Evaluating Biotransformation Efficiency of Oleic Acid to10-Hydrodroxystearic Acid by Two Secondary Alcohol Dehydrogenase Knockout Mutants of *Nocardia cholesterolicum* NRRL5767

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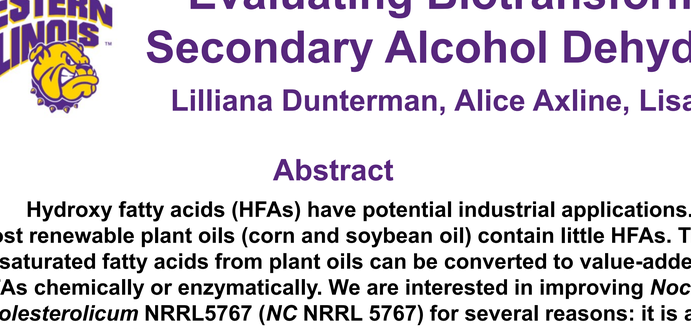
Abstract

Hydroxy fatty acids (HFAs) have potential industrial applications. Most renewable plant oils (corn and soybean oil) contain little HFAs. The unsaturated fatty acids from plant oils can be converted to value-added HFAs chemically or enzymatically. We are interested in improving *Nocardia cholesterolicum* NRRL5767 (*NC* NRRL 5767) for several reasons: it is a stable industrial microorganism, it converts oleic acid and linoleic acid to corresponding HFAs in high yield with minor keto fatty acids. We intend to improve NC NRRL 5767 by CRISPR/Cas9 genome editing to knockout the secondary alcohol dehydrogenase (2º-ADH) gene, thus blocking the conversion of hydroxy fatty acids to their keto derivatives.

We have recently obtained two knockout mutants of 2º-ADH of *NC* NRRL5767. We are now evaluating the biotransformation efficiency of oleic acid to10-hydrodroxystearic acid (10-HSA) by these two knockout mutants. The two mutants and wild-type *NC* NRRL 5767 were cultured. Individual cell pellet was used to set up biotransformation reaction containing one mL of 100 mM sodium phosphate buffer (pH 6.8) and 5 µL oleic acid (as substrate). Biotransformation was carried at 37ºC for 8 hrs. The yield of biotransformation products was analyzed by TLC and will be further quantified by Gas chromatography (GC). Our preliminary results from TLC analysis indicated that there is a similar yield of 10-HSA from the knockout mutants and the wild-type *NC* NRRL 5767.

2º-*ADH* is the target for gene knockout

The 10-HSA is an industrial valuable product. Knocking out the 2º-*ADH* gene may enhance the production of the 10-HSA from oleic acid.



Methods

The 2º-ADH knockout mutants and control cells were cultured in LB or BHI media

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The same amount of each culture was centrifuged. The pellet was suspended in 1 mL sodium phosphate, pH 6.8

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Biotransformation reaction was carried out with or without oleic acid at 37ºC, 250 rpm, 8 hours

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Acidified biotransformation products were extracted with ether and analyzed by TLC

Various clones used in this study

- Wild-type NC NRRL 5767
- 1-p-11: NC NRRL 5767 transformed with pCRISPomyces-2
- 1-3-17 and 2-3-52: 2º-ADH knockout mutants created previously by CRISPR/Cas9 genome editing technology
- 1-3-3: NC NRRL 5767 transformed with CRISPR/Cas9/sgRNA designed to target the 2º-ADH gene, but failed

TLC analysis results of biotransformation products produced by knockout mutants and controls using oleic acid as the substrate

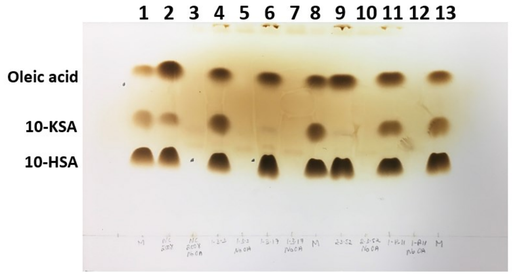


Figure 1. Biotransformation products produced by the knockout mutants or 1-p-11 cultured in LB broth.
Lanes 1, 8, 13: fatty acids standard.
Lanes 2-3: *NC* NRRL5767 with or without oleic acid
Lanes 4-5: 1-3-3 with or without oleic acid
Lanes 6-7: mutant, 1-3-17, with or without oleic acid
Lanes 9-10: mutant, 2-3-52, with or without oleic acid
Lanes 11-12: 1-p-11 with or without oleic acid

