Supplementary Information

A Comparative Study of Two Aldehyde Dehydrogenases from *Sphinobium* sp.: Substrate Spectrum and Catalytic Mechanism

Siyi Chen, Jieyu Zhou, Xiangyuan Gu,Ye Ni**

Key laboratory of industrial Biotechnology, Ministry of Education, School of Biotechnology,

Jiangnan University, Wuxi 214122, Jiangsu, China

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Table S1. Molar absorbance coefficient (ε) at different pH conditions.

	pH	equation	R^2	ε($\mu L \cdot \mu mol^{-1} \cdot cm^{-1}$)
citric acid/sodium citrate buffer (pH 5.0-6.5, 100 mM)	5.0	y = 0.790x + 0.029	0.984	1.271
	5.5	y = 0.784x + 0.028	0.990	1.260
	6.0	y = 0.948x + 0.034	0.983	1.524
	6.5	y = 1.666x + 0.028	0.985	2.679
phosphate buffer (pH 6.0-8.0, 50 mM)	6.0	y = 1.110x + 0.024	0.982	1.785
	6.5	y = 2.140x - 0.001	0.984	3.441
	7.0	y = 4.886x - 0.066	0.994	7.856
	7.5	y = 6.113x + 0.006	0.993	9.828
	8.0	y = 7.140x + 0.025	0.999	11.479
Tris–HCl (pH 7.0-9.0, 50 mM)	7.0	y = 6.311x - 0.107	0.992	10.146
	7.5	y = 5.883x + 0.057	0.995	9.458
	8.0	y = 6.377x + 0.065	0.998	10.252
	8.5	y = 6.933x + 0.094	0.998	11.147
	9.0	y = 8.001x + 0.054	0.999	12.863
Glycine-NaOH (pH 8.5-11.0, 50 mM)	8.7	y = 7.890x + 0.039	0.998	12.685
	9.0	y = 8.022x + 0.036	0.998	12.897
	9.6	y = 7.206x + 0.070	0.999	11.586
	10.5	y = 7.310x + 0.094	0.999	11.753
	11.0	y = 7.115x + 0.142	0.999	11.439
	11.5	y = 6.905x + 0.210	0.999	11.102

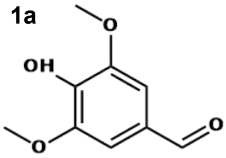
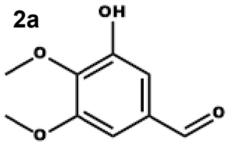
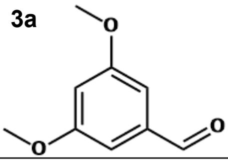
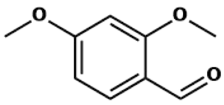
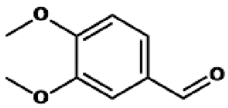
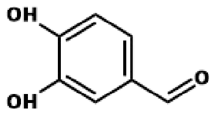
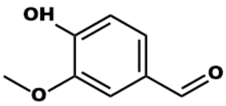
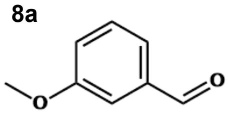
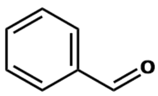
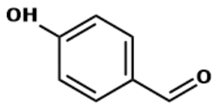
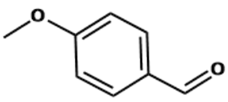
Table S2. Coupling reaction between ALDHs and NOX (600μl reaction system (35℃))

	Final concentration
1a	20 mM
NAD^+	0.5 mM
NOX	0.5 mg/mL
*Sp*ALDH1/*Sp*ALDH2	0.1 mg/mL
Citric acid/sodium citrate buffer (100mM, pH 6.5) /Tris-HCl (100mM, pH 8.0)	/

Table S3. Coupling reaction between ALDHs and NOX (600μl reaction system (35℃))

	Final concentration
1a	20 mM
NAD^+	0.5 mM
NOX	1.5 mg/mL
*Sp*ALDH1/*Sp*ALDH2	0.3 mg/mL
Citric acid/sodium citrate buffer (100mM, pH 6.5) /Tris-HCl (100mM, pH 8.0)	/

Table S4. Biocatalytic oxidation of aromatic aldehydes to carboxylic acids catalyzed by ALDHs.

