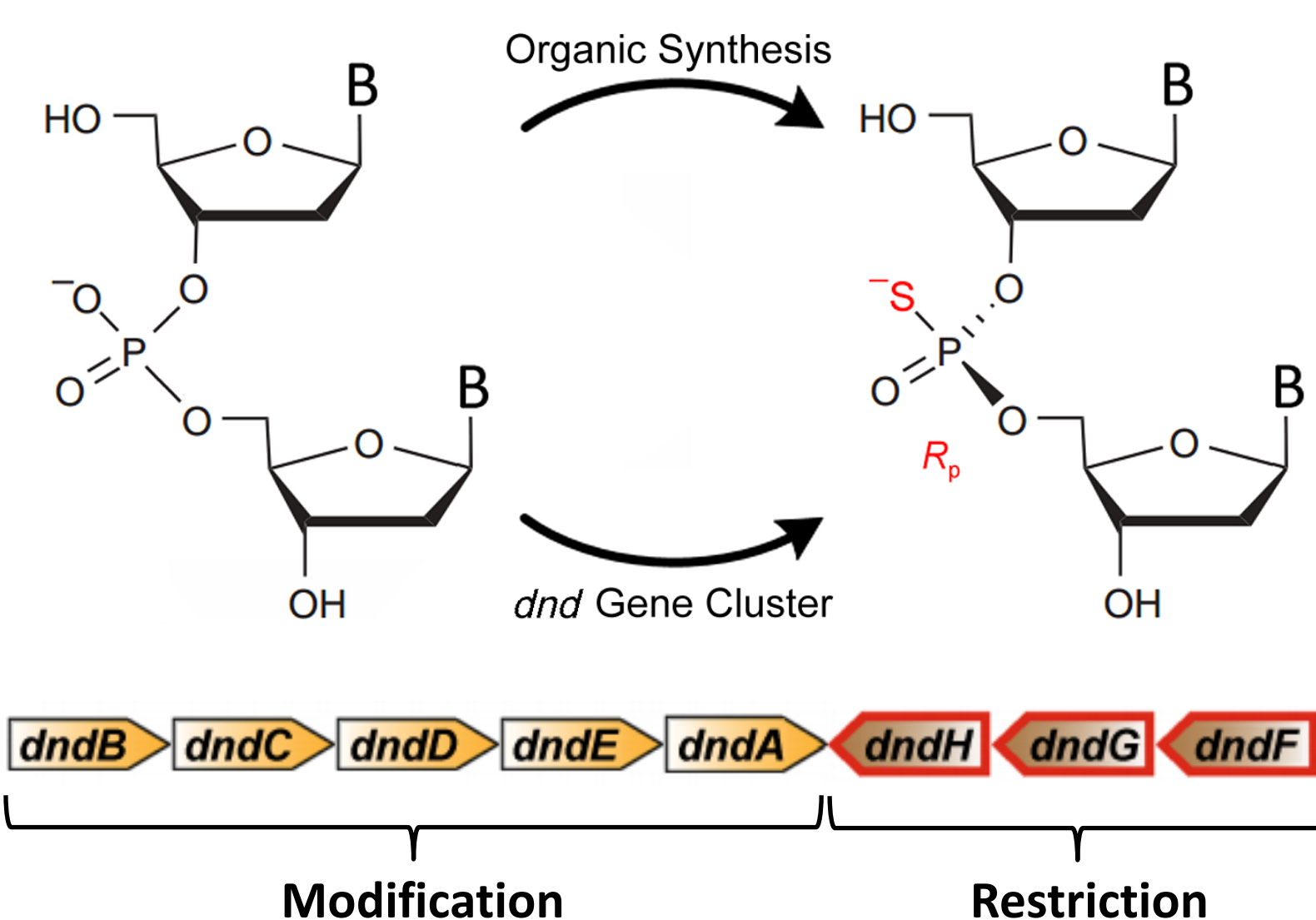


# DNA Phosphorothioation Enhances Bacterial Stress Response and Confers Partial Antibiotic Resistance

Brandon S. Russell<sup>1</sup>, Bo Cao<sup>1</sup>, Sasilada Sirirungruang<sup>2</sup>, Aislyn Schlack<sup>1</sup>, Michael S. DeMott<sup>1</sup>, Megan E. McBee<sup>1</sup>, James G. Fox<sup>1,3,4</sup>, and Peter C. Dedon<sup>1,3</sup>

