



COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR
AND CELLULAR BIOLOGY

Announcement: All interested members of the university community are invited to attend the Final Oral Examination for the degree of **Master of Science** of

STEPHANIE GILBERT, on Wed. December 13, 2017 at 9:30 a.m. in SSC 3317

(Advisor: Dr. S. Seah)

Thesis Title: Characterization of an aldolase-hydratase complex found within the cholesterol degradation pathway of *Mycobacterium tuberculosis*.

Examination Committee:

Dr. J. Vessey, Dept. of Molecular and Cellular Biology (Chair)

Dr. S. Seah, Dept. of Molecular and Cellular Biology

Dr. M. Kimber, Dept. of Molecular and Cellular Biology

Dr. G. Cox, Dept. of Molecular and Cellular Biology

Abstract: The heteromeric acyl-CoA dehydrogenase, FadE28-FadE29 and the enoyl CoA hydratase ChsH1-ChsH2, respectively, catalyze the dehydrogenation and subsequent hydration of the 3-carbon side chain cholesterol metabolite to form 17-hydroxy-3-oxo-4-pregnene-20-carboxyl-CoA (17-HOPC-CoA) in *M. tuberculosis*. The gene downstream of *chsH2*, *ltp2*, was introduced into *Rhodococcus jostii* RHA1 and the recombinantly produced His-tagged Ltp2 co-purified with untagged ChsH1-ChsH2, ChsH2, or the C-terminal domain of ChsH2 that contains a domain of unknown function (DUF35). Ltp2-ChsH1-ChsH2 or Ltp2-DUF35 complexes catalyzed the retro-aldol cleavage of 17-HOPC-CoA to form androst-4-ene-3,17-dione and propionyl-CoA. Ltp2-DUF35 complex has optimal activity at pH 7.5 with K_m of 6.54 ± 0.90 mM and k_{cat} of 159 ± 8.50 s⁻¹. ChsH1-ChsH2 alone can only hydrate about 30% of its substrate, but its association with Ltp2 enables complete hydration. Conditions for crystallizing Ltp2-DUF35 were formulated which may facilitate future structure determination by X-ray crystallography.

Curriculum Vitae: Stephanie obtained her B.Sc. (Hons.) in Molecular Biology and Genetics at the University of Guelph in the spring of 2015 and began her M.Sc. graduate studies in the lab of Dr. Stephen Seah in September of the same year.

Publications: Gilbert, S.E., Hood, L., and Seah, S.Y.K. (2017). Characterization of an aldolase involved in cholesterol side chain degradation in *Mycobacterium tuberculosis*. *J. Bacteriol.* (Accepted 26th October 2017). DOI: 10.1128/JB.00512-00517.