fabI Gene Alteration as a Mechanism for Triclosan Resistance in *Enterobacter cloacae*

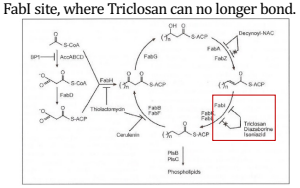
Khoula Jaber* and Dr. K. Joy Karnas
Department of Biological Sciences
Cedar Crest College, 100 College Drive, Allentown, PA 18104

Abstract

In response to increased exposure to triclosan, *Enterobacter cloacae* has developed resistance to the compound. The underlying changes to the genome were investigated focusing on the *fabI* gene, which codes for an enzyme involved in the lipid biosynthesis pathway in *En. cloacae* and is the target for triclosan. Triclosan binds to the target, blocking lipid biosynthesis in *En. cloacae* and therefore blocking growth of the bacteria. We found that with continued exposure to triclosan, *En. cloacae* became increasingly insensitive to the compound, and eventually developed complete resistance. We analyzed the specific genetic changes, focusing on the *fabI* gene in different strains with different levels of resistance. In the completely resistant strain, a known *fabI* mutation had occurred, but in the moderately resistant strain, there were no signs of the same mutation. There must be another mechanism causing partial resistance in that strain. Further research in this strain suggests exploration in other genetic mutations and possible gene duplication occurrences in the *fabI* gene.

Background

Triclosan resistance is becoming increasingly prevalent in *Enterobacter cloacae*. Triclosan is an antibiotic compound that inhibits lipid biosynthesis in *En. cloacae* at the enoyl-acyl carrier protein reductase, at the FabI step. The formation of the FabI-NAD^+-triclosan complex is the important factor for triclosan effectiveness. This complex is formed by triclosan binding to the enoyl substrate site on FabI, causing interactions between the NAD^+ and the protein. Leads to the formation of the FabI-NAD^+-triclosan ternary complex. Triclosan resistance develops in the *fabI* gene due to a missense mutation in the gene, and thus changing the FabI site, where Triclosan can no longer bond.



Heath, R. J. and C. O. Rock 2004 Fatty acid biosynthesis as a target for novel antibacterials

Figure 1: Lipid Biosynthesis pathway, the boxed region is the FabI region which is the target for triclosan when inhibiting this process.

Methods

Strain used: ATCC 13047 (*Enterobacter cloacae*)

1. Achieving Complete Resistance:
   - Kirby-Bauer using 20mM Triclosan/NaOH mix as the antibiotic compound and 20mM NaOH in form of paper disks. This process was repeated until the bacteria developed complete resistance to the antibiotic.

2. Assessing Genetic Mutations at partially resistant strain and completely resistance strain:
   - RFLP assay done to compare the original strain with both the resistant strain and completely resistant strain. PCR was done using the EndoFNae primer to amplify the *fabI* gene. The samples were separated through gel electrophoresis (1% gel)using the NaeI enzyme to cut at the *fabI* gene site.

3. Intermediate Testing:
   - Determining if the mutation had occurred in just the completely resistant strain, compared the completely resistant strain to 5 strains prior to complete resistance and to the original strain. RFLP assay was done using the same methods described above and samples were separated using gel electrophoresis (1% gel)

RFLP Mechanism by Gel Electrophoresis

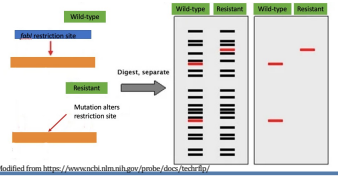


Image Modified from https://www.ncbi.nlm.nih.gov/probe/docs/techrflp/

Results

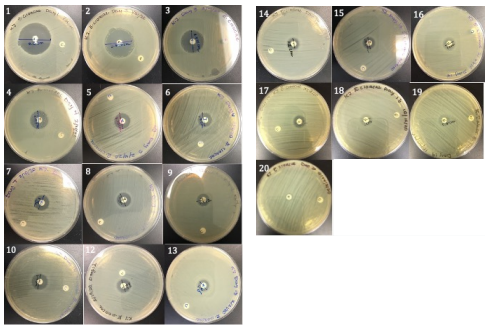
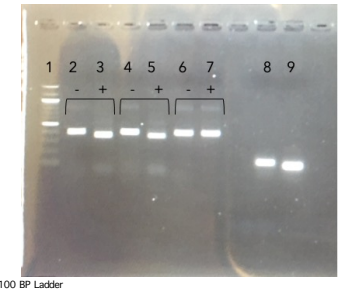


Figure 2: Increased resistance of *Enterobacter cloacae* to triclosan through persistent exposure.