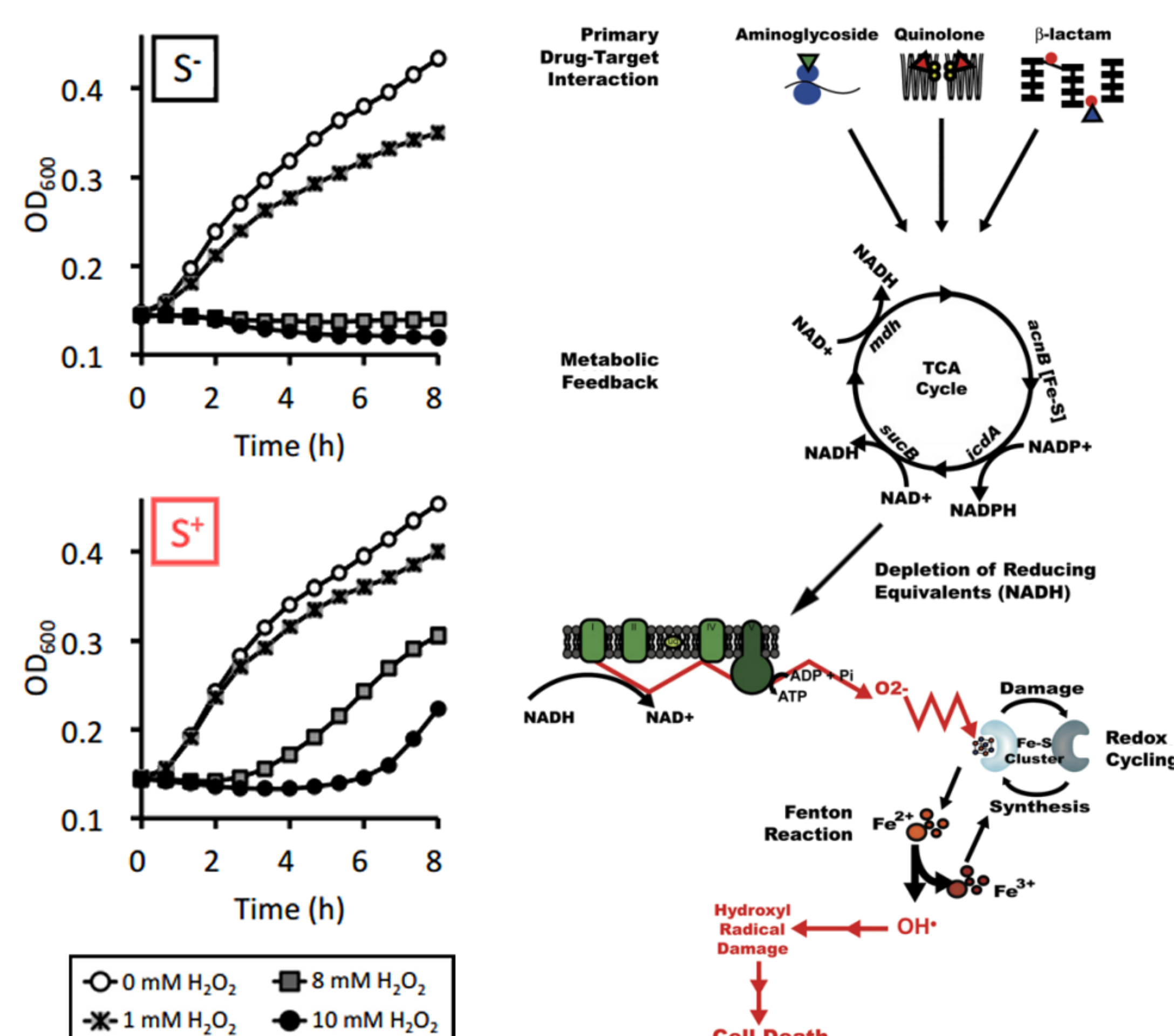
Departments of <sup>1</sup>Biological Engineering & <sup>2</sup>Chemistry, <sup>3</sup>Center for Environmental Health Sciences, & <sup>4</sup>Division of Comparative Medicine – MIT, Cambridge, MA

