

Study of ALDH from *Thermus thermophilus* – expression, purification and characterisation of the non-substrate specific, thermophilic enzyme displaying both dehydrogenase and esterase activity

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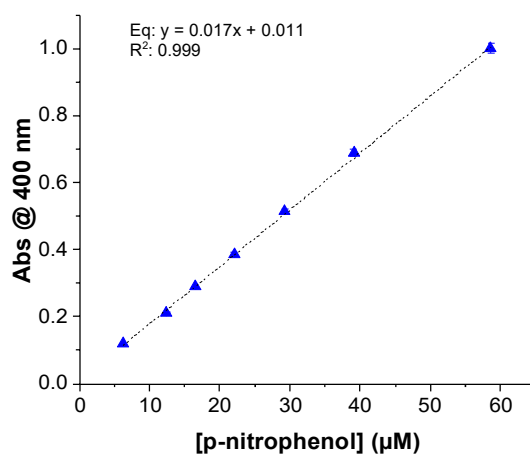


Figure S1: Calibration curve of p-nitrophenol in 10 mM potassium phosphate pH 8, 0.1 % acetone.

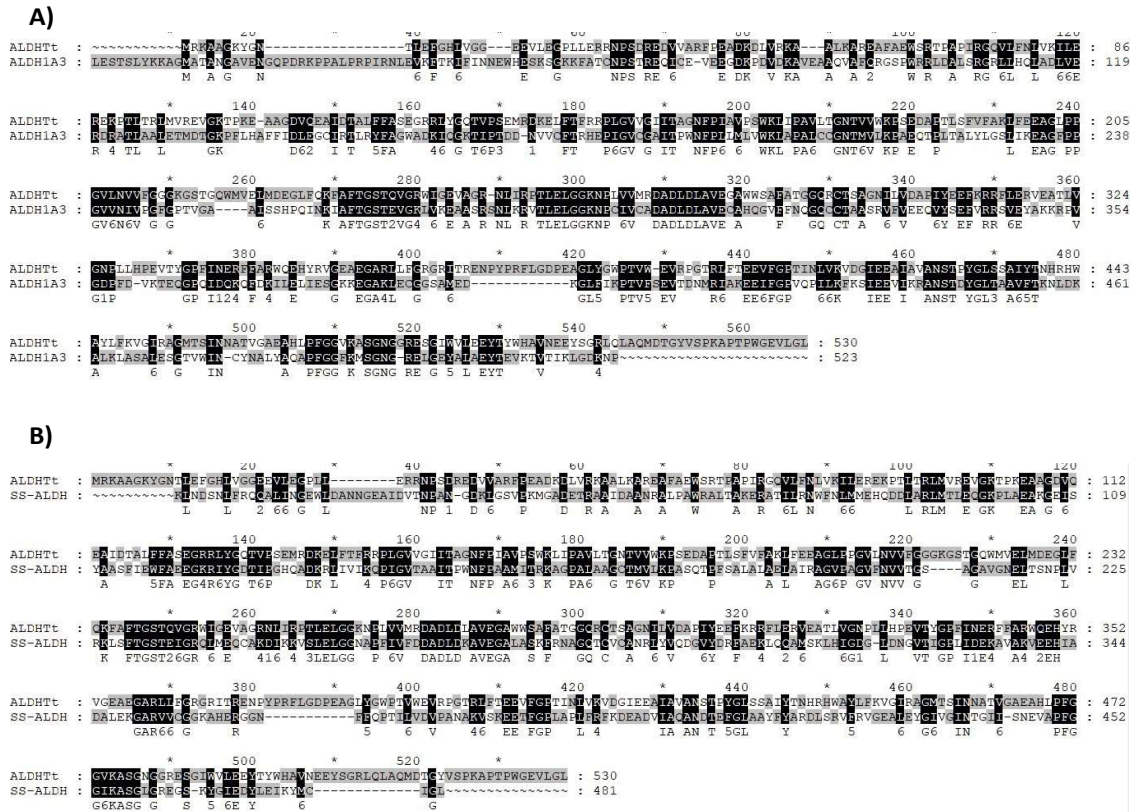
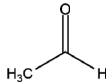
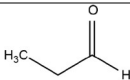
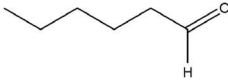
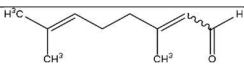
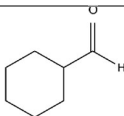
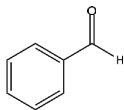
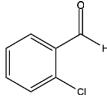
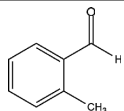
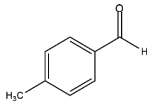
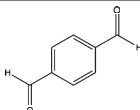
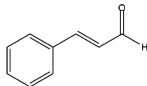
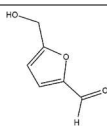


Figure S2: Protein sequence alignment of ALDH_{Tt} (PDB: 6FJX) and A) ALDH1A3 (PDB: 5FHZ) and B) succinate semialdehyde dehydrogenase (SS-ALDH) from *E. coli* (PDB: 3JZ4).

