Proteus mirabilis urease and human effort to inhibit it

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Objectives

- Define *P.mirabilis* and its role in urolithiasis.
- Define *P.mirabilis* urease constitution and its regulation.
- Explain the stone formation in the urinary tract focusing on the *P.mirabilis* persistence.
- Explain different urease inhibitors to treat the urolithiasis.

Introduction [1]

- *P.mirabilis* is a motile gram-negative bacterium that differentiates from a short vegetative rod to an elongated highly flagellated form.
- It is common in long-term catheterization and it is able to form biofilms on catheters.
- Because of the activation of urease, *P. mirabilis* can cause complicated urinary tract infections, like urolithiasis, that is based on the formation of stones through crystallization.

Materials and Methods [2], [3], [4]

- *P. mirabilis* strain was isolated and it was maintained on a slant of tryptic soy agar overnight at 37ºC. Then, it was suspended in an artificial urine.
- Artificial urine contains: $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, NaCl, Na_2SO_4, KH_2PO_4, KCl, NH_4Cl, $Na_3C_6H_5O_7$, $C_2Na_2O_4$, $(NH_2)_2CO$, $C_4H_9O_2N_3$ and tryptic soy broth.
- To test the urease inhibitors, it should be added at different concentrations to artificial urine.

Proteus mirabilis urease [5]

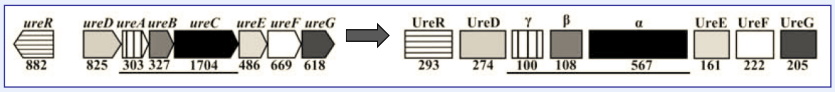

Fig. 2. Scheme of genetic organization of urease gens and structural composition of urease. Modification of Ref. [5].

REGULATION [1]:

- (+) ⟶ UreR
- (–) ⟶ H-NS

- *P. mirabilis* urease is a cytoplasmic metaloenzim
- It is a multimeric enzyme composed by three structural subunits (γ, β and α). These polypeptides are encoded by three structural genes (*ureA, ureB* and *ureC* respectively).
- Additional proteins (UreD, UreE, UreF and UreG), products of accessory genes, are required for urease activation

Urease activation

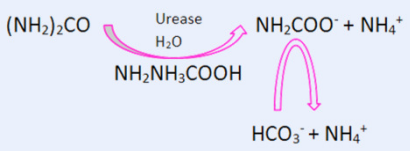

Fig. 3. Urea degradation. Modification of Ref. [5].

Stone formation (Urolithiasis)

Under alkaline conditions, an increase in the concentration of the NH_4^+, CO_3^{2-} and PO_4^{3-} ions occurs [6].

$$CO_3^{2-} + 10Ca^{2+} + 6PO_4^{3-} \xrightarrow{pH \geq 6,8} Ca_{10}(PO_4)_6CO_3 - \text{Carbonate apatite}$$

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \xrightarrow{pH \geq 7,2} MgNH_4PO_4 \cdot 6H_2O - \text{Struvite}$$

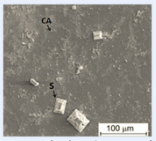

Fig. 4. Carbonate apatite (CA) and Struvite (S) grown from artificial urine in the presence of *P.mirabilis*. Modification of Ref. [6].

P. mirabilis persistence in stones

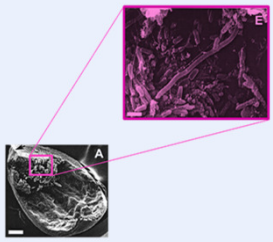

Fig. 5. *P.mirabilis* persistence in the stones. Modification of Ref. [7].

Results

- The basic crystal morphology is coffin-like (Fig.6, panels s1 and s2).
- When pH increases, crystals frequently form twins (Fig.6, panels t1 and t2).
- For highest value of pH 9.5, dendritic structures appear (Fig.6, panels d1 and d2).

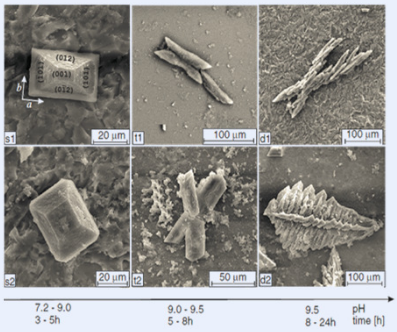

Fig. 6. Struvite morphology depending on pH changes on time. Ref. [6].

- The typical treatment of urolithiasis concerns antibiotic therapy. However, the widespread bacterial resistance shows the need of the **development of effective inhibitors** with safe and more potent profiles [2].

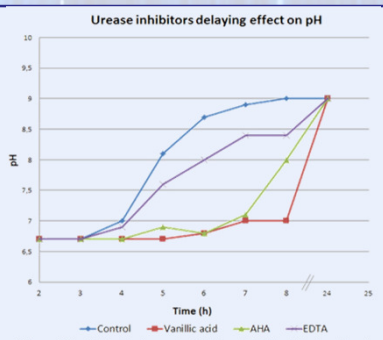

Fig. 7. Acid vanillic, AHA (Hydroxamic Acid) and EDTA (Ethylenediamine Tetraacetic Acid) delaying effect on pH. Inhibitors were used at the concentration 0.5 mg/ml (3 mM). Based on Ref. [2] and [3].