## 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



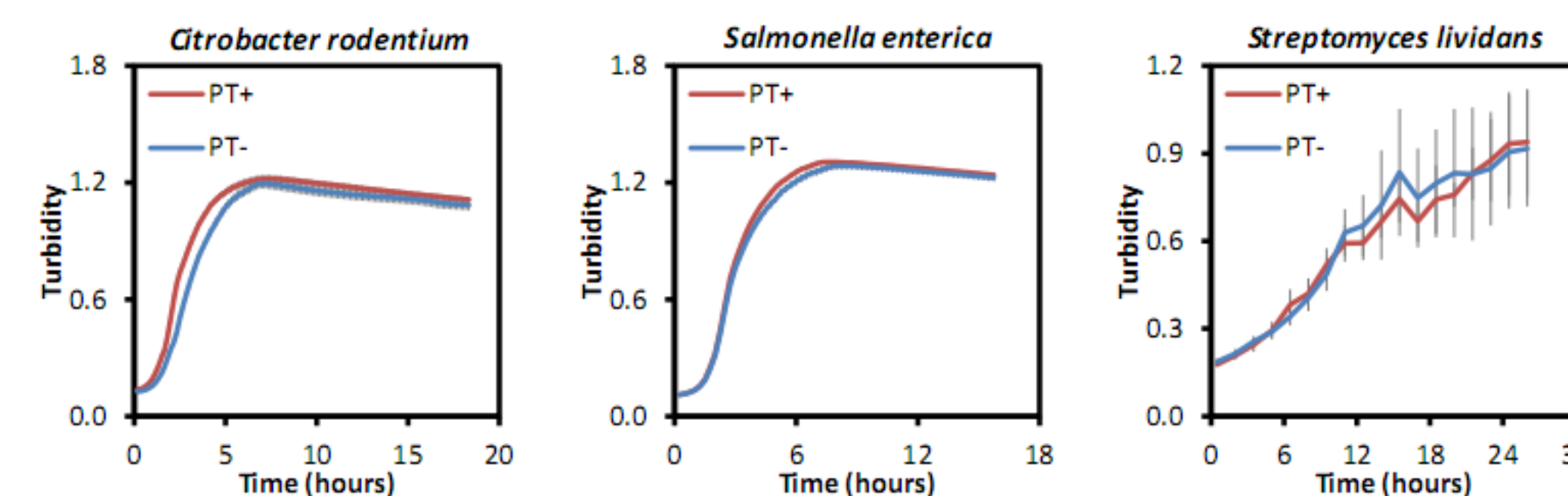
- Phosphorothioation confers H<sub>2</sub>O<sub>2</sub> resistance<sup>1</sup>
- Bactericidal antibiotics may cause oxidative death<sup>2</sup>
- Does *dnd* and/or PT confer antibiotic resistance?

