bio-protocol

Published: May 05, 2022

Expression, Purification, and *in vitro* Enzyme Activity Assay of a Recombinant Aldehyde Dehydrogenase from *Thermus thermophilus*, Using an *Escherichia coli* Host

Kim Shortall, Edmond Magner and Tewfik Soulimane*

Department of Chemical Sciences, Bernal Institute, University of Limerick, V94 T9PX, Limerick, Ireland
*For correspondence: Tewfik.soulimane@ul.ie

Abstract

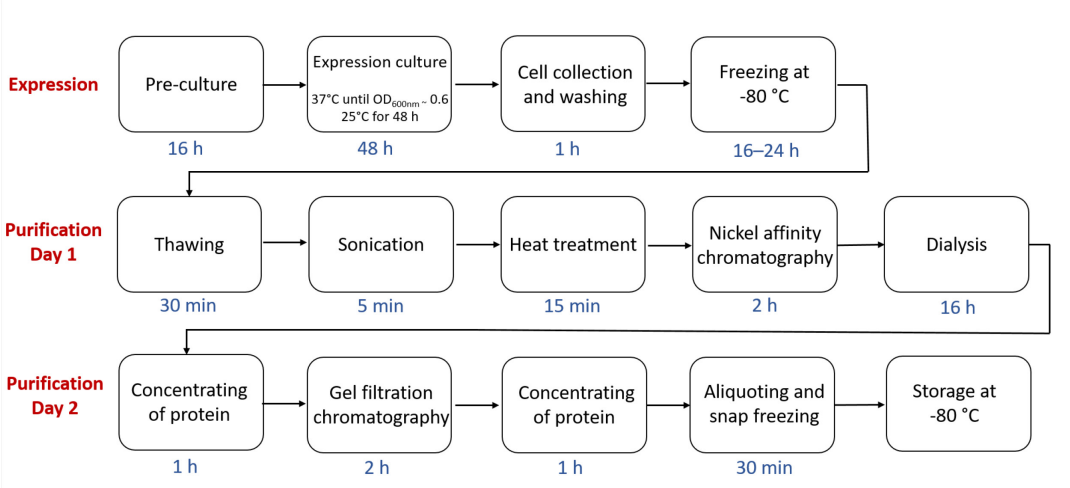
Based on previous in-depth characterisation, aldehyde dehydrogenases (ALDH) are a diverse superfamily of enzymes, in terms of both structure and function, present in all kingdoms of life. They catalyse the oxidation of an aldehyde to carboxylic acid using the cofactor nicotinamide adenine dinucleotide (phosphate) ($NAD(P)^+$), and are often not substrate-specific, but rather have a broad range of associated biological functions, including detoxification and biosynthesis. We studied the structure of $ALDH_{Tt}$ from *Thermus thermophilus*, as well as performed its biochemical characterisation. This allowed for insight into its potential substrates and biological roles.

In this protocol, we describe $ALDH_{Tt}$ heterologous expression in *E. coli*, purification, and activity assay (based on Shortall *et al*., 2021). $ALDH_{Tt}$ was first copurified as a contaminant during caa_3-type cytochrome oxidase isolation from *T. thermophilus*. This recombinant production system was employed for structural and biochemical analysis of wild-type and mutants, and proved efficient, yielding approximately 15–20 mg/L $ALDH_{Tt}$. For purification of the thermophilic his-tagged $ALDH_{Tt}$, heat treatment, immobilized metal affinity chromatography (IMAC), and gel filtration chromatography were used. The enzyme activity assay was performed via UV-Vis spectrophotometry, monitoring the production of reduced nicotinamide adenine dinucleotide (NADH).

Keywords: Aldehyde dehydrogenase, Auto-induction media, Cell culture, Nickel affinity chromatography, Gel filtration chromatography, Heat treatment purification, UV-vis spectrophotometry, Enzymatic activity

This protocol was validated in: Cells (2021), DOI: 10.3390/cells10123535

Graphical abstract:



Flow chart outlining the steps in $ALDH_{Tt}$ expression and purification, highlighting the approximate time required for each step.

