Supplemental material

Nicotine Dehydrogenase Complexed with 6-Hydroxypseudooxynicotine Oxidase Involved in the Hybrid Nicotine-degrading Pathway in *Agrobacterium tumefaciens* S33

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Running title: NdhAB-Pno complex involved in nicotine degradation (50<54)

Key words: Nicotine dehydrogenase; 6-hydroxypseudooxynicotine oxidase; biochemical mechanism; nicotine degradation; *Agrobacterium tumefaciens*

TABLE S1 The primers used for gene disruption experiments

Primers	Sequence (5′-3′)
ndhA-A	CGCGGATCCATGAGCGTCTCTGTCAATCGT[a]
ndhA-B	ATTTGTATACGACCTTCTCA GCTAACGGAGGAGTCAG
ndhA-C	TGAGAAGGTCGTATACAAATAAGGACCTTGAGCAGTACGAC
ndhA-D	CCGCTCGAGCTATACTAACGACAGTTCCTT[b]
ndhB-A	CGCGGATCCATGAAAGTCGATTTTACTGTTAATG[a]
ndhB-B	AAAATATCGGATACGGCCATGATCATGCCGGACTGGCAATAGCCGC
ndhB-C	CATGGCCGTATCCGATATTTTATGAGCGTCTCTGTCAATCGT
ndhB-D	CCGCTCGAGTGCAAAGAGATAATGTGCGGAC[b]
pno-A	CGCGGATCCATGCGAGATCCACGTTATGAC[a]
pno-B	TTTTTACATCAAGGAAGTCCTGCAGAAGGGACAAATTGTGAG
pno-C	AGGACTTCCTTGATGTAAAAAACTTACCAAGCGGTGAGG
pno-D	CCGCTCGAGCTAACGACCGGTACCGCAAAATTC[b]

[a] The BamHI restriction site is underlined;

[b] The XhoI restriction site is underlined.

TABLE S2 The primers used for genes complementation

Primers	Sequence (5′-3′)
ndhA-F	CGCCTCGAGATGAGCGTCTCTGTCAATCGT[a]
ndhA-R	CCGGAATTCCTATACTAACGACAGTTCCTT[b]
ndhB-F	CGCCTCGAGATGAAAGTCGATTTTACTGTTAATG[a]
ndhB-R	CCGGGATCC TGCAAAGAGATAATGTGCGGAC[c]
pno-F	CGCCTCGAGATGCGAGATCCACGTTATGAC[a]
pno-R	CCGGGATCCCTAACGACCGGTACCGCAAAATTC[c]

[a] The XhoI restriction site is underlined;

[b] The EcoRI restriction site is underlined;

[c] The BamHI restriction site is underlined.

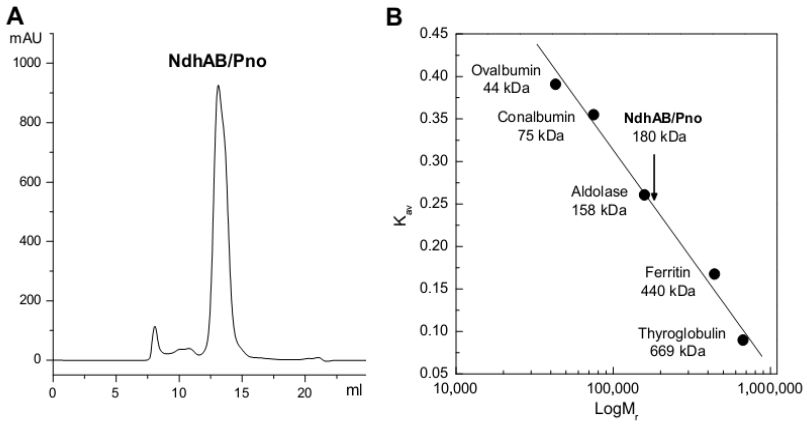


FIG S1 Determination of the apparent molecular mass of NdhAB-Pno complex from *A. tumefaciens* S33 by gel filtration on Superdex 200 (1.0 cm × 30 cm). (A) Elution profile of NdhAB-Pno complex. (B) Calibration curve for Superdex 200 (1.0 cm × 30 cm). The column was calibrated with gel filtration calibration kit (High molecular weight) from GE Healthcare according to the manual. The buffer used was 50 mM sodium phosphate buffer (pH 7.0) containing 150 mM NaCl. The flow rate was 0.5 ml/min.