## Test Strains & Genotypes

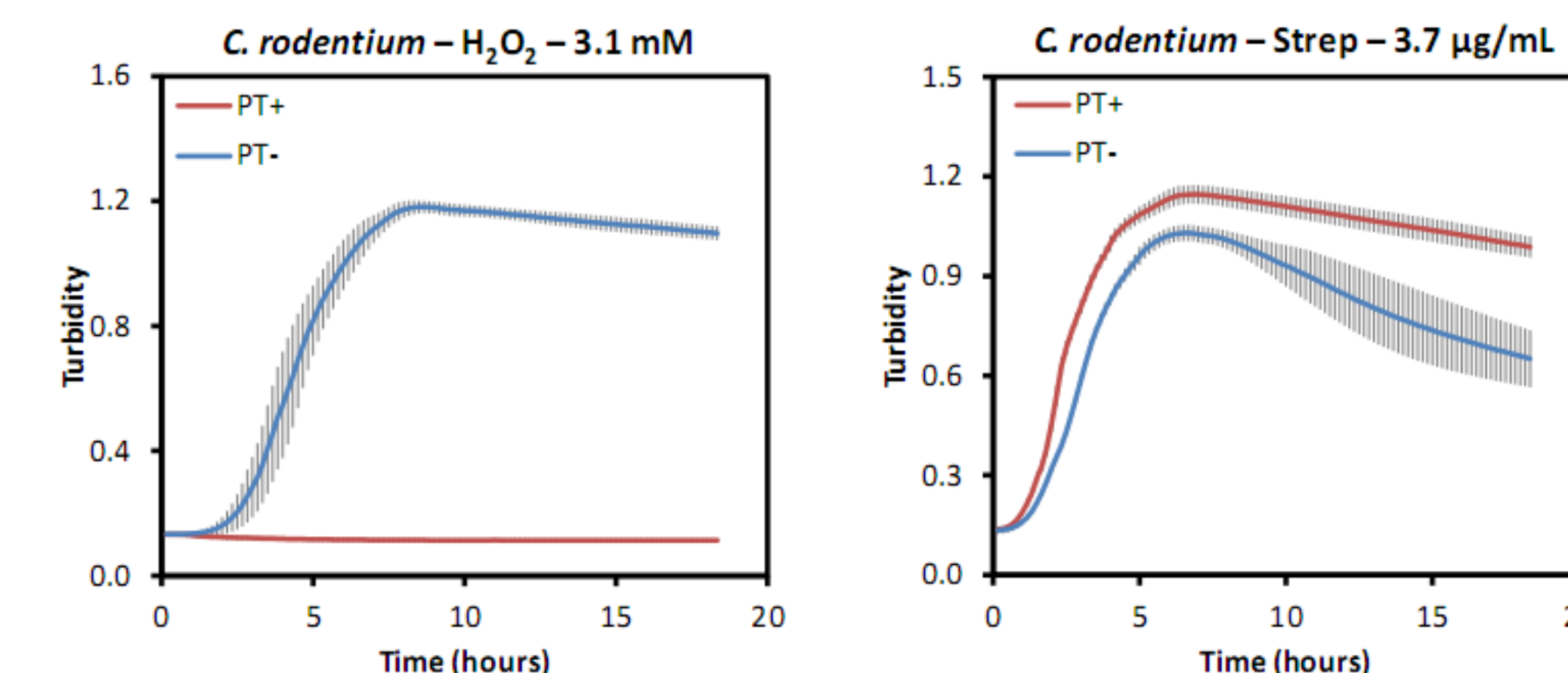
Organism	WT	Strain	Status	<i>dnd</i> Genes
<i>Citrobacter rodentium</i>	PT -	DBS 805	PT+	A,B,C,D,E
		DBS 803	PT-	—
<i>Salmonella enterica</i>	PT +	Cerro 87	PT+	A,B,C,D,E,F,G,H
		XTG 103	PT-	—
<i>Streptomyces lividans</i>	PT +	ZX 1	PT+	A,B,C,D,E
		Δ <i>dndA</i>	PT-	B,C,D,E

