Thymoquinones inhibitory effects on pARA-R transformed *Escherichia Coli*

Purpose: Investigate the extent of thymoquione's antibacterial properties and determine if pARA-R and beta lactamase in particular has any effect on thymoquinone.

Abstract

With the rise of antibiotic resistant bacteria and the creation of superbugs, there has been an unparalleled need for new antibiotics. Thymoquinone, the active antibacterial ingredient in black cumin, has proven to be a potent molecule as it is relatively harmless and it targets ATP synthase—no antibiotics currently target ATP synthase. In this study, thymoquinone's inhibitory effects were compared in wild type and ampicillin resistant *E.coli.*Thymoquione was tested in 4 different concentrations and the *E.coli's* growth was measured through O.D.'s. Comparative results showed that thymoquinone's growth inhibitory properties were similar in both types of *E.coli.*

Introduction

The black seeds in the *Nigella sativa* plant, more commonly known as black cumin, have long been cultivated in Asia, Europe, and North Africa and used for medicinal purposes for centuries[3]. It originated from Southeastern Asia and was also used in ancient Egypt, Greece, Middle East and Africa[3]. In Islam, it is regarded as one of the greatest forms of healing medicine available[3]. It was used to cure asthma, hypertension, diabetes, inflammation, bronchitis, headaches, fever, dizziness and influenza[4]. N. Sativa's success as a medicine and food preservative has been explained by its active ingredient thymoquinone(TQ). Researchers have found that TQ inhibits the growth of multiple cancer cell lines and has proven to have antioxidant, anti inflammatory, antidiabetic, antibacterial, antifungal, antitussive, and neuroprotective properties[3,4]. With the development of antibiotic resistant bacteria and need for new antibiotics, TQ has shown promise as a natural remedy for multiple studies have proven *N.Sativa* and TQ's antibacterial effect. While TQ has proven to inhibit growth of both gram positive and gram negative bacteria, it has shown to better inhibit growth of gram positive bacteria.

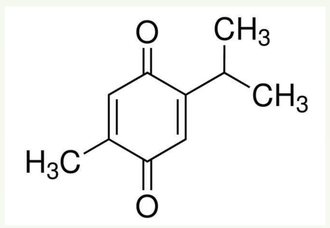

Figure 1[2]: TQ's molecular structure. It's molecular formula is C10H1002 and it has 2 methyl groups connected to the 2-Carbon,1 methyl group on the 5-Carbon and a carbonyl group on the 1 and 4 Carbon.

Hypothesis

The pARA-R plasmid should not alter TQ's active site, ATP synthase,or break down TQ's molecular structure, so TQ should successfully inhibit the transformed *E.coli's* growth.

Methods/Materials

Materials

- 500mg of 99% TQ purchased (Caymen Chemicals, #15039)
- 99% DMSO (Amazon)
- Petri Dishes ,LB Agar, LB broth (Lab)
- E.coli K12 beta strain (Lab)
- pARA-R plasmid (80ng/ml, Amgen Biotech Experience)
- Ampicillin (Caymen Chemicals,#14417)
- Kanamycin (Caymen Chemicals,#16140)
- Spectrophotometer (Lab)
- Shaker (Lab)
- 15 ml Falcon Tubes(Lab)
- DI Water (Lab)
- Serological Pipette/Micropipette (Lab)

Creation of 7.5 ml 250mM TQ cocktail mixture

1) 99% pure TQ was bought from Cayman Chemicals and 98% pure DMSO was bought from Amazon
2) 410mg of TQ was added to 5ml of 99% DMSO and 2.5ml of water to create a 250 mM stock solution.
3) The 250 mM solution was left in a 42℃ water bath and left in dark as TQ is light sensitive for 20 minute intervals and vortexed for 30 seconds after each 20 minute interval to help dissolve the solution.
4) Half of the solution was kept at 0℃ and covered
5) Half of the solution was kept at -18℃ and covered when not used

Optical Densities

1) The Optical Densities(O.D.'s) of transformed and wild type *E.coli* were taken. 20µLof ampicillin was added to the transformed *E.coli*'s solutions for the purpose of selection
2) 7 15ml Falcon tubes were obtained and 100µLof *E.coli* and 10ml of broth was added to every tube
3) Preparation of 7 different solutions:

–Tube 1:Negative Control

–Tube 2: 250µL of 1 M DMSO added to create a 2% DMSO solution

–Tube 3: 7µL 250 mM TQ added to create a .175mM solution[2]

–Tube 4:65.2µL of 250 mM TQ added to create a 2.6mM solution

–Tube 5:123µL of 250 mM TQ added to create a 3.12mM solution[1]

–Tube 6:250µL of 250m MTQ added to create a 6.24mM solution

–Tube 7:20 µL of ampicillin added to wild type and 20 µL of kanamycin added to transformed as positive control

4) All 7 O.D.'s taken at 0 time, shaken at 37℃ and O.D's were taken a second time after 24 hours.

