**Supplementary Information**

# Evolutionary, computational, and biochemical studies of the salicylaldehyde dehydrogenases in the naphthalene degradation pathway

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Running title: A comprehensive analysis of salicylaldehyde dehydrogenases

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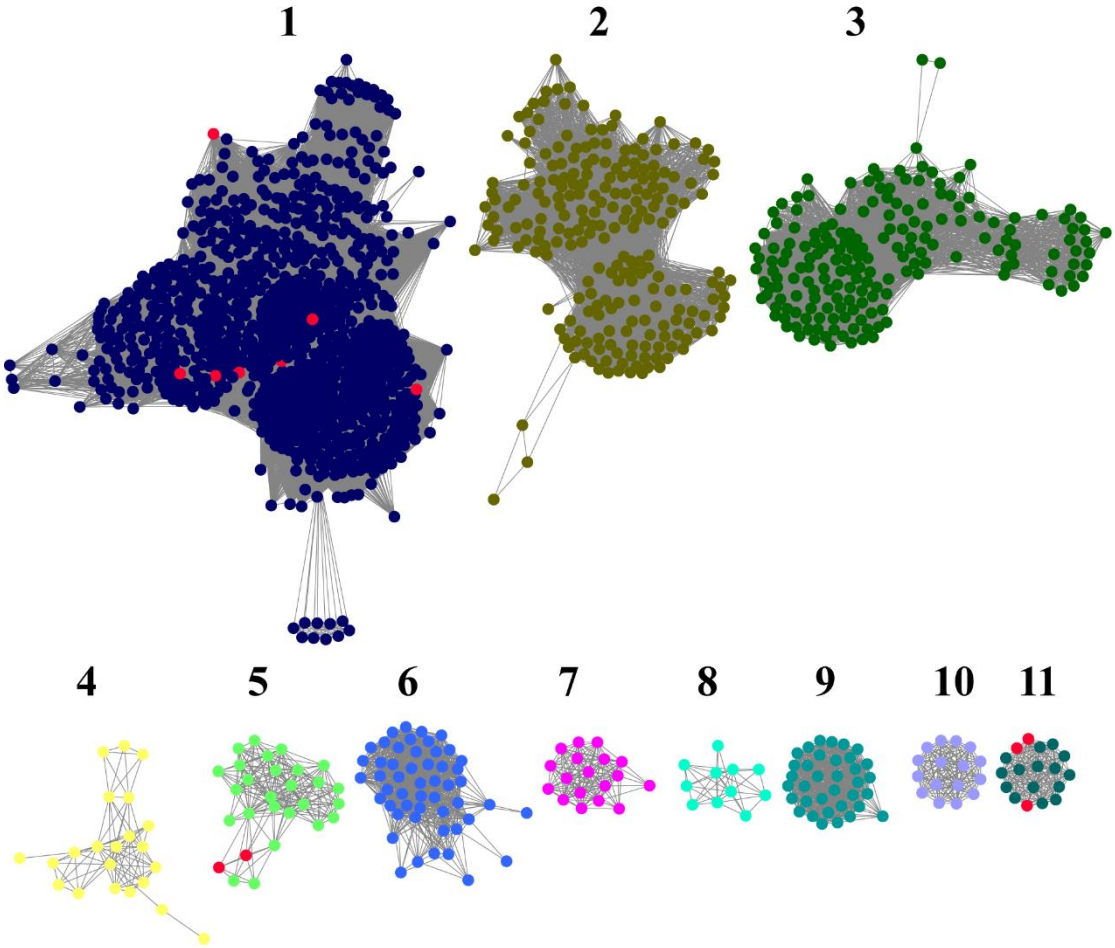
**Supplementary Table 1.** Molar absorption coefficient (ε) at 340 nm of aromatic aldehyde and acid and in 50 mM HEPES pH 7.5, and 150 mM NaCl.

| **Substrate** | ***ε*, $M^{-1}$ $cm^{-1}$** | **Product** | ***ε*, $M^{-1}$ $cm^{-1}$** | **Correction factor** |
|---|---|---|---|---|
| Salicylaldehyde | 2530 | Salicylic acid | 52 | 1.67 |
| Benzaldehyde | 51 | Benzyl acid | 38 | 1.00 |
| 2-Chlorobenzaldehyde | 63 | 2-Chlorobenzoic acid | 59 | 1.00 |
| 3-Chlorobenzaldehyde | 74 | 3-Chlorobenzoic acid | 63 | 1.00 |
| 4-Chlorobenzaldehyde | 88 | 4-Chlorobenzoic acid | 76 | 1.00 |
| 4-Nitrobenzaldehyde | 682 | 4-Nitrobenzyl acid | 654 | 1.00 |
| 2-Naphthaldehyde | 3542 | 2-Naphthoic acid | 352 | 2.13 |

