Characterisation of sterol and amino acid biosynthesis pathways in *Acanthamoeba* species

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Acanthamoeba is normally a free-living protist that can cause severe disease in humans, including *Acanthamoeba* keratitis, a severe sight-threatening infection of the eye and the almost universally fatal granulomatous amoebic encephalitis. More effective treatments and diagnostic tests are required. We have now characterized a number of biochemical pathways that may offer new insights and encouraging new drug targets against *Acanthamoeba* species, as they do not exist in the mammalian host. These include sterol and amino acid biosynthesis pathways. All these pathways possess conserved enzymes that are also found in fungi and/or plants, but some *Acanthamoeba* pathways differ in their genetic structure. For example, we confirm that *A. castellanii* and *A. polyphaga* have a unique heptafunctional polypeptide encoding seven (7) enzymes of histidine biosynthesis, and that *A. castellanii* has has a novel complement of shikimate pathway enzymes including unique gene fusions, two Type I and one Type II DAHP synthases (for which their likely sensitivities to feedback inhibition by phenylalanine, tyrosine and tryptophan has been modelled) and a canonical chorismate synthase. In sterol biosynthesis, we have demonstrated that the major sterol of *Acanthamoeba castellanii* is ergosterol and identified novel putative precursors and intermediate sterols in its production. Unlike previously reported, we found no evidence of 7-dehydrostigmasterol or any other phytosterol in *Acanthamoeba*.