Enzymatic Inactivation of Antibiotics to Combat Antimicrobial Resistances

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Introduction

- Antibiotics are used to treat diseases in humans and animals
- Many antibiotic are widely used in animal farming and aquaculture [1,2]
- Antibiotic waste in the environment promotes emergence of antibiotic resistances [3,4]
- Currently, there is no environmentally-friendly method to inactivate antibiotics

- **PROBLEM: How can antibiotics be effectively inactivated?**

Project

- Investigation of the enzymatic inactivation of the antibiotic florfenicol
- Improvement of a hydrolytic enzyme discovered by a Korean research group [5]
- Comparison of selected enzyme variants
- Immobilization of the optimized hydrolase mutant on different materials
- Test of florfenicol inactivation in different media such as saltwater and milk

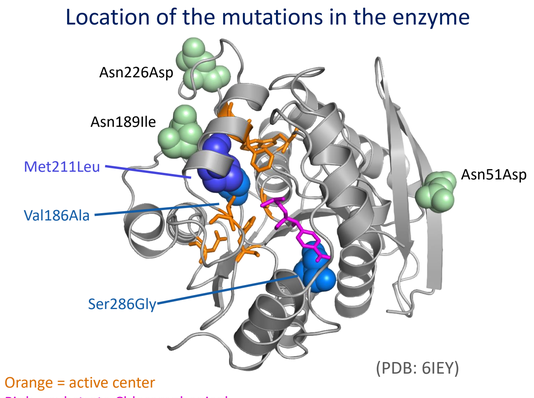
➢ **SOLUTION: Enzymatic inactivation as eco-friendly strategy**

Enzyme Optimization and Characterization for Florfenicol Cleavage

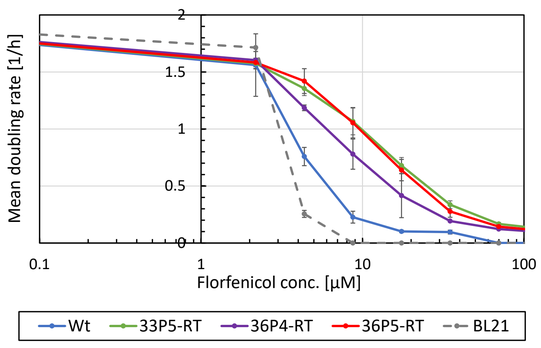
Selection of Hydrolase Mutants and Characterization

- Wild-type hydrolase (EstDL136) shows only insufficient activity for florfenicol cleavage
- Optimization by error-prone PCR (epPCR) combined with selection of mutants in florfenicol containing media
- Sequencing of the enriched mutants and localization of the mutations in the 3D structure [6]

Location of the mutations in the enzyme



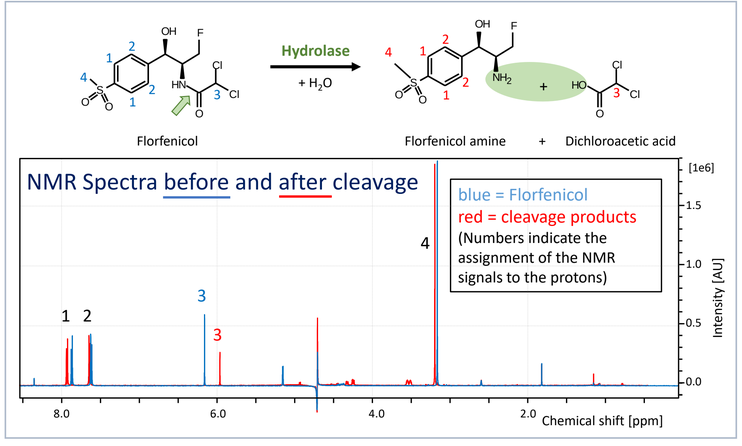
Growth assay of *E. coli* expressing different mutants in florfenicol-containing media



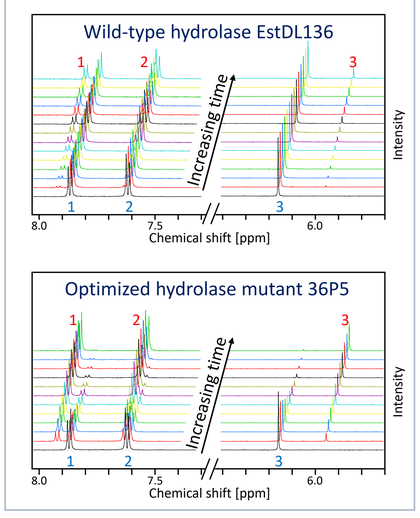
➢ Identical mutants were found multiple times; most mutations close to the active center
➢ Selected hydrolase mutants perform better than wild-type hydrolase

Assessing Catalytic Activity by NMR Spectroscopy

- Mixture of purified hydrolase (0.25 µM) with florfenicol (0.66 mM)
- Measurement of the NMR spectra every 4 min
- Comparison of wild-type hydrolase and mutant 36P5



