The substrate specificity & cofactor preference of different Flavin-Containing Monooxygenases

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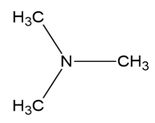
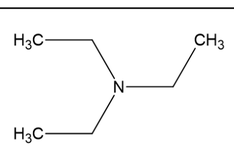
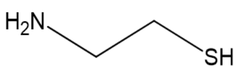
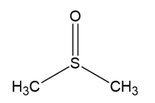
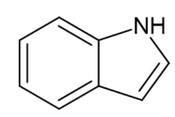
Introduction

After fish has been filleted and processed, there are fish heads, organs and skin left as by-products. Said by-products can be processed with enzymes into a highly nutritional fish protein hydrolysate, offering a new source of food (Kristinsson et al., 2000 & Olsen et al, 2014). Unfortunately, bitter taste and unpleasant odor from trimethylamine (TMA) makes it unfit for human consumption (Hebard et al, 1982). One solution to the issues is using flavin-containing monooxygenase (FMO) to convert TMA into the odorless trimethylamine N-oxide (TMAO) (Goris et al, 2020). A thermostable variant of FMO called mFMO_20/T0110 has been made for industrial use, but it uses nicotinamide adenine dinucleotide phosphate (NADPH) which is expensive as a co-factor (Goris et al, 2023). My aim was to find a variant of FMO that uses the cheaper nicotinamide adenine dinucleotide (NADH) as co-factor. Possible candidates were discovered in a previous master project, and now I want to verify the results and find other potential uses for FMOs (Vargas, 2023). T0110, which is known to work with TMA & NADPH, was used as positive control and benchmark.

Methods:

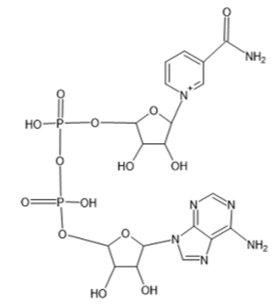
Escherichia coli bacteria were transformed with plasmids encoding polyhistidine-tagged enzymes of interest. The bacteria were induced to express the enzymes with arabinose in the mid-log phase (OD ~ 0.6). The bacteria were harvested, and the enzymes were purified by affinity chromatography. Purity was analyzed using SDS-PAGE. The protein concentration was found using a standard curve based on BSA and analysed on wavelength 660 nm.

Enzyme activity was assessed by the consumption of co-factor (NADH/NADPH), measured by the decrease in absorbance at wavelenght 340 nm. All the reactions had a cofactor concentration on 250 µM. Since the exchange between cofactor and product is 1:1, the product formation can be infered from change in cofactor concentration.

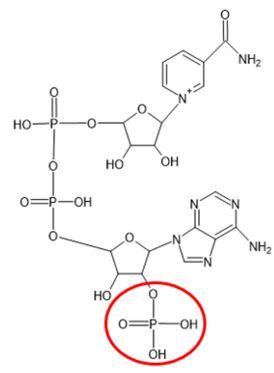
Substrate	Abbreviation	Structure
Trimethylamine	TMA	
Triethylamine	TEA	
Cysteamine	CSA	
Dimethyl sulfoxide	DMSO	
Indole	-	

Cofactors

Nicotinamide adenine dinucleotide (NADH)

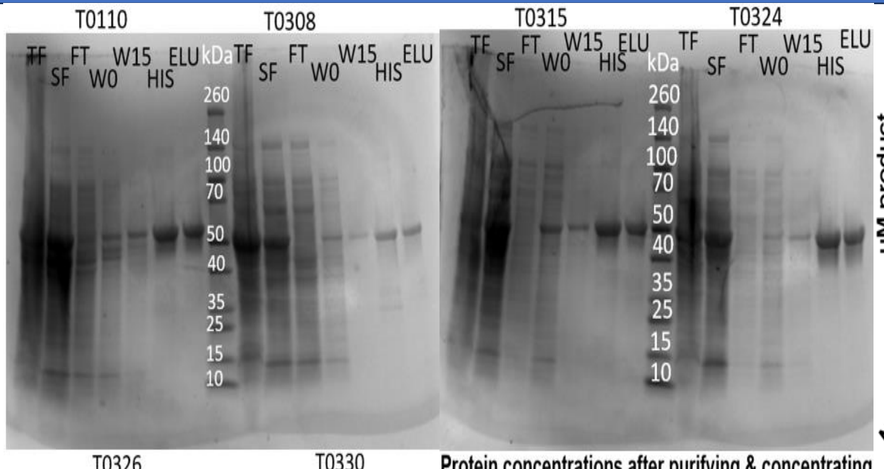


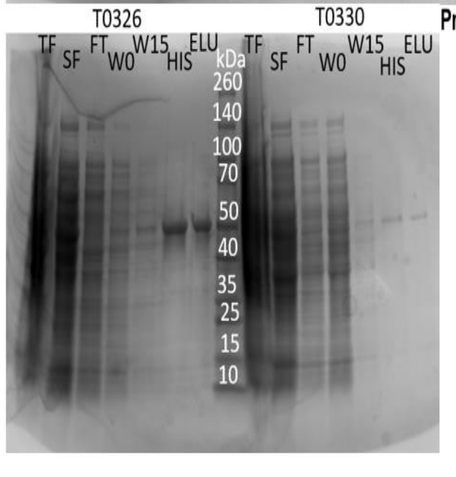
Nicotinamide adenine dinucleotide phosphate (NADPH)



