrading free oxalate thereby decreasing its concentration in the gut (1). To date, there have been no studies performed on the activities of anaerobic glyoxylate-degrading bacteria in the human gut. Furthermore, there has yet to be an anaerobic glyoxylate-degrading bacterium isolated from the human gastrointestinal tract.

Objectives

- To determine glyoxylate degradation and the formation of glyoxylate derived products by bacterial populations form the human gut
- To establish enrichment cultures of intestinal anaerobic glyoxylate-degrading bacteria
- To isolate an anaerobic glyoxylate-degrading bacterium from maintained enrichment cultures

Figure 1 – Examples of Kidney Stones

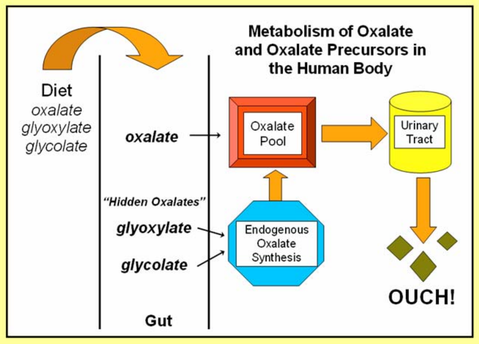


Calcium Oxalate Monohydrate- 97%, protein and blood 3%

Calcium Oxalate Monohydrate- 98%, protein- 2%

Source: L. C. Herring & Co. Laboratory

Figure 2 - Metabolism of Oxalate and Oxalate Precursors in the Human Body



Materials and Methods

Subjects and Fecal Collection

Fecal samples were collected from five subjects (Table 1). Five grams of a fecal sample was added to a serum bottle containing 50 ml of anaerobic dilution solution. Bottles were sealed and shaken at room temperature for 15 minutes at 175 rpm.

Table 1 – Subject Data

Subject	Ethnicity	1	2	3
1. Male 21-30	Caucasian	No	No	No
2. Male 21-30	African-American	No	No	No
3. Female 21-30	African-American	Yes	No	Yes
4. Male 41-50	Norwegian-German	Yes	No	No
5. Female 41-50	Caucasian	No	No	No

Legend (Questions asked to subjects)
1 – Has had kidney stones before
2 – Had a history of kidney stones in family
3 – Had recently (within the previous 2 months) taken antibiotics

Culture Preparation

One milliliter of the fecal slurry from each bottle was removed with the use of a 1 mL syringe and an 18-gauge needle. The 1 mL from each was then injected into 50 mL of an undefined medium (0.1% yeast extract, trace minerals; metals) containing 10 mM glyoxylate. All cultures were incubated at 37°C.

Sample Preparation and Analysis

Cultures were analyzed by high performance liquid chromatography (HPLC). This was done by transferring 1 mL of the culture to a microfuge tube. The microfuge tube was centrifuged at 14,000 rpm for 4 minutes. The supernatant was removed and filtered into an HPLC vial using a 4 mm nylon syringe filter. The sample was then subjected to HPLC analysis. A culture was considered positive if glyoxylate concentrations were at 1 mM or less.

Enhancement of Glyoxylate Consumption

Enhancement of glyoxylate consumption was performed with cultures from subject #2 (refer to results). This was done by the addition of extra nutrients (1% yeast extract or 10 mM glucose) to the undefined medium. An active glyoxylate-degrading culture (<1mM glyoxylate) was transferred into each of the enhanced enrichment media.

Results

All five subjects tested positive for the presence of fecal glyoxylate-degrading bacteria in initial enrichment cultures (Table 2). Subsequent transfers were made from these initial enrichment cultures and four of the five cultures remained active in glyoxylate degradation (Figure 3). Only the culture from subject 3 failed to degrade the glyoxylate. Enrichment cultures from subjects 1, 2, 4, and 5 were maintained for more than 15 sequential transfers.

When subject 2 enrichment culture was transferred to the enhanced enrichment media, the glucose, supplemented medium showed immediate consumption of glyoxylate (Figure 4). The 1% yeast extract enrichment medium also enhanced glyoxylate consumption when compared to the 0.1% yeast extract medium.

Table 2 – Initial Positive Cultures

Subjects	Glyoxylate (initial culture)
1. Male 21-30	+ (14)
2. Male 21-30	+ (12)
3. Female 21-30	+ (13)
4. Male 41-50	+ (13)
5. Female 41-50	+ (15)

Legend:
(#) = Number of days until culture was positive
(+) = Culture's substrate concentration was <1mM
(-) = Culture's substrate concentration was >1mM

Figure 3 – Glyoxylate Degradation in Subjects: 1, 2, 4, and 5

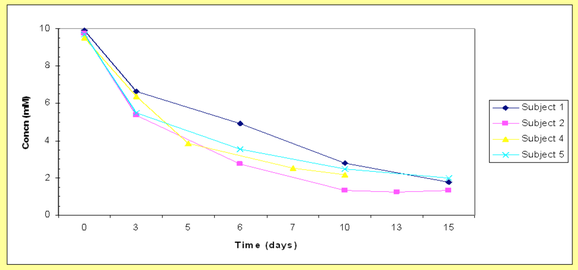
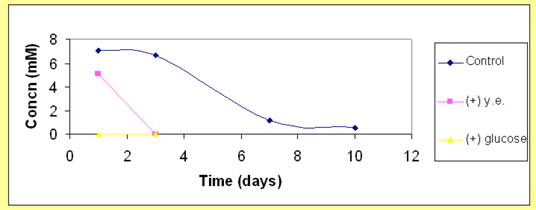


Figure 4 - Glyoxylate Degradation with Yeast Extract and Glucose



Conclusions

- In this study, the undefined medium with the addition of glucose served as the best enrichment medium for glyoxylate-degrading bacteria.
- High levels of yeast extract also stimulated glyoxylate degradation in enrichment cultures.
- With the enrichment media developed in this study, it will now be possible in future projects to isolate and characterize glyoxylate degrading anaerobes in the human gastrointestinal tract.
- Isolation of glyoxylate-degrading bacteria from the gut will have important medical implications since these bacteria may serve as a probiotic for the prevention of calcium oxalate stones in humans.

References

(1) Allison, Milton J., Daniel, Steven L., and Cornick, Nancy A. 1995. Calcium Oxalate in Biological Systems 7: 131-168.

(2) Cornick, N.A., and Allison, M.J. 1996. Assimilation of oxalate, acetate, and CO2 by Oxalobacter formigenes. Canadian Journal of Microbiology 42: 1081-1086.

(3) Harris, Kim S.; Richardson, K.E.. 1980. Glycolate in the Diet and Its Conversion to Urinary Oxalate in the Rat. Investigative Urology 18 (1): 106-109.

(4) Ogawa, Yoshihide; Miyazato, Tomonori; and Hatano, Tadashi. 1999. Importance of Oxalate Precursors for Oxalate Metabolism in Rats. Journal of the American Society of Nephrology 10: 341-344.

(5) Richardson, Keith E., and Farinelli, Michael P. The Pathways of Oxalate Biosynthesis. Ohio State University 855-863.

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