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D.49- Heterologous expression in E. coli and purification of the Leishmania major NADP+-dependent Glutamate Dehydrogenase (NADP+-LmGDH)

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Leishmania has a complex life cycle, alternating between the insect vector and the mammalian vertebrate host. During the life cycle, the parasite passes through different stages, such as promastigote and amastigotes. These different forms of the parasite must adapt through the changes of the environment inside the vector and the host, being able to utilize the available amino acids as the main carbon and energy source. Glutamate Dehydrogenases (GDHs) are essential enzymes for the metabolism of amino nitrogen in most organisms, from bacteria to mammals. There are three types of GDH: those that are cofactor specific for NAD(H) (EC 1.4.1.2), those that are specific for NADP(H) (EC 1.4.1.4) and those which can use both cofactors (EC 1.4.1.3). In bacteria, some protozoa, fungi and plants are present the NAD(H) and NADP(H) GDH, being the former responsible for the biosynthesis and the latter for the catabolic role. The objective of this project was to produce the LmGDH in E. coli to enable structural and functional characterization. The region containing the GDH coding sequence (TritrypDB: LmjF.28.2910) was amplified from L. major genomic DNA by PCR and cloned into pET28a expression vector. The pET28aGDH construct was used for protein expression in E. coli BL21(DE3) and the recombinant LmGDH (rLmGDH) containing a His6-tag was expressed as soluble form and subsequently purified from the cell lysate by affinity chromatography using a Ni-NTA resin. The secondary and tertiary structure of rLmGDH was evaluated by circular dichroism and intrinsic tryptophan fluorescence spectroscopy respectively. The activity of rLmGDH was evaluated using the consumption of NADPH during the reductive amination of a-ketoglutarate and NADPH formation through the oxidative deamination of glutamate. The purification yield showed that 11,54% of total reductive amination activity (382,8U) was retained by the Ni-NTA column, yielding a final activity purification of 35,6 fold. No activity was detected when using NAD+, demonstrating that rLmGDH (LmjF.28.2910) has a predominant NADP+-dependent activity in vitro. Keywords: Enzyme, Glutamate Dehydrogenase, Leishmania

D.50- Engineering of an Aromatic Polyester Degrading Enzyme.

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Polyethylene terephthalate (PET), a widely utilized polymer, causing significant environmental challenges due to its prevalent use and rapid disposal. Microbial biorecycling has emerged as a potential solution, particularly after discovering a PET-degrading bacterium, Ideonella sakaiensis. This discovery has led to identifying enzymes capable of breaking down PET polymers, such as PETase and MHETase. Conduct site-specific mutations in a thermophilic aromatic polyesterase (PETase) from Hidrogenobacter thermophilus and improve its catalytic capacity. Site-specific mutations were introduced using the KLD Enzyme kit (NEB) and transformed into Escherichia coli DH5a. Upon sequencing confirmation, enzyme expression, purification, and a series of biochemical and biophysical will be conducted. We conducted molecular dynamics analyses and explored the effect of different mutations. Four mutant versions of enzymes were designed. Site-specific mutations have been carried out. The findings of this study aim to advance the understanding of thermophilic PET-degrading enzymes, thereby encouraging initiatives towards economically viable applications of this technology.
Keywords: Biodegradation, Recombinant enzyme, Site-specific mutation