

Finding New Enzymes From Modified TIM Barrel

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Funds: UC Toxic Substances Research
and Training program

The BIG Picture

To create biocatalysts that would be able to break down toxic substances.

Specific Research Goals

Find enzymes that degrade antibiotics

Improve antibiotic degradation efficiency

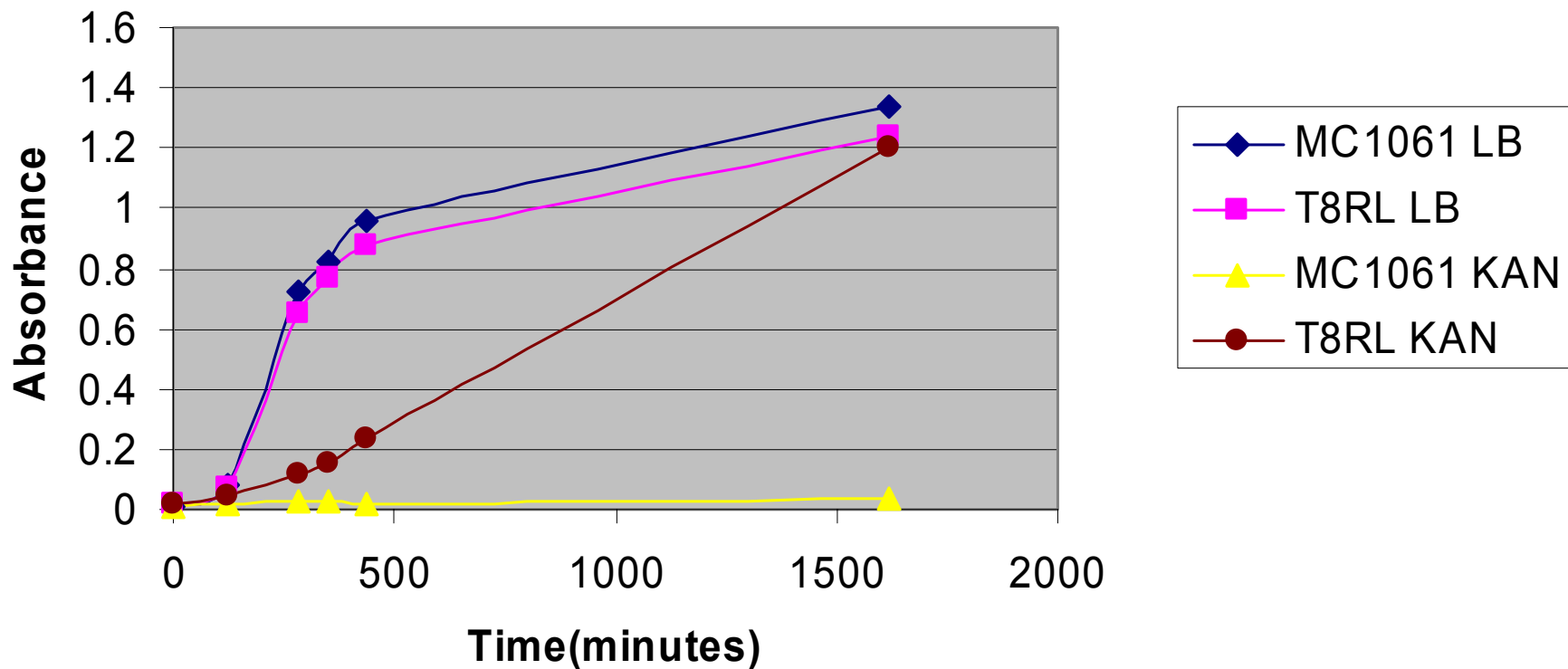
Compare enzymes to see how they evolved

Experimental Method

