

Annotation of *Propionibacterium acne* Locus Tags PPA_RS00195, PPA_RS00205, PPA_RS00215 and PPA_RS00220

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Abstract

A group of four genes from the microorganism *Propionibacterium acne* were annotated using the collaborative genome annotating site GENI-ACT.org. For as far as we have researched locus tags PPA_RS00195, RS00215 and RS00220, the proposed gene annotations matched that of proposed in Genbank. For PPA_RS00205, results showed it was a cytoplasmic protein instead of the proposed hypothetical protein. Our bacteria has research being done to see if it could help build human's innate immunity, thanks to specific gene properties.



Introduction

P. acne is a gram positive organism that does not require oxygen to grow. It is involved in the development of human acne. *P. acne* is very prevalent, affecting more than 45 million people in the United States alone. It is estimated that 20% of all dermatology visits are related to the treatment of acne. Research is being done on *P. acne* because scientists have found that it can act as an immune-stimulant in humans with severe acne.

In the early 80's *P. acne* was commonly used to stimulate a non-specific immune response in mice and human cells against cancer. Scientists discovered an important cytokine interleukin (IL)N-18 was discovered from *P. acne*. Research is still being done on *P. acne* to see whether or not it can play a role in protection against life-threatening diseases like malaria.

The purpose of our research in the *P. acne* bacteria is to discover the specific proteins that this organism codes for and to determine if these proteins could assist in bettering humans' innate immune system. Through the majority of this project we will take in-depth look at the genome of the organism *P. acne*.

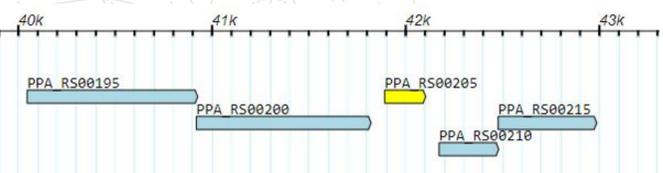


Figure 1. The locus tags and relative position of the genes under investigation in this research include PPA_RS00195 (Lizzie Hojnoski, Josiah Jones, Natacha Valley), PPA_RS00205 (Karina Morling, Maisy Booth), PPA_RS00215 (Christopher Graham, Westin Perry), PPA_RS00220 (Jaiden Weitzel, Lindsey Simons).

Methods

Modules of the GENI-ACT (<http://www.geni-act.org/>) were used to complete *Propionibacterium acne* 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

RS00195-The initial proposed product of this gene by GENI-ACT was a membrane protein. This gene product proposal was supported by the COG test which identified the proteins as Flp pilus assembly protein TadB. This is an intracellular trafficking secretion and vesicular protein. The second COG hit showed identified it as a type 2 secretion (T2ss), Protein F. Because the sequence coded for a trafficking protein we can guess that it is found in the membrane in order to let certain things in and out of the cell. Upon further investigation, the LipoP results indicated this gene codes for a lipo protein with a cleavage site between amino acid 18 and 19 and amino acid G in position +2 after the cleavage site.

RS00205-The initial proposed product of this gene by GENI-ACT was a hypothetical protein. This gene product proposal was inconclusive because when we ran the COG test there were no results found. We think this is because our protein is hypothetical and no wet laboratory research has been done to back up the initial proposal. Upon further research, according to the signalP our protein did contain a not signal peptide. pSORTb predicted it was a cytoplasmic membrane protein, and TMHMM predicted it to have 4 transmembrane helices (Figure 2).

RS00215- The initial proposed product of this gene by GENI-ACT was a membrane protein. This gene product proposal was supported by the COG test which identified the proteins that act to assemble or export an Flp pilus. These flp-like genes are required for the production of pili, which are involved in the adherence to surfaces. Upon further investigation, the LipoP results indicated this gene codes for a lipo protein with a cleavage site between amino acid 18 and 19 and amino acid G in position +2 after the cleavage site.

RS00220- The initial proposed product of this gene by GENI-ACT was a peptidoglycan-binding protein. This gene product proposal was supported by the COG which identified this portion of the gene to be a LysM domain which is a general peptidoglycan-binding module. Though mainly used for Gram - bacteria, LipoP predicted a potential n-terminal signal peptide I cleavage site, when SignalP did not predict a signal peptide to be present.