Background

The aldehyde dehydrogenase $ALDH_{Tt}$ was first identified and isolated as a contaminant, during cytochrome oxidase caa_3-type native purification (Lyons *et al.*, 2012) from *Thermus thermophilus*, which led to its further investigation. One of the common roles of ALDH family members in mammals is their inherent biosynthesis of retinoic acid assisted by cytochrome oxidases, which shows the possibility of such a role for this enzyme in *T. thermophilus,* due to the close relation between these two enzymes. Native purification of enzymes from *T. thermophilus* and other bacterial sources can be cumbersome, difficult, and lengthy, resulting in low protein yields (Soulimane, 2010; Robin *et al.*, 2011). Therefore, recombinant protein production may be an attractive alternative route for the production of proteins for structural characterisation and functional analysis, allowing for high protein purity and yields. In 2018, the $ALDH_{Tt}$ was first recombinantly expressed, purified, and its crystal structure determined (Hayes *et al.*, 2018), revealing a novel C-terminal extension in the form of a tail, which contributes to active site regulation, thermostability and the oligomerization mode, aspects not before seen in the ALDH superfamily. The production protocol, employing a 48-h expression culture, heat treatment, immobilized metal affinity chromatography (IMAC) (Figure 1), and gel filtration chromatography, allows for yields of 15–20 mg of highly pure $ALDH_{Tt}$ per litre of culture (Figure 2) (Shortall *et al.*, 2021).

ALDHs are often not substrate specific, and can be characterised by their activity for the oxidation of aldehydes using the cofactor nicotinamide adenine dinucleotide (phosphate) ($NAD(P)^+$) to the corresponding carboxylic acid, and nicotinamide adenine dinucleotide (phosphate) hydrate (NAD(P)H). Like other dehydrogenase enzymes, their activity is monitored most commonly via UV-Vis spectrometry for the production of NAD(P)H at 340 nm (Figure 3). $ALDH_{Tt}$ can oxidise a range of aldehydes, including aliphatic, aromatic, and cyclics, at elevated temperatures, with the highest catalytic activity achieved with hexanal at 50°C (Shortall *et al.*, 2021).

Conditions detailed here allowed for the production of a pure, soluble, and active form of $ALDH_{Tt}$. The protocol described can also serve as a starting strategy to express and purify similar proteins.

Materials and Reagents

Note: All reagents were stored at room temperature, unless otherwise stated here.

1. Ice
2. MilliQ water

A. Protein Expression

1. 1.5 mL centrifuge tubes (Eppendorf, Sigma-Aldrich, catalog number: EP0030120086-1PAK)
2. 50 mL sterile centrifuge tubes (Corning, Sigma-Aldrich, catalog number: CLS430290)
3. 100 mm Petri dishes (Sigma-Aldrich, catalog number: P5731-500EA)
4. Cell spreader, sterile (Sigma-Aldrich, catalog number: HS8171A-500EA)
5. 2 L cell culture Erlenmeyer flasks
6. 10 mL serological pipettes, sterile (Sigma-Aldrich, catalog number: CLS4488-50EA)
7. 25 mL serological pipettes, sterile (Sigma-Aldrich, catalog number: CLS4251-200EA)
8. Aluminium foil
9. pET22b(+)-ALDH$_{Tt}$ (constructed in the lab by Hayes *et al*. (2018)), store at -20°C
10. BL21(DE3) chemically competent cells (prepared in the lab), store at -80°C
11. LB agar (Fisher Scientific, catalog number: BP9724-500)
12. LB broth (Sigma-Aldrich, catalog number: L3022)
13. Ampicillin sodium salt (Fisher Scientific, catalog number: A0166), store at 4°C
14. Tryptone (Fisher Scientific, catalog number: 1278-7099)
15. Yeast extract (Fisher Scientific, catalog number: 10225203)
16. Ammonium sulfate ($(NH_4)_2SO_4$) (Sigma-Aldrich, catalog number: A4418)
17. Potassium phosphate monobasic (KH_2PO_4) (Sigma-Aldrich, catalog number: P0662)
18. Sodium phosphate dibasic (Na_2HPO_4) (Sigma-Aldrich, catalog number: S9763)
19. Glycerol (Fisher Scientific, catalog number: BP229-4)
20. (D)-(+)-Glucose (Sigma-Aldrich, catalog number: G7528)
21. α-Lactose monohydrate (Sigma-Aldrich, catalog number: L2643)
22. Magnesium sulfate heptahydrate ($MgSO_4·7H_2O$) (Sigma-Aldrich, catalog number: 230391)
23. ZY Media (see Recipes)
24. 20× NPS (see Recipes)
25. 50× 5052 (see Recipes)
26. 1 M $MgSO_4$ (see Recipes)
27. ZYP-5052 Auto-induction Media (see Recipes)