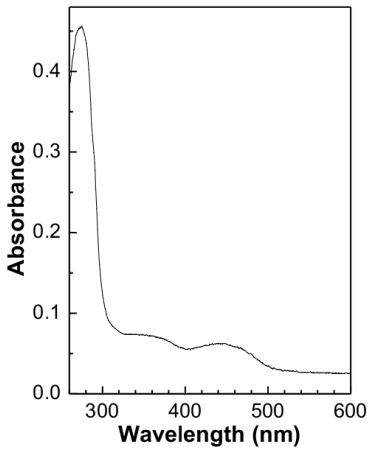


FIG S2 UV-visible absorption spectrum of the purified Ndh-Pno complex from *A. tumefaciens* S33.

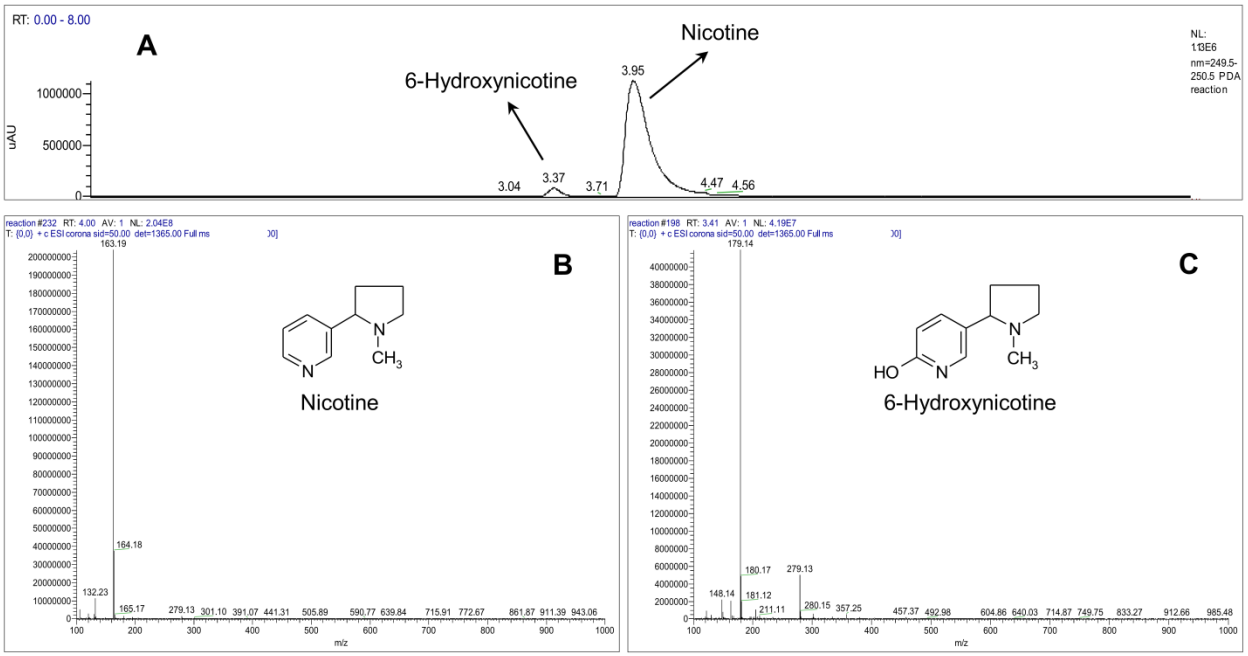


FIG S3 LC-MS profiles of the reaction catalyzed by purified Ndh-Pno complex from *A. tumefaciens* S33. (A), HPLC profile monitored with PDA detector; (B) and (C), mass spectra of substrate nicotine (*m/z* 163.19; $C_{10}H_{14}N_2$, cal. MW 162.1157) and product 6-hydroxynicotine (*m/z* 179.14; $C_{10}H_{14}ON_2$, cal. MW 178.1106), respectively.

