Predicting Phage Preferences: Lytic vs. Lysogenic Lifestyle from Genomes

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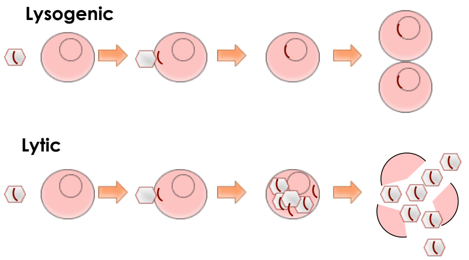
INTRODUCTION

Viruses that that infect bacteria are called phage. There are two distinct lifestyles of phages: lytic and lysogenic. A lytic lifestyle is when a phage infects a bacteria; replicates itself many times; lyses the host bacteria; and releases all the newly created phage. A lysogenic lifestyle is when a phage infects a bacteria; inserts its DNA into the bacteria genome; and replicates when the host bacteria divides.



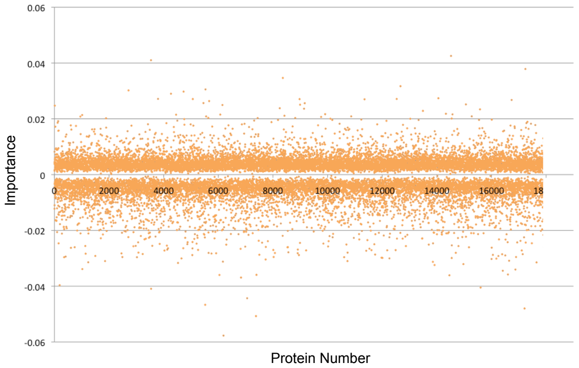
The lifestyle of a phage is important to more accurately classify and understand phages and their environments. Shotgun sequencing now allows us to sequence entire environmental communities, where isolation of individual phages is impossible. Also of great importance are the fields of phage therapy and bio-control. With the increase if antibiotic resistant bacteria and ever increasing food needs, both fields are seeing a resurgence. Since only lytic phage are useful for these treatments, rapidly identifying and excluding lysogenic phage is of great importance. Determining the lifestyle a newly sequenced phage is currently determined by using standard culturing techniques. This method is not only difficult but time consuming.

Using phage genomes to classify is problematic since phage genomes are highly mosaic and there is no single gene that is present in all phage. Thus you cannot simply compare one genome to another. However each phages' proteome has differing characteristics that cause it to enter into one of the two difference lifestyles. These phage proteomes can be compared to the proteome of an unknown phage and a computational classifying scheme can be used to decide if a given unknown phage is lytic or lysogenic.

Currently there are 656 phage in The SEED[1] database that are fully sequenced. Only 228 of these phage have their lifestyle annotated. A Phage Classification Tool Set was written to computationally determine the lifestyle for these unknown phage as well as for any newly sequenced phage.

METHODS

There are 47,376 unique proteins that belong to fully sequenced phages in The SEED[1] database. Not all of these proteins are useful to identify the lifestyle of a phage. Using the RandomForest[3] algorithm, available in the R programming language[2], I calculate how important each protein is towards classifying phage lifestyles. I exclude any proteins that are less then two standard deviations above the mean. The Random Forest algorithm generates many classification trees from a training set of data. These trees are used to predict the class of a testing set by finding the mode of the predictions of all the individual trees.



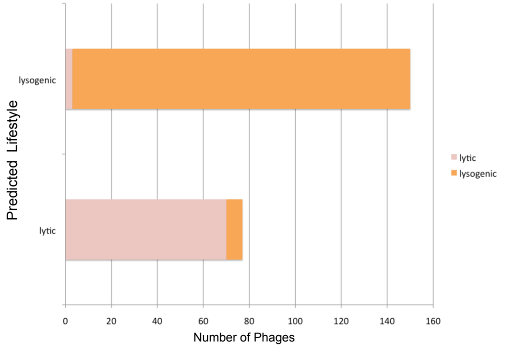
Basic Computational Algorithm of PHACTS:

1. 50 random lytic phage and 50 random lysogenic phage are selected.
2. 300 important lytic proteins and 300 important lysogenic proteins are selected at random.
3. Each of the 600 proteins is then queried against each of the 100 random phage proteomes, and a the highest FASTA similarity value is returned.
4. These 100 vectors, of 600 similarity scores, become a training set for the Random Forest algorithm to create a classification scheme.
5. A testing set is created by querying each of the previous 600 proteins against the unknown phage proteome.
6. Using the classification scheme created by the Random Forest algorithm, the lifestyle of the unknown phage is predicted.

The output of the Random Forest prediction is a probability score for each class that is calculated by dividing the number of decision trees predicting that class by the total number of decision trees. To better account for error in the Random Forest model and random protein selection, I repeat steps 1-6 ten times and average the prediction probabilities.

RESULTS

To test the accuracy of the predictions I sequentially removed each known phage from the database. Treating each removed phage as an unknown, I predicted the lifestyle of each phage. Out of the 228 known phages in my database I was able to correctly predict the lifestyle of 218 phages.



Seven of the phages that were incorrectly classified were the phages that had the lowest probability scores out of all 228 predictions. Subsequently the phage that was incorrectly classified with the highest probability score, was a lytic Lactococcus phage that was predicted to be a temperate phage. The reason for such a high certainty score was because this phage genome contains a functional integrase. Integrases are important proteins for temperate phage to incorporate themselves into host genomes. Cross over events like this are just one of the reasons that make genomic phage analysis difficult.

REFERENCES

1.) The SEED Database
www.theseed.org

2.) R programming language
www.r-project.org

3.) Random Forest Code
www.berkeley.edu/users/breiman/RandomForests/

4.) FASTA
http://fasta.bioch.virginia.edu/