B. Protein Purification

1. 1 L Super-Speed Centrifuge Bottles with Sealing Closure, Nalgene (ThermoFisher, Fisher Scientific, catalog number: 3140-1006)
2. 50 mL Oak Ridge High-Speed Polycarbonate Centrifuge Tubes w/Sealing Cap (ThermoFisher, Fisher Scientific, catalog number: 3138-0050PK)
3. 10 mL serological pipettes, sterile (Sigma-Aldrich, catalog number: CLS4488-50EA)
4. 50 mL sterile centrifuge tubes (Corning, Sigma-Aldrich, catalog number: CLS430290)
5. 0.45 µm syringe filters, nylon (Fisher Scientific, catalog number: 15131499)
6. 20 mL plastic syringes (Fisher Scientific, catalog number: 15889152)
7. 500 mL glass beaker
8. 4 L glass beaker
9. Magnetic stir bars
10. Dialysis clips
11. Dialysis tubing, Biodesign™ Cellulose Dialysis Tubing Roll, 8000 Da MWCO (Fisher Scientific, catalog number: 12707486), store at 4°C
12. Amicon Ultra-15 centrifugal filters, 50 kDa MWCO (Merck Millipore, Sigma-Aldrich, catalog number: UFC9050)
13. 200 µL PCR tubes (Sigma-Aldrich, catalog number: BR781301)
14. Liquid nitrogen (LN_2)
15. Lysozyme from chicken egg white (~ 70,000 U/mg) (Sigma-Aldrich, catalog number: 62971), store at 4°C
16. Deoxyribonuclease I from bovine pancreas (Sigma-Aldrich, catalog number: DN25), store at -20°C
17. Magnesium chloride ($MgCl_2$) (Sigma-Aldrich, catalog number: M8266)
18. Trizma base (Sigma-Aldrich, catalog number: T6066)

19. Hydrochloric acid (HCl) (Fisher Scientific, catalog number: 10053023)
20. β-mercaptoethanol (Sigma-Aldrich, catalog number: M3148)
21. Imidazole (Sigma-Aldrich, catalog number: I2399)
22. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S7653)
23. Chelating Sepharose fast flow (Sigma-Aldrich, catalog number: GE17-0575-01), store at 4–30°C
24. Ethylenediamine tetraacetic acid (EDTA) (Sigma-Aldrich, catalog number: E9884)
25. Nickel sulfate hexahydrate ($NiSO_4 \cdot 6H_2O$) (Sigma-Aldrich, catalog number: 227676)
26. Sodium acetate ($C_2H_3NaO_2$) (Sigma-Aldrich, catalog number: S2889)
27. Acetic acid (CH_3COOH) (Sigma-Aldrich, catalog number: 695092)
28. Lysis Buffer (see Recipes)
29. Buffer A (see Recipes)
30. Buffer B (see Recipes)
31. Dialysis Buffer (see Recipes)
32. Gel Filtration Buffer (see Recipes)