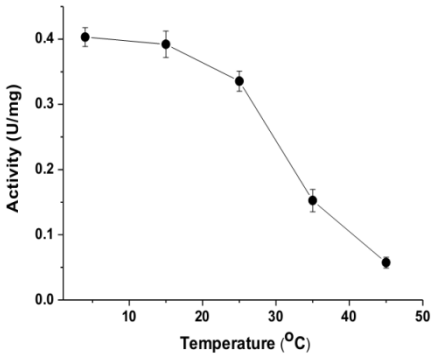


FIG S4 Thermal stability of the purified NdhAB-Pno complex from *A. tumefaciens* S33. The enzyme (0.66 U/mg) was incubated at different temperature for 30 min, then assayed at 30°C as described in Material and Methods section.

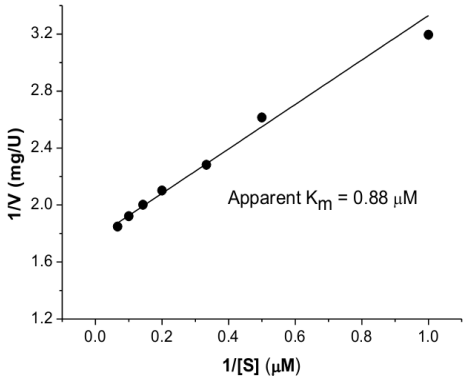


FIG S5 Estimation of the apparent K_m of the purified NdhAB-Pno complex from *A. tumefaciens* S33 for nicotine.

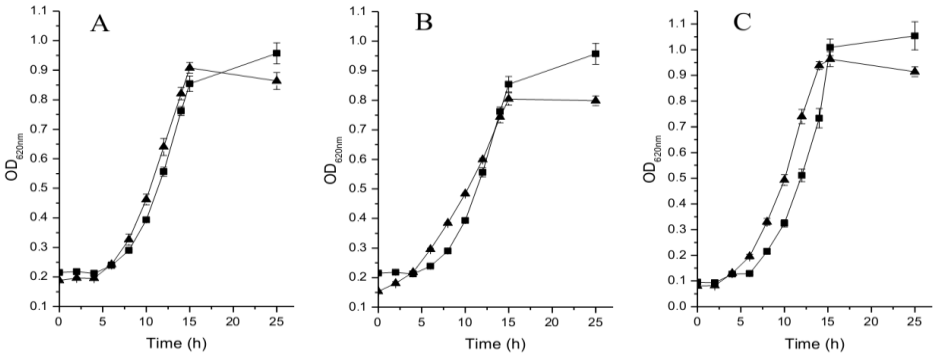


FIG S6 Growth of complementation strains of S33 with disrupted genes (▼) and wild type strain (■) with nicotine as the sole source of carbon and nitrogen. A, S33-Δ*ndhA*-C; B, S33-Δ*ndhB*-C; C, S33-Δ*pno*-C.

