By Christopher Eeles

Introduction

In this laboratory we will be performing a BlastN search to find six human pathogens with sequences similar to the erythromycin-resistance gene *ermB*, which is associated with *Streptococcus agalactiae*—a Gram-positive bacterium commonly found on cow utters.¹ These sequences will then be aligned using ClustalOmega, a web-based multiple sequence alignment tool, to evaluate similarity between each respective gene.¹,² ClustalOmega is scalable and widely viewed as one of the fastest online multiple sequence alignment tools; speed and accuracy for a small number of sequences are similar to other high quality sequence aligners, but the tool provides significance performance gains in comparison of large data sets with hundreds of thousands of sequences.² The output from the ClustalOmega sequence alignment will be used to compare the structures of erythromycin resistance genes in human pathogen to *ermB*.¹ In doing so we hope to determine if horizontal gene transfer from live-stock associated bacteria in ingested food products to human gut microbes plays a role in the development of antibiotic resistance in human pathogens.

Results

The initial search via BlastN was conducted using the *ermB* gene accession number DQ355148.1 with optimization set to "Somewhat similar sequences". The search yielded complete cds sequences for *Listeria monocytogenes, Enterococcus faecium, Bacillus cereus, Enterococcus faecalis, Staphylococcus intermedius* and *Streptococcus pyogenes* which were confirmed to be human pathogens via a Google search. The sequence data was downloaded in FASTA format and reformatted to list the species name as the identifier instead of the accession number, the FASTA sequence of *S. agalactiae* was also included.

The ClustalOmega search was conducted with standard parameters, results below:

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Discussion and Conclusion

The results of the ClustalOmega alignment of the six pathogens with the *ermB* gene from *S. agalactiae* yielded near perfect matches, with all but one position aligned identically (indicated by the star below the position) with the entire *ermB* coding sequence across all species. The position lacking a perfect alignment contained a purine transition from A to G, and only occurred in two of the six sequences aligned. In horizontal gene transfer we would expect to see a high degree of similarity across the *ermB* gene between species and strains of bacteria: this is exactly what was observed in our results. These findings are highly suggestive of horizontal gene transfer with *S. agalactiae* in *L. monocytogenes, E. faecium, B. cereus, E. faecalis, S. intermedius* and *S. pyogenes*. While confirmation of transfer facilitated by gut microbiota would require additional alignments in known gut microbes to complete the hypothesized transfer chain, it is clear that antibiotic resistance in live-stock associated bacteria is resulting the propagation of these trains in human pathogens. Sequence of transfer aside, such effects significantly increase the risk of antibiotic resistant infections in humans and thus constitute a serious risk of causing harm in human populations. Such risks justify further investigation of this process and likely necessitate increased regulation, and possible ban, of routine antibiotic use in lives-stock populations.

References

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