C. $ALDH_{Tt}$ Enzyme Assay

1. Plastic cuvettes, Fisherbrand Macrocuvettes (Fisher Scientific, catalog number: FB55923)
2. Hexanal (Sigma-Aldrich, catalog number: 115606)
3. β-nicotinamide adenine dinucleotide sodium salt (NAD^+) (Sigma-Aldrich, catalog number: N0632), store at -20°C
4. Potassium phosphate monobasic (KH_2PO_4) (Sigma-Aldrich, catalog number: P0662)
5. Potassium phosphate dibasic (K_2HPO_4) (Sigma-Aldrich, catalog number: P3786)

Equipment

1. Biological safety cabinet
2. Autoclave
3. Lab balances
4. Water bath
5. Corning® 5 × 7 Inch Top PC-420D Stirring Hot Plate with Digital Displays, 120V/60Hz (Corning, catalog number: 6795-420D)
6. Shaking incubator (Eppendorf, New Brunswick Scientific Inova 40, Sigma-Aldrich, catalog number: EPM1299-0094)
7. -80°C freezer, New Brunswisk Scientific Ultra Low Temperature Freezer
8. Large scale centrifuge (ThermoFisher Scientific Sorvall RC6+ Centrifuge, Fisher Scientific, catalog number: 12121680)
9. ThermoFisher Scientific, Heraeus Megafuge 16R Centrifuge (ThermoFisher Scientific, Fisher Scientific, catalog number: 75004230)
10. VWR Microstar12 microcentrifuge (VWR, catalog number: 521-1651)
11. Probe sonicator (Bandelin Sonoplus HD 2200, catalog number: 2531)
12. Peristaltic Pump P-1 (Cytivia Life Sciences, catalog number: 18111091)
13. ÄKTAprime plus (Cytiva Life Sciences)
14. XK16 chromatography column (Sigma-Aldrich, catalog number: GE28-9889-37)
15. HiLoad 16/60 superdex 200 pg gel filtration column (Cytiva, Sigma-Aldrich, catalog number: GE28-9893-36)
16. NanoDrop™ ND-1000 (ThermoFisher Scientific, catalog number: ND-ONE-W)
17. SDS-PAGE apparatus (Biorad Mini-PROTEAN Tetra Cell, catalog number: 1658005EDU)
18. Cary 60 UV-vis spectrophotometer (Agilent) equipped with a temperature controller (TC-1 temperature controller) (Quantum Northwest, catalog number: TC 1-MAN-2.2)
19. pH meter, Thermo Scientific Orion 2-star benchtop pH meter (Fisher Scientific, catalog number: Meter Kit 1111001)

Procedure

A. Protein Expression

1. Transformation: Transform the construct DNA pET-22b(+)-ALDH$_{Tt}$ (Hayes *et al.*, 2018) into *E. coli* BL21(DE3) chemically competent cells, via a standard heat shock in a water bath at 42°C for 30 s. Plate the resultant transformation on an agar plate supplemented with 100 µg/mL ampicillin, and grow at 37°C overnight.
2. Preculture: Innoculate 10 mL of LB broth supplemented with 100 µg/mL ampicillin with one colony from the transformation plate, and incubate with agitation at 250 rpm and 37°C for 16 h.
3. Main culture: Innoculate 1 L of ZYP-5052 auto-induction media (see Recipes below) supplemented with 50 µg/mL ampicillin with 10 mL of preculture (1% v/v), and incubate with agitation at 200 rpm and 25°C for 48 h.
   Note: The main culture for expression is split, so as to have no more than 500 mL in a 2- L shake flask, for aeration purposes.

