Investigating the Possible Role of a Glycosyl Transferase Protein in the Biosynthesis of Long-Chain Hydrocarbons in *Shewanella oneidensis*

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Abstract

In the search for alternative sources of energy, new organisms are being looked at as potential biofuel producers. It has been shown that the long-chain hydrocarbons produced by certain bacteria can be broken down into usable fuel. *Shewanella oneidensis*, a gram-negative bacterium, may be an ideal organism for producing these long-chain hydrocarbons. The hydrocarbons are formed as a result of a head-to-head fatty acid condensation via the enzyme OleA. Because previous attempts to overproduce these long-chain hydrocarbons in recombinant *S. oneidensis* strains containing the *oleA* gene from *Stenotrophomonas maltophilia* have not yielded a significant increase in production over the wild type strain, the question has been raised as to whether or not other proteins might play a role, either directly or indirectly, in the production process. In my thesis work, I deleted the gene *SO_3174*, which was interrupted in a transposon mutagenesis screen for increased hydrocarbon production, from *S. oneidensis*. *SO_3174* encodes a putative glycosyl transferase protein. I then tried to show that deleting *SO_3174* resulted in an increase in hydrocarbon production just as the interruption of the gene had. The deletion strain showed an increased fluorescence in the presence of Nile Red dye, a hydrophobic dye that can be used to indirectly detect hydrocarbon levels. However, the deletion strain did not exhibit increased hydrocarbons during direct analysis of nonpolar extractions. These same results were obtained from a strain containing the *SO_3174* deletion and expressing OleA from *S. maltophilia*. The *SO_3174* deletion strain was shown to have lower levels of extracellular polysaccharides than wild type *S. oneidensis* based on a Congo Red binding assay. From these results, I hypothesized that the lower levels of extracellular sugars resulting from the absence of the glycosyl transferase may have made the membrane of the deletion strain more permeable to the Nile Red dye. Overall, I found that the protein encoded by *SO_3174* most likely does not play a role in hydrocarbon biosynthesis in *S. oneidensis*.

S. oneidensis and Hydrocarbons

One of the sources of biofuel being examined by researchers is bacteria. Certain strains of bacteria, including *S. oneidensis* have been found to produce long-chain hydrocarbons and ketones that can be broken down for fuel. These hydrocarbons are produced as a result of a head-to-head condensation of fatty acids by the enzyme OleA.

Mechanism of OleA

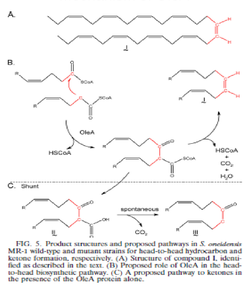
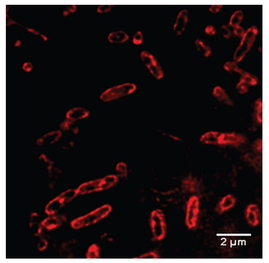


FIG. 5. Product structures and proposed pathways in *S. oneidensis* MR-1 wild-type and mutant strains for head-to-head hydrocarbon and ketone formation, respectively. (A) Structure of compound I, identified as described in the text. (B) Proposed role of OleA in the head-to-head biosynthetic pathway. (C) A proposed pathway to ketones in the presence of the OleA protein alone.

From Sukovich et al. (2010b)

Studies have shown a correlation between hydrocarbon production levels and fluorescence in the presence of Nile Red dye. Nile Red is known to localize to the membrane space where the hydrocarbons and ketones are found.



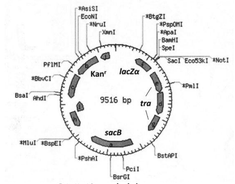
S. Oneidensis cells exposed to Nile Red dye

From Pinzon et al. (2011)

Aims and Hypothesis

The goal of my study was to find a protein that is involved either directly or indirectly in the biosynthesis of long-chain ketones and hydrocarbons. The other goal was to validate the theory of using Nile Red fluorescence to detect changes in hydrocarbon production. My hypothesis was that deleting the gene *SO_3174*, interrupted in a transposon mutagenesis screen for increased hydrocarbon production, from *S. oneidensis* and adding *Stenotrophomonas maltophilia oleA* would result in increased production of hydrocarbons.

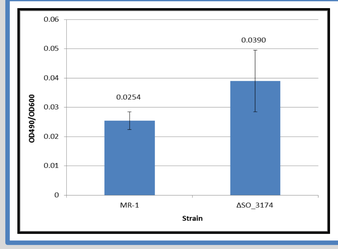
Δ*SO_3174* Construction



pSMV3 suicide vector

The regions 1 kb upstream and downstream of *SO_3174* were cloned into the pSMV3 suicide vector using EcoRI, BamHI, and SacI. The deletion construct was mated into wild type *S. oneidensis*. Selective media was used to force homologous recombination which resulted in deletion of *SO_3174*.