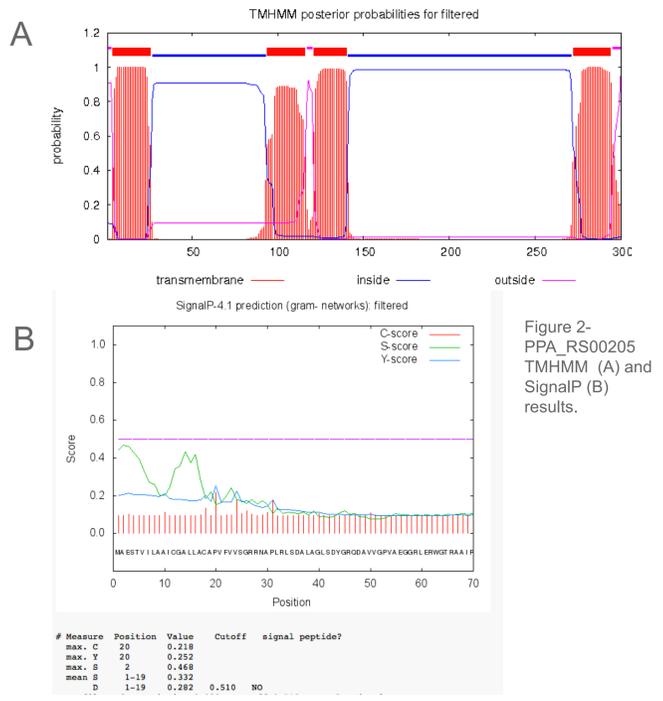


Figure 2- PPA_RS00205 TMHMM (A) and SignalP (B) results.

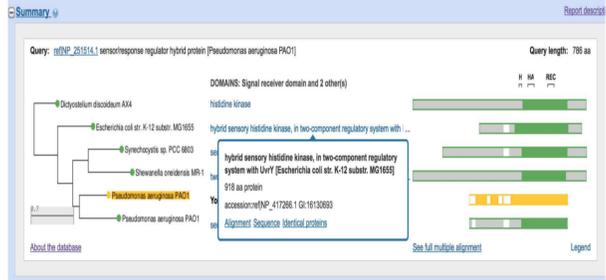


Figure 3 -PPA_RS00220 Evolutionary tree

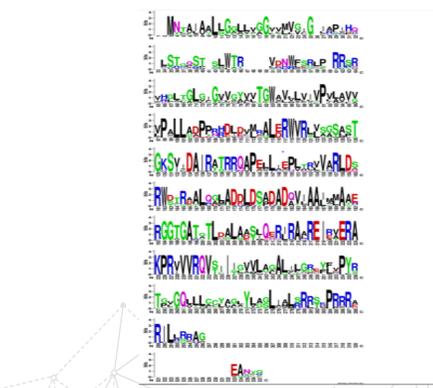


Figure 4- PPA_RS00195 Web logo

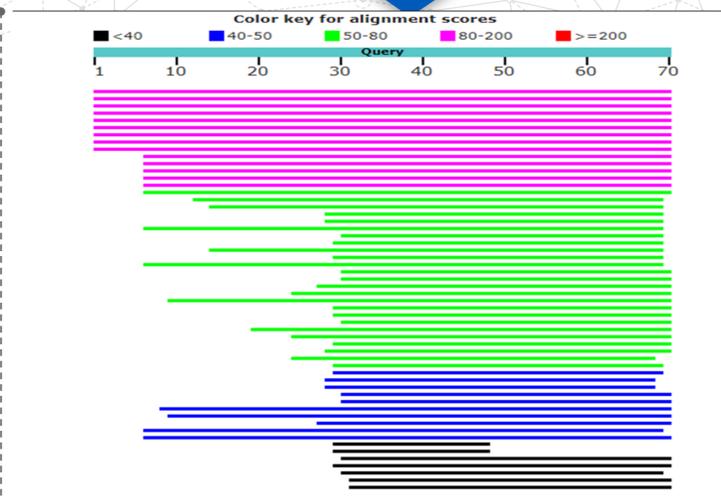


Figure 5- PPA_RS00205 blast alignment

Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.

Download Phylogenetic Tree Data
Branch length: Cladogram Real

PPA_RS00195_0
PPA_RS00195_1_0

Conclusion

The GENI-ACT proposed gene product differed in PPA_RS00205, but in the other three genes we analyzed the product matched our results.

Gene Locus	GENI-ACT Gene Products	Proposed Annotations
00195	Membrane Protein	Membrane Protein
00205	Hypothetical Protein	Cytoplasmic Protein
00215	Membrane Protein	Membrane Protein
00220	Peptidoglycan-binding protein	Peptidoglycan-binding protein

References

<https://www.ncbi.nlm.nih.gov/books/NBK83685/>

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