Substrate	ALDH	Conv. [%]	Substrate	ALDH	Conv. [%]
1a 	*Sp*ALDH1	＞99	**2a** 	*Sp*ALDH1	＞99
	*Sp*ALDH2	＞99		*Sp*ALDH2	＞99
3a 	*Sp*ALDH1	＞99	**4a** 	*Sp*ALDH1	3.2
	*Sp*ALDH2	＞99		*Sp*ALDH2	＞99
5a 	*Sp*ALDH1	＞99	**6a** 	*Sp*ALDH1	5.3
	*Sp*ALDH2	＞99		*Sp*ALDH2	＞99
7a 	*Sp*ALDH1	＞99	**8a** 	*Sp*ALDH1	＞99
	*Sp*ALDH2	＞99		*Sp*ALDH2	＞99
9a 	*Sp*ALDH1	＞99	**10a** 	*Sp*ALDH1	＞99
	*Sp*ALDH2	＞99		*Sp*ALDH2	＞99
11a 	*Sp*ALDH1	＞99			
	*Sp*ALDH2	＞99			

Experimental: 600 μL in buffer, T = 35 ℃, 100 rpm, [substrate] = 5 mM, [ALDH] = 0.2 mg/mL, [NAD^+] =10 mM. Citrate buffer (100 mM, pH 6.5) was used to test *Sp*ALDH1, while glycine-NaOH buffer (50 mM, pH 10.5) was employed for *Sp*ALDH2. Conversions were measured by HPLC.

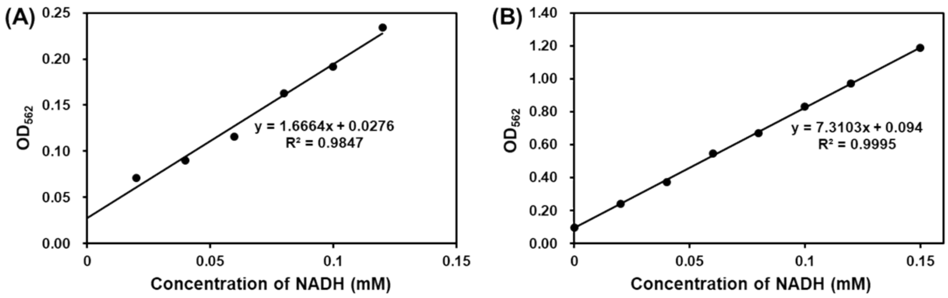


Fig S1. Molar absorbance coefficient (ε) at different pH conditions. (A) Citric acid/sodium citrate buffer (pH 6.5, 100 mM); (B) Glycine-NaOH (pH 10.5, 50 mM).