Table S1: Aldehyde substrates used for catalysis by ALDH_{TI} highlighting their associated specific activity.

Chemical Structure	Substrate	Specific activity (U/mg)	
		25 °C	50 °C
	Acetaldehyde	0.006 ± 0.0003	0.098 ± 0.009
	Propanal	0.01 ± 0.002	0.40 ± 0.01
	Hexanal	0.09 ± 0.002	1.08 ± 0.03
	Citral	-	-
	Cyclohexanecarboxaldehyde	0.004 ± 0.007	0.11 ± 0.008
	Benzaldehyde	0.03 ± 0.002	0.09 ± 0.008
	2-chlorobenzaldehyde	-	0.005 ± 0.004
	<i>o</i> -tolualdehyde	-	-
	<i>p</i> -tolualdehyde	0.02 ± 0.003	0.23 ± 0.08
	Terephthalaldehyde	0.09 ± 0.005	0.88 ± 0.05
	<i>trans</i> -Cinnamaldehyde	0.02 ± 0.01	0.12 ± 0.03
	5-hydroxymethyl-2-furfural	-	-

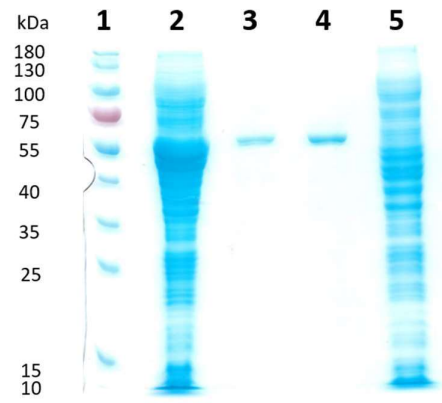


Figure S3: SDS-PAGE of ALDH_{T1} expression and purification fractions omitting the heat treatment step. Lane 1: PageRuler prestained protein ladder (ThermoFisher), lane 2: *E. coli* BL21(DE3) cell lysate expressing ALDH_{T1}, lane 3: Ni affinity chromatography 200 mM imidazole elution, lane 4: purified ALDH_{T1}, lane 5: Ni affinity chromatography flow through.

