Annotation of the Kytococcus sedentarius Genome DNA Locus Tags Ksed_02660, Ksed_00940, Ksed_03530 and Ksed_00070

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Abstract

A selection of 5 genes from the microorganism Kytococcus sedentarius were annotated, using the collaborative genome annotation website GENI-ACT. The Genbank proposed a gene product name for each gene that was assessed. The genes were evaluated in terms of the general genomic information, amino acid sequence-based similarity data, structure-based evidence from the amino acid sequence, cellular localization data, and potential alternative open reading frames. The Genbank proposed gene product name did not differ significantly from the proposed gene annotation for each of the genes in the group. As such, the genes appear to be correctly annotated by the database based on the modules that were completed.

Introduction

We were annotating the organism *Kytococcus sedentarius* through the GENI-ACT program. We met once a week for about an hour. To help us increase efficiency we received print copies of the training manuals and worked at our own pace. When doing general research about the organism we come across the following information on one of the program websites, NCBI PubMed.gov :

"K. sedentarius produces two extracellular enzymes that independently degrade natural, insoluble human callus. Both enzymes are serine proteases and have cleavage preference sites that are present in a range of human keratins. The identification, in K. sedentarius cultures, of two enzymes which can degrade human callus strengthens the hypothesis that this organism is responsible for the pitting in human epidermis observed in pitted keratolysis. These enzymes may be of commercial use in the biodegradation of a range of keratin polymers, biological washing powders and in the treatment of unwanted callus on human skin." (Longshaw 2002).

Methods

Modules of 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?

Ksed 00070: We investigated Locus Tag Ksed 00070 that has a gene ID of 644990323. When performing BLAST the protein was identified by the computer as DNA gyrase subunit A. This gene has coordinates of 8710..11337 in the forward direction. While conducting our research, our data has supported that the the computer prediction is accurate. When conducting WebLogo, the picture in Figure 1 supports that the larger letters represent the amino acids similar to the protein that we are coding for. Thus concludes that the proteins must have a function comparing to others.

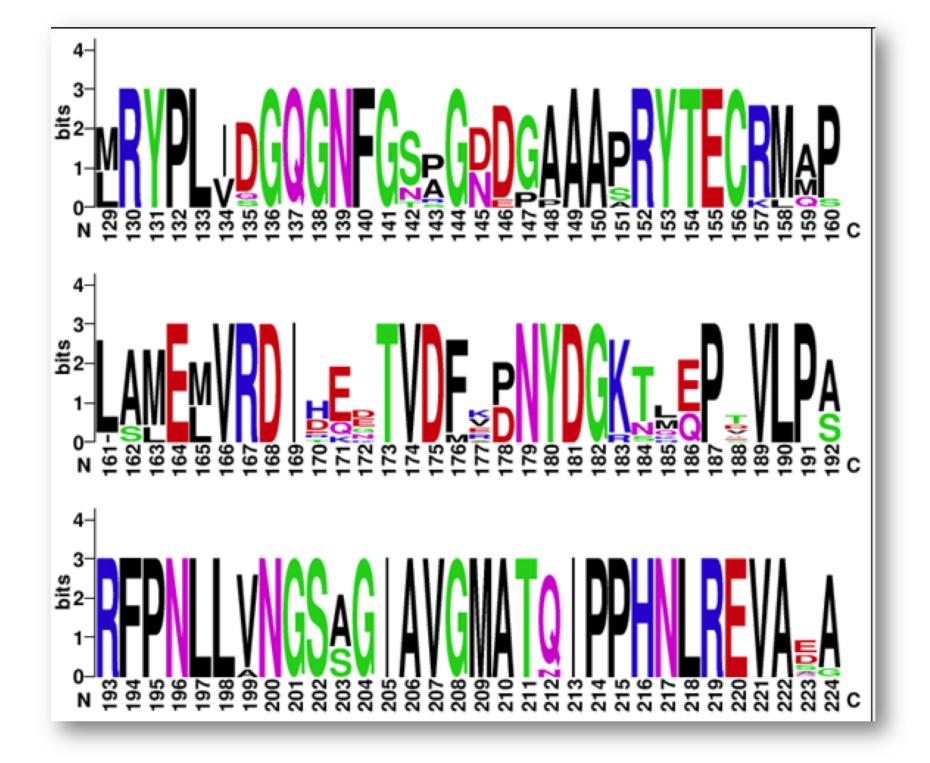


Figure 1 – A portion of the WebLogo showing the similarities between amino acids in different organisms.

