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Screening Inhibitors of Methylerythritol Phosphate (MEP) Pathway Enzymes

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Screening Inhibitors of Methylerythritol Phosphate (MEP) Pathway Enzymes

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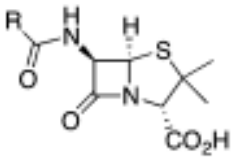
Abstract:

With various antibiotics becoming illegitimate due to bacteria building up resistance to them, it becomes imperative to find new antibiotics to replace the ones that no longer work. In theory, this can be accomplished by inhibiting the IspF enzyme which can be found within the Methylerythritol phosphate (MEP) pathway. Four different cell cultures, one treated and containing no IPTG, one treated and containing IPTG, a control with IPTG, and a control lacking IPTG were found to have grown successfully at a steady pace over a period of 5 hours. This means that the overexpression of IspF within the cell cultures does not affect their ability to grow at a steady rate. An overexpression of IspF resulted in a large peak pertaining to CMP, whereas an unaltered sample lacked a defined peak. This should be useful in future work because it will be easier to see if potential inhibitors are successful.

Introduction:

Medicine changed forever in 1928 with the realization that *Penicillin notatum* could stop bacteria from growing. In the early 1940s, patients suffering from life-threatening bacterial infections started to receive penicillin as treatment and as a result usually made a speedy recovery.[1] However, bacteria have the capability to quickly become resistant to antibiotics and this has caused some to die from infections once again. Since bacteria reproduce so rapidly, scientists must create and test new antibiotics to replace the ones that are in use now. The Methylerythritol Phosphate (MEP) pathway has recently taken center stage in the production of new antibiotics.



Penicillin

Figure 1: Penicillin[2]

By utilizing a two-step method involving screening and time-based studies, an in-vivo cell growth and cell lysis method compatible with High Performance Liquid Chromatography (HPLC) is capable of being developed. This methodology is used to test potential inhibitors of the MEP pathway. Use of the MEP pathway in drug development is beneficial because this pathway is exclusive to bacteria. Since the MEP pathway is absent in humans, plants, and animals, they must rely more heavily on the mevalonate (MVA) pathway.[4] The MEP pathway, as seen in Figure 2, is made up of seven different enzymes that a potential antibiotic can be designed to target particularly.[5] The enzyme this experiment will focus specifically on is IspF. IspF is necessary for the transformation of CDPME2P into MEcDP to occur. When this transition takes place, the compound CMP is given off as a by-product of the reaction. This becomes advantageous when one realizes that potential inhibitors should block this compound from forming. By implementing LC/MS, cells containing potential drugs can be screened to see whether they contain CMP or not. If the data lacks a peak for CMP, then the antibiotic works.

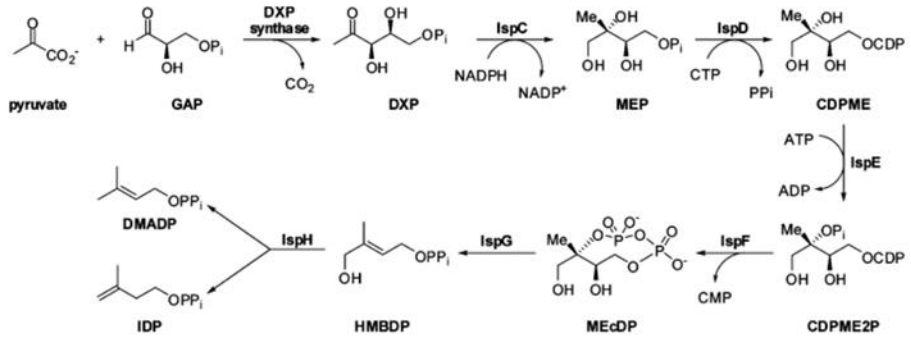


Figure 2: The MEP Pathway[3]

Experimental Procedure:

By following the protocol for cell lysis, *E. coli* cells containing IspF from the pathogen *Burkholderia pseudomallei* that were held in a frozen cell pellet were allowed to dissolve in 30 mL of 100 mM Tris lysis buffer with a pH of 8.0. A digital sonicator was utilized to break open the cells. These cells were then placed into a centrifuge at 15,000 x g for 20 minutes at 4ºC.

Afterwards, organic solvent was added and the resulting mixture was placed into the centrifuge once again. Then the cell supernatant was collected for future LC/MS analysis of the cells. In a separate experiment, the optical density was recorded for four different cell cultures including a control containing no IPTG, a control with IPTG, one treated culture containing IPTG, and one treated culture containing no IPTG after 0 hours, 1 hour, 3 hours, and 5 hours. The resulting data was studied to determine how similar or dissimilar the cell growth was for each of the cell cultures.

Results and Discussion:

The four different cell cultures were graphed together so that a clear picture for the cell growth over time could be ascertained. The following depiction illustrates that the overexpression of IspF within cell cultures does not have any negating effects on cell growth rate.