Reaction Rate



NMR measurements were done in collaboration with Dr. Ruslan Nedielkov and Prof. Dr. Heiko Möller, Institute of Chemistry, University of Potsdam

➢ Optimized hydrolase mutant 36P5 can cleave florfenicol significantly more efficiently

Enzyme Immobilization and Test Strategies

Strategies for Repeated Use

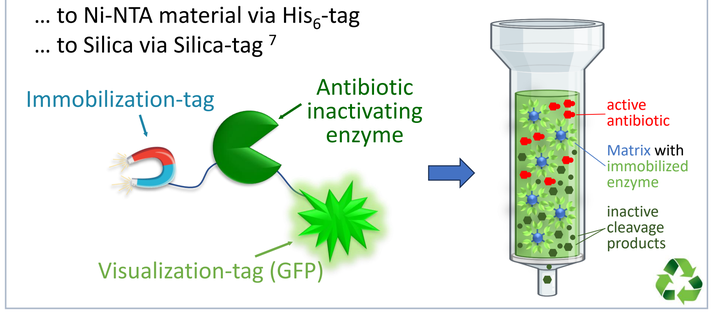
Purified enzyme

Encapsulation

…within semipermeable membrane

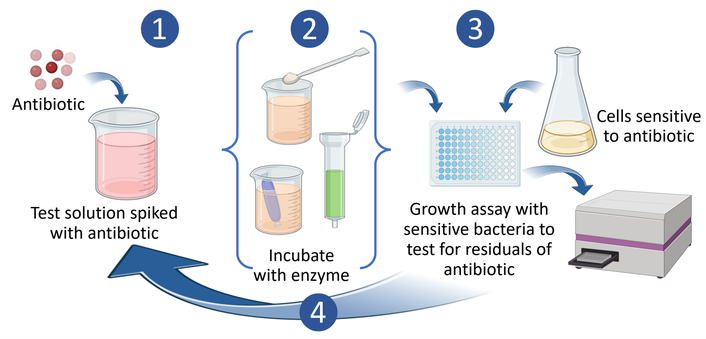
Immobilization onto carrier material

… to Ni-NTA material via His_6-tag
… to Silica via Silica-tag [7]



Assay Setup for Performance Test

1) Imitate use scenarios by spiking different media with antibiotics
2) Incubate antibiotic-containing media with inactivating enzyme in solution, encapsulated or immobilized
3) Check for residual antibiotics by comparing cell growth of susceptible bacteria in media before and after treatment
4) Repeat assay to test reuse of the materials

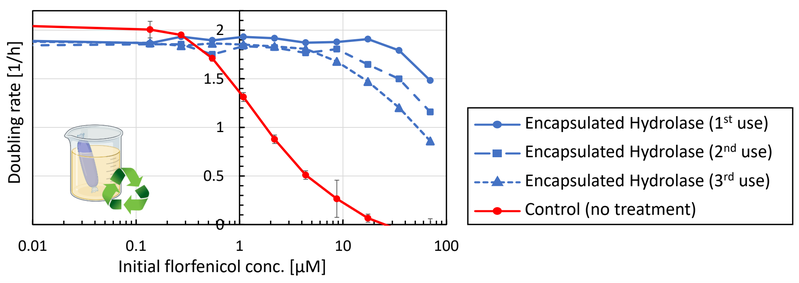


Antibiotic Inactivation in Different Application Areas

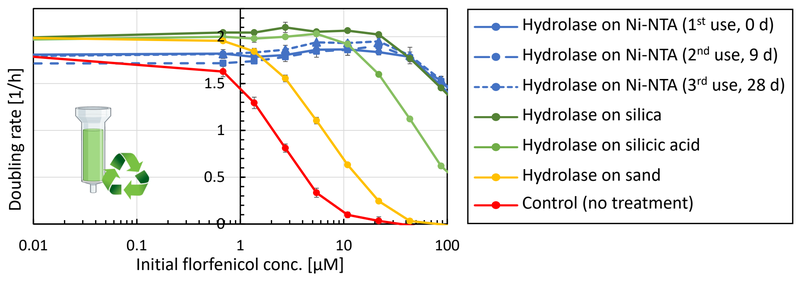
Application: Florfenicol Inactivation in Saltwater

PROBLEM: Antibiotics in aquaculture contaminate the water
SOLUTION: Use of hydrolase for florfenicol inactivation

Antibiotic inactivation in saltwater with encapsulated hydrolase

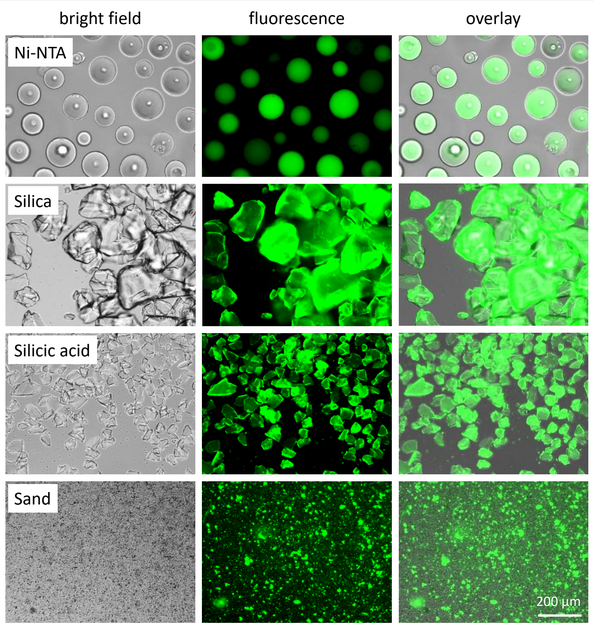


Antibiotic inactivation with hydrolase immobilized to different carrier materials



