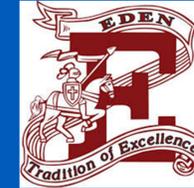


Annotation of the *Kytococcus sedentarius* Genome from Locus Tags

Ksed_RS00750 to Ksed_RS00765

Matt Grapes, Brianna Keller, Jillian Weber and Keith Kwass
Eden Jr/Sr High School Eden, N.Y. and The Western
New York Genetics in Research and Health Care Partnership



Abstract

A group of four consecutive genes from the microorganism *Kytococcus sedentarius*, Ksed_RS00750 – Ksed_RS00765 (formerly Ksed_01560 – Ksed_01590) were annotated using the collaborative genome annotation website GENI-ACT. The Genbank proposed gene product name for each gene was assessed in terms of the general genomic information, amino acid sequence-based similarity data, structure-based evidence from the amino acid sequence, cellular localization data, potential alternative open reading frames, enzymatic function, presence or absence of gene duplication and degradation, the possibility of horizontal gene transfer, and the production of an RNA product. The Genbank proposed gene product name did not differ significantly from the proposed gene annotation for each of the genes in the group and as such, the genes appear to be correctly annotated in the computer database.

Introduction

Kytococcus sedentarius is a strictly aerobic, non-motile, non-encapsulated, and non-endospore forming gram positive coccoid bacterium, found predominantly in tetrad formation. This organism is classified as a chemoheterotroph, as it requires methionine and several other amino acids for growth. Originally isolated from a microscope slide submerged in sea water in 1944, *Kytococcus sedentarius* grows well in sodium chloride at concentrations less than 10% (w/v).

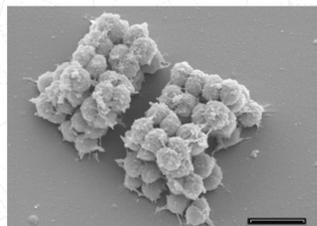


Figure 1. Scanning electron micrograph of *K. sedentarius* strain 541T (Manfred Rohde, Helmholtz Centre for Infection Biology, Braunschweig)

According to Sims et al. (2009), *K. sedentarius* is of interest for several reasons. It is known for the production of oligoketide antibiotics as well as for its role as an opportunistic pathogen causing valve endocarditis, hemorrhagic pneumonia, and pitted keratolysis. It is strictly aerobic and can only grow when several amino acids are provided in the medium. The strain described in this report is a free-living, nonmotile, Gram-positive bacterium, originally isolated from a marine environment. (Sims et al., 2009).

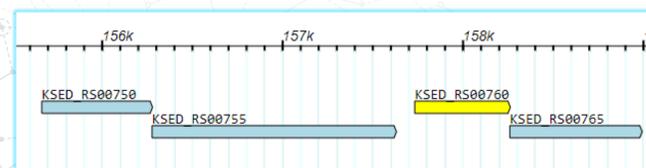


Figure 2. The locus tags of Ksed_RS00750 – Ksed_RS00765 and relative position of the genes under investigation in this research

Methods

Modules of the GENI-ACT (<http://www.geni-act.org/>) were used to complete *Kytococcus sedentarius* genome annotation. The modules are described below:

Modules	Activities	Questions Investigated
Module 1- Basic Information Module	DNA Coordinates and Sequence, Protein Sequence	What is the sequence of my gene and protein? Where is it located in the genome?
Module 2- Sequence-Based Similarity Data	Blast, CDD, T-Coffee, WebLogo	Is my sequence similar to other sequences in Genbank?
Module 3- Cellular Localization Data	Gram Stain, TMHMM, SignalP, PSORT, Phobius	Is my protein in the cytoplasm, secreted or embedded in the membrane?
Module 4- Alternative Open Reading Frame	IMG Sequence Viewer For Alternate ORF Search	Has the amino acid sequence of my protein been called correctly by the computer?
Module 5- Structure-Based Evidence	TIGRFam, Pfam, PDB	Are there functional domains in my protein?
Module 6- Enzymatic Function	KEGG, MetaCyc, E.C. Number,	In what process does my protein take part?
Module 7- Gene Duplication/ Gene Degradation	Paralog, Pseudogene	Are there other forms of my gene in the bacterium? Is my gene functional?
Module 8- Evidence for Horizontal Gene Transfer	Phylogenetic Tree,	Has my gene co-evolved with other genes in the genome?
Module 9- RNA	RFAM	Does my gene encode a functional RNA?