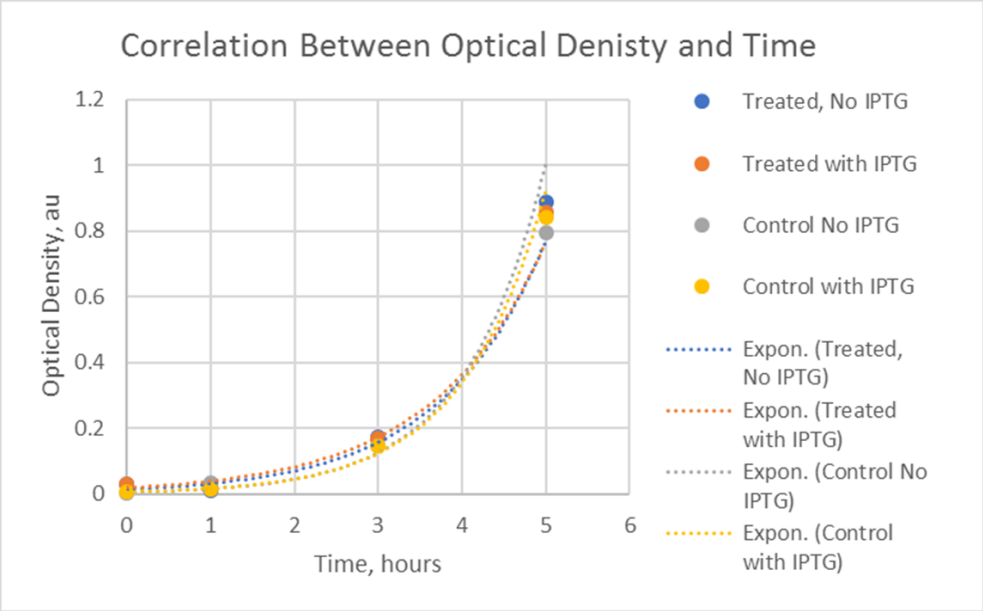


Figure 3: Optical Density vs. Time

Figure 4 was created to show what spectra could be expected to look like if BpIspF in the XL-1 Blue cells was not overexpressed and no inhibitor was present.

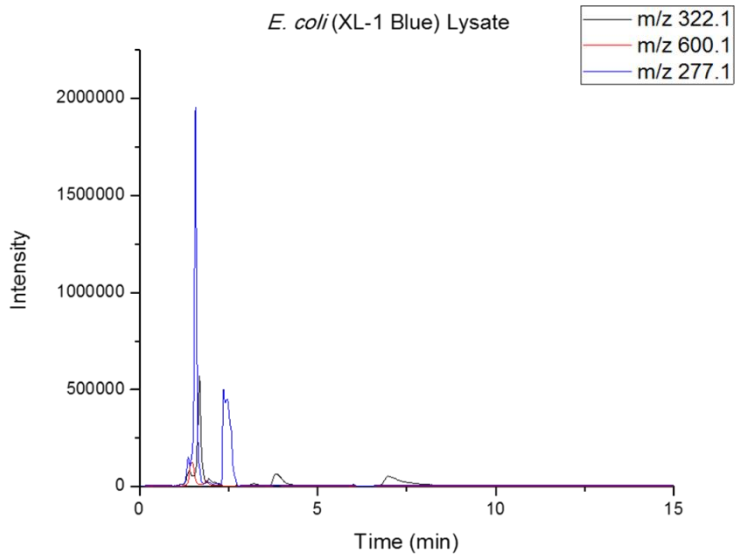


Figure 4: E. Coli Lysate

Figure 5 was generated to show what the spectra would look like once the overexpression of IspF in the sample had occurred. A larger peak for CMP in the spectra was found to occur after the overexpression of IspF. This was considered to be logical since CMP is a product of IspF. Thereby, more IspF would make the signal for CMP larger and clearer. This will be useful in later experiments because it will make it easier to tell whether the potential IspF inhibitors work or not. If the inhibitor works, then there should not be a large peak of CMP present in the spectra.

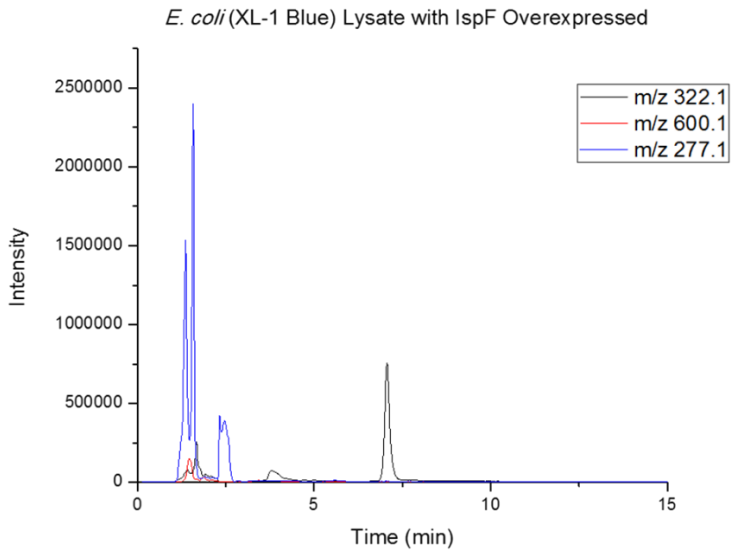


Figure 5: E. Coli Lysate with IspF overexpressed

Conclusion:

The cells were found to have grown steadily and successfully over the span of 5 hours. A large peak pertaining to CMP from the overexpression of IspF in *E. Coli* was also observed. This meant that if the inhibitor had success targeting the IspF enzyme within the MEP pathway, there would be a marked difference between its spectra and that of an inhibitor that did not work. In other words, if the inhibitor was found to be effective, then no CMP peak would be seen in the resulting spectra. Future work should encompass varying the conditions of the *E. coli* cell cultures containing *Burkholderia pseudomallei* IspF. These constraints could include whether the cell cultures contain an inhibitor or not, the concentration, and the type of inhibitor utilized.

Acknowledgments:

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