Investigating the Potential of *Escherichia coli* to Promote Kidney Stone Formation

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Abstract

*Escherichia coli* is commonly isolated from the urine of kidney stone patients and has been observed to be embedded within the nidus of calcium oxalate kidney stones. A possible association between urinary tract infections and calcium oxalate crystallogenesis was investigated using an agarose plate assay. Calcium oxalate crystallization was observed to be directly proportional to the density of *E. coli*, suggesting that *E. coli* may directly or indirectly promote the formation of calcium oxalate crystals.

**Keywords:** *Escherichia coli, Proteus mirabilis*, renal calculi, kidney stones, calcium oxalate, urinary tract infection, crystallogenesis.

Introduction

Renal calculi, or kidney stones, may either be classified as metabolic stones or as infection-induced stones (Gomez-Nunez et al. 2009) and both types tend to be associated with urinary tract infections (UTIs). The strong correlation of UTI’s with nephrolithiasis may simply demonstrate the increased susceptibility to infection of tissue damaged by a pre-existing stone, but, in some cases, the UTI may be able to serve as the direct or indirect cause of stone formation itself (Burall et al. 2004, Gomez-Nunez et al. 2009, Lindberg 2001, Pearson et al. 2008).

As renal calculi form bacterial cells may become embedded within the stone matrix and recent studies have suggested that the location of these embedded bacteria within the matrix may be indicative of the timing of the infection relative to the onset of stone formation (Tavichakorntrakoo et al. 2012). If the infection preceded stone formation the bacterial cells would most likely be embedded within the stone nidus, whereas bacterial cells located in the stone periphery may best correlate with those microorganisms present during a secondary UTI. Those bacteria isolated from the stone nidus are of particular interest for their potential role as causative agents of nephrolithogenesis.

While the urea-splitting bacterium, *Proteus mirabilis*, is commonly associated with stuvite kidney stones and is generally accepted as a significant risk factor for struvite nephrolithogenesis, *Escherichia coli* is the single most common bacterium isolated from the stone matrices and urine of patients who have kidney stones, accounting for approximately one-third of all bacterial isolates (Tavichakorntrakoo et al.
The predominance of *E. coli* in both urine and throughout stone matrices, including the stone nidus, suggests a possible correlation between this bacteria and nephrolithogenesis (Tavichakorntrakoo et al. 2012). However, the direct role of *E. coli* in kidney stone formation, particularly in calcium oxalate stone formation, has not been investigated. The purpose of this research was to investigate the role of *E. coli* in calcium oxalate crystallogenesis.

**Material and Methods**

*Bacterial cells and culture conditions.* *E. coli* strain ER2420 (New England Biolabs, Inc.) was maintained on LB media at 37°C for all routine manipulations. Prior to incorporating *E. coli* into agarose pour plates, *E. coli* cells were harvested from an overnight culture by centrifugation (10,000 rpm, 5 min, 4°C) and washed 3 times with sterilized isotonic saline (0.9% NaCl). Washed *E. coli* cells were resuspended in sterile 0.9% NaCl to a standardized cell density of 2 x 10⁹ CFU/mL. Agarose pour plates (1% (w/v) agarose, 10 mM Tris, 90 mM NaCl, pH7.4) were prepared to contain 5 x 10⁴, 5 x 10⁵, 5 x 10⁶, and 5 x 10⁷ CFU/mL *E. coli* cells by tempering agarose to 45°C, adding culture, and pouring to an even depth of 8 mm.

*Calcium oxalate crystallogenesis assays.* All assays were performed in quadruplicate. Two diverging wells were created by cutting 1 x 5 cm trenches in the agar such that the wells were positioned 1 cm apart on one end and 3 cm apart on the other (Figure 1). To one well 1 mL sodium oxalate solution (50 mM sodium oxalate, 10 mM Tris (pH 7.4), 90 mM NaCl) was added; 1 mL calcium chloride solution (50 mM calcium chloride, 10 mM Tris (pH 7.4), 90 mM NaCl) was added into the second well. All plates were incubated at 37°C for up to 48 hours, whereupon crystal formation was observed in a region between the two wells. A gel image of each plate was recorded using the ImageQuant 4000 gel documentation system.
Figure 1. Two diverging 5 cm trenches were cut into the agarose as indicated.

Results

With the agarose plates set up as described, the calcium chloride and sodium oxalate solutions diffuse outward and combine in the region between the two wells; a zone of precipitation is observed as calcium oxalate crystals are formed. The density of the calcium oxalate precipitate was observed to be directly proportional to the density of *E. coli* in the agarose plates (Figure 2) suggesting that *E. coli* cells directly or indirectly promote calcium oxalate crystal formation.

Figure 2: Crystallization of calcium oxalate increased as the volume of *E. coli* suspension incorporated in agarose plate increased. A) Bacteria-free control, B) $5 \times 10^4$ CFU/mL *E. coli*, C) $5 \times 10^5$ CFU/mL *E. coli*, D) $5 \times 10^6$ CFU/mL *E. coli*, E) $5 \times 10^4$ CFU/mL *E. coli*.
Discussion

*Escherichia coli* is the most common bacterium isolated from stone matrices and from the urine of kidney stone patients. This correlation suggests a possible role of *E. coli* in promoting the formation of calcium oxalate crystals *in vivo* and indicates that *E. coli*-based UTIs may play a role in kidney stone formation *in vivo* (Tavichakorntrakoo et al. 2012). The agarose plate assay described in this report allows crystals to become trapped upon formation and forms the basis for a qualitative assay of the association between bacterial isolates and kidney stone formation. This assay demonstrates a strong correlation between *E. coli* concentration and calcium oxalate crystallogenesis. These observations suggest that some feature of the bacterial cell may serve as a nucleus for crystal formation to promote the accumulation and aggregation of calcium oxalate crystals, or that some byproduct of *E. coli* metabolism may alter the pH or otherwise affect the local environment to promote crystallization.

The assay developed herein also presents several interesting opportunities for further study of crystallogenesis, especially in that it can easily be adapted to study the inhibition of crystal formation. The introduction an additional well half way between the two diverging wells (Figure 3), for example, would allow for various solutions or extracts to be tested to gauge their effects, both positive and negative, on calcium oxalate crystal formation. Further investigation could be performed by substituting *E. coli* with other bacterial species commonly identified in stone matrices or the urine of kidney stone patients, to gauge their ability to promote crystal formation.

![Figure 3](image)

Figure 3. Adaptation of the plate crystallogenesis assay for testing potential inhibitors of calcium oxalate formation.
References