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

## PT Does Not Alter Growth Rate

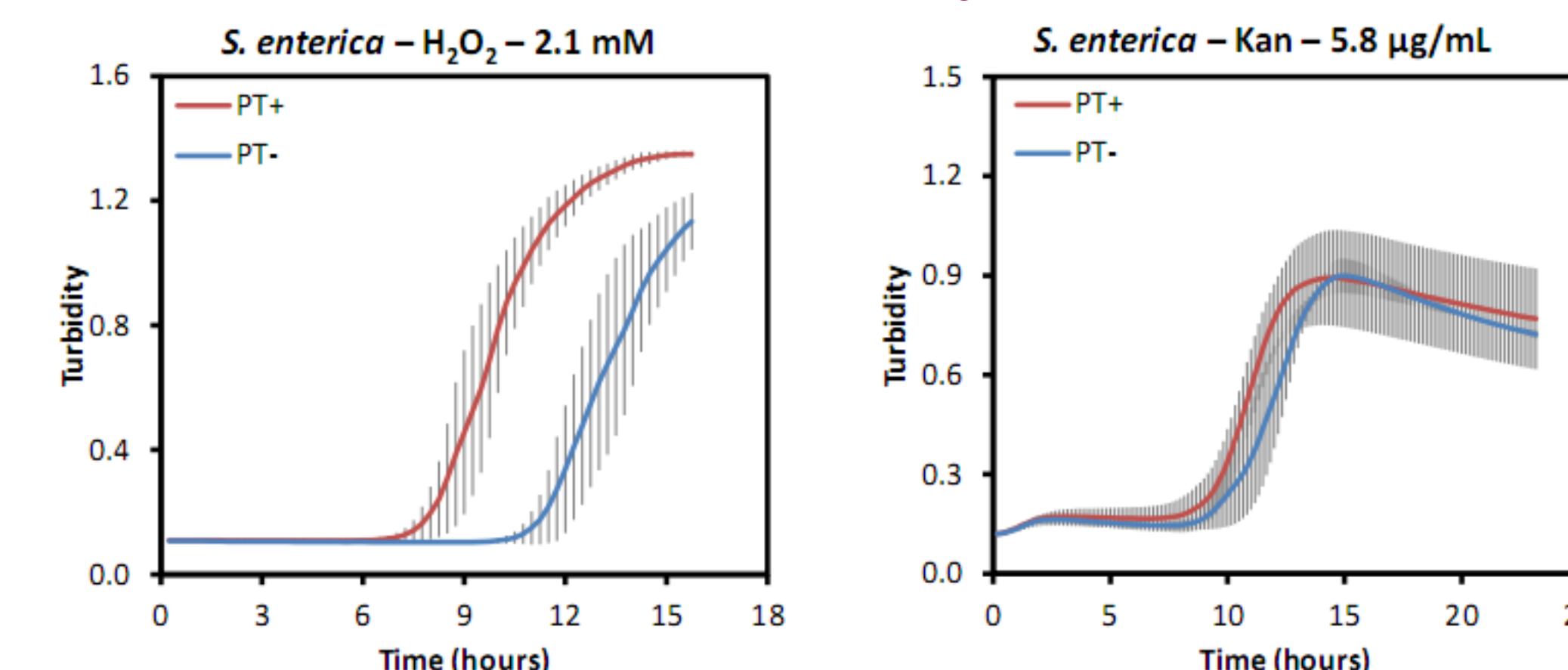


## C. rodentium is Partially Resistant



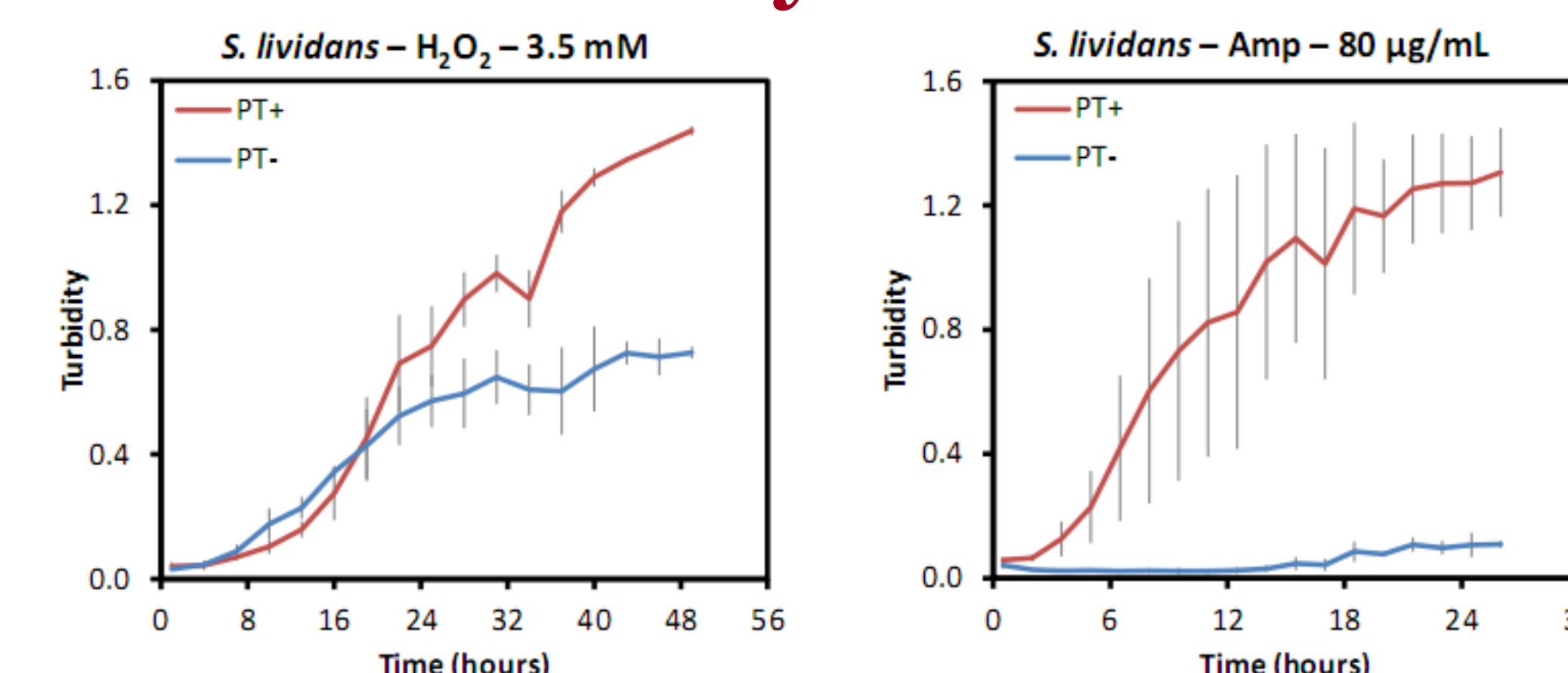
- PT+ strain is much more sensitive to H<sub>2</sub>O<sub>2</sub> stress
- PT+ strain is more resistant to antibiotic stress

## S. enterica is Partially Resistant



- PT+ strain is more resistant to H<sub>2</sub>O<sub>2</sub> stress
- Strains are equally sensitive to antibiotic stress

