IMPROVING 1,3-BUTANEDIOL PRODUCTION IN *E. COLI* USING A PROTEIN ENGINEERING APPROACH

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Traditional chemical production processes have high yields but require harsh reaction conditions and use non-renewable feedstocks derived from petroleum [1, 2]. These processes have a negative impact on the environment, which motivates the development of more sustainable processes as replacements [2]. Advances in systems metabolic engineering over the past thirty years have given rise to bioprocesses where engineered microbes make chemicals from natural feedstocks under mild reaction conditions [1]. The promise of the field has also resulted in financial resources being made available to the development and commercialization of bioprocess. According to a recent report by Ontario Genomics [3], global investment in the field is projected to be at $38.7B in 2020, a 12-fold increase from what it was at in 2013.

Recently, a novel aldolase-based pathway for producing 1,3-butanediol (BDO) in *E. coli* was reported by Nemr et. al [4, 5]. 1,3-BDO is a commercially viable product as it is used in formulations in cosmetics products, and as a precursor for pharmaceuticals [2]. This pathway involves the conversion of pyruvate to acetaldehyde via the EutE enzyme from *E. coli*, followed by the conversion of acetaldehyde to 3- hydroxybutanal via the enzyme BH1352 – a Deoxyribose-phosphate aldolase (DERA) – from *Bacillus halodurans* and subsequently by the conversion of 3-hydroxybutanal to 1,3-BDO via the enzyme PA1127 (an aldo-keto reductase) from *Pseudomonas aeruginosa* [5].

We examined the crystal structure of BH1352, which revealed key residues involved in catalytic activity in the substrate binding pocket. We show that two DERA mutants F160Y and F160Y/M173I improve the production of 1,3-BDO 5-fold and 6-fold respectively in bench-scale bioreactors [6].

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