

BIF701 Lab 4 Multiple Sequence Alignment: Horizontal Gene Transfer of Antibiotic Resistance

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 udders.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 examine 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:

```
Enterococcus_faecalis      AATCGTTAACTCTGTATTATTATAGATGTATACCTCAGGAGTGATTACGAAC 285
Staphylococcus_intermedius AATCGTTAACTCTGTATTATTATAGATGTATACCTCAGGAGTGATTACGAAC 306
Streptococcus_pyogenes    AATCGTTAACTCTGTATTATTATAGATGT-ATCACTCAGGAGTGATTACGAAC 306
Enterococcus_faecium      AATCGTTAACTCTGTATTATTATAGATGTATACCTCAGGAGTGATTACGAAC 359
Streptococcus_agalactiae -----ATGAGC 6
Listeria_monocytogenes    AATCGTTAACTCTGTATTATTATAGATGTATACCTCAGGAGTGATTACGAAC 339
Bacillus_cereus           -----ATGAGC 6

Enterococcus_faecalis      AAAAATATAAAATTTCTCAAAACTTTTAAAGAGTAAAGATCTCAACCAATAATA 345
Staphylococcus_intermedius AAAAATATAAAATTTCTCAAAACTTTTAAAGAGTAAAGATCTCAACCAATAATA 346
Streptococcus_pyogenes    AAAAATATAAAATTTCTCAAAACTTTTAAAGAGTAAAGATCTCAACCAATAATA 346
Enterococcus_faecium      AAAAATATAAAATTTCTCAAAACTTTTAAAGAGTAAAGATCTCAACCAATAATA 419
Streptococcus_agalactiae AAAAATATAAAATTTCTCAAAACTTTTAAAGAGTAAAGATCTCAACCAATAATA 66
Listeria_monocytogenes    AAAAATATAAAATTTCTCAAAACTTTTAAAGAGTAAAGATCTCAACCAATAATA 399
Bacillus_cereus           -----ATGAGC 6

Enterococcus_faecalis      AAACATTAAGTTTAAAGAGAACCGATACGTTTACGAATTTGGAACAGTTAAAGGGCAT 405
Staphylococcus_intermedius AAACATTAAGTTTAAAGAGAACCGATACGTTTACGAATTTGGAACAGTTAAAGGGCAT 426
Streptococcus_pyogenes    AAACATTAAGTTTAAAGAGAACCGATACGTTTACGAATTTGGAACAGTTAAAGGGCAT 426
Enterococcus_faecium      AAACATTAAGTTTAAAGAGAACCGATACGTTTACGAATTTGGAACAGTTAAAGGGCAT 479
Streptococcus_agalactiae AAACATTAAGTTTAAAGAGAACCGATACGTTTACGAATTTGGAACAGTTAAAGGGCAT 459
Listeria_monocytogenes    AAACATTAAGTTTAAAGAGAACCGATACGTTTACGAATTTGGAACAGTTAAAGGGCAT 459
Bacillus_cereus           -----ATGAGC 6

Enterococcus_faecalis      TTAACGAGCAAACTGGCTAAATTAAGTAAGCAAGTAAAGCTGATTGAATAGACAGTGT 465
Staphylococcus_intermedius TTAACGAGCAAACTGGCTAAATTAAGTAAGCAAGTAAAGCTGATTGAATAGACAGTGT 286
Streptococcus_pyogenes    TTAACGAGCAAACTGGCTAAATTAAGTAAGCAAGTAAAGCTGATTGAATAGACAGTGT 286
Enterococcus_faecium      TTAACGAGCAAACTGGCTAAATTAAGTAAGCAAGTAAAGCTGATTGAATAGACAGTGT 539
Streptococcus_agalactiae TTAACGAGCAAACTGGCTAAATTAAGTAAGCAAGTAAAGCTGATTGAATAGACAGTGT 186
Listeria_monocytogenes    TTAACGAGCAAACTGGCTAAATTAAGTAAGCAAGTAAAGCTGATTGAATAGACAGTGT 519
Bacillus_cereus           -----ATGAGC 6

Enterococcus_faecalis      CTATTCAACTTATGTCAGAAAATTTAAACGTGACATCTGTCCTTTAATTCACCA 525
Staphylococcus_intermedius CTATTCAACTTATGTCAGAAAATTTAAACGTGACATCTGTCCTTTAATTCACCA 346