B. Protein Purification

1. Transfer *E. coli* cells to 1 L centrifuge bottles, and harvest the cells via centrifugation at 6,000 × *g* and 4°C for 15 min.
   Note: Prior to cell collection, weigh the empty centrifuge bottles, to allow for determination of the cell mass after centrifuging.
2. Discard the supernatant, and autoclave the waste.
3. Weigh the cell pellet (typically ~35 g/L culture).
4. Supplement the lysis buffer with 0.25 mg/mL lysozyme, 20 µg/mL DNase I, and 200 mM $MgCl_2$. Resuspend the pellet in lysis buffer (see Recipes below), adding 5 mL of lysis buffer for every 1 g of cells present.
   Note: Resuspension of cells is carried out on ice using a 10-mL serological pipette for drawing the buffer-cell suspension up and down. If carried out at room temperature, this should be performed in a timely manner, as native cell proteases may degrade the desired protein.
5. Store the cell lysate in aliquots of 50 mL in Falcon tubes at -80°C overnight.
   Note: Storage of cell lysate at -80°C overnight can be extended for up to 1 week.
6. Thaw the cell lysate in a water bath at 37°C.
7. Sonicate the cells using a probe sonicator with the power set between 60–70% (ultrasonic nominal output maximum of 200 W). Sonicate for 30 s, rest for 30 s, and repeat this process three times.
   Note: Sonication was carried out in a cold room at 4°C, with the cell lysate solution surrounded by ice.
8. Heat the cell lysate in a water bath to 65°C for 15 min. Omission of this step results in an approximate 40% decrease in protein yield.
9. Collect the cell debris via centrifugation at 34,000 × *g* and 4°C for 30 min. Remove the supernatant that contains the ALDH$_{Tt}$, and store on ice. Autoclave the collected cells prior to disposal.
   Note: Following the 30-min centrifugation for collection of the soluble fraction containing ALDH$_{Tt}$, the sample should be collected immediately after the centrifuge stops, to ensure the cells don't re-enter solution.
10. Filter the collected soluble fraction through a 0.45 µm nylon syringe filter, and store in a 50 mL Falcon tube on ice. Keep 1 mL of sample for SDS-PAGE and Western blot analysis.
11. Pack an XK 16/20 column with approximately 10 mL of chelating Sepharose fast flow resin, and pre-equilibrate as follows. Using a peristaltic pump, deliver the following quantities of buffer to the column, at a rate of 1–3 mL/min: 0.5 column volume (CV) of 0.2 M EDTA, 0.5 M NaCl, pH 7, 2 CV of 0.5 M NaCl, 2 CV of MilliQ water, 0.2 CV of 0.2 M $NiSO_4$, 5 CV of MilliQ water, 5 CV of 20 mM sodium acetate, 0.5 M NaCl pH 4, and finally 2 CV of buffer A (see Recipes below).
12. Load the entire filtered soluble fraction (approximately 80–100 mL from 1 L culture) onto the Ni-affinity column. Keep the solution on ice during loading. Collect the flow through for SDS-PAGE and Western blot analysis.
13. Connect the column to the Akta Prime system, and start the IMAC purification.

14. Wash the column with buffer A at 3 mL/min, until a stable (close to zero) Abs_{280nm} is achieved.
15. Elute proteins at 3 mL/min, via an imidazole step gradient of 50, 100, 200, and 500 mM (Figure 1), using a combination of buffer A and buffer B (see Recipes below). Only increase the concentration of buffer B, and thus imidazole, when the Abs_{280nm} obtained from the previous concentration is stable. Collect a fraction from each elution for SDS-PAGE and Western blot.
    Note: $ALDH_{Tt}$ should elute from Ni-affinity chromatography in the 200 mM imidazole fraction.

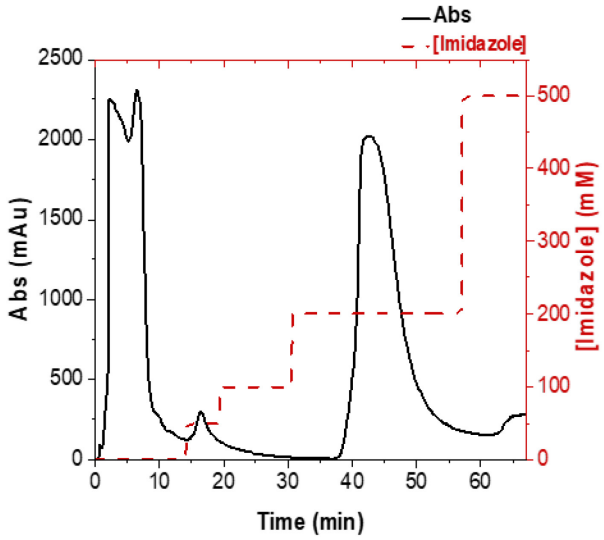


Figure 1. Elution profile of $ALDH_{Tt}$ via Ni-affinity chromatography.
Chromatogram displaying purification of $ALDH_{Tt}$ via Ni-affinity chromatography using a step gradient of imidazole from 50–500 mM. *E. coli* host proteins are eluted from 10–50 mM imidazole in the first two peaks, while $ALDH_{Tt}$ is eluted at 200 mM imidazole in the third peak.

