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Annotation of the *Kytococcus sedentarius* Genome from Locus Tags Ksed_08190 to Ksed_08230

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Abstract

A group of 5 consecutive genes from the microorganism *Kytococcus sedentarius* (Ksed_08190 – Ksed_08230) 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, a sit 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).

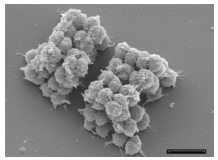


Figure 2. Scanning electron micrograph of K. sedentarius strain 541T (Manfred Rohde, Helmholtz Centre for Infection Biology, Braunschweig)

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).

Kytococcus sedentarius Gene region 08190-08230

Methods and Materials

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

***Kytococcus sedentarius* 08190:** acetyl/propionyl CoA carboxylase - In this cycle the ATP-dependent carboxylation of acetyl-CoA (biotin-dependent) to malonyl-CoA and propionyl-CoA to methylmalonyl-CoA, represent the key CO_2 fixation reactions. It is the first step in fatty acid synthesis.

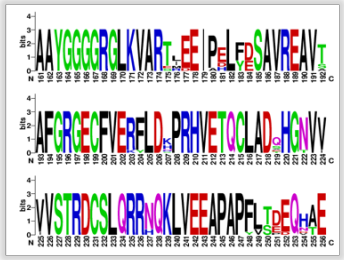


Figure I – *Kytococcus sedentarius* 08190 gene WebLogo showing that it is a well conserved protein amongst various species.

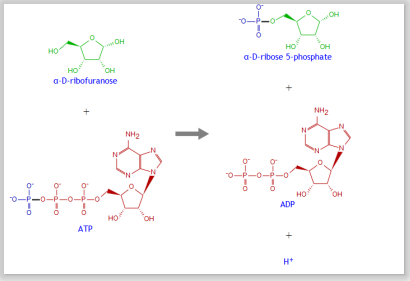
***Kytococcus sedentarius* 08200:** a ribokinase (EC 2.7.1.15) is an enzyme that catalyzes the chemical reaction

ATP + D-ribose ←→ ADP + D-ribose 5-phosphate

This enzyme belongs to the family of transferases, specifically those transferring phosphorus-containing groups (phosphotransferases) with an alcohol group as acceptor. The systematic name of this enzyme class is ATP:D-ribose 5-phosphotransferase.

Results

Other names in common use include deoxyribokinase, ribokinase (phosphorylating), and D-ribokinase. This enzyme, Phosphofructokinase B (PfkB) family of carbohydrate kinases participates in pentose phosphate pathway.



***Kytococcus sedentarius* 08210 :** butyryl-CoA dehydrogenase is an enzyme that catalyzes the chemical reaction

butyryl-CoA + acceptor ←→ 2-butenoyl-CoA + reduced acceptor

This enzyme participates in 3 metabolic pathways: fatty acid metabolism, valine, leucine and isoleucine degradation, and butanoate metabolism. It employs one cofactor, FAD.

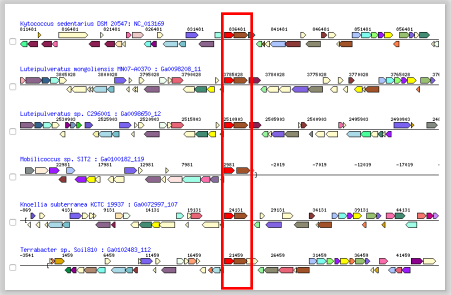


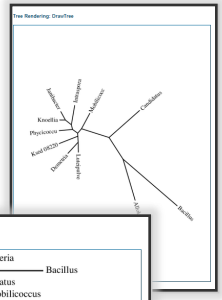
Figure II – *Kytococcus sedentarius* 08210: Ortholog Neighborhood Region of Ksed_08210 shows similarities amongst different bacteria.

***Kytococcus sedentarius* 08220 :** acetyl-CoA carboxylase, carboxyltransferase component (subunits alpha and beta).
Component of the acetyl coenzyme A carboxylase (ACC) complex. First, biotin carboxylase catalyzes the carboxylation of biotin on its carrier protein (BCCP) and then the CO_2 group is transferred by the carboxyltransferase to acetyl-CoA to form malonyl-CoA. This biotin-dependent enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA through its two catalytic activities, biotin carboxylase (BC) and carboxyltransferase (CT). ACC is a multi-subunit enzyme in most prokaryotes and in the chloroplasts of most plants and algae. The most important function of ACC is to provide the malonyl-CoA substrate for the biosynthesis of fatty acids.

Results

***Kytococcus sedentarius* 08220**
Phylogentic tree results show a close relationship with *Demetria* and *Luteipulveratus*.

Two other Genera that were consistent with our other genes were *Dermacoccus* and *Serinicoccus*.



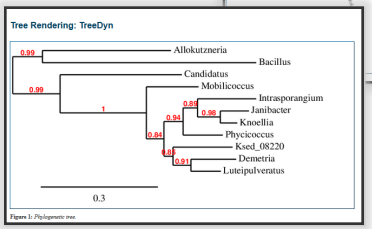
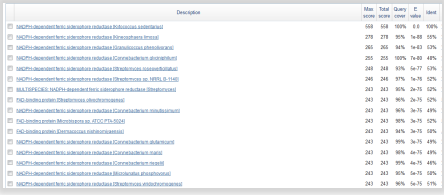


Figure 3: Phylogenetic tree

***Kytococcus sedentarius* 08230:** Siderophore-interacting protein, FAD-binding domain. Iron limitation is one major constraints of microbial life, and plenty of microbes use siderophores for high affinity iron acquisition.



BLAST results strongly supporting results of Ksed_08230 as a NADPH-dependent ferricsiderophore reductase.

Conclusion

The GENI-ACT proposed gene product did not differ significantly from the proposed gene annotation for each of the genes in the group. These genes appear to be correctly annotated by the computer database. This region appears to be involved in the synthesis fatty acids or possibly fatty acid β-oxidation.

References

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

Acknowledgments

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