Streptococcus_pyogenes    CTATTCAACTTATGTCAGAAAATTTAAACGTGACATCTGTCCTTTAATTCACCA 346
Enterococcus_faecium      CTATTCAACTTATGTCAGAAAATTTAAACGTGACATCTGTCCTTTAATTCACCA 346
Streptococcus_agalactiae CTATTCAACTTATGTCAGAAAATTTAAACGTGACATCTGTCCTTTAATTCACCA 246
Listeria_monocytogenes    CTATTCAACTTATGTCAGAAAATTTAAACGTGACATCTGTCCTTTAATTCACCA 579
Bacillus_cereus           -----ATGAGC 6

Enterococcus_faecalis      GATATTCTACAGTTTCAATTCCTCAACAAACAGAGGTATAAATTTGTGGGAGATTCTCT 585
Staphylococcus_intermedius GATATTCTACAGTTTCAATTCCTCAACAAACAGAGGTATAAATTTGTGGGAGATTCTCT 406
Streptococcus_pyogenes    GATATTCTACAGTTTCAATTCCTCAACAAACAGAGGTATAAATTTGTGGGAGATTCTCT 659
Enterococcus_faecium      GATATTCTACAGTTTCAATTCCTCAACAAACAGAGGTATAAATTTGTGGGAGATTCTCT 306
Streptococcus_agalactiae GATATTCTACAGTTTCAATTCCTCAACAAACAGAGGTATAAATTTGTGGGAGATTCTCT 639
Listeria_monocytogenes    GATATTCTACAGTTTCAATTCCTCAACAAACAGAGGTATAAATTTGTGGGAGATTCTCT 306
Bacillus_cereus           -----ATGAGC 6

Enterococcus_faecalis      TTAAGTACCGTACTTATGAGCAAGTATTGTCTATTTTAAATAGTTATCTATTATTAAC 1005
Staphylococcus_intermedius TTAAGTACCGTACTTATGAGCAAGTATTGTCTATTTTAAATAGTTATCTATTATTAAC 826
Streptococcus_pyogenes    TTAAGTACCGTACTTATGAGCAAGTATTGTCTATTTTAAATAGTTATCTATTATTAAC 826
Enterococcus_faecium      TTAAGTACCGTACTTATGAGCAAGTATTGTCTATTTTAAATAGTTATCTATTATTAAC 1079
Streptococcus_agalactiae TTAAGTACCGTACTTATGAGCAAGTATTGTCTATTTTAAATAGTTATCTATTATTAAC 726
Listeria_monocytogenes    TTAAGTACCGTACTTATGAGCAAGTATTGTCTATTTTAAATAGTTATCTATTATTAAC 1059
Bacillus_cereus           -----ATGAGC 6

Enterococcus_faecalis      GGAGAGAAATTAATCTATGAGTGCTTTTAAATTTGGAAGTTACAGTTACTAAAGG 1065
Staphylococcus_intermedius GGAGAGAAATTAATCTATGAGTGCTTTTAAATTTGGAAGTTACAGTTACTAAAGG 886
Streptococcus_pyogenes    GGAGAGAAATTAATCTATGAGTGCTTTTAAATTTGGAAGTTACAGTTACTAAAGG 886
Enterococcus_faecium      GGAGAGAAATTAATCTATGAGTGCTTTTAAATTTGGAAGTTACAGTTACTAAAGG 1091
Streptococcus_agalactiae GGAGAGAAATTAATCTATGAGTGCTTTTAAATTTGGAAGTTACAGTTACTAAAGG 738
Listeria_monocytogenes    GGAGAGAAATTAATCTATGAGTGCTTTTAAATTTGGAAGTTACAGTTACTAAAGG 1095
Bacillus_cereus           -----ATGAGC 6
```

BIF701 Lab 4 Multiple Sequence Alignment: Horizontal Gene Transfer of Antibiotic Resistance

By Christopher Eeles

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.¹ 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 traits 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 live-stock populations.

References

1. School of Biological Sciences and Applied Chemistry. (2018). *BIF701 Lab 4: Horizontal Gene Transfer of Antibiotic Resistance*. Toronto, ON: Seneca College.
2. Wikipedia Community. (2018). Clustal Omega. In *Clustal*. Retrieved 2018 October 6 from https://en.wikipedia.org/wiki/Clustal#Clustal_Omega.