

# **Molecular and Genetic analysis of *Desulfovibrio***

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I certify that all material in this thesis which is not my own work has been identified and  
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## ABSTRACT

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The current population explosion has resulted in an ever-increasing demand for petroleum-based fuels, consequently global fossil fuel reserves are diminishing at record pace. To provide a sustainable future for the next generation, renewable alternatives to current fuels products are required. *Desulfovibrio* spp. has been reported to microbially synthesise hydrocarbons of similar structure to that found in petroleum-based fuel products. Exploration of the hydrocarbon synthesis pathway through transcriptomic analysis highlights the genes and proteins involved. Comparative RNA-seq analysis between two homologous strains of *Desulfovibrio*; *Desulfovibrio desulfuricans* 8326 and *Desulfovibrio salexigens* 2638 provided growth characterisation and the development of a reliable RNA extraction method when cultivated in Postgate medium B. Bioinformatic analyses are currently pending to identify components accountable for hydrocarbon synthesis. Complementary C<sub>18</sub> alkane and 16S genetic analysis confirmed *D.desulfuricans* hydrocarbon synthesis but highlighted contamination of *D.salexigens* cultures resulting in false-positive alkane production. Additional transformation investigations of *D.desulfuricans* confirmed natural resistance markers. Supplementary work to generate a highly transformable strain lacking the *hsdR* gene examined two methods of gene deletion; TargeTron and Cre-lox. Neither methodology provided viable transformants. Future work in developing a 'tool box' for genetic manipulation using a highly transformable strain of *D.desulfuricans* would allow control of the hydrocarbon synthetic pathway through regulation of genes discovered in the RNA-seq analysis. This new insight would improve our knowledge and enhance the future viability of renewable microbial-derived hydrocarbons as a replacement for the current non-renewable petroleum-based fuels.

**Keywords:** *Desulfovibrio desulfuricans* 8326, *Desulfovibrio salexigens* 2638, comparative transcriptomics, C<sub>18</sub> alkanes, Postgate medium B, 16S, RNA, transformation, TargeTron, Cre-lox, *hsdR*.

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## TABLE OF CONTENTS

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Abstract.....	II
Acknowledgements.....	III
Index of Figures .....	VII
Index of Appendixes .....	VIII
Abbreviations .....	VIII
Chapter 1 Introduction .....	1
1.1: Economic and Political Drivers behind improving Biofuel Production .....	1
1.2: Biofuels Overview.....	2
1.3: First Generation Biofuels .....	2
1.4: Second Generation Biofuels.....	3
1.5: Advanced biofuels .....	5
1.6: Metabolic Engineering .....	6
1.7: <i>Desulfovibrio</i> species.....	10
1.8: Hypothesis and scope of project .....	13
1.9: Experimental Plan .....	14
Chapter 2 Materials and Methods .....	15
2.1: Strains, Media and Culturing Methods .....	15
2.2 Growth Analysis.....	16
2.3 Comparative Transcriptome Analysis.....	16
2.3.1: RNA extraction and purification .....	16
2.3.2: RNA Analysis.....	17
2.3.3: RNA-seq method .....	17
2.3.4: RNA transcriptomic analysis.....	17
2.4: Alkane Analysis .....	17
2.4.1: Sample Preparation.....	17

2.4.2: GC/MS .....	17
2.4.3: GC/GC .....	18
2.4.4: 16S gene Sequencing .....	18
2.5: Transformation .....	18
2.5.1: Antibiotic resistance test.....	18
2.5.2: TargeTron Plasmid construction.....	19
2.5.3: Cre-lox $\Delta$ hsdR DNA preparation .....	19
2.5.3: Electroporation .....	20
Chapter 3: <i>D.desulfuricans</i> and <i>D.salexigens</i> growth characterisation and optimisation.....	21
3.1: Optimisation of growth analysis.....	21
3.2: Characterisation of <i>D.desulfuricans</i> and <i>D.salexigens</i> growth ....	25
Chapter 4 Effects of Media On C18 Alkane synthesis in <i>Desulfovibrio</i> .....	27
4.1: <i>Desulfovibrio</i> Genus Screen:.....	27
4.2: <i>D.salexigens</i> metal toxicity medium analysis .....	31
4.3: Affect of $\text{KH}_2\text{PO}_4$ and Ascorbic acid on alkane synthesis in <i>D.desulfuricans</i> and <i>D.salexigens</i> .....	33
4.4: Analysis of bacterial culture integrity .....	34
Chapter 5 RNA Extraction and Comparative Transcriptome Analysis .....	35
5.1: RNA Extraction Optimisation .....	35
5.2: Comparative Transcriptome Analysis.....	35
Chapter 6 Generating a <i>D.desulfuricans</i> Strain with High Transformation Efficiency	36
6.1: Antibiotic Sensitivity Test.....	36
6.2: <i>hsdR</i> Nucleotide sequence .....	39
6.3: TargeTron Transformation.....	39
6.4: Cre-lox Transformation.....	39
Chapter 7 Discussion.....	40
7.1: Growth Analysis and Characterisation of <i>D.desulfuricans</i> and <i>D.salexigens</i> .....	40

7.2: C <sub>18</sub> Alkane Synthesis in <i>Desulfovbrrio</i> .....	41
7.3: Comparative Transcriptome Analysis.....	42
7.4: Generating a <i>D.desulfuricans</i> strain with high transformation efficiency.....	43
Chapter 8 Conclusion .....	45
References .....	46
Appendices .....	51

## INDEX OF FIGURES

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Figure: 1.1.....	7
Figure: 1.2.....	9
Figure: 1.3.....	12
Figure: 1.4.....	13
Figure: 3.1.....	22
Figure: 3.2.....	23
Figure: 3.3.....	24
Figure: 3.4.....	26
Figure: 4.1.....	28
Figure: 4.2.....	29
Figure: 4.3.....	30
Figure: 4.4.....	31
Figure: 6.1.....	37
Figure: 6.2.....	37
Figure: 6.3.....	38
Figure: 6.4.....	38

## INDEX OF APPENDIXES

---

Appendix I.....	51
Appendix II.....	53
Appendix III.....	55
Section I: <i>Desulfovibrio</i> Genus Screen.....	55
Section II: <i>D. salexigens</i> metal toxicity medium analysis .....	56
Section III: <i>D. salexigens</i> metal toxicity medium analysis .....	61
Appendix IV .....	69
Appendix V .....	74
Appendix VI .....	77

## ABBREVIATIONS

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GHG	Greenhouse gas
PGB	Postgate Medium B
MT	Metal Toxicity
<i>D. desulfuricans</i>	<i>Desulfovibrio desulfuricans</i> 8326
<i>D. salexigens</i>	<i>Desulfovibrio salexigens</i> 2638
<i>D. desulfuricans</i> G20	<i>Desulfovibrio desulfuricans</i> G20
Abs <sub>600</sub>	Absorbance at 600 nm
GC/MS	Gas chromatography- mass spectrometry
GC/GC	Two dimensional gas chromatography
H <sup>2</sup>	Deuterium
D <sub>2</sub> O	Deuterium oxide (heavy water)
m/z	Mass to charge ratio