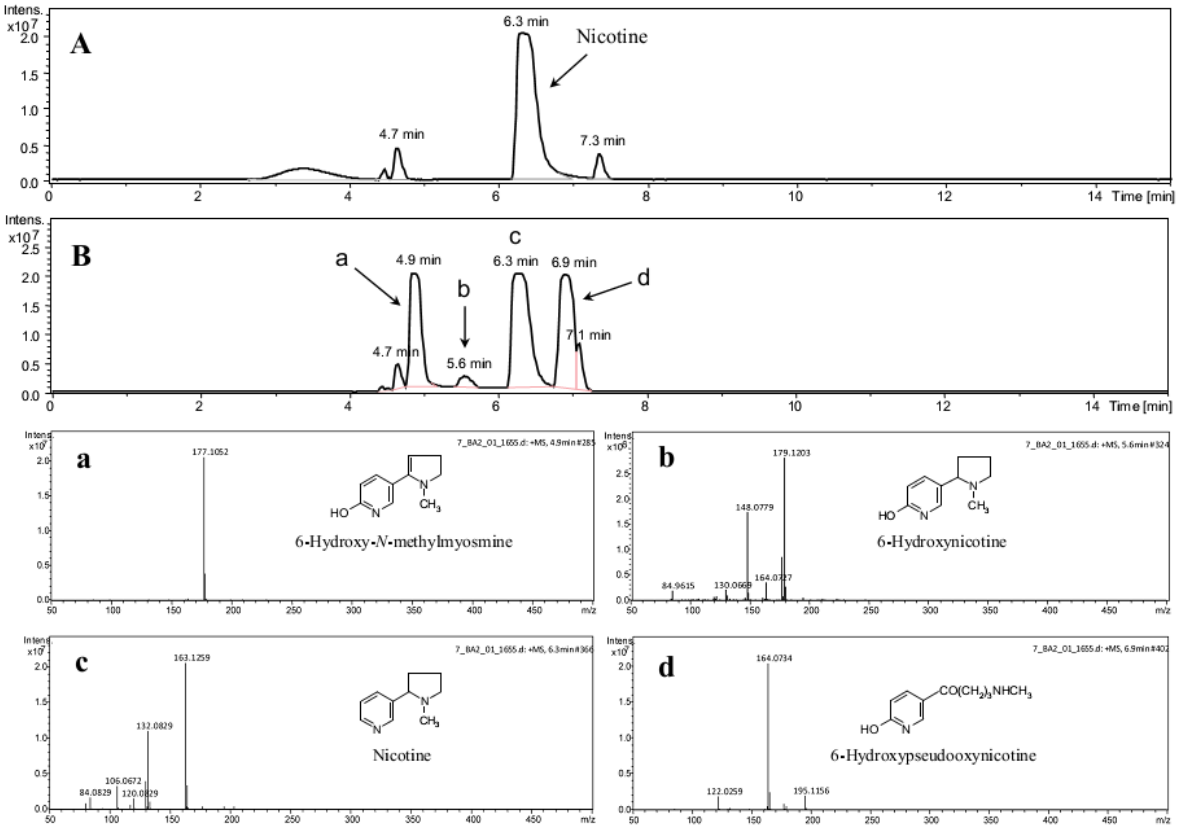


FIG S7 LC-MS profiles of the reaction catalyzed by resting cells of S33-Δ*pno* with nicotine as substrate. A, total ion chromatograph of the reaction mixture without adding resting cells as control; B, total ion chromatograph of the reaction mixture after adding resting cells for 12 h; a-d, mass spectra of the substrate nicotine (c, 6.3 min, *m/z* 163.1259; $C_{10}H_{14}N_2$, cal. MW 162.1157), and the products 6-hydroxynicotine (b, 5.6 min, *m/z* 179.1203; $C_{10}H_{14}N_2O$, cal. MW 178.1106), 6-hydroxy-*N*-methylmyosmine (a, 4.9 min, *m/z* 177.1052; $C_{10}H_{12}N_2O$, cal. MW 176.0950) and 6-hydroxypseudooxynicotine (d, 6.9 min, *m/z* 195.1156; $C_{10}H_{14}N_2O_2$, cal. MW 194.1055) .

