Effects of *hepA*, *rfaY*, and *cspC* Genes on Benzoate Tolerance and Antibiotic Resistance in Benzoate Evolved *Escherichia coli*

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Abstract

Benzoate-evolved *Escherichia coli* have been shown to be tolerant to benzoate and can grow in media containing this compound. In addition, these evolved strains have become tolerant to a variety of antibiotics. A recent study showed that *hepA*, *rfaY*, and *cspC* genes are present in the evolved strains. To determine if these genes contribute to benzoate tolerance and antibiotic resistance, we tested the evolved strains for growth on benzoate and in the presence of antibiotics.

Methods

*Construction of Knockouts.* The targeted gene was integrated by homologous recombination through *FLP* (degenerate). See *Figure 3.*

*Colony PCR.* To verify the mutation introduction, PCR was used to confirm the presence of the *FLP* cassette in a sample of cells that contained the *hepA* knockout strain. The *FLP* cassette was introduced into the genome of the *hepA* knockout strain using *FLP* recombination.

*Conclusions.*

- The *hepA* knockout strain showed increased growth upon exposure to 10 μM benzoate compared to *wild type* (*Figure 3*). The *wild type* strain showed decreased growth upon exposure to 2 μM benzoate compared to *wild type* (*Figure 3*).

- The *rfaY* knockout strain showed decreased growth upon exposure to 2 μM benzoate compared to *wild type* (*Figure 3*).

- The *cspC* knockout strain showed increased growth upon exposure to 10 μM benzoate compared to *wild type* (*Figure 3*).

Ablation of *cspC* and *hepA* resulted in increased growth on 2 μM benzoate compared to *wild type* (*Figure 3*).

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References

