Department of Molecular and Cellular Biology

Graduate Seminar MCB*6500

Friday, Jan. 20, 2017 in SSC 1511 @ 12 noon

presented by:

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Characterization of hydratases in the bile acid degradation pathways of *Comamonas testosteroni* KF1 and *Rhodococcus jostii* RHA1

Bile acids, such as cholate, are water soluble, surface active steroid compounds that aid in the emulsification and absorption of dietary fats in mammals. Only bacteria are able to breakdown and utilize steroids as sole carbon and energy sources for growth. The 5-carbon steroid side chains of bile acids are thought to be degraded in a process mirroring the β-oxidation of fatty acids and involve reactions catalyzed by acyl-CoA dehydrogenases, enoyl-CoA hydratases, hydroxyacyl-CoA dehydrogenases and thiolases. The two rounds of β-oxidation required for complete catabolism of the bile acid side chain will yield the central metabolites acetyl-CoA and propionyl-CoA. In the Gram negative bacteria, *Comamonas testosteroni* KF1, a multi-functional enzyme complex, FadIJ, is encoded within the steroid degradation gene cluster. The hydratase domain of FadJ shares homology to hydratases from the crotonase family of enzymes. The Gram positive bacteria *Rhodococcus jostii* RHA1 on the other hand contains two genes, *casD* and *casQ*, located in the cholate degradation gene cluster and sharing homology to the MaoC family of hot-dog fold hydratases. We hypothesize that these hydratases are responsible for hydrating the 5-carbon side chain of bile acid metabolites during the first cycle of β-oxidation. To test this hypothesis, FadJ, CasD and CasQ will be separately overexpressed, purified and characterized to confirm their ability to hydrate bile acid side chains. Genes encoding each of these enzymes will also be deleted in their respective hosts and the resultant knockout strains will be tested for growth in media containing bile acids as a sole carbon and energy source. Culture supernatants will be analyzed by GC-MS to confirm the accumulation of a 5-carbon side chain bile acid metabolite in these knockout strains.