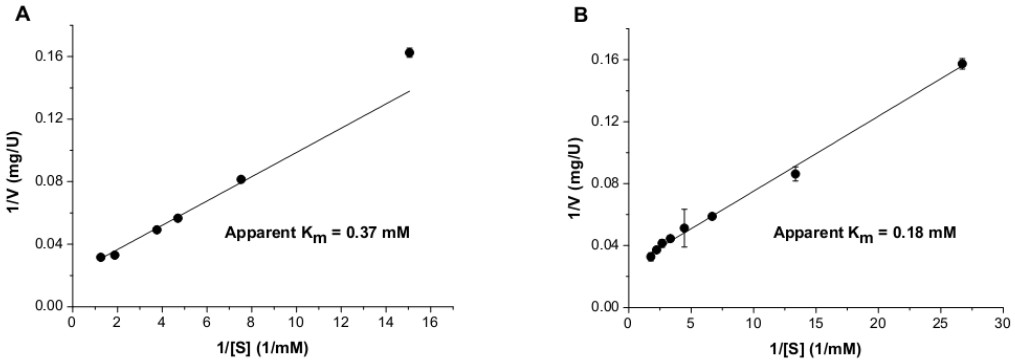
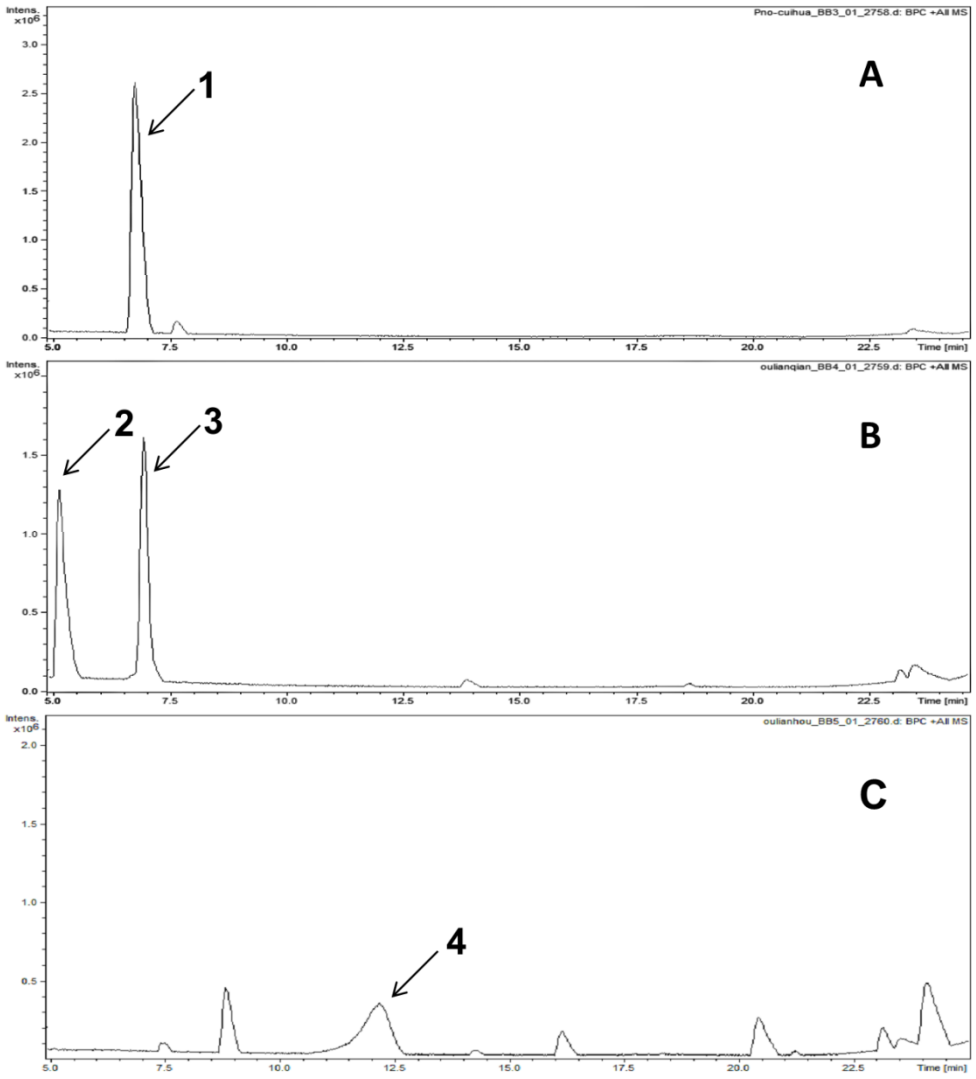


FIG S8 Estimation of the apparent K_m of purified wild NdhAB-Pno complex from *A. tumefaciens* S33 (A) and recombinant Pno (B) for 6-hydroxypseudooxynicotine.



(FIG S9 is continued.)

