Introduction

- According to the CDC, < 23,000 people die annually from antibiotic resistant bacterial infections (1).
- MdTK is a transmembrane protein that functions as a multidrug efflux pump with the ability to export several antimicrobial agents including ciprofloxacin, ethidium bromide and doxorubicin (2).
- Residue F285 has been shown to be important for cation binding and export function (3).
- We have recently shown that E. coli can develop resistance to the aminoglycoside Kasugamycin (Ksg) through a single phenylalanine to valine mutation in MdTK at the F285 residue. The E. coli Ksg relationship is a novel mechanism that does not rely on an increased MdTK synthesis but rather a gain of function mutation that is predicted to increase the pump’s affinity for Ksg, thus empowering greater drug export.
- Given that there are only the wild type and a single mutant available for further analysis, there is a lack of understanding of the contributions of the 18 other possible amino acids at this location and their effect on E. coli Ksg resistance.
- Additionally, crystal structures are available for the Vibrio cholerae MdTK protein and it is predicted that F285 is a cation binding site.
- The purpose of this study is to generate MdTK proteins containing the most structurally relevant amino acids and determine any changes in the pump’s substrate specificity profile.

Hypothesis

We hypothesize that amino acids with a similar structure to valine will result in similar if not raised export of kasugamycin while the amino acids that are closer in structure to phenylalanine will result in a lower export of kasugamycin or in a nonfunctional protein.

Results

Figure 3. Generation of site directed mutants of E. coli mdTK

The Phe (m7a) residues were mutated using site-directed overlap extension PCR. The resulting amplimers were ligated into plasmid pET and transformed into E. coli DH10B cells. Transformants containing the appropriate 1.7-kb insert were sequenced at the MU DNA Core facility. Shown above are the chromatograms of the region containing the mutated residue. Two new mutations were obtained, together with two different synonymous mutations of the valine mutation that was identified as the ksgB mutation.

Figure 4. Site-directed mutagenesis of E. coli MdTK residue 285. The Phe (m7a) residue was mutated using site-directed overlap extension PCR. The resulting amplimers were ligated into plasmid pET and transformed into E. coli DH10B cells. Transformants containing the appropriate 1.7-kb insert were sequenced at the MU DNA Core facility. Shown above are the chromatograms of the region containing the mutated residue. Two new mutations were obtained, together with two different synonymous mutations of the valine mutation that was identified as the ksgB mutation.

Bioinformatic Analysis

Figure 5. The Phe (m7a) residue is highly conserved in MdTK proteins. The F285 region of MdTK proteins from eight Gram-negative pathogens were aligned. Amino acid residues that are identical in 4 or more representatives are highlighted with a black background. The F285 residue (marked by a red spot) is conserved in all eight of the examined species. The colored strip indicates the conservation.

Figure 6. The genomic environment of mdTK in Gram negative pathogens. All genes boxed in yellow represent different genes encoding transport systems. T is representative of RNA genes. Ec: Escherichia coli; St: Salmonella enteritidis; Yp: Yersinia pestis; Ps: Pseudomonas mirabilis; Kp: Klebsiella pneumoniae; Cs: Clostridium sakazakii; Et: Edwardsiella tarda; Vc: Vibrio cholerae; Nm: Neisseria meningitidis; Cb: Clostridium butylicum; Ps: Pseudomonas aeruginosa; Tp: T. pallidum; H: Haemophilus influenzae; Lp: Legionella pneumophila.

Results: To date, there have been no published comparative analyses on the genetic synteny of mdTK in Entero bacteriaceae, or more distantly related Gram-negative taxa. Through a BLAST (1) and a BLAST (2) of the genome surrounding the mdTK gene, it was found that the nearest two genes surrounding mdTK in other non-Entero bacteriaceae Gram negative species are not conserved, with the exception of the predicted RNA gene. A second comparison of the genome surrounding mdTK in Haemophilus influenzae. It was also found that in non-Entero bacteriaceae, mdTK is monocistronic (based on gene orientation) while in non-Entero bacteriaceae Gram negative species mdTK is found to be either monocistronic (e.g. Haemophilus influenzae) or had the potential to be polycistronic (e.g. Clostridium butylicum).

Conclusions

We hypothesize that amino acids with a similar structure to valine will result in similar if not raised export of kasugamycin while the amino acids that are closer in structure to phenylalanine will result in a lower export of kasugamycin or in a nonfunctional protein.

Future Directions

This investigation will continue with verified plasmids being transformed into E. coli with a mdTK knockout mutation from the Keio collection (6). A minimum inhibitory concentration (MIC) determination will then be run on the transformed cells to determine the effect the mutation has on the ability of the bacteria to resist antibiotics. This procedure allows for the direct testing of our hypothesis as well as obtaining a better understanding of the differences between species pertaining to a gain or loss of function mutation of a cation binding site (5).

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References