## S. lividans is Fully Resistant

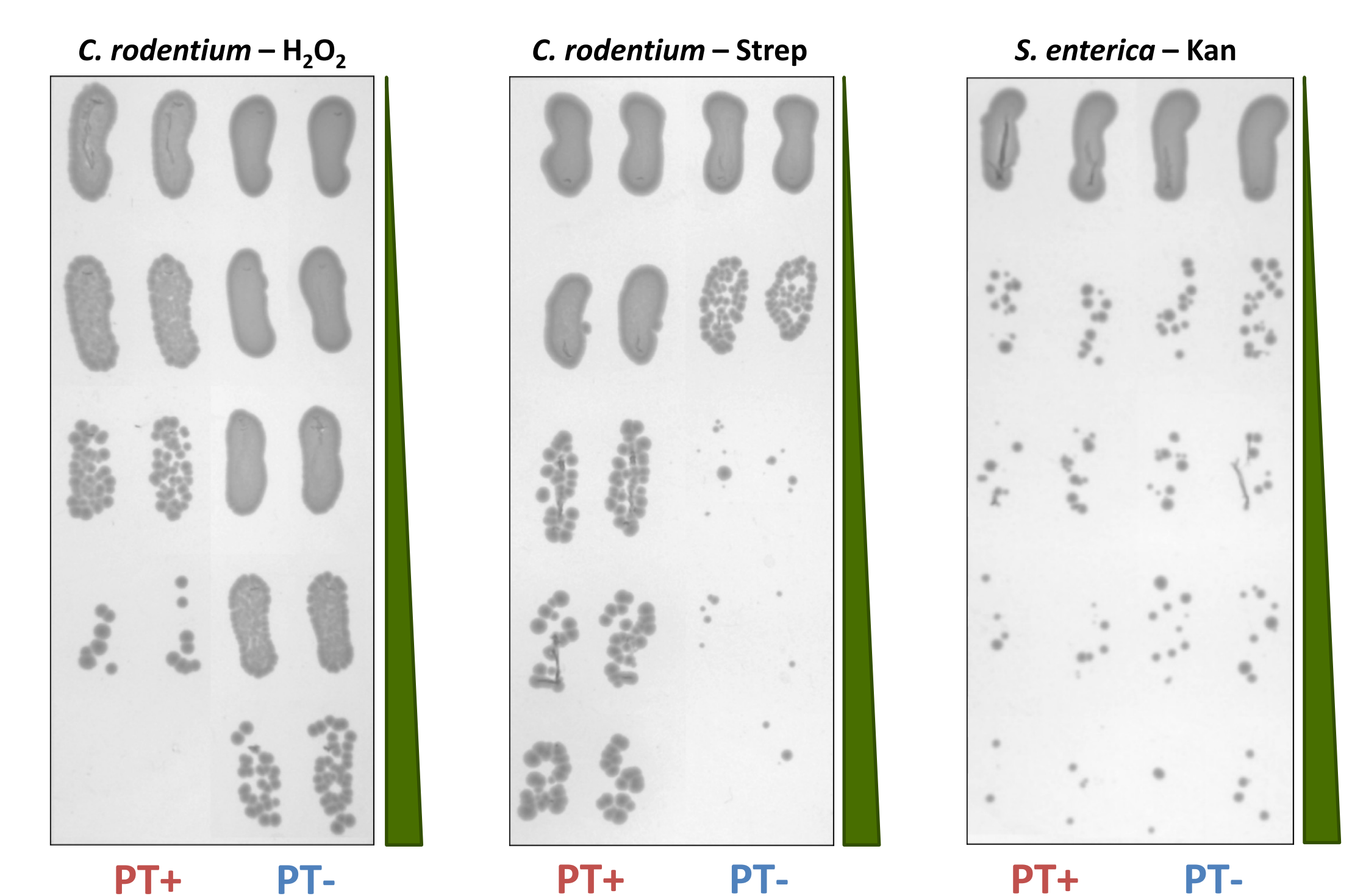


- PT+ strain is more resistant to H<sub>2</sub>O<sub>2</sub> stress
- PT+ strain is more resistant to antibiotic stress