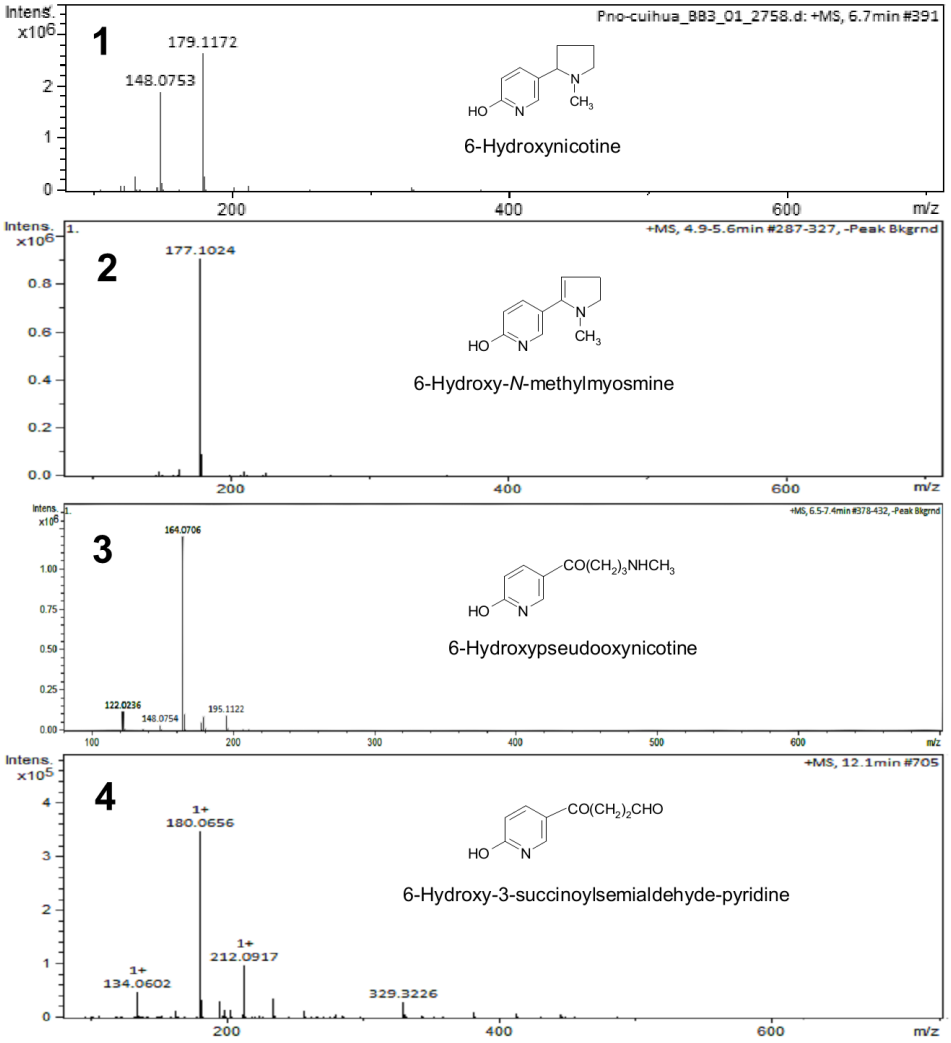


FIG S9 LC-MS profiles of the reaction catalyzed by recombinant Pno coupled to 6-hydroxynicotine oxidation by Hno from *A. nicotinovorans*. A, HPLC profile of the reaction mixture containing 6-hydroxynicotine before adding Hno and Pno; B, HPLC profile of the reaction mixture after adding only Hno; C, HPLC profile of the reaction mixture after adding both Hno and Pno; 1-4, mass spectra of the substrate 6-hydroxynicotine (1, *m/z* 179.1172; $C_{10}H_{14}N_2O$, cal. MW 178.1106), the intermediates 6-hydroxy-*N*-methylmyosmine (2, *m/z* 177.1024; $C_{10}H_{12}N_2O$, cal. MW 176.0950) and 6-hydroxypseudooxynicotine (3, *m/z* 195.1122; $C_{10}H_{14}N_2O_2$, cal. MW 194.1055), and final product 6-hydroxy-3-succinoylsemialdehyde-pyridine (4, *m/z* 180.0656; $C_9H_9NO_3$, cal. MW 179.0582) and its methanol adduct (4, *m/z* 212.0917; $C_{10}H_{13}NO_4$, cal. MW 211.0844).