

LIST OF FIGURES

FIGURE 1. Life cycle of <i>Leishmania</i> spp.	3
FIGURE 2. Purification scheme of amastigote isolation from infected macrophages.	8
FIGURE 3: Oxidation of polyunsaturated fatty acids.	15
FIGURE 4: Correlation between the status of free fatty acids and cholesterol derivatives and transcription in the example of a macrophage.	17
FIGURE 5: Sterol regulatory binding protein (SREBP) and cholesterol regulation of gene transcription.	18
FIGURE 6. Proteome separated by 2-DE of <i>L. mexicana</i> ::DsRed amastigotes	47
FIGURE 7. Comparison of 2-DE-separated proteome of <i>L. mexicana</i> ::DsRed pro- and amastigotes.	48
FIGURE 8. Complementary protein sets identified by MALDI MS/MS and nano-LC ESI-MS/MS.	49
FIGURE 9. Amastigotes contain more proteins with basic pI.	50
FIGURE 10. Codon bias in abundantly expressed ORFs.	51
FIGURE 11. Pie charts of predicted subcellular localization of <i>L. major</i> reference genome ORFs (left) and ORFs corresponding to identified proteins from this study (right).	53
Figure 12. Predicted functional classification of <i>L. major</i> reference genome ORFs (left) and ORFs corresponding to proteins identified in <i>L. mexicana</i> promastigotes (middle) and amastigotes (right).	55
FIGURE 13. Comparison of identified proteins with two published datasets.	61
FIGURE 14. Distribution of identified proteins containing one or more predicted trans-membrane helices (TM).	62
FIGURE 15. Representation of predicted functional classification.	63
FIGURE 17. Comparative analysis of gluconeogenic and glycolytic pathways.	67
FIGURE 18. Screen shot of proteome-to-genome visualization tool.	68
FIGURE 19. Comparative analysis of predicted secreted proteins detected in three proteomic studies.	70
Figure 20. ClustalW protein sequence alignment of putative <i>Leishmania</i> DECR.	76
FIGURE 21. Overlay of <i>E. coli</i> FADH structure and the modelled DECR of <i>L. mexicana</i>	78
FIGURE 22. Model of <i>L. mexicana</i> DECR with differences to <i>E. coli</i> FADH structure highlighted.	79
FIGURE 23. Active site of <i>L. mexicana</i> DECR model.	80
Figure 24. Expression vector pET28a carrying <i>decr</i> open reading frame.	81
FIGURE 25. Complementation assay of leishmanial DECR in 2,4 Dienoyl-CoA reductase deficient <i>S. cerevisiae</i> cells.	83
FIGURE 26. Southern blot analysis of <i>decr</i> locus of <i>L. mexicana</i>	84
FIGURE 27. Cloning strategy of vectors for targeted gene replacement of <i>decr</i> in <i>L. major</i> and <i>L. mexicana</i>	86
FIGURE 28. Gene replacement strategy and <i>decr</i> locus of <i>L. major</i> (top) and <i>L. mexicana</i> (bottom).	90
FIGURE 29. Southern blot analysis of <i>L. major</i> 173 wt parasites as well as parasites with single <i>decr</i> gene replacement and with double <i>decr</i> allele replacement.	92
FIGURE 30. Southern blot analysis of <i>L. mexicana</i> wt parasites as well as parasites with single and double <i>decr</i> allele replacement.	94
FIGURE 31. Growth curve of <i>L. major</i> 173 wt and of <i>decr</i> null mutants clones.	95
Figure 32. Infection of BALB/c mice with <i>L. major</i> 173 wt or <i>L. major</i> 173 <i>decr</i> deficient mutant promastigotes.	97
FIGURE 33. Change of mRNA levels in macrophages upon <i>L. mexicana</i> amastigotes infection.	101

Figure 34. Protein amount of fatty acid binding protein 4, 5 and 7 in with <i>L. mexicana</i> amastigotes infected and uninfected bmdm.....	104
FIGURE 35. Distribution of free cholesterol in uninfected and <i>L. mexicana</i> ::DsRed infected MΦ.....	106
FIGURE 36. Flow chart indicating origin of aliquots in anlysis of the fate of cholesterol in <i>L. mexicana</i> amastigote infected bmdm compared to beads and non infected bmdms.	107
FIGURE 37. Model of leishmanial DECR highlighting substrate binding site.....	128
FIGURE 38. Altered lipid homeostasis and immune function of <i>Leishmania</i> infected MΦ.....	137

LIST OF TABLES

TABLE 1: Species of Leishmania that cause human disease	4
TABLE 2. List of primers used for sequencing and cloning of <i>L. major</i> and <i>L. mexicana</i> DECR, respective 5' and 3' flanking regions and selection markers used to generate replacement constructs	30
TABLE 3. Primers used in qRT-PCR	31
TABLE 4. Pipetting scheme SDS-mini-gels	32
TABLE 5. Proteins identified solely in amastigotes	57
TABLE 6. Sequence motifs in 3'-UTR characteristic of differentially expressed ORFs	59
TABLE 7. The 20 most abundant hypothetical proteins putatively secreted.	71
TABLE 8: NCBI-Blast result of nucleotide sequence of <i>L. mexicana</i> 2,4 Dienoyl-CoA Reductase	73
TABLE 9: NCBI-Blast result of protein sequence of <i>L. mexicana</i> 2,4 Dienoyl-CoA Reductase	73
TABLE 10. Results of homologous recombination of selection marker genes to replace decr in <i>Leishmania</i>	89
TABLE 11. Result of homologous recombination to replace second decr allele	89
TABLE 12. Uptake and retention of cholesterol in uninfected bmdm and amastigotes or beads exposed bmdm	108

ACKNOWLEDGEMENTS

I want to thank Toni Aebischer for giving me the opportunity to do this diverse PhD project, and especially as I was able to do the project in Edinburgh. And thanks to the rest of the Leishmaniacs, it has been a good time with you guys over the years.

Thanks to all people who have been in the ever changing office 2.54. I've seen many people come and go, that was not because I took my time.

Further I want to thank the Matthews lab, where I could always come and ask for things and I almost always left with what I wanted. I'd especially like to thank Paula from the Matthews lab for helping me with some of the Stats.

I also want to thank Stefan, who was a great inspiration on the model of DECR and for all the other helpful hints and advice he gave me.

Thanks to the rest of 3IR for giving me a fun and pleasurable time. And not forgetting Bette, who made life so much easier in the lab: "Do you need anything Daniel....".

Thank you to my parents and all the rest of my family who supported me emotionally and also financially when I still went to University. I surely wouldn't be here without you.

My warmest thanks to Cat, who read and corrected this thesis more than one time and without her I probably would not be at this stage yet. She kept me alive by making sure I'm eating enough and pushed me to keep going with the writing.