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Structure and Function of Proteins Important in Mycobacterium tuberculosis Energy Metabolism

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB). A unique feature of Mtb is that it can remain dormant within the human host for years (persistence), and can survive in hypoxic and nutrient-depleted media [1]. Coenzyme F420, a flavin analogue has been hypothesized to be associated with Mtb viability in anaerobic conditions and in persistence [2]. This hydride carrier also acts as a redox sensor in Mtb by converting NO2 to NO released by Mtb-infected macrophages under aerobic condition [3]. At least three genes are involved in the biosynthesis of F420; F420 biosynthesis A, B, and C (fbiA, fbiB and fbiC). This PhD project will explore F420 biosynthesis using biophysical techniques. The fbiA and fbiB genes were cloned in a pET-Duet vector to test for protein interaction. While co-expression was unsuccessful, single expression of the genes produced soluble protein. FbiB has been purified and crystallized as small needles. Purification of a GST-tagged construct to eliminate proteolytic degradation and further fine screening is ongoing to obtain better quality crystals for X-ray diffraction. FbiC cloning into Gateway vectors is ongoing. Further biochemical and biophysical tests hopefully obtained in the near future will assist in our understanding of this unique coenzyme.

References:

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Microbial production of food grade pigments – Screening and metabolic pathway analysis

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Until recently, the use of plant pigments as natural food colourant is expensive and uncompetitive to synthetic dyes due to their high production costs. Such disadvantages could be overcome with the development of microbial food grade pigments which are likely to cut down the high production cost, thus leading to a cheaper source of natural food colourants. The production of natural colourant by microbial fermentation has a number of advantages over plant-based systems such as higher yields within a shorter period of time and no seasonal variations. We, therefore, have screened 285 microbial strains from our New Zealand environmental microorganism collection to find new sources of natural food grade pigments. Among those, 166 strains produced pigments when growing in liquid medium including 28 extracellular pigments and 90 intracellular pigments. HPLC results showed that 33 purified pigments were water-soluble. Molecular identification of 39 selected strains using ribosomal DNA sequencing showed the presence of representatives from 19 different genera. Interestingly, a number of pigments have shown biological activity against filamentous fungi. We are currently carrying out pigment stability tests on selected microbial strains. Further to this study, metabolomics and metabolic engineering tools will be used for improvement of pigment yield of the best candidate strain with potential for industrial applications.