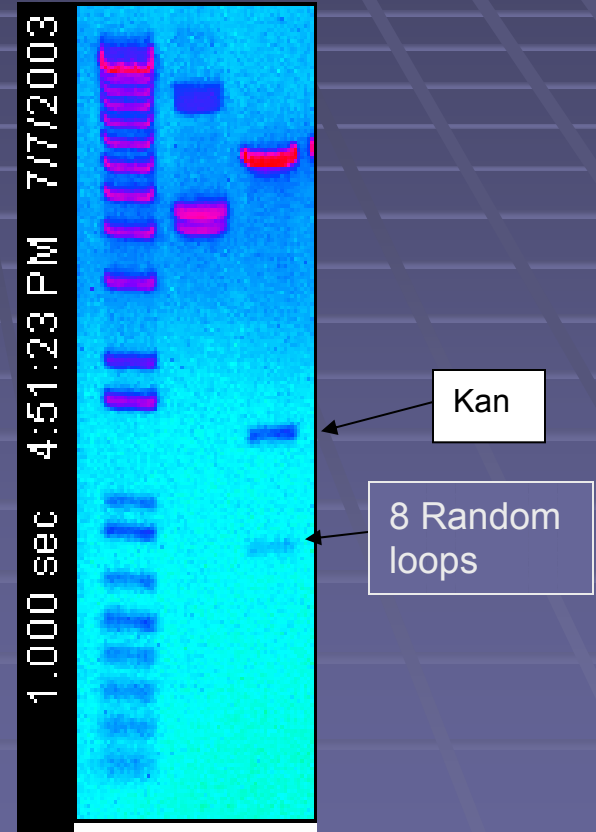
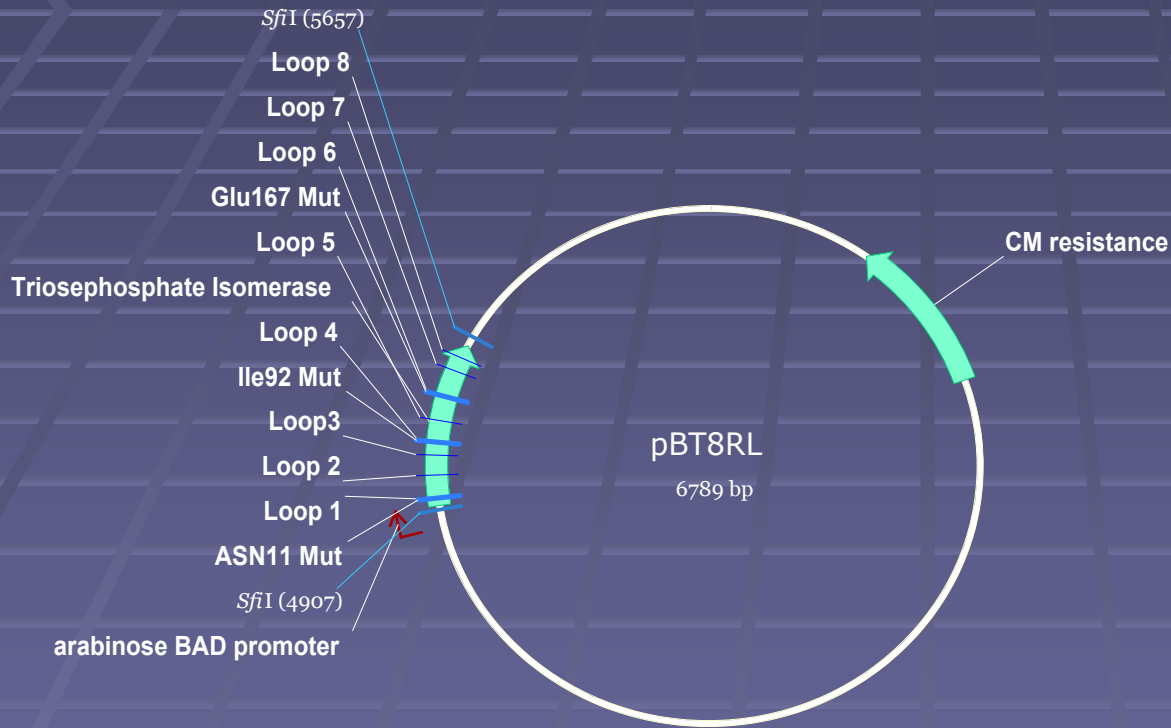
- Determine Killing curve for MC1061 bacterial cells in pre-chosen antibiotics.
- Determine growth curve for induced enzyme library from TIM barrel.
- Screen enzyme library for antibiotic degrading enzymes.
- Isolate degrading enzyme bacteria and retransform into fresh MC1061 cells.
- Repeat screen mutagenesis to improve enzyme degradation efficiency.
- Sequence clones from library that degrade antibiotics.