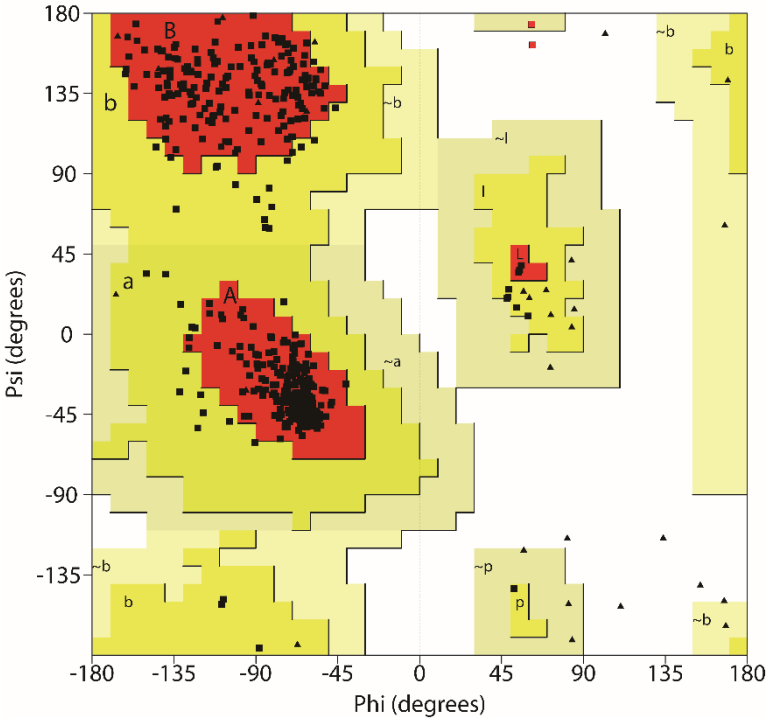
The correction factor was calculated by the equation:
correction factor=$\varepsilon$(NADH)/( $\varepsilon$(NADH)- $\varepsilon$(substrate)+ $\varepsilon$(product)), where $\varepsilon$(NADH)=6200 $M^{-1}$ $cm^{-1}$.

**Supplementary Figure 1**



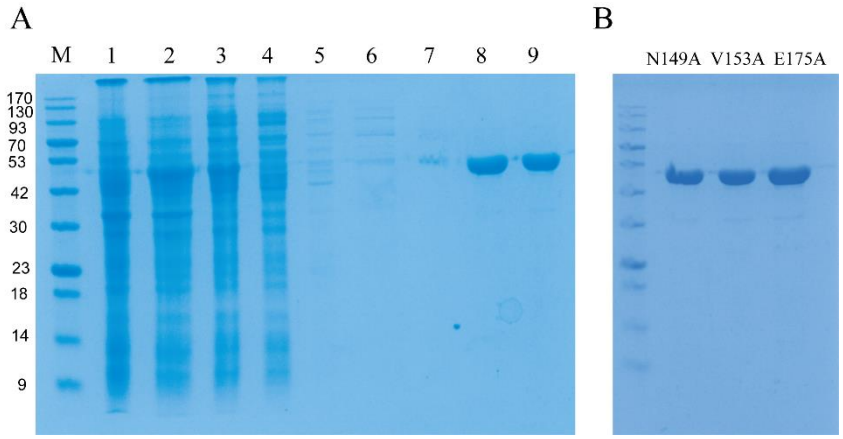

**Supplementary Figure 1.** Protein sequence similarity network of SALDs from Uniprot and NCBI metagenomics protein database. The network was generated using an e-value of $10^{-150}$ (> 60% sequence identity). The proteins from Uniprot database were classified into 11 clusters and each cluster was assigned a different color. Proteins from NCBI metagenomics protein database grouped into cluster 1, 5, and 11 were painted by red color. The accession number of proteins from NCBI metagenomics protein database were listed in Supplementary Dataset 2.