Enzyme	Species	Preferred cofactor	Yield [µM]	Purity
T0110	*Methylophaga aminisulfidivorans*	NADPH	58.9	High
T0308	*Adineta steineri*	NADPH	32.6	Medium
T0315	*Rotaria sp*	NADH	26.4	High
T0324	*Stylophora pistillata*	NADH	29.8	High
T0326	*Macrostomum lignano*	NADH	19.4	High
T0330	*Hypsibius dujardini*	NADPH	0.5	Low

Results:





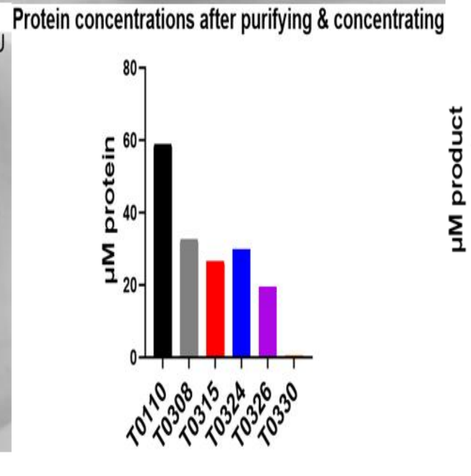


Figure 1: Purity and quantitative control of enzymes.

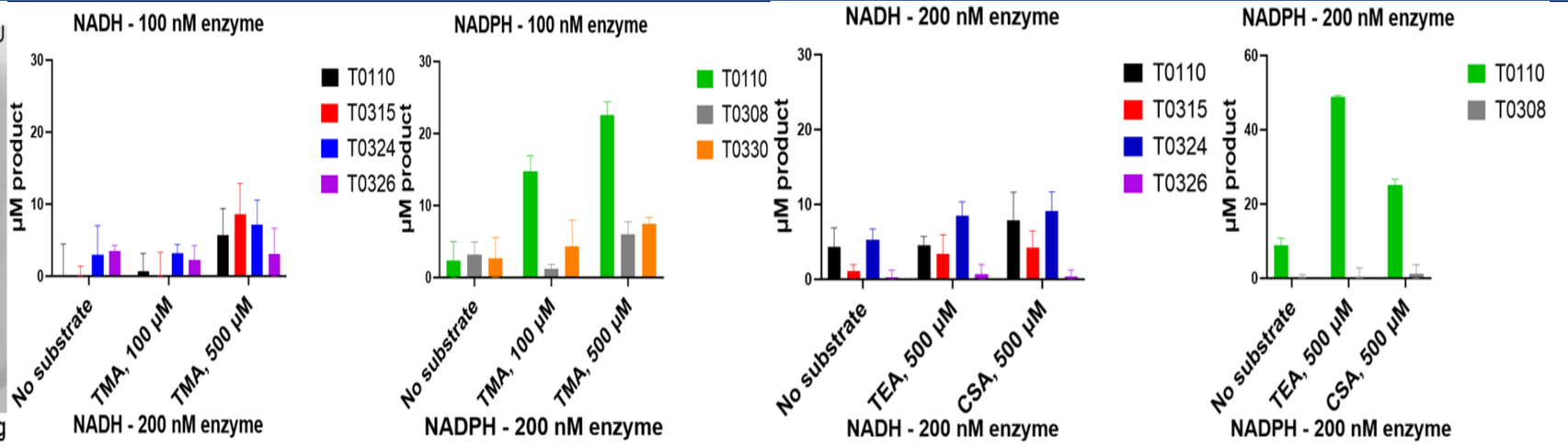


Figure 2: Activity of enzymes with TMA as substrate.