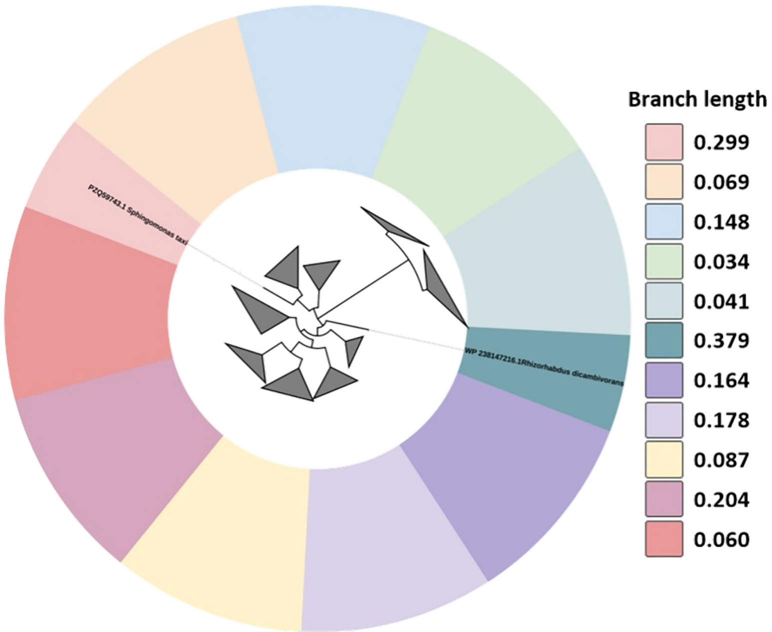
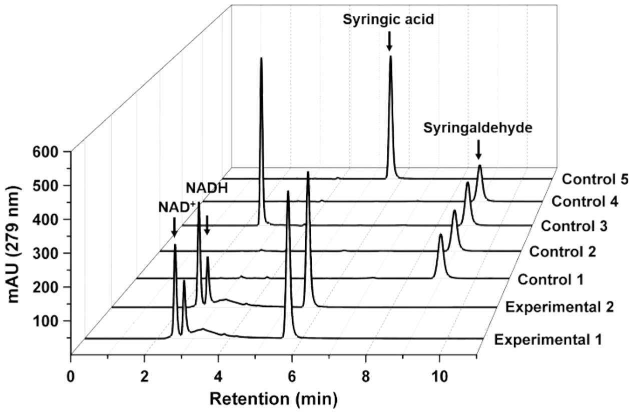


Fig. S2 Phylogenetic analysis of *Sp*ALDH1, *Sp*ALDH2 and other bacterial ALDHs. Use differences in branch lengths as a basis for coloring. Auto collapse clade is set to be 0.5.



	*Sp*ALDH1	*Sp*ALDH2	NAD^+	Syringaldehyde	Syringic acid	Buffer
Experimental 1	+	−	+	+	−	+
Experimental 2	−	+	+	+	−	+
Control 1	+	−	−	+	−	+
Control 2	−	+	−	+	−	+
Control 3	−	−	+	+	−	+
Control 4	−	−	−	+	−	+
Control 5	−	−	−	−	+	+

Fig. S3 HPLC analysis of syringaldehyde conversions by purified ALDHs. Controlled experiments of ALDHs biocatalyzed oxidation of syringaldehyde.

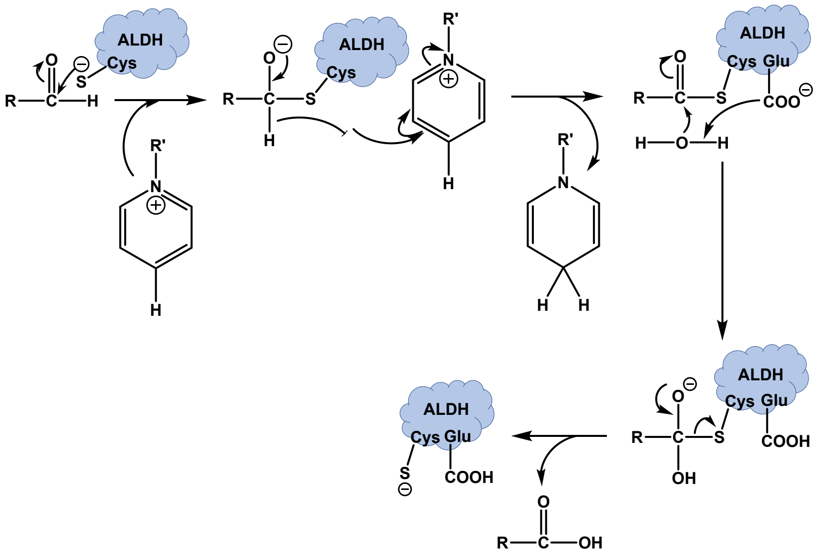


Fig S4. Catalytic mechanism of ALDHs toward aldehyde substrates.