Results

T Test used for statistical analysis

All 4 TQ solutions showed lower O.D.'s after 2 hours than both the negative control and DMSO(Figure 2). 1.6, 3.12, and 6.24 mM had lower growth than the .175 mM solution. After 22 hours, the 1.6, and 6.24 mM solutions still showed growth inhibition, but the 6.24 mM solution grew less than the 1.6 mM solution(Figure 2). The rate of growth of every solution increased after 2 hours(Figure 3). The transformed *E.coli* showed significantly less growth at 6.24 mM compared to Ampicillin alone as shown in Figure 5($P<0.05$) and less growth at 1.6,3.12, and 6.24 mM in Figure 4. The 1.6 mM and 3.12 mM solutions had similar growth, but the 6.24 mM solution had higher growth than the 1.6 and 3.12 mM solutions(Figure 4).

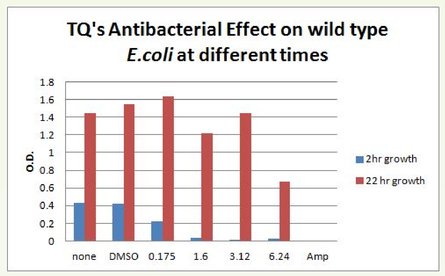

Figure 2: Thymoquinone's growth inhibitory effects on Wild Type *E.coli* at both 2 hours and 22 hours.

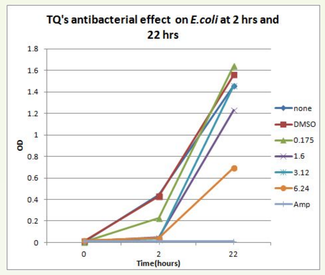

Figure 3: Wild Type *E.coli*'s growth curve after at 2 hours and 22 hours.

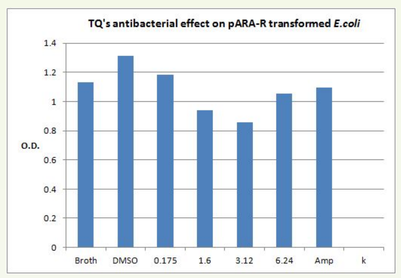

Figure 4: Thymoquionone's growth inhibitory effects on transformed *E.coli* after 24 hours.

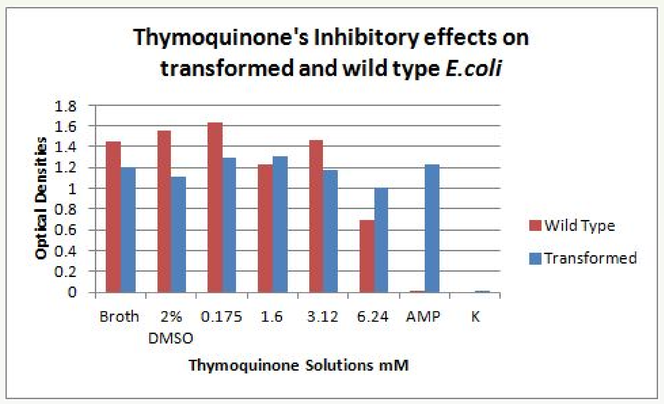

Figure 5: Comparative graph of thymoquinone's growth inhibitory effects after 24 hours on both wild type and transformed *E.coli*

Discussion

TQ showed antibacterial properties in both the wild type and transformed *E.coli* as shown by Figure 5. While the .175 mM solution showed some growth inhibition at 2 hours, it failed to inhibit growth after 24 hours in all 3 trials. Furthermore, TQ seems to inhibit growth to a limit as Figure 2 shows that after 2 hours, the growth inhibition of both the 3.12 mM and 6.24 mM solution are very similar, yet the 6.24 mM solution inhibits growth to a greater extent. Thus, it seems that TQ simply delays the growth of bacteria. As shown by Figure 2, it seems that under ideal conditions, TQ has the ability to inhibit *E.coli's* growth up to 52.5% after 24 hours and the general trend shows that the 1.6 mM solution's inhibitory effects begin to end after 24 hours as the *E.coli* reaches its lag phase. Looking at both the wild type and transformed *E.coli,* it seems that pARA-R and more specifically beta lactamase has no effect on TQ because both the transformed and wild type *E.coli* followed the same growth patterns(Figure 5). This makes sense as beta lactamase targets the beta-lactam structure of ampicillin, but TQ doesn't have a beta-lactam ring. TQ has shown its ability to combat ampicillin resistant bacteria, but its effects are solely growth inhibiting and it lacks a killing mechanism. With further research, it could be possible to develop a new antibiotic by paring TQ with another molecule. With further research on both TQ's active site, ATP synthase, and ways bacteria could become resistant to TQ, it could become a powerful antibiotic.

Conclusion

Figure 5 shows that TQ inhibited the growth of both the wild type and transformed *E.coli*. Thus, it seems that pARA-R and beta lactamase has no effect on TQ.

Acknowledgements

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