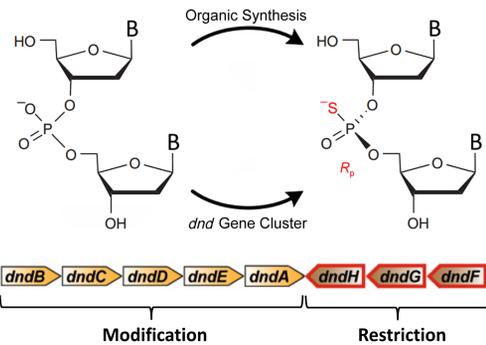


DNA Phosphorothioation Enhances Bacterial Stress Response and Confers Partial Antibiotic Resistance

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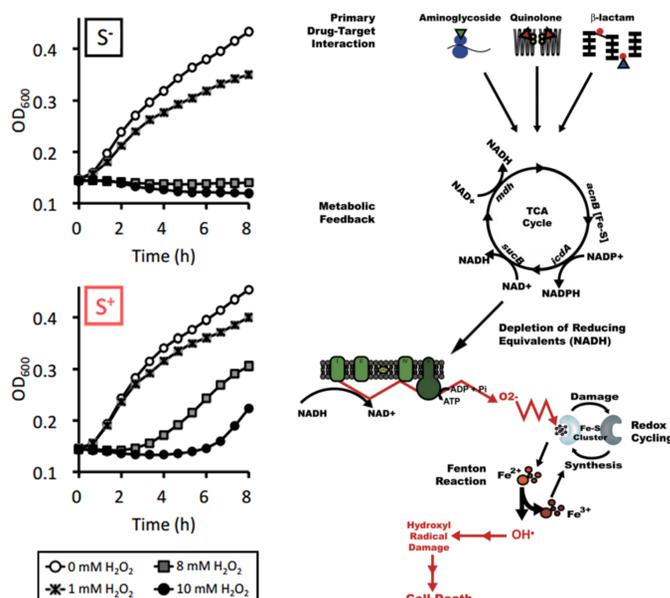
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Phosphorothioate (PT) History



- Sulfur replaces non-bridging oxygen to make PT
- Five- or eight-gene mobile genetic island *dnd*
- Five modification genes; three restriction genes
- Found by homology in many human pathogens

Antibiotic Resistance Link



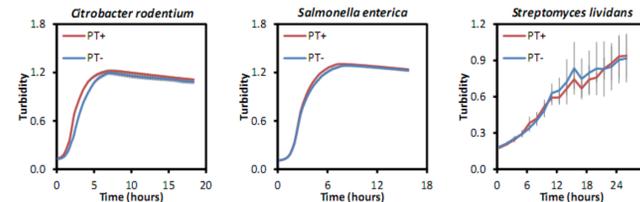
- Phosphorothioation confers H₂O₂ resistance¹
- Bactericidal antibiotics may cause oxidative death²
- Does *dnd* and/or PT confer antibiotic resistance?

Test Strains & Genotypes

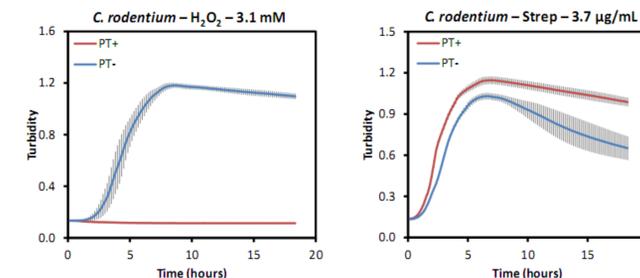
Organism	WT	Strain	Status	<i>dnd</i> Genes
<i>Citrobacter rodentium</i>	PT-	DBS 805	PT+	A,B,C,D,E
		DBS 803	PT-	–
<i>Salmonella enterica</i>	PT+	Cerro 87	PT+	A,B,C,D,E,F,G,H
		XTG 103	PT-	–
<i>Streptomyces lividans</i>	PT+	ZX 1	PT+	A,B,C,D,E
		Δ <i>dndA</i>	PT-	B,C,D,E

- Both native and artificially inserted PT systems
- With and without *dndF-H* genes for restriction

