

Annotation of Locus Tags Ksed_07750, Ksed_07840, Ksed_07810, Ksed_07790 of the Kytococcus sedentarius Genome

Achutha Ramesh, Rohit Marwah, Matthew Cowie, Chase Boss and Caitlin Ulock Pittsford Mendon High School and the Western New York Genetics in Research Partnership

A group of genes from the microorganism Kytoco ccus sedentarius (Ksed 07750. Ksed 07840. -Ksed 07810. and Ksed 07790) were annotated using the collaborative genome annotation website GENI-ACT. The Genbankproposed gene productname for each gene was assessed in terms of the general genomic information, amino add sequence-based similarity data, structure-based evidence from the amino acid sequence, cellular localization data, potential alternative open reading frames. The Genbankproposed 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 by in the nr database.

Kyto coccus sedentarius is a marine dwelling. Gram positive bacterium that produces the polyketide antibiotic against pathogens. It is aerobic and can only grow when certain amino acids are present. It is in the Dermacoccaceae family and is primarily found as part of human skin and groundwater. It is well-known as a producer ofantibiotics such as monesin A and monesin B. Its optimum growth temperature is between 25-37 degrees celsius (Figure 1).

Kyto coccus sedentarius is mostly known as a pathogen that creates pitted keratolysis which commonly affects feet and hands (Figure 2). The benefit of actually annotation of genes of Kytococcus sedentarius is that it can help us better understand how specific

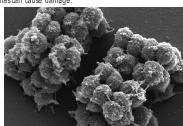


Figure 1. A scanning electron micrograph of Kytococcus sedentarius (Taken from Sims et al. 2009)



Figure 2. An image of a food showing the appearance of pitted keratolysis. (Taken with http://www.med-h.ealt.h.net/Pitte.d-K.e.ra.tolysis.ht.ml)

Methods and Materials

Modules of the GENI-ACT (http://www.geni-act.org/) were used to complete Kyto coccus 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?

Results

Kytococcus sedentarius 07750:

The initial proposed product of this gene by GENI-ACT was a alcohol dehydrogenase [Nocardia vinacea]. This gene product proposal was supported by the top BLAST hits for the amino acid sequence, the presence of well-curated protein functional domains within the amino acid sequence, the transmembrane topography of the amino acid sequence, and the cellular location of the amino acid sequence. As such, the proposed annotation is a alcohol dehydrogenase. I have discovered that my protein has two transmembrane helixe sas seen in Figure 3.

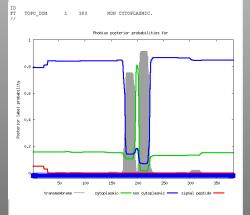


Figure 3- Phobius probibility graph for Kytococcus sedentarius 07750 showing the presence of 2 transmembra ne helixes.

Kytococcus sedentarius 07810:

The initial proposed product of this gene by GENI-ACT was a phosphoribosylaminoimidazolecarboxamideformyltran sferase. This gene product proposal was supported by the top BLAST hits for the amino acid sequence, the presence of well-curated protein functional domains within the amino acid sequence, the transmembrane topography of the amino acid sequence, and the cellular location of the amino acid sequence. As such, the proposed annotation is phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cydohydrolase.

Prediction of Ksed 07810

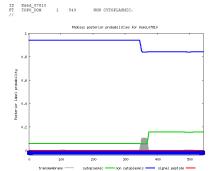


Figure 4- Phobius probability graph for Kytococcus sedentarius 07810, showing the protein lacks both transmembrane helixes and and a signal peotide

Kytococcus sedentarius 07790:

The initial proposed product of this gene by GENI-ACT was Peptidase A24. This gene product proposal was supported by the top BLAST hits for the amino acid sequence, the presence of wellgurated protein functional domains within the amino acid sequence. the transmembrane topography of the amino acid sequence, and the cellular location of the amino acid sequence. As such, the proposed annotation is Peptidase A24. I have discovered that my protein strand is within the Cytoplasmic Membrane, with seven transmembrane helixes, as shown on my Phobius Probability Graph (Figure 5).

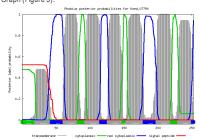


Figure 5- Phobius probability graph for Kytococcus sedentarius 07790, showing the has 7 transmembran e helixes.

Kytococcus sedentarius 07840:

The initial proposed product of this gene by GENI-ACT was malate dehydrogenase . This gene product proposal was supported by the top BLAST hits for the amino acid sequence, the presence of wellcurated protein functional domains within the amino acid sequence, the transmembrane topography of the amino acid sequence, and the cellular location of the amino acid sequence. As such, the proposed annotation is malate dehydrogenase



Figure 6. WebLogo of Ksed 07840. From the amino terminus to the carboxyl terminus, the letters are mainly hin telling us that that the amino acids are mainly similar between the divisions in the sequence. The amino acid sequence is conserved (mainly big letters). The protein is found somewhere in the hacteria It won't he found in the cytonlasmic membrane

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 genesappear to be correctly annotated by the computer

Gene Locus	Geni-Act Gene Products	Proposed Annotation
07750	Alcohol deydrogenase [Nocardia vinacea]	Alcohol dehydrogenase [Nocardia vinacea]
07840	Malate dehydrogenase	Malate dehydrogenase
07810	phosphoribosylaminoimidazo lecarboxamideformyltransfer ase	phosphoribosylaminoimidazo lecarboxamide formyltransferase/IMP cyclohydrolase
07790	Peptidase A24	Peptidase A24

Sims et al. (2009). Complete genome sequence of Kytococcus sedentarius type strain (541T). Standards in Genomic Sciences, 12 - 20.

Acknowledgments

Supported by NSF ITEST Strategies Award Number 1311902