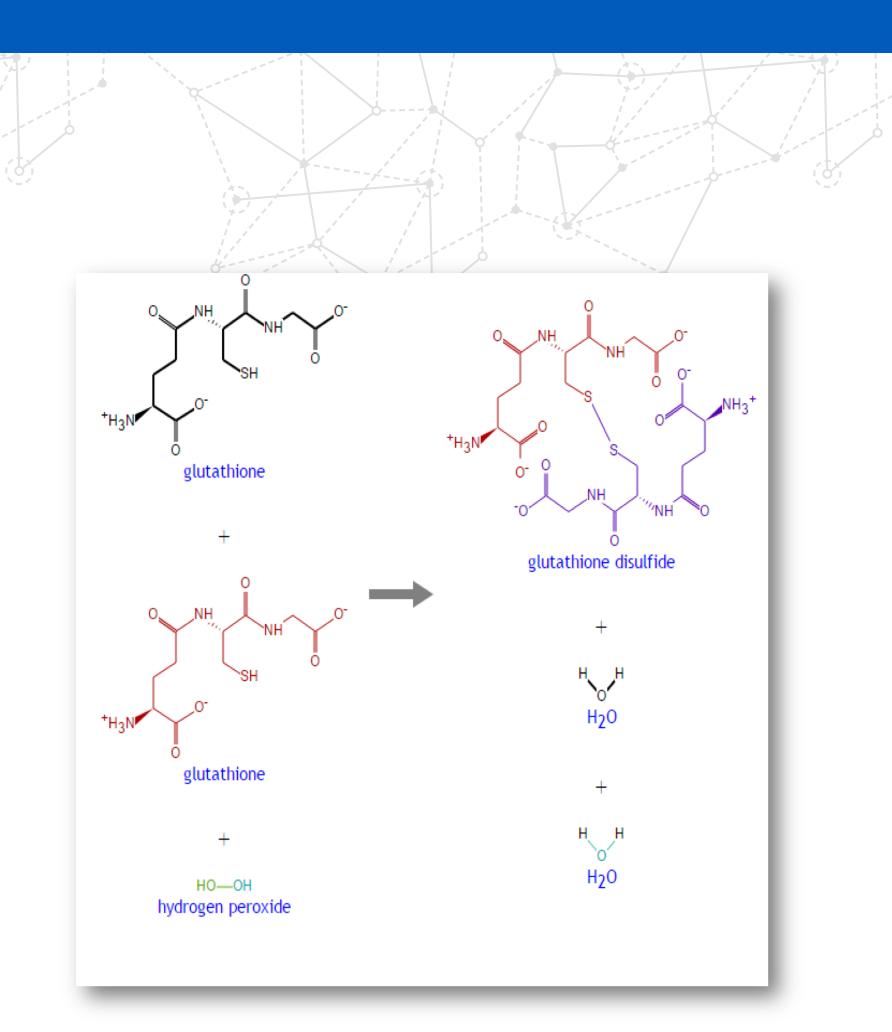
Ksed 02660:

Results

Based off our research our protein, Ksed 02660, coded for an enzyme named glutathione peroxidase. The information that the computer called for was supported by our research. The BLAST, TIGRFAM, Pfam, and enzyme numbers matches the computer's original results. Our E.C. number was 1.11.1.9, if it was not an enzyme it would not have had an E.C. number. Our enzyme, is a catalyst to make glutathione disulfide and two water molecules, from two glutathione molecules and a hydrogen peroxide molecule. The main purpose of the our enzyme glutathione peroxidase is to convert hydrogen peroxide into water to neutralize the harmful effects of the peroxide, and protecting an organism from getting any type of oxidative damage. Glutathione is also known as one of the most potent natural antioxidants. As an antioxidant, glutathione gives up an electron and gives it to an unpaired electron known as a free radical to complete the pair. Free radicals are connected to causing cancer, Alzheimer's, Parkinson's disease, the effects of aging, and other illnesses because of the effect they have on the cell when they take the electron away.