- With a 0,5 mg/ml (3 mM) concentration of **AHA**, 7-8 hours are necessary to form the first struvite crystals. It needs 5 mg/ml to inhibit struvite stone formation. AHA causes side effects [2].
- With a 0,5 mg/ml (3 mM) concentration of **vanillic acid**, 24 hours are necessary to form the first struvite crystals (smaller but with the same morphology) [2].
- With a 0,5 mg/ml (3 mM) concentration of **EDTA**, 4,5 hours are necessary to form the first struvite crystals (smaller and with different morphology, without dendrites) [3].

UREASE INHIBITOR	CRYSTALLIZATION INFLUENCE
Fluorofamide [8]	• Fails to inhibit cytoplasmic ureases.
Curcumine [4]	• With a concentration of 1 mM, 10 hours are required to form the first struvite crystals (fewer and smaller but with the same morphology). • Low solubility.
TA and PNPG [9]	• There are a quorum sensing antagonists. • In the presence of 100 – 200 µg/ml, biofilms possess a significantly lower number of bacterial cells, decreasing the pH of 8.3-8.4 to 6.1-6.7.
GAGs [10]	• ChSC increases the crystallization. • All GAGs intensified the struvite stones adhesion to ureter and kidney urothelium. Adhesion to bladder urothelium was not promoted neither by HS nor ChSB. • ChSA showed an increment of adherence to three urotheliums (ureter, bladder and kidney).

Table 1. Crystallization effect of different urease inhibitors. TA (Tannic Acid), PNPG (p-nitrophenyl glycerol), GAGs (Glycosaminoglycans), ChSC (Chondroitin Sulfate C), HS (Heparan Sulfate), ChSB (Chondroitin sulfate B), ChSA (Chondroitin Sulfate A).

Discussion

- In an artificial urine (with *P.mirabilis*) without inhibitors, the first struvite crystals are formed at 3 - 4 hours. When inhibitors are added, the time to form the first struvite crystals is increases.
- Less curcumine concentration is needed than AHA, EDTA and vanillic acid to delay the struvite stones formation.
- Despite AHA is a good urease inhibitor, it is discarded because of side effects.
- So, it could be say that curcumine is the most effective inhibitor. However, it is not because of its low solubility.
- Vanillic acid is more effective than EDTA because with 3 mM of concentration, it allows a greater delay on the formation of the first struvite crystals. Nevertheless, EDTA is also effective because the stones that forms do not have dendrites, therefore they are easier to remove.
- Quorum sensing antagonists seem to be good candidates for the urolithiasis treatment because they decrease urease activity.
- Glycosaminoglycans (GAGs) influence the crystallization but no compound inhibit this process. So, GAGs don't prevent the formation of struvite crystals. GAGs are molecules that have an influence on the adhesion of crystals to urothelium.

Conclusions

- Crystals morphology are mainly influenced by the rate of change in pH with minor influence on the value of pH.
- Aggregation is one of the main causes of stone formation in the urinary tract.
- Molecules such as polyphenols, quorum sensing antagonists and other chemical molecules are replacing the role of antibiotics in the treatment and prevention of urolithiasis.
- The use of GAGs replacement therapy is investigated for the treatment of urolithiasis.
- Fluorofamide fails to inhibit cytoplasmic urease, so it is not useful for treating urolithiasis produced by *P. mirabilis.*
- Research about urease inhibitory mechanism of quorum sensing antagonists is required.
- **One of the urease inhibitors seems to be the most effective for the treatment of urolithiasis is vanillic acid, followed by EDTA.**

References

[1] Armbruster CE, Mobley HL. Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. Nat Rev Microbiol. 2012;10(11):743-54.
[2] Torzewska A, Rozalski A. Inhibition of crystallization caused by *Proteus mirabilis* during the development of infectious urolithiasis by various phenolic substances. Microbiological Research. 2014;169(7–8):579-84.
[3] Prywer J, Marcin O, Torzewska A, Ewa M-B. Comparative in vitro studies on disodium EDTA effect with and without *Proteus mirabilis* on the crystallization of carbonate apatite and struvite. Journal of Crystal Growth. 2014;395:123-31.
[4] Prywer J, Torzewska A. Effect of Curcumin Against *Proteus mirabilis* During Crystallization of Struvite from Artificial Urine. Evidence-Based Complementary and Alternative Medicine. 2012;2012:7.
[5] Konieczna I, Zarnowiec P, Kwinkowski M, Kolesinska B, Fraczyk J, Kaminski Z, et al. Bacterial Urease and its Role in Long-Lasting Human Diseases. Current Protein & Peptide Science. 2012;13(8):789-806.
[6] Prywer J, Torzewska A, Plocinski T. Unique surface and internal structure of struvite crystals formed by *Proteus mirabilis*. Urol Res. 2012;40(6):699-707
[7] Li X, Zhao H, Lockatell CV, Drachenberg CB, Johnson DE, Mobley HL. Visualization of *Proteus mirabilis* within the matrix of urease-induced bladder stones during experimental urinary tract infection. Infect Immun. 2002;70(1):389-94.
[8] Follmer C. Ureases as a target for the treatment of gastric and urinary infections. J Clin Pathol. 2010;63(5):424-30.
[9] Jones SM, Dang TT, Martinuzzi R. Use of quorum sensing antagonists to deter the formation of crystalline *Proteus mirabilis* biofilms. Int J Antimicrob Agents. 2009;34(4):360-4.
[10] Torzewska A, Rozalski A. In vitro studies on the role of glycosaminoglycans in crystallization intensity during infectious urinary stones formation. Apmis. 2014;122(6):505-11.