Results

Ksed_RS00750:

The initial proposed product of this gene by GENI-ACT was riboflavin synthase. Riboflavin synthase is an enzyme that catalyzes the final reaction of riboflavin biosynthesis:

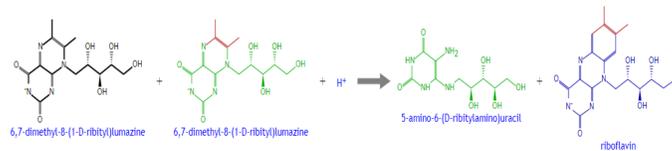
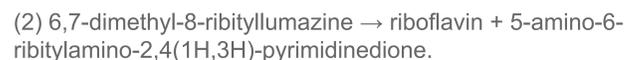


Figure 3. Metacyc Pathway map of riboflavin biosynthesis

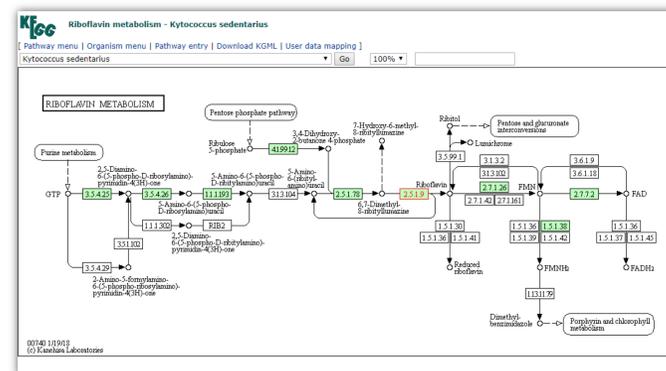


Figure 4. Kegg Pathway of riboflavin metabolism in *Kytococcus sedentarius*. Showing the enzymes associated with Ksed_RS00750 – Ksed_RS00760 (2.5.1.9; 4.1.99.12; 3.5.4.25; 2.5.1.78)

Ksed_RS00755:

The initial proposed product of this gene by GENI-ACT was bifunctional 3,4-dihydroxy-2-butanone-4-phosphate synthase/GTP cyclohydrolase II. Blast results supported this finding. Web logo showed a great deal of matching amino acids with a space in the middle. This gene seems to be two separate genes. The Pfam picture seems to support this showing two different families. Sometimes the GTP cyclohydrolase II and 3,4-DHBP synthase are fused in a single hybrid enzyme coded by the *ribBA* gene but that does not seem to be the case here.

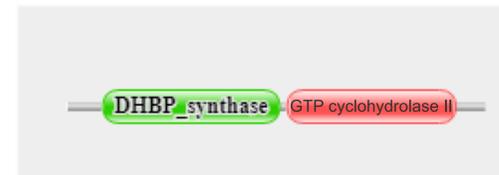


Figure 5. Pfam picture of Ksed_RS00755 clearly showing two distinct enzymes.

Ksed_RS00760:

The initial proposed product of this gene by GENI-ACT was a 6,7-dimethyl-8-ribityllumazine synthase. This is involved in riboflavin biosynthesis. Again highly supported by Blast, TIGRFam, and Pfam results.