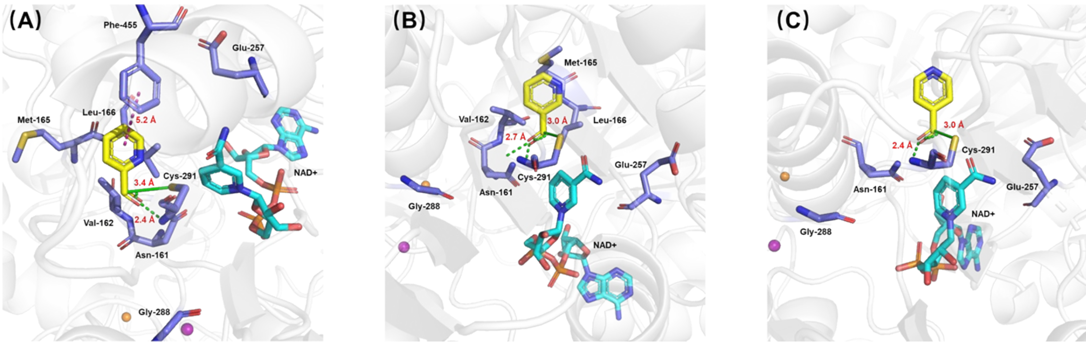


Fig S5. Molecular docking of *Sp*ALDH1 with pyridine-formaldehydes. (A) 22a; (B) 23a; (C) 24a. Active site residues, substrates and NAD^+ cofactor are showed as sticks, represented by slate (carbon atom), yellow (carbon atom) and cyan (carbon atom), respectively. The ALDHs are depicted as grey. Oxygen atoms (red); Nitrogen atoms (blue); Sulfur atoms (yellow orange); Potassium ions (purple ball); Sodium ions (orange ball). The hydrogen bonds are showed as green dotted lines; the pi-pi stacking interactions are showed as light magenta dotted lines.

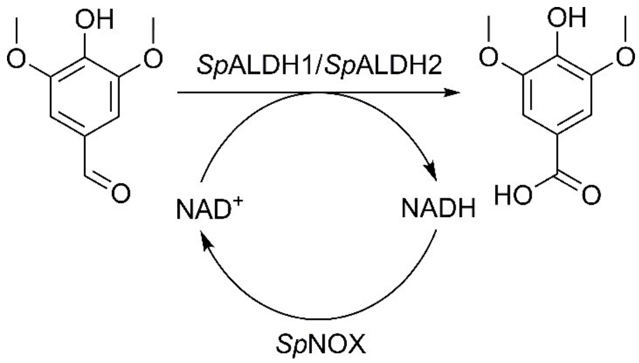


Fig. S6 Preparation of syringic acid by coupling ALDHs with *Sp*NOX.

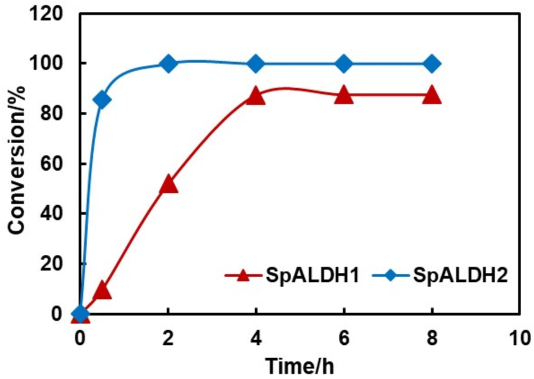


Fig S7. Coupled reaction catalyzed by ALDHs and NOX at substrate **1a** concentration of 20 mM.