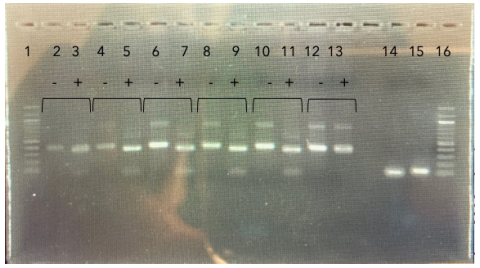
Percent Change in Zone of Inhibition		
Day	Zone Size (cm)	Percent Change (%)
1	3.5	0
2	2.875	17.86
3	2.375	32.14
4	2	42.86
5	1.75	50
6	1.5	57.14
7	1.6	54.29
8	1.5	57.14
9	1.3	62.86
10	1.7	51.43
11	1.3	62.86
12	1.2	65.71
13	1.2	65.71
14	1.2	65.71
15	1.3	62.86
16	1	71.43
17	1.3	62.86
18	1	71.43
19	0.8	77.14
20	0	100

Figure 3: Table showing the percent change of the zone of inhibition of the Kirby-Bauer.



Lane 1: 100 BP Ladder
Lane 2: Day 1 no enzyme
Lane 3: Day 1 with enzyme
Lane 4: Day 16 no enzyme
Lane 5: Day 16 with enzyme
Lane 6: Day 20 no enzyme
Lane 7: Day 20 with enzyme
Lane 9: Day 16 Sample with positive FabI control primer
Lane 10: Day 16 Sample with RpoB primer

Figure 4: Gel showing restriction enzyme sensitive and restriction enzyme resistant strains of *Enterobacter cloacae* comparing Days 1, 16 , and 20.



Lane 1: 100 BP Ladder
Lane 2: Day 1 no enzyme
Lane 3: Day 1 with enzyme
Lane 4: Day 16 no enzyme
Lane 5: Day 16 with enzyme
Lane 6: Day 17 no enzyme
Lane 7: Day 17 with enzyme
Lane 8: Day 18 no enzyme
Lane 9: Day 18 with enzyme
Lane 10: Day 19 no enzyme
Lane 11: Day 19 with enzyme
Lane 12: Day 20 no enzyme
Lane 13: Day 20 with enzyme
Lane 14: Day 16 with positive FabI control primer
Lane 15: Day 16 with RpoB primer
Lane 16: 100 BP Ladder

Figure 5: Gel showing restriction enzyme sensitive and restriction enzyme resistant strains of *Enterobacter cloacae* comparing Days 1, 16 , 17, 18, 19, 20.

Results Analysis

Enterobacter cloacae developed complete resistance at Day 20. The table displays the percent change of the zone of inhibition, the percent change is negative as the zone of inhibition decreases over time. The first gel compares the original ATCC (Day 1), Day 16 and Day 20 strains, each strain is represented by 2 bands, where the first band was a sample with no enzyme, and the second had enzyme added. The final two lanes show Day 16 strain with control FabI primer and RpoB (qPCR) primer. The second gel is similar, but with additional samples added to the gel, including Days 17, 18, and 19 in addition to Days 1, 16, and 20. The final two bands are the control FabI and RpoB primers with the Day 16 strains.

Discussion

Figure 4 shows that Day 16 did not show signs of mutation since the restriction enzyme cut at the FabI site, whereas Day 20 did not cut at the FabI site, there is no smaller band indicating the smaller strand of DNA after the enzyme had cut. Day 16 shows a difference in band sizes comparing the large strands of the cut and uncut bands. This is clear evidence that the partially resistant strain did not exhibit a FabI mutation, and there must be another mechanism causing increased resistance in this strain. As seen in Figure 5, Days 17, 18, and 19 did not exhibit the mutation in the *fabI* gene due to the cutting patterns of the bands, where only Day 20 shows no restriction enzyme activity, indicating the mutation is only in the completely resistant Day 20 strain . Other mechanisms to be assessed are gene duplication occurrences of the *fabI* gene and mutations in other genes such as *fabK* or *fabL*

Future Direction

- Gene duplication assay (qPCR)
- Explore mutations in other genes besides *fabI* to assess earlier resistance patterns
- Observe strains similar to *Enterobacter cloacae* for similar antibiotic resistance mechanisms.

Acknowledgments

•Dr. K. Joy Karnas
•Cedar Crest College Department of Biology

References

Heath, R. J. and C. O. Rock 2004 Fatty acid biosynthesis as a target for novel antibacterials. Curr Opin Investig Drugs **5:**1-13
Heath, R. J., J. R. Rubin, D. R. Holland, E. Zhang, M. E. Snow, and C. O. Rock 1999 Mechanism of Triclosan Inhibition of Bacterial Fatty Acid **274:** 11110-11114