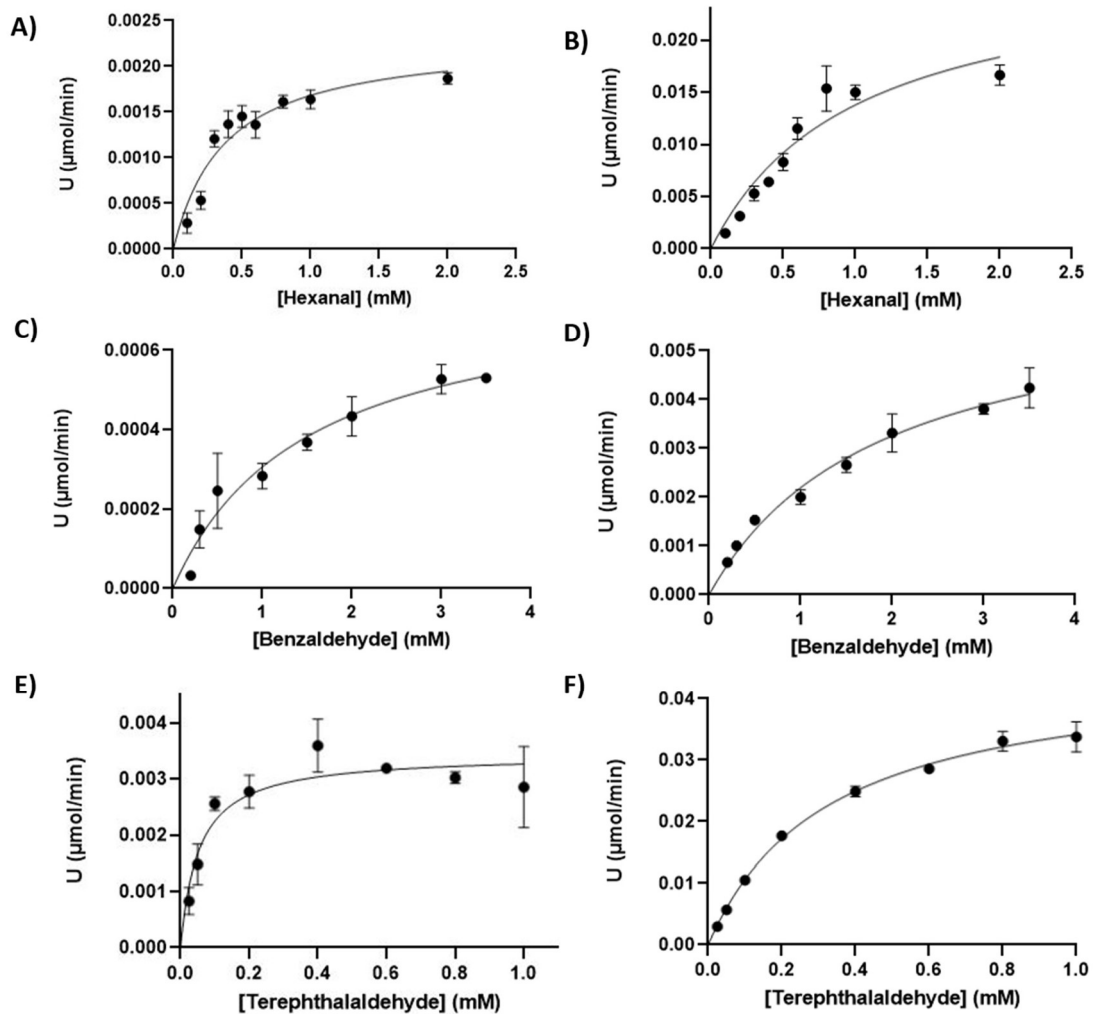


Figure S4: Michaelis-menten kinetic plots for ALDH_{T1} with different substrates, A) hexanal 25 °C, B) hexanal 50 °C C) benzaldehyde 25 °C, D) benzaldehyde 50 °C, E) terephthalaldehyde 25 °C, F) terephthalaldehyde 50 °C.

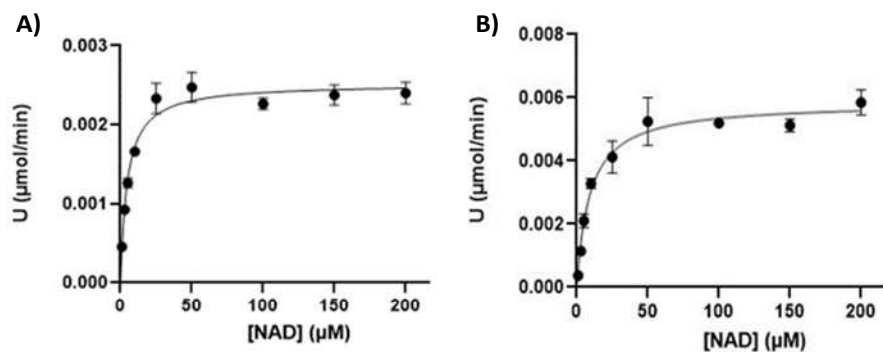


Figure S5: Michaelis-menten kinetics plot varying $[NAD^+]$ for ALDH_{T1} using hexanal at A) 25 °C and B) 50 °C.

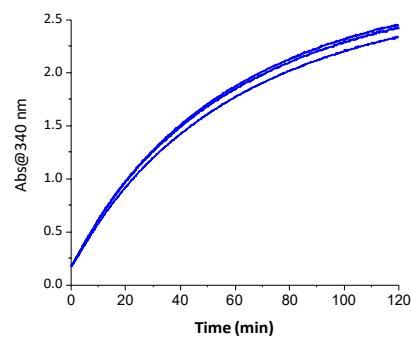


Figure S6: ALDH_{T1} reaction course over 2 h using hexanal with NADH production monitored at 340 nm.