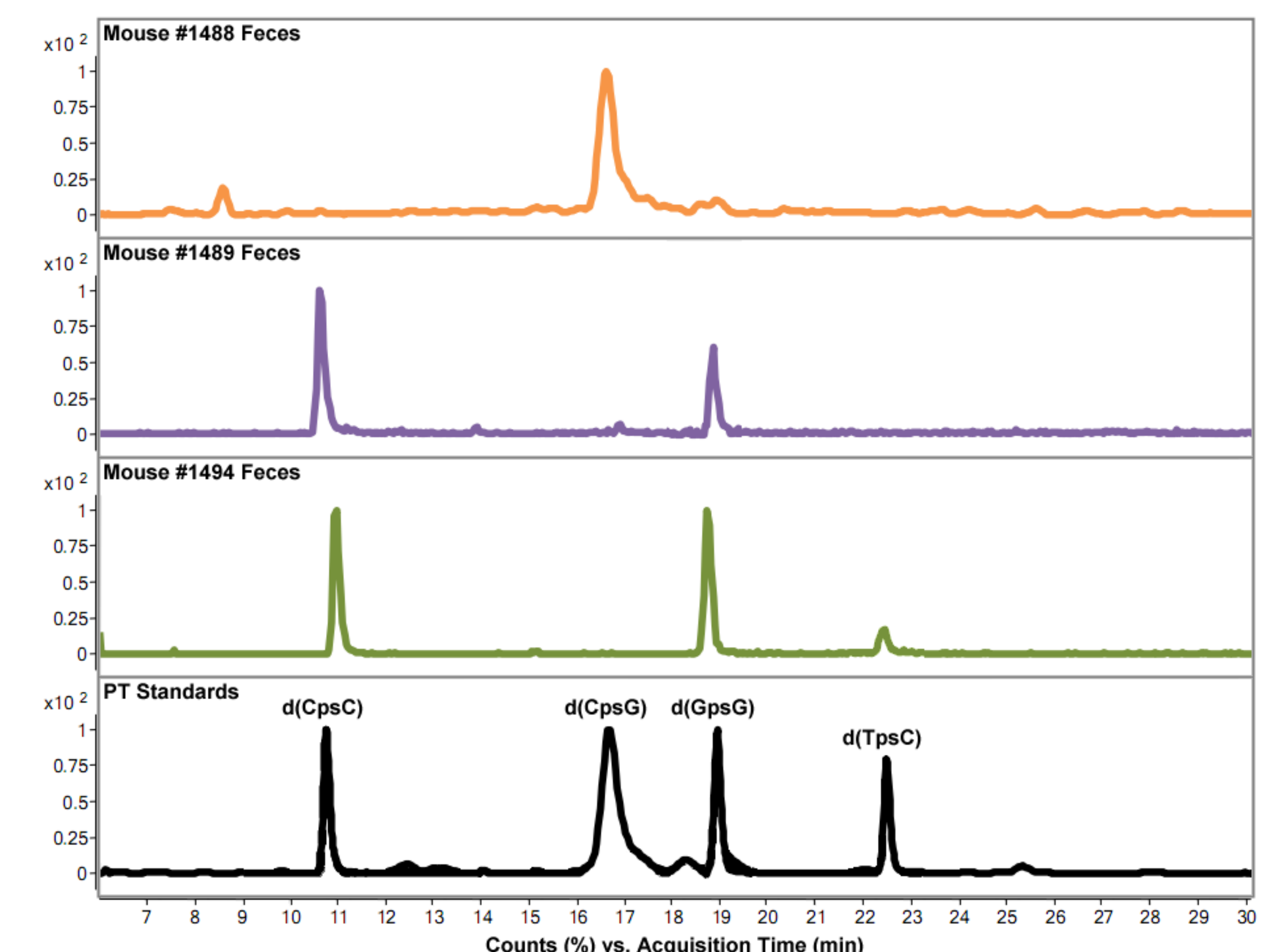
## Summary of Resistance Patterns

Organism	Source	<i>dndF-H</i>	H <sub>2</sub> O <sub>2</sub>	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