**Supplementary Figure 2**



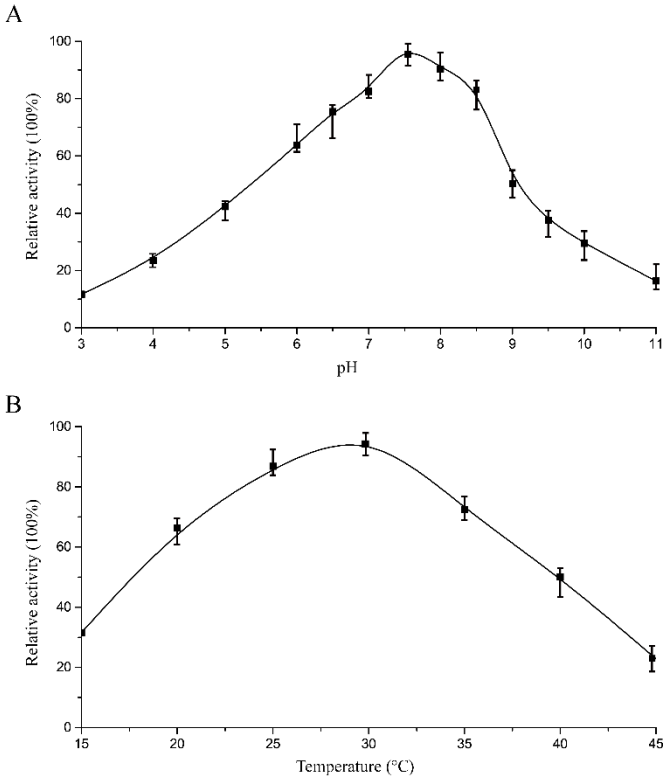

**Supplementary Figure 2.** Validation of protein structure of SALDan using Ramchandran plot. The Ramachandran plot revealed that 90.3% of amino acid residues from modeled structure were incorporated in the favored regions (A, B, and L) of the plot. 9.2% of the residues were in allowed regions (a, b, l, and p) of the plot.

