Annotation of the *Kytococcus sedentarius* Genome from DNA Coordinates 500258 to 504432
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Abstract

*Kytococcus sedentarius* is a gram positive species of bacteria that is responsible for causing several diseases including infective endocarditis, hemorraghic pneumonia, and pitted keratolysis. Although it can be a harmful pathogen it is also a beneficial biotechnology organism known to produce Monensin A and B, important oligoketide antibiotics. The entire genome of *Kytococcus sedentarius* has been annotated using computer software and models; however, manual annotation is necessary to confirm computerized annotation. Manual annotation was performed using the GENI-ACT (Genomics Education National Initiative Annotation Collaboration Toolkit) notebook and basic biotechnology and bioinformatics resources. The computer called coordinates for all five genes annotated were accepted.

Introduction

Even though the entire genome of *Kytococcus sedentarius* has been annotated using computer software and models (Sims et al., 2009), it is important to manually annotate this genome to fully understand the pathways and functions of the different proteins encoded for by the genes and find potential cellular targets. This project brought together several students from Rochester STEM high school with varying backgrounds and interests, including health science, engineering, and information technology. While we all knew very little about annotating genes in the beginning, through this unique experience we were able to collaborate with one another, sharing and gaining valuable knowledge about concepts in biology, informatics, and technology all while contributing to a current and global research project.

Methods and Materials

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Results

The manual annotation of *Kytococcus sedentarius* genes was performed using the GENI-ACT (Genomics Education National Initiative Annotation Collaboration Toolkit) notebook. Our team used different tools, including BLAST (Basic Local Alignment Search Tool), T-Coffee Multiple Sequence Alignment Tools, and WebLogo to find similar sequences in other organisms and possible gene functions. To learn where our proteins were located in the bacterium, we used the utilities of TMHMM (prediction of transmembrane helices in proteins), PSORT-b, and Phobius. Some of the group members were also able to analyze if there were functional domains in their protein, if there was any enzymatic function related to their protein, as well as possible alternate open reading frames. One member even explored the possibilities of paralogs and pseudogenes related to their particular gene. We found that all genes had been correctly called by the computer. As a group of genes they varied in cellular localization and function.

Figure 1. Scanning electron micrograph of K. sedentarius (Sims et al., 2009).

Figure 2. Pitted keratolysis forming on the heel of an individual (The Health Science, 2013).

Figure 3. TMHMM (top) results for Ksed_05030 predicting 10 transmembrane helices. Phobius (bottom) for Ksed_05030 confirming the results of the TMHMM test for transmembrane helices. However, the Phobius is predicting a total of 12 helices. These results suggest that the protein is an integral membrane protein.

Figure 4. The transmembrane topology graph for Ksed_05060 (top left) shows no evidence of any transmembrane helices. The Phobius probability graph for Ksed_05060 (top right) confirms the results that the protein is not crossing the cytoplasmic membrane. Although, these two analytical tools do not predict a transmembrane protein, the PSORT-b localization prediction tool (bottom left) gives a final prediction of the protein’s location in the cytoplasmic membrane with a high score of 9.55 (highlighted in red box). Based on these results, it is possible that the protein may be a peripheral membrane protein, embedded in only the inside part of the membrane and not making it through all the way to the outside portion of the membrane.

Figure 5. Phobius results for Ksed_05040 showing no transmembrane helices. But the PSORT-b results predict a cytoplasmic membrane with a score of 9.55. This could possibly mean that the protein sits on the edge of the membrane.

Figure 6. The computer called start codon (in red) for Ksed_05050 has a Shine-Dalgarno region 5’ to 15 base pairs upstream, but, this potential start codon is GTG coding for the amino acid Valine (V) rather than the typical ATG sequence codes for the amino acid methionine (M) that signals the start of transcription and translation. Four possible alternate open reading frames were explored, however, none of these gave better scores or e-values in BLAST than the start codon.

References


Longshaw, et al. (2004). Fatal hemorraghic pneumonia caused by infection due to *Kytococcus sedentarius*—a pathogen or passenger? Archives of Hematology, 93, 447-449.

Levenga, et al. (2004). Fatal hemorraghic pneumonia caused by infection due to *Kytococcus sedentarius*—a pathogen or passenger? Archives of Hematology, 93, 447-449.


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Key verbiage to readers

"Annotation of the *Kytococcus sedentarius* Genome from DNA Coordinates 500258 to 504432"