### Proteus mirabilis urease

**Fig. 1.** Proteus mirabilis urease.

**Materials and Methods**

- *P. mirabilis* strain was isolated and then maintained on a slant of tryptic soy agar overnight at 37°C. Then, it was suspended in an artificial urine.
- Artificial urine contains: CaCl$_2$·2H$_2$O, MgCl$_2$·6H$_2$O, Na$_2$HPO$_4$·7H$_2$O, KCl, Na$_2$SO$_4$, Na$_2$HPO$_4$, (NH$_4$)$_2$CO, CaCl$_2$·2H$_2$O, and tryptic soy broth.
- To test the urease inhibitors, it should be added at different concentrations to artificial urine.

**Results**

- The basic crystal morphology is coffin-like (Fig.6, panels a and b).
- When pH increases, crystals frequently form twin plates (Fig.6, panels 1 and 2).
- For higher value of pH, 9.5, dendritic structures appear (Fig.6, panels d1 and d2).

**Conclusion**

- *P. mirabilis* is an environmental 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.

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- **Fig. 2.** Scheme of genetic organization of urease genes and structural composition of urease. Modification of Ref. [2].

**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 curcumin 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 curcumin 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. It needs 5 mg/ml to inhibit struvite stone formation. AHA causes side effects.
- With a 0.5 mg/ml (3 mM) concentration of vanillic acid, 24 hours are necessary to form the first struvite crystals (smaller with the same morphology).
- With a 0.5 mg/ml (3 mM) concentration of EDTA, 4.5 hours are necessary to form the first struvite crystals (smaller but with the same morphology).

**Table 1.** Crystalization effect of different urease inhibitors. TK (Tryptic Acid), PNP (p-nitrophosphoglyceride), GAGs (Glycosaminoglycans), CDS (Chondroitin sulfate C), HS (Heparan Sulfate), CDF (Chondroitin sulfate B), CHA (Chondroitin sulfate A).

**References**


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

- 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 replacements 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.

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

**Fig. 5.** Persistence of the crystals on the stones. Modification of Ref. [7].

- *P. mirabilis* is a multi-enzyme composed by three structural subunits (γ, β and α). These polypeptides are encoded by three structural genes (*ureA, ureB* and ureC, respectively).
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