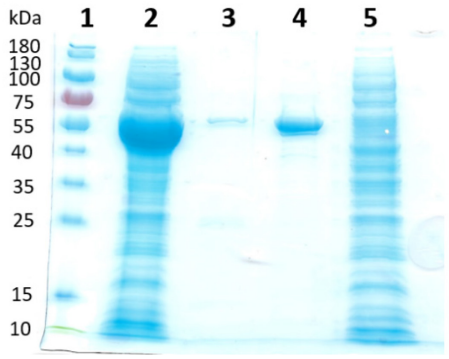


Figure 2. SDS-PAGE of $ALDH_{Tt}$ expression and purification samples.
Lane 1: PageRuler Pre-stained protein ladder (ThermoFisher Scientific), lane 2: *E. coli* BL21(DE3) cell lysate expressing $ALDH_{Tt}$, lane 3: Ni-affinity chromatography 200 mM imidazole elution, lane 4: purified $ALDH_{Tt}$ following gel filtration chromatography, lane 5: Ni-affinity chromatography flow through (adapted from Shortall *et al*., 2021).

16. Take the fractions containing the $ALDH_{Tt}$ (typically 30–40 mL), and dialyse against 4 L of dialysis buffer (see Recipes below). Dialyse with gentle magnetic stirring at 4°C overnight.
17. Concentrate the protein sample using Amicon Ultra-15 centrifugal filters, 50 kDa MWCO (Merck

Millipore), at 3,500 × *g* using 5 min spins, until a volume of approximately 1 mL (containing approximately 15–20 mg/mL) is obtained.

18. Gel filtration chromatography: Pre-equilibrate the HiLoad 16/60 Superdex 200 pg column with 2 CV (240 mL) of gel filtration buffer (see Recipes below) at 1 mL/min. Load approximately 1 mL of concentrated $ALDH_{Tt}$ protein sample onto the column using the injection loop on the Akta Prime system, and run at 1 mL/min for 120 min, with collection of the protein peak.
Note: $ALDH_{Tt}$ should begin to elute from gel filtration chromatography at approximately 65 mL. There should only be one peak on the gel filtration chromatogram. A slight shoulder at the start of the peak (on the left) can occur, but should not be collected.
19. Pool the fractions from gel filtration chromatography (approximately 20 mL), and concentrate to 25–30 mg/mL (or as desired) using Amicon Ultra-15 centrifugal filters, 50 kDa MWCO (Merck Millipore), at 3,500 × *g* using 5 min spins, until the desired concentration is obtained.
20. Monitor protein concentration by UV absorbance at 280 nm on a NanoDrop™ 1000 spectrophotometer, using a sequence-derived extinction coefficient of 1671 M^{-1} cm^{-1}. Use gel filtration buffer as the blank.
21. Prepare 30-µL aliquots, snap freeze in liquid nitrogen (LN_2), and store at -80°C until further use. If the protein is to be used immediately, aliquot as desired, and store on ice until further use.