Figure 2 – MetaCyc image of reaction catalyzed by the enzyme glutathione peroxidase.

The computer predicted that the bacterial protein is called cytosine/adenosine deaminase. Though my Blast Generator gene product says that the bacterial protein is called nucleoside deaminase. Even though the names are different, I noticed many similarities that could link the different bacteria names together. For instance, in Module 1, the computer generated the gene's DNA coordinates as 99499..100026(-). When I generated my data it came up as the same. Another example being that when the computer generator caught the Pfam name as Cytidine and deoxycytidylate deaminase zincbinding region for my gene, while when I generated the Pfam name, I got the same result. Some other results that are the same by comparing my information to the computer's information are: Pfam Number: (PF00383), Helix Presence: None and Signal Peptide Presence: None. Wondering what Nucleoside meant and what made it different from the name that the computer generated for my bacteria, I looked up the definition; Nucleoside: a compound (e.g., adenosine or cytidine) commonly found in DNA or RNA, consisting of a purine or pyrimidine base linked to a sugar. Purine bases are the chemicals Cytosine and Adenine that make up a nucleotide in DNA and RNA. The same goes for pyrimidine.



Ksed 00940:

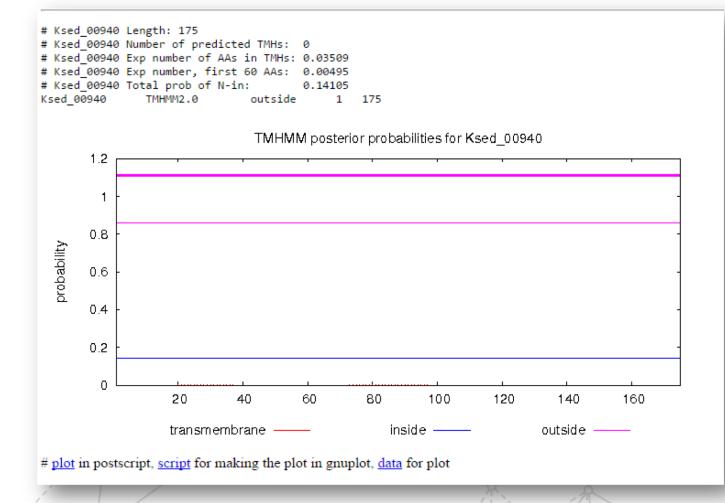


Figure 3 – The TMHMM graph shows that there is no helix present in the bacterial protein.

Ksed 03530: In the organism Kytococcus sedentarius, with the gene/ Ksed_03530 the computer is predicting the gene codes for a protein called polyphosphate kinase which my research is supporting as shown with the information below. This is shown being supported because the BLAST results are shown below, the top hit's name is similar to the result that the computer gave me.

The table below summarizes the individuals' conclusions based on each of their work in GENI-ACT.

Gene

References Longshaw et al. (2002). Kytococcus sedentarius, the organism associated with pitted keratolysis, produces two keratin-degrading enzymes. Journal of Applied Microbiology, 93(5):810-6.

Smirnova, G. V., and O. N. Oktyabrsky. "Glutathione in Bacteria." Biochemistry (Moscow), vol. 70, no. 11, 2005, pp. 1-2. Springer Link, link.springer.com/article/10.1007/s10541-005-0248-3. Accessed 12 May 2018.