Kanamycin Data

**Kanamycin Selection
at 15 $\mu\text{g}/\text{mL}$**

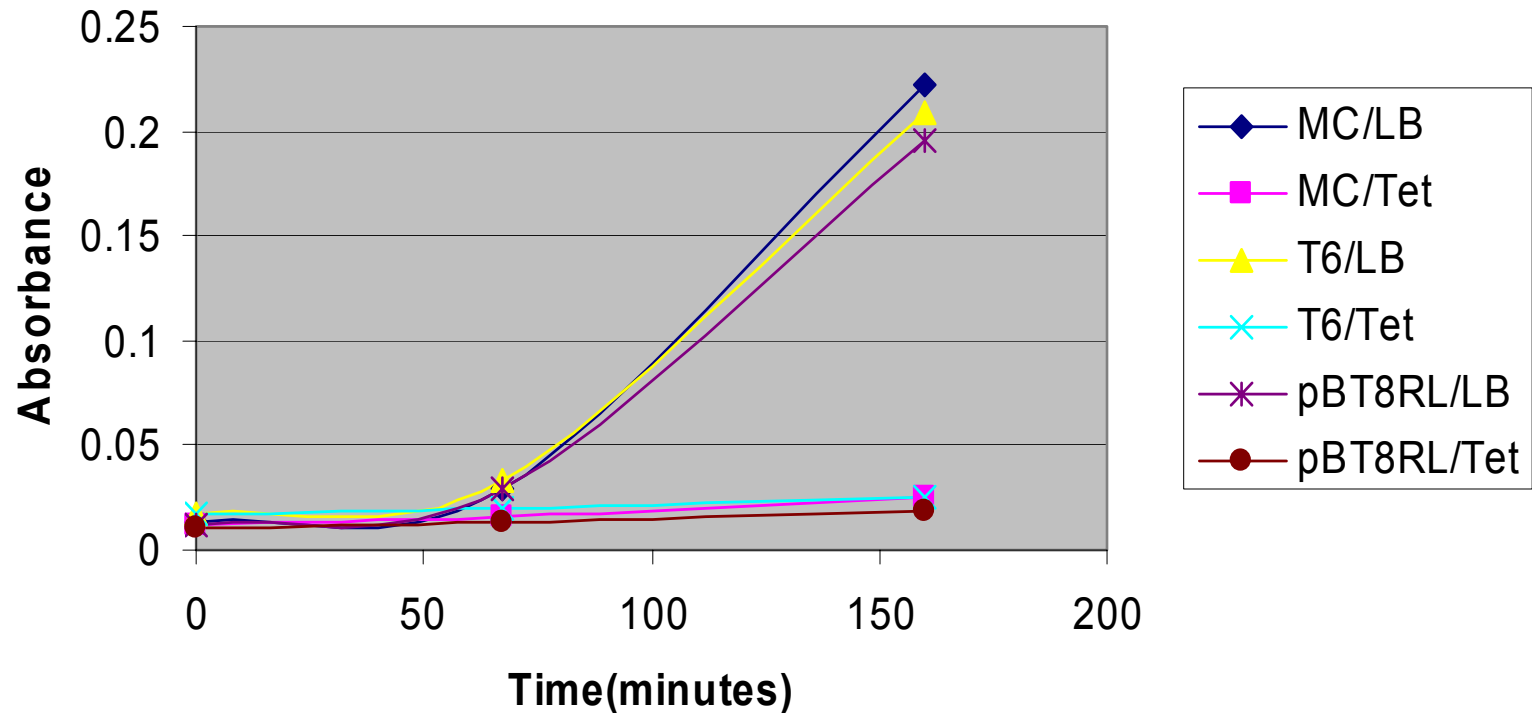


Kanamycin Data



Tetracycline Data

Tetracycline Growth Curve



Tetracycline Data

- DNA transformation was successful
- Cell growth occurred in tetracycline
- Cell growth did not occur after retransforming DNA into fresh bacteria cells
- Mutation in original cells caused cells to grow in tetracycline
- Experiment didn't work

Summary

- Library gene doesn't work in Kanamycin or Tetracycline
- Bacteria is highly susceptible to mutations and other problems

Special Thanks

- Test protein libraries in different antibiotics for degradation
- Isolate any enzymes that successfully degrade antibiotics

Kevin Bauliore

INSET Company and Staff