Immobilization

- Fluorescence microscopy of enzyme on carrier materials
- Enzyme between immobilization tag and GFP

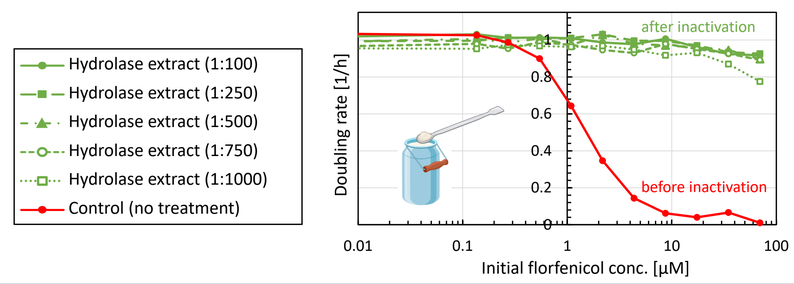


➢ Different carrier materials are possible

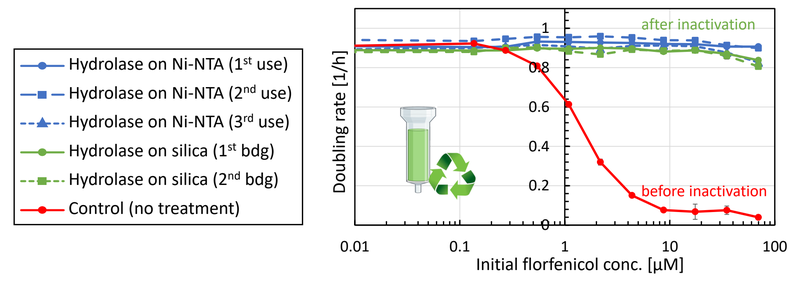
Application: Florfenicol Inactivation in Milk

PROBLEM: Antibiotic treatment of dairy cattle leads to waste milk [8]
SOLUTION: Use of hydrolase for florfenicol inactivation in milk

Antibiotic Inactivation in milk with hydrolase extract



Antibiotic Inactivation in milk with immobilized hydrolase



➢ The optimized hydrolase can successfully inactivate florfenicol in salt water and milk
➢ The immobilized and encapsulated hydrolase is stable and can be used repeatedly

Conclusions:

- ✓ Hydrolase enzyme from literature (EstDL136) was successfully optimized for efficient florfenicol inactivation
- ✓ Optimized hydrolase was immobilized on different materials (Ni-NTA, silica, sand) and could be used repeatedly
- ✓ Different use scenarios were successfully tested using soluble, immobilized or encapsulated hydrolase for florfenicol inactivation in saltwater and milk

References:

[1] Miranda CD *et al.*, Current Status of the Use of Antibiotics and the Antimicrobial Resistance in the Chilean Salmon Farms. *Front. Microbiol.* 2018, 9:1284.
[2] Subbiah M *et al.*, Beta-Lactams and Florfenicol Antibiotics Remain Bioactive in Soils while Ciprofloxacin, Neomycin, and Tetracycline Are Neutralized. *Appl. Environ. Microbiol.* 2011, 77:7255–7260.
[3] Singh AK *et al.*, Animal-Use Antibiotics Induce Cross-Resistance in Bacterial Pathogens to Human Therapeutic Antibiotics. *Curr. Microbiol.* 2019, 76, 1112–1117.
[4] Zhao Q *et al.*, Prevalence and Abundance of Florfenicol and Linezolid Resistance Genes in Soils Adjacent to Swine Feedlots. *Sci. Rep.* 2016, 6, 32192.
[5] Tao W *et al.*, Inactivation of Chloramphenicol and Florfenicol by a Novel Chloramphenicol Hydrolase. Appl. Environ. Microbiol. 2012, 78:6295–6301.
[6] Kim SH *et al.*, Crystal Structure of Chloramphenicol-metabolizing Enzyme EstDL136 from a Metagenome. *PLoS One* 2019, 14:e0210298.
[7] Taniguchi K *et al.*, The Si-Tag for Immobilizing Proteins on a Silica Surface. *Biotechnol. Bioeng.* 2007; 96:1023–1029.
[8] Ricci A *et al.*, Risk for the development of Antimicrobial Resistance (AMR) due to feeding of calves with milk containing residues of antibiotics. EFSA J. 2017, 15, 4665.
Schematic diagrams created with BioRender.com
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