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RNA degradosome polyphosphate kinase [Kytococcus sp. HMSC28H12] Sequence ID: <u>WP_070703513.1</u> Length: 759 Number of Matches: 1 See 1 more title(s)							
Range 1	: 45	to 759 GenPept Graphics		V Next Mate	h 🔺 Previous Match		
Score		Expect Method	Identities	Positives	Gaps		
1038 bi	its(2						
Query	5	PAPDGELPADRFLERETSWLQFNERVLELAADPAVPL			1		
Sbjct 4	45	P DG+LPADRFLERETSWLQFNERVLELAAD +PL PGADGDLPADRFLERETSWLQFNERVLELAADRTIPL			34		
Query	65	GLKRRIATGLAVRSASGQEPQEVLSMVGRSAHALMTE			24		
Sbjct	105	GL+RRIATGLAVR+ SGQ+P EVL+ VGR AHALM E GLQRRIATGLAVRTPSGQDPHEVLATVGRCAHALMDE			54		
Query	125	KDLQDAERDAFEEFFVRDVYPVLTPLAVDPAHPFPYI ++L E+ FE+ FV VYPVLTPLAVDPAHPFPYI			34		
Sbjct	165	EELAAHEKQYFEDLFVEQVYPVLTPLAVDPAHPFPYI			24		
Query	185	VKVPPALPRLLRVSHVLGTDESDEQIQESEHGIRFLP VKVPPALPRLLRV H LG ESDE + SE GIRFLP			14		
Sbjct	225	VKVPPALPRLLRVCHGLGVHESDEVLVRSESGIRFLP			34		
Query	245	FRVTRNEDLEVEEDEAENLLTALERELTRRRFGPAVR FRVTRNEDL ++ED +ENLL ALERELTRRR GP VR			34		
Sbjct	285	FRVTRNEDLALDEDGSENLLPALERELTRRRQGPPVR			14		
Query	305	HEVYVLPAPLDLRSMNTVADLRISDLRWPAFRTHTHP +VYVLP PLDLR + T+ADL + LRWP + HTH			54		
Sbjct	345	PDVYVLPGPLDLRGLETLADLHLMPLRWPVMQAHTHS			34		
Query	365	LHHPYDSFSTSVQRFLEQAAADPHVLAIKQTLYRTSG LHHPYDSFSTSVQRFLEQAAADP VLAIKQTLYRT+G			24		
Sbjct 4	405	LHHPYDSFSTSVQRFLEQAAADPDVLAIKQTLYRTNG			54		
Query	425	IKARFDEENNITWARQLERAGVHVVYGQVGLKTHAKL IKARFDEENNI+WAR+LERAGVHVVYGOVGLKTHAK+			34		
Sbjct 4	465	IKARFDEENNISWARELERAGVHVVYGQVGLKTHAKV			24		
Query	485	PKTARLYEDLGLLSTDPQVTEDVTRLFNQLSGMAPRS P TAR++EDLGLL+ DP+VTEDVTRLFNQLSG+AP +			14		
Sbjct	525	PGTARVFEDLGLLTCDPEVTEDVTRLFNQLSGIAPGA			34		
Query	545	AARARKGHPARVRVKANSIVDEAFIDACYRASQAGVQ ARAR GH ARVR+K N +VD+ +DA YRAS AGV+			34		
Sbjct	585	CARARAGHRARVRLKGNGLVDQEVVDALYRASGAGVE			14		
Query	605	VRSVLGRFLEHSRVVVAGIPGDPANPLRALIGSADMM VRS++GR LEHSR +V G+PGDP NP+RA IGS DMM	DMM RNLDRRVEAMV L+	+D HV H+	54		
	645	VRSIVGRLLEHSRALVVGVPGDPHNPVRAFIGSPDMM			34		
		DELFELAFSPDVATWHLDAEGVWTRHHLDEAGEPLKD D LF+ AF+PD A W LD +G+WTR H D G PLK+	+ FE+EK Y + R++	RR			
Sbjct	705	DWLFDTAFAPDTACWELDGDGLWTRRHTDGEGRPLKE	MHFEMEKHYTRARKE	SRR 759			

Figure 4 – Top hit of BLAST results shown above for Ksed_03530.

Conclusion

e Locus Tags	Module Completed *based on Geni-Act Manual	Conclusion
sed_02660	<u>Module 6</u>	Predicted to be a protein called glutathione peroxidase
sed_00940	Module 4	Predicted to be a protein called cytosine/adenosine deaminase
sed_03530	Module 4	Predicted to be a protein called polyphosphate kinase
sed_00070	Module 3	Predicted to be DNA gyrase

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