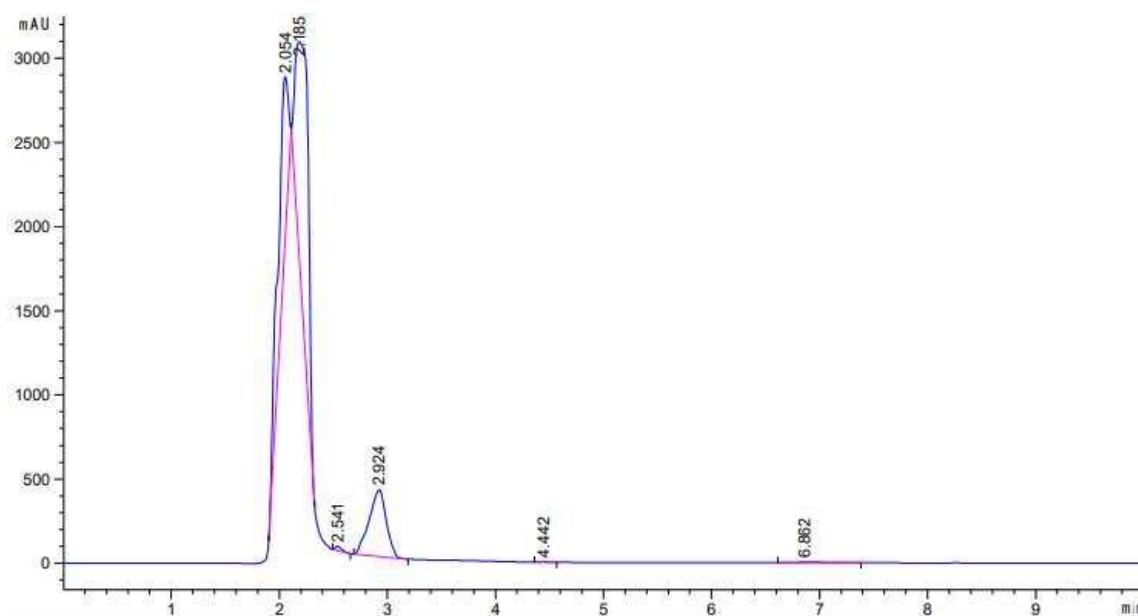
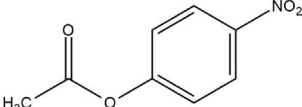
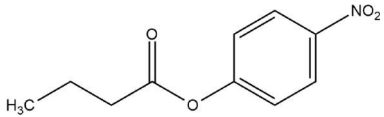


Figure S7: HPLC chromatogram for the detection of hexanoic acid from 2 h ALDH_{T1} assay with a retention time of 6.86 min.

Table S2: Esterase substrates catalysed by ALDH_{T1} highlighting their associated specific activity.

Chemical Structure	Substrate	Specific Activity (U/mg)
	<i>p</i> -nitrophenyl acetate	0.033 ± 0.006
	<i>p</i> -nitrophenyl butyrate	0.013 ± 0.0002

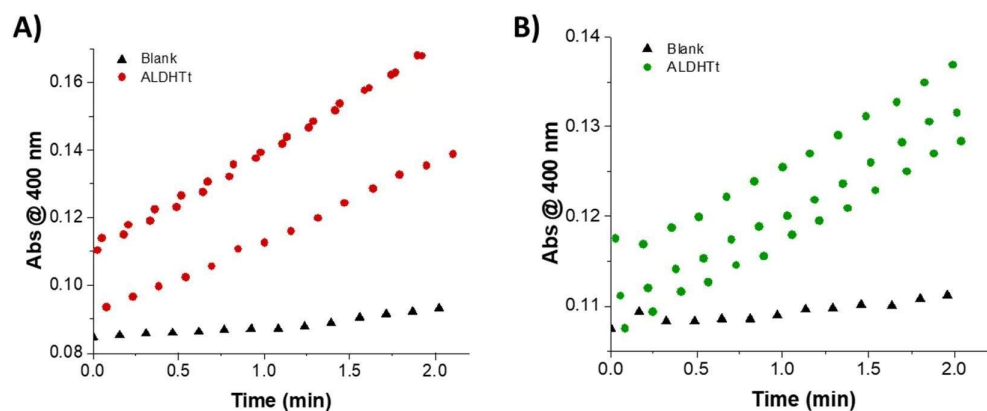


Figure S8: Absorbance vs. time for esterase activity monitored at 400 nm at 25°C using A) PNP-acetate and B) PNP-butyrate.

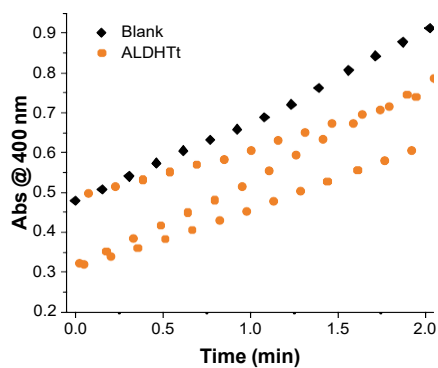


Figure S9: Absorbance vs. time monitored at 400 nm for esterase activity at 50°C using PNP-acetate.