**Supplementary Figure 3**



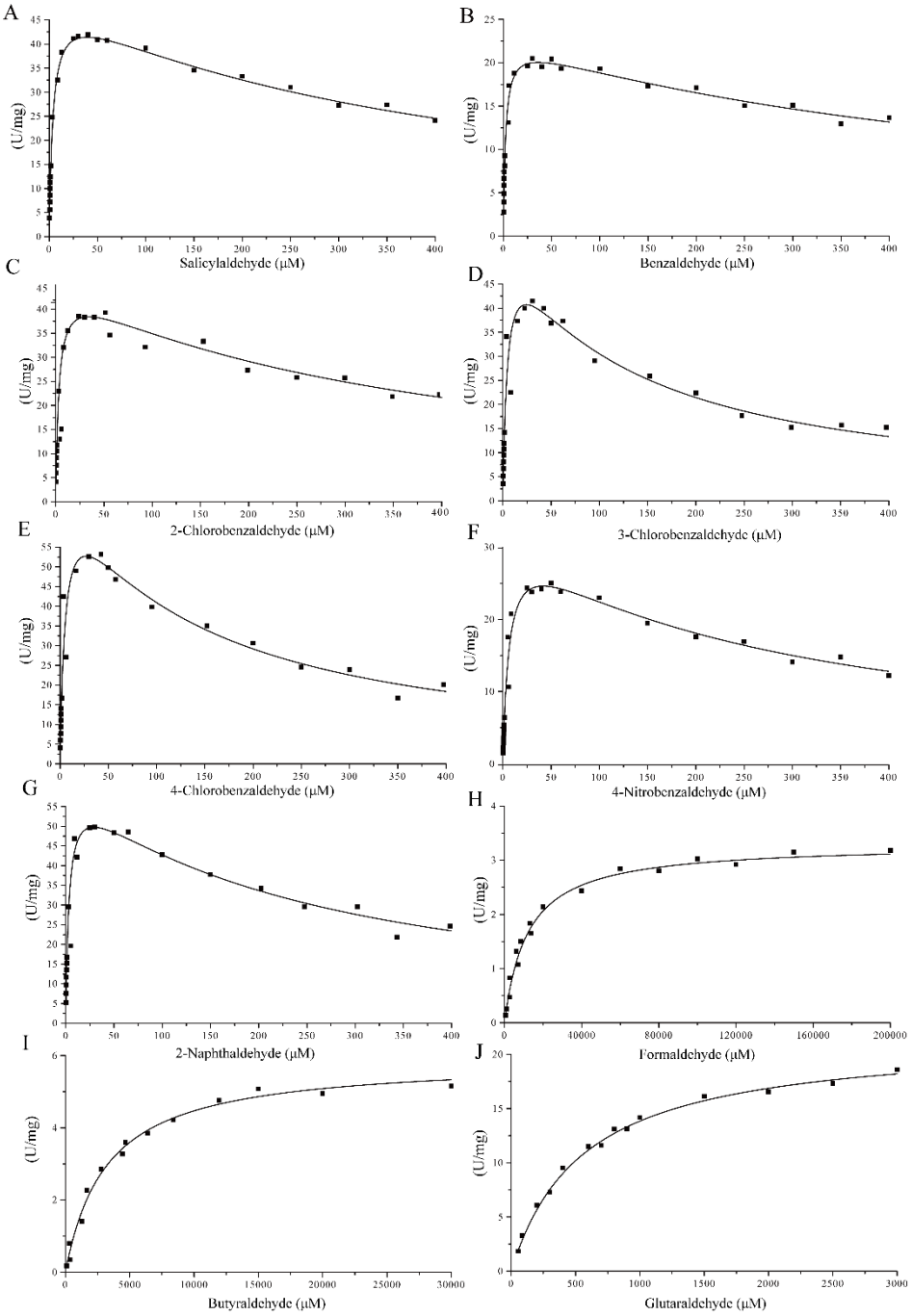

**Supplementary Figure 3.** Expression and purification of SALDan and the mutants from *Escherichia coli*. A Purification of SALDan, Lane M, protein marker; the molecular mass standards are indicated at the left. Lane 1, crude protein extract from non-induced cells; Lane 2, crude protein extract from IPTG-induced cells; Lane 3, soluble extract from IPTG-induced cells; Lane 4, unbound proteins eluted from the Ni-NTA column; Lane 5-7, proteins eluted with 20 mM, 50 Mm, and 100 mM imidazole, respectively; Lane 8, proteins eluted with 300 mM imidazole. B Purification of the mutant of N149A V153A, and E175A.

**Supplementary Figure 4**



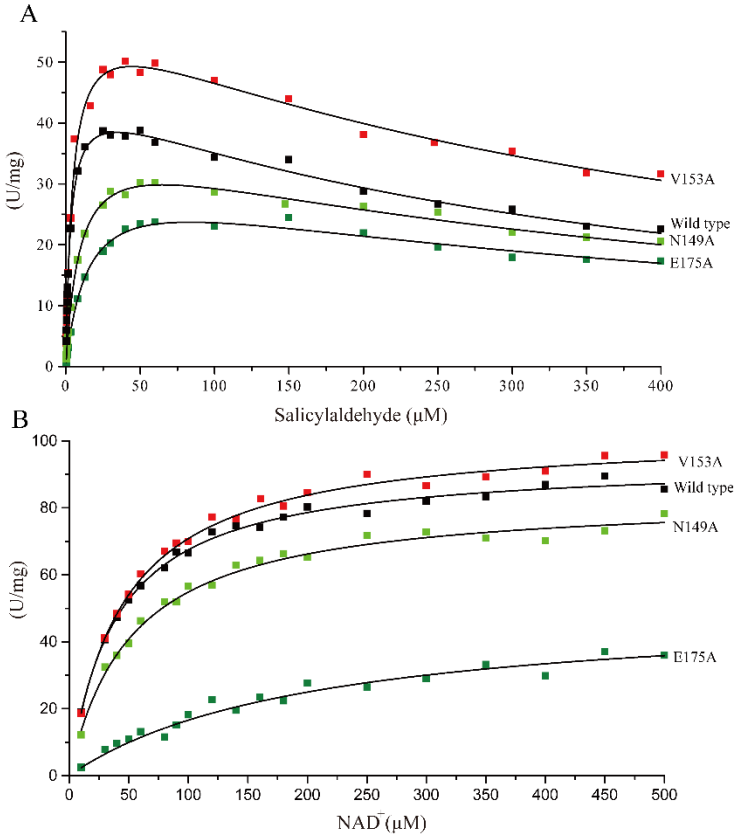

**Supplementary Figure 4.** A Optimal pH of SALDan. Different buffers were used for the different pH solutions in this assay. Sodium acetate buffer was used for pH 3.0–5.0; MES buffer was used for pH 5.0–7.5; HEPES buffer was used for pH 8 and 8.5; glycine buffer was used for pH 9.0 and 10.0; and $Na_2HPO_4$ buffer was used for pH 11.0. Values obtained at pH 7.5 were set as 100%. B Optimal temperature of SALDan. Values obtained at 30°C were set as 100%.

**Supplementary Figure 5**




**Supplementary Figure 5.** Effects of substrates (names shown under X-axis) concentration on the velocity of the SALDan. Assays were performed as described in Section "Materials and Methods."

**Supplementary Figure 6**




**Supplementary Figure 6.** Kinetics characteristics of wild-type SALDan (black), V153A (red), N149A (green), and E175A (blue) with salicylaldehyde (A) and $NAD^+$ (B) as substrates.