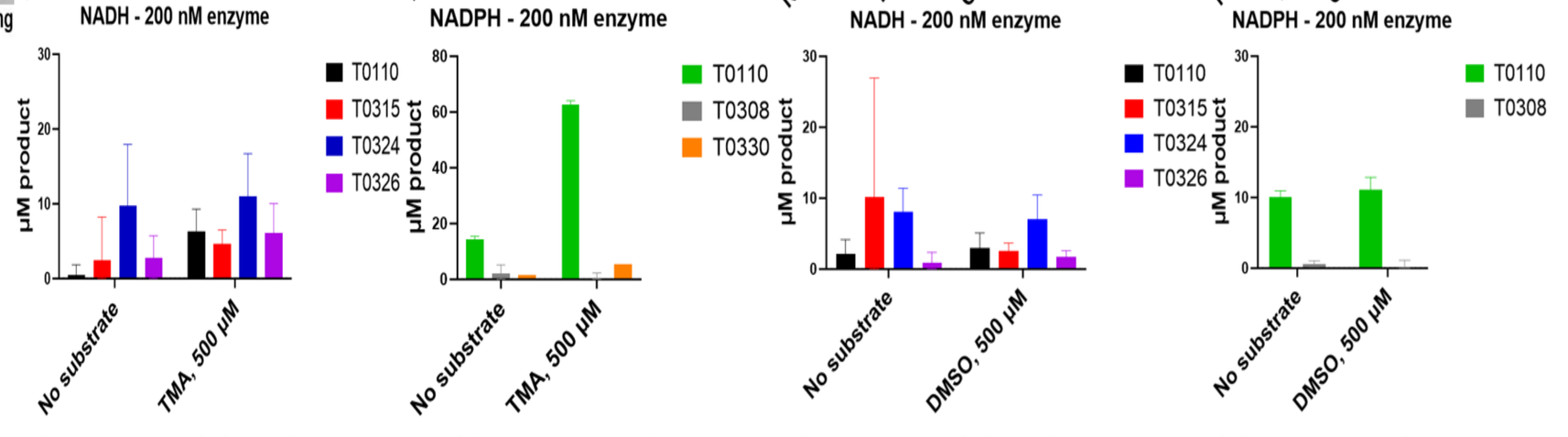


Figure 3: Activity of enzymes with TEA, CSA & DMSO as substrates.

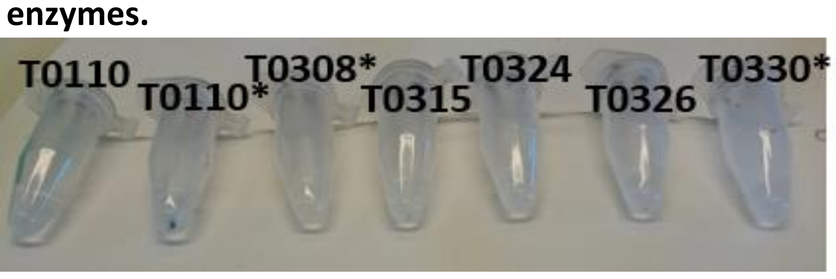


Figure 4: Activity of enzymes with indole as substrate. Indole is turned in the reaction into indigo, which is blue. The reaction had an enzyme concentration on 100 nM and an indole concentration on 2.4 mM. The enzymes marked with * had NADPH as co-factor, while the rest had NADH.The image was taken approx. 25 hours after start of reaction, and the samples had been centrifuged.

Conclusion:

- T0110 was quite easy to purify and get a relatively large yield, while T0330 was hard to purify and gave close to no yield at all. The rest of the enzymes gave a moderate yield.
- Regarding TMA, T0110 with NADPH had the strongest activity. T0308 & T0330 had overall little activity with TMA. T0110 could work with NADH, but it was less effective. T0315, T0324 & T0326 effectivity depended on enzyme concentration.
- T0110 with NADPH had a strong activity with TEA and less strong one with CSA. T0308 & T0326 had no effect on TEA and CSA. T0315 & T0324 had some effect on TEA and CSA. T0110 with NADH only had effect on CSA.
- All enzymes had little to no effect on DMSO.
- Only T0110 with NADPH had effect on indole.

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References:

Goris M, et al. 2023. Increased Thermostability of an Engineered Flavin-Containing Monooxygenase to Remediate Trimethylamine in Fish Protein Hydrolysates. Appl Environ Microbiol e0039023

Goris M, et al. 2020. Use of Flavin-Containing Monooxygenases for Conversion of Trimethylamine in Salmon Protein Hydrolysates. Appl Environ Microbiol 86:e02105-20.

Hebard CE, et al. 1982. Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish, p 149 –304. In Martin RE (ed), Chemistry and biochemistry of marine food products

Kristinsson HG, et al. 2000. Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 40:43– 81.

Olsen RL, et al. 2014. Challenges and realistic opportunities in the use of by-products from processing of fish and shellfish. Trends Food Sci Technol 36:144 –151.

Vargas TP. 2023. Bioprospecting and Characterization of Novel Eukaryotic Flavin-Containing Monooxygenases (FMOs). Department of Biological Sciences, Faculty of Mathematics and Natural Sciences, University of Bergen.

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