PT Does Not Alter Growth Rate

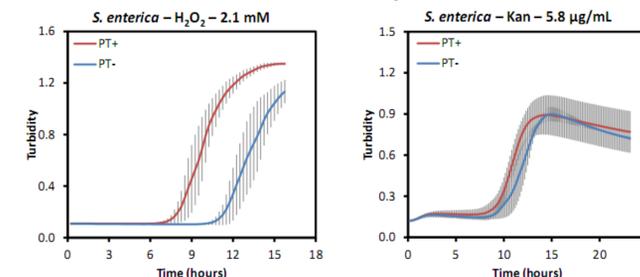


C. rodentium is Partially Resistant



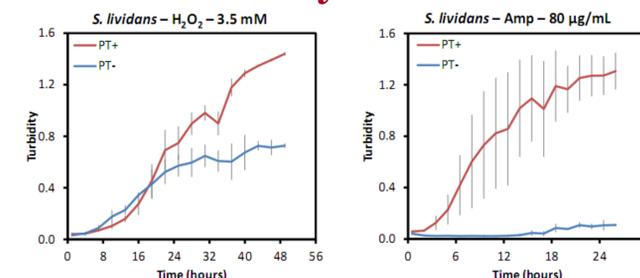
- PT+ strain is much more sensitive to H₂O₂ stress
- PT+ strain is more resistant to antibiotic stress

S. enterica is Partially Resistant



- PT+ strain is more resistant to H₂O₂ stress
- Strains are equally sensitive to antibiotic stress

S. lividans is Fully Resistant

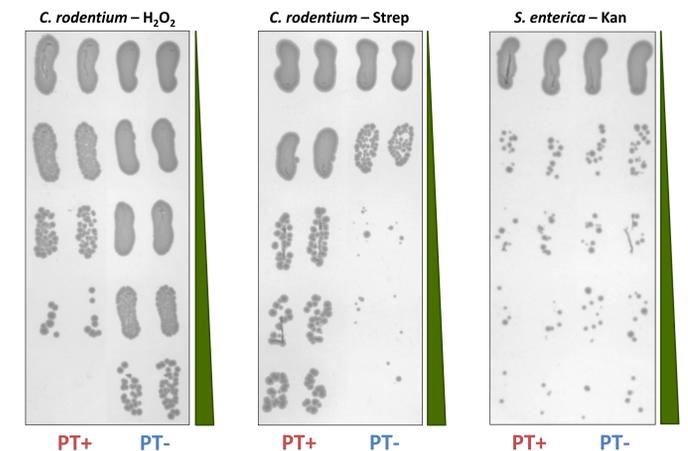


- PT+ strain is more resistant to H₂O₂ stress
- PT+ strain is more resistant to antibiotic stress

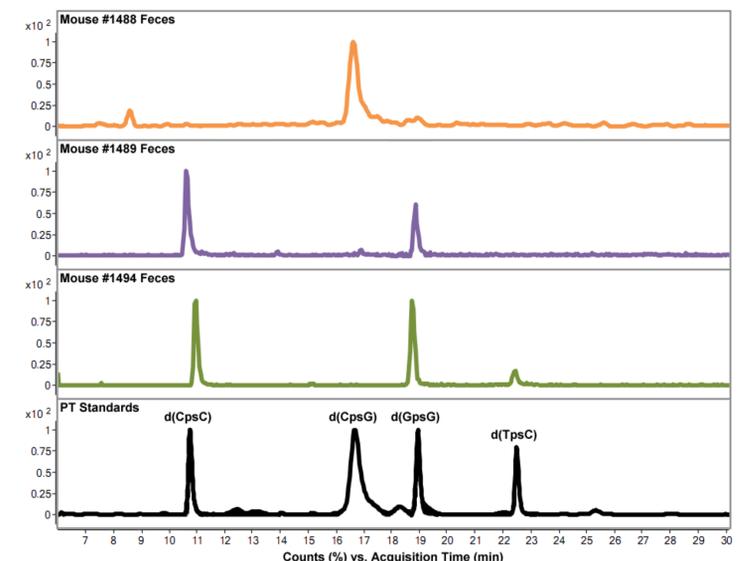
Summary of Resistance Patterns

Organism	Source	<i>dndF-H</i>	H ₂ O ₂	Antibiotic
<i>C. rodentium</i>	Artificial	No	Sensitive	Resistant
<i>S. enterica</i>	Native	Yes	Resistant	Sensitive
<i>S. lividans</i>	Native	No	Resistant	Resistant

Effect Depends on Cell Death



PT is Found in Gut Microbiome



- DNA extracted from healthy mouse feces, purified by HPLC, & detected by QQQ vs. standards
- Could affect microbiome restructuring following disease or antibiotic treatment
- Could serve as a reservoir of antibiotic resistance for invading pathogens

Ongoing Work

- Additional species, strains, drugs, and stresses
- Effect of PT on *in vivo* infection animal model
- Effect of disease or antibiotics on microbiome PT

Acknowledgements

- Xie *et al.* 2012 *Nucl. Acids Res.* 1-10
- Kohanski *et al.* 2007 *Cell* 130: 797-810
- Strains from Dr. Liangrong Wang & Dr. Delin You