C. $ALDH_{Tt}$ Enzyme Assay

1. Assay the $ALDH_{Tt}$, using hexanal as the substrate (2 mM) and NAD^+ as the cofactor (2 mM), in 10 mM potassium phosphate pH 8.
Note: Hexanal is only slightly soluble in water, so stock solutions at low concentrations should be prepared. Solubility in water is ~6 g/L (60 mM). Shelf life of the hexanal solution prepared in buffer is one week.
2. Program the spectrophotometer equipped with a temperature controller to analyse at 340 nm and 50°C for 2 min. Add a pre-equilibration step of 1 min, to ensure all solutions are kept at 50°C. A short analysis time of 2 min is utilised to avoid loss of volatile aldehyde substrates due to evaporation.
3. Heat solutions of 5 mM hexanal, 10 mM NAD^+, and 10 mM potassium phosphate pH 8 to 50°C, in a water bath.
Note: The enzyme activity of $ALDH_{Tt}$ can be analysed at 20–50°C. However, with decreased temperature a decrease in enzymatic activity is observed.
4. Thaw a 30-µL aliquot of $ALDH_{Tt}$ on ice.
5. Dilute the $ALDH_{Tt}$ to obtain a concentration of 0.38 mg/mL using gel filtration buffer, and centrifuge at 4,050 × *g* for 5 min.
Note: Diluting the $ALDH_{Tt}$ to a concentration of 0.38 mg/mL for the enzyme assay should be carried out using gel filtration buffer. For example, a 1:70 dilution of $ALDH_{Tt}$ at a stock concentration of 26.65 mg/mL can be performed, by adding 10 µL of $ALDH_{Tt}$ to 690 µL of gel filtration buffer.
6. To a plastic cuvette, add 360 µL of 10 mM NAD^+, 680 µL of 10 mM potassium phosphate pH 8, and 40 µL of $ALDH_{Tt}$ at 0.38 mg/mL. Insert the cuvette into the spectrophotometer, to undergo equilibration at 50°C for 1 min.
7. Add 720 µL of 5 mM hexanal to the cuvette and mix gently. Monitor the absorbance at 340 nm for 2 min, for the production of NADH.
Note: Enzymatic assaying of $ALDH_{Tt}$ should result in an increase in absorbance at 340 nm (a positive slope) when monitored at 50°C (Figure 3).
8. Calculate enzyme activity employing Beer-Lambert's Law, using the 6,220 M^{-1} cm^{-1} extinction coefficient of NADH at 340 nm (Equation 1).

$$U\ mg^{-1} = \frac{slope \times 10^6 \times vol}{6220\ M^{-1}cm^{-1} \times l \times mg\ of\ ALDH_{Tt}} \qquad \textbf{Equation 1}$$

Note: One enzyme unit is equal to the production of 1 µmol/min NADH. l is equal to the path length of the cuvette, in this case l = 1 cm. Vol is equal to the volume of the cuvette, in this case vol = 1.8 mL.

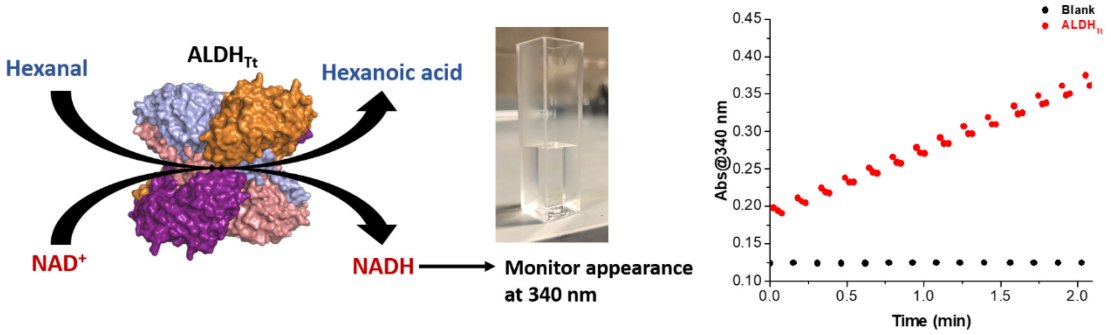


Figure 3. Principle for $ALDH_{Tt}$ assaying using hexanal and NAD^+ with activity monitored spectrophotometrically via the appearance of NADH. An example of the cuvette used and results obtained are demonstrated.

Data analysis

The original research article for the above expression, purification, and enzyme activity analysis was published in Shortall *et al*. (2021), https://doi.org/10.3390/cells10123535.