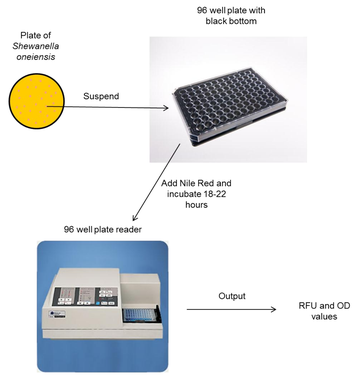
Sugar Quantification

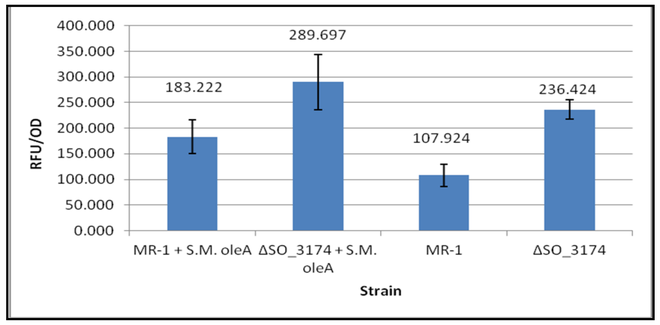


Congo Red Binding Assay results in terms of OD_{490}/OD_{600} of culture supernatant for wild type *S. oneidensis* and the Δ*SO_3174* deletion strain. The deletion resulted in an increase in OD_{490}/OD_{600} of supernatant, or a decrease in extracellular polysaccharides.

Screening for Hydrocarbons

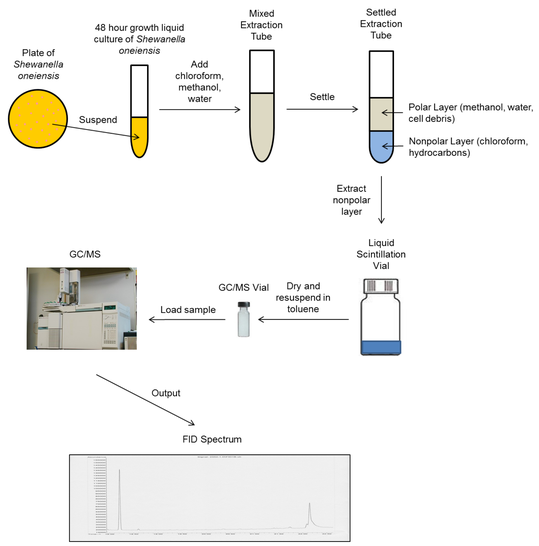
Indirect – Nile Red Screening

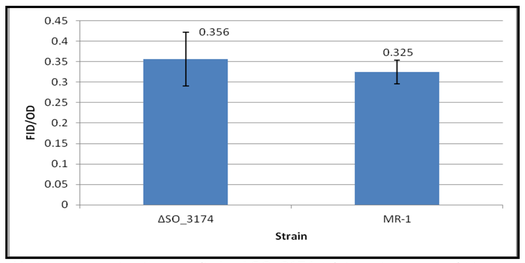




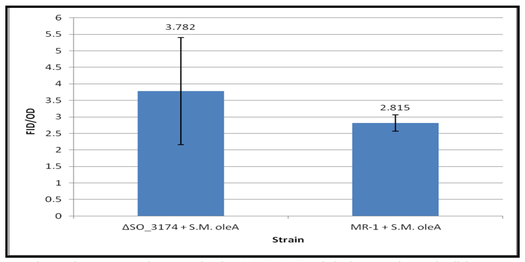
Nile Red screen results for Δ*SO_3174* deletion strain and wild type *S. oneidensis* with and without *S. maltophilia* (S.M.) OleA in terms of RFU/OD_{600}. For both comparisons, the deletion resulted in a higher Nile Red signal.

Direct – Chloroform / Methanol Extractions





Hydrocarbon extraction results for Δ*SO_3174* deletion strain and wild type *S. oneidensis* in terms of FID/OD_{600}. The deletion did not result in significantly higher hydrocarbons.



Hydrocarbon extraction results for Δ*SO_3174* deletion strain and wild type *S. oneidensis* with S.M. OleA in terms of FID/OD_{600}. The deletion did not result in significantly higher hydrocarbons.

Conclusions

In summary, it was found that the deletion of *SO_3174* resulted in an increase in Nile Red signal but no significant difference in hydrocarbon production. One possible hypothesis for this is that the reduction in extracellular polysaccharides resulting from the absence of the glycosyl transferase made the membrane more permeable to the Nile Red dye. This claim can be backed up by the fact that a Congo Red binding assay showed the deletion strain had less extracellular sugars than wild type. The next step from here would be to repeat this experiment with another one of the genes found interrupted in the transposon mutagenesis screen and see if deleting it has any effect on hydrocarbon production.

Acknowledgments

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References

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