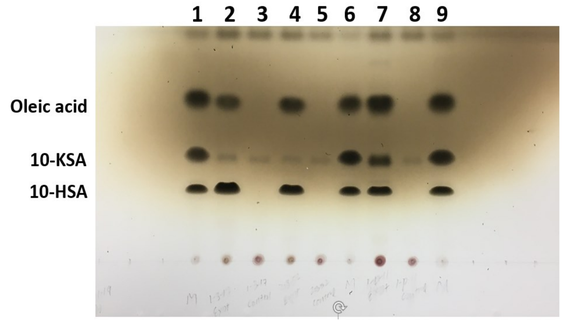


Figure 2: Biotransformation products produced by the knockout mutants or 1-p-11 cultured in LB broth.
Lanes 1, 6, and 9: Fatty acid standard
Lanes 2 and 3: mutant 1-3-17 with or without oleic acid
Lanes 4 and 5: mutant 2-3-52 with or without oleic acid
Lanes 7 and 8: 1-p-11 with or without oleic acid

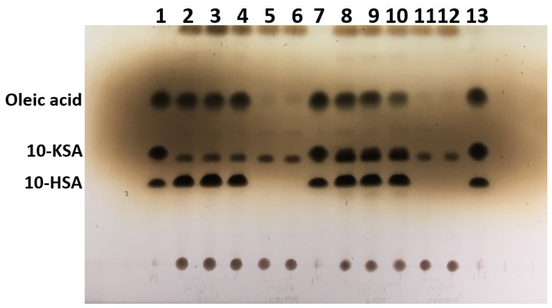


Figure 3. Biotransformation products produced by knockout mutant 1-3-17 or 1-p-11 cultured in BHI medium.
Lanes 1, 7, and 13: Fatty acids standard.
Lanes 2-4: mutant 1-3-17 with oleic acid (triplicate).
Lanes 5-6: mutant 1-3-17 without oleic acid (duplicate).
Lanes 8-10: 1-p-11 with oleic acid (triplicate).
Lanes 11-12: 1-p-11 without oleic acid (duplicate).

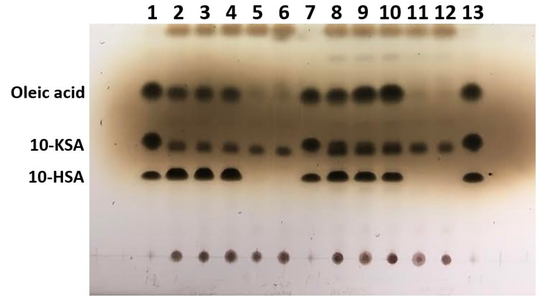
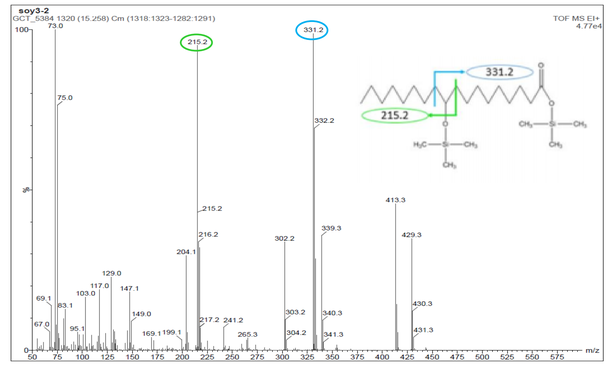


Figure 4. Biotransformation products produced by knockout mutant 2-3-52 or wild type NC NRRL (2008) cultured in BHI medium.
Lanes 1, 7, and 13: Fatty acids standard.
Lanes 2-4: mutant 2-3-52 with oleic acid (triplicate)
Lanes 5-6: mutant 2-3-52 without oleic acid (duplicate)
Lane 8-10: wild-type NC NRRL 5767 with oleic acid (triplicate).
Lanes 11-12: wild-type NC NRRL 5767 without oleic acid (duplicate).

Identification of Silylated 10-HSA by GC-MS



Summary

1. We have demonstrated that mutants 1-3-17 and 2-3-52 lack 2º-ADH activity through biotransformation experiments using two different culture media (LB and BHI) with oleic acid as the substrate.
2. The TLC analysis indicated that there is a similar yield of 10-HSA from the knockout mutants and the wild-type *NC* NRRL 5767 or clone 1-p-11.
3. The 10-HSA product was confirmed by GC-MS.
4. The detailed quantitative analysis of 10-HSA produced by these clones will be examined by GC analysis

Acknowledgements

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Selected References

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