Recipes

Note: All solutions were prepared using MilliQ water (18.2 MΩ cm).

1. **ZY Media**
   10% typtone
   5% yeast extract

2. **20× NPS (NPS = 100 mM PO_4, 25 mM SO_4, 50 mM NH_4, 100 mM Na, 50 mM K)**
   0.5 M $(NH_4)_2SO_4$
   1 M KH_2PO_4
   1 M Na_2HPO_4

3. **50× 5052 (5052 = 0.5% glycerol, 0.05% glucose, 0.2% α-lactose)**
   25% glycerol
   2.5% glucose
   10% α-lactose
   Note: Preparation of 50× 5052 can be assisted by gentle heating with magnetic stirring. 50× 5052 can be difficult to dissolve, and should not be autoclaved until all components are dissolved. If the solution is visibly displaying a brown tinge after autoclaving, it should be re-prepared.

4. **1 M $MgSO_4$**

5. **ZYP-5052 Auto-induction Media**
   ~928 mL of ZY media
   1 mL of 1 M $MgSO_4$
   20 mL of 50× 5052
   50 mL of 20× NPS
   50 μg/mL ampicillin

Note: Solutions for ZYP-5052 auto-induction media are prepared and autoclaved separately. These are combined to make the media on the day of the expression culture.

6. **Lysis Buffer**
   20 mM Tris-HCl pH 7.5
   5 mM β-mercaptoethanol
   10 mM imidazole
   500 mM NaCl

7. **Buffer A**
   20 mM Tris-HCl pH 7.5
   5 mM β-mercaptoethanol
   10 mM imidazole
   200 mM NaCl

8. **Buffer B**
   20 mM Tris-HCl pH 7.5
   5 mM β-mercaptoethanol
   1 M imidazole
   200 mM NaCl

9. **Dialysis Buffer**
   50 mM Tris-HCl pH 7.5
   5 mM β-mercaptoethanol
   250 mM NaCl

10. **Gel Filtration Buffer**
    50 mM Tris-HCl pH 7.5
    5 mM β-mercaptoethanol
    150 mM NaCl

Acknowledgments

This protocol is an extension of that described in Shortall *et al*. (2021) and adapted from Hayes *et al*. (2018). This research was funded by European Union's Horizon 2020 Research and Innovation programme, Oyster (Open characterisation and modelling environment to drive innovation in advanced nano-architectured and bio-inspired hard/soft interfaces) under grant agreement No. 760827. Funding is also acknowledged from the Department of Chemical Sciences, University of Limerick and The Higher Education Authority, Ireland.

Competing interests

There are no conflicts of interest or competing interests.

References

Hayes, K., Noor, M., Djeghader, A., Armshaw, P., Pembroke, T., Tofail, S. and Soulimane, T. (2018). The quaternary structure of Thermus thermophilus aldehyde dehydrogenase is stabilized by an evolutionary distinct C-terminal arm extension. *Sci Rep* 8(1): 1-14.

Lyons, J. A., Aragão, D., Slattery, O., Pisliakov, A. V., Soulimane, T. and Caffrey, M. (2012). Structural insights into electron transfer in caa 3-type cytochrome oxidase. *Nature* 487(7408): 514-518.

Robin, S., Arese, M., Forte, E., Sarti, P., Giuffre, A. and Soulimane, T. (2011). A sulfite respiration pathway from Thermus thermophilus and the key role of newly identified cytochrome c_{550}. *J Bacteriol* 193(15): 3988-3997.

Shortall, K., Durack, E., Magner, E. and Soulimane, T. (2021). Study of ALDH from *Thermus thermophilus*-Expression, Purification and Characterisation of the Non-Substrate Specific, Thermophilic Enzyme Displaying Both Dehydrogenase and Esterase Activity. *Cells* 10(12): 3535.

Soulimane, T. (2010). Thermus thermophilus encodes an archaeal-like fructose-1,6-bisphosphatase: purification of native and recombinant protein for structural studies. *Protein Expr Purif* 74(2): 175-180.