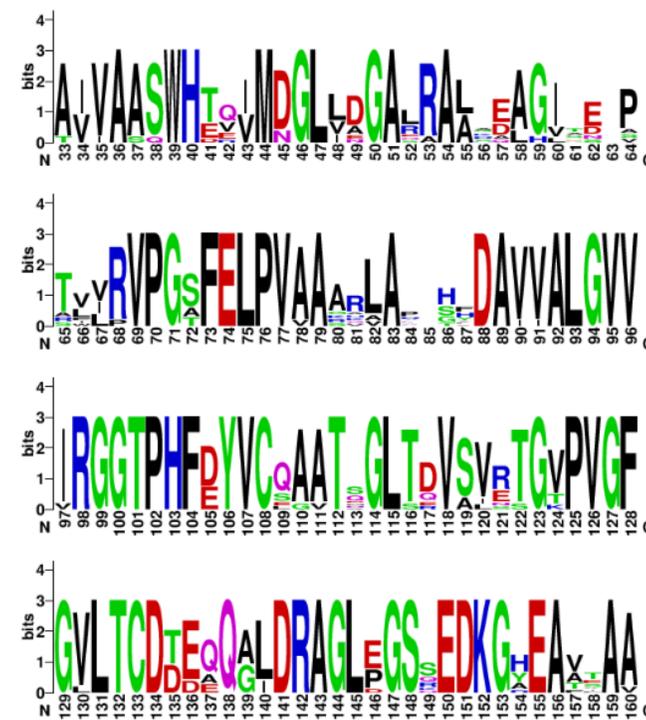


Figure 6. Partial WebLogo of Ksed_RS00760 showing the amino acid sequences are highly conserved across ten different species

Ksed_RS00765:

The initial proposed product of this gene was a nicotinamide riboside transporter PnuC. All of the modules supported this finding. TMHMM and Phobius clearly showed that this is a transmembrane protein. While we know this is a transmembrane protein, we are not sure if this protein moves molecules in or out. It is possible that it moves the riboflavin out of the membrane to continue on to the FMN – FAD path. Another possibility is that it transports riboflavin into the cell. This tends to be the case with most bacteria. When riboflavin is externally available they can import it instead of produce it thus conserving energy on the biosynthetic pathway.

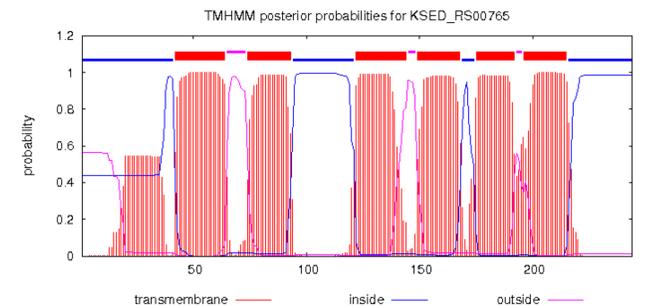


Figure 7. TMHMM transmembrane topology graph

Conclusion

The GENI-ACT proposed gene product did not differ significantly from the proposed gene annotation for each of the genes in the group and as such, the genes appear to be correctly annotated by the computer database. This region appears to be involved in the biosynthesis of riboflavin. This combination of genes are often referred to as the riboflavin biosynthetic pathway or RBP genes. Ksed_RS00750 – Ksed_RS00760 are directly involved with this synthesis while Ksed_RS00765 is a transporter. It is possible that the transporter takes in riboflavin when it is externally available but it is also possible that this transporter exports the synthesized riboflavin, an essential micronutrient required by all life forms. Riboflavin is known as vitamin B-2. This pathway is nonexistent in humans and perhaps *Kytococcus sedentarius* could serve as a source for the production of riboflavin with these genes.

References

- Sims et al. (2009). Complete genome sequence of *Kytococcus sedentarius* type strain (541T). Standards Genomic Sciences, 12 - 20.
- García-Angulo, Victor Antonio. Overlapping riboflavin supply pathways in bacteria. Journal-Critical Reviews in Microbiology Volume 43, 2017 - Issue 2, Pages 196-209 | Received 26 Jan 2016, Accepted 18 May 2016, Published online: 08 Nov 2016.

Acknowledgments

Special thanks to Dr. Stephen Koury and Dr. Rama Dey-Rao for their assistance with this project. This work was supported by an NIH Science Education Partnership (SEPA) Award - R25OD010536