Supplemental Materials

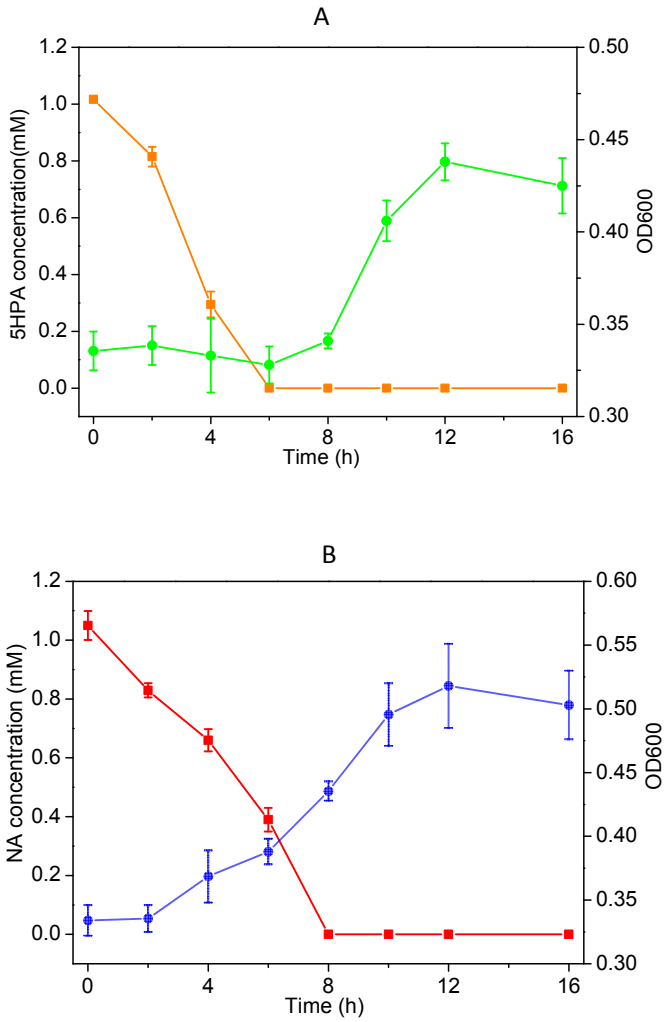


Fig. S1 (A) Degradation of 5HPA (■) and cell growth (●) of *Alcaligenes faecalis* JQ135. (B) Degradation of NA (■) and cell growth (●) of *A. faecalis* JQ135.

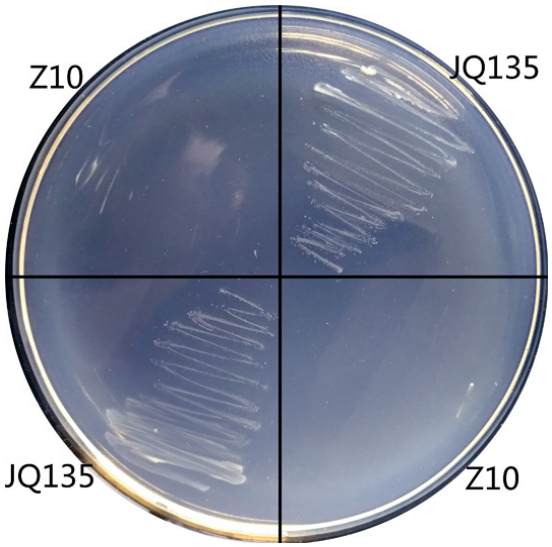


Fig. S2 Growth phenotype of the wild type *A. faecalis* JQ135 and mutant Z10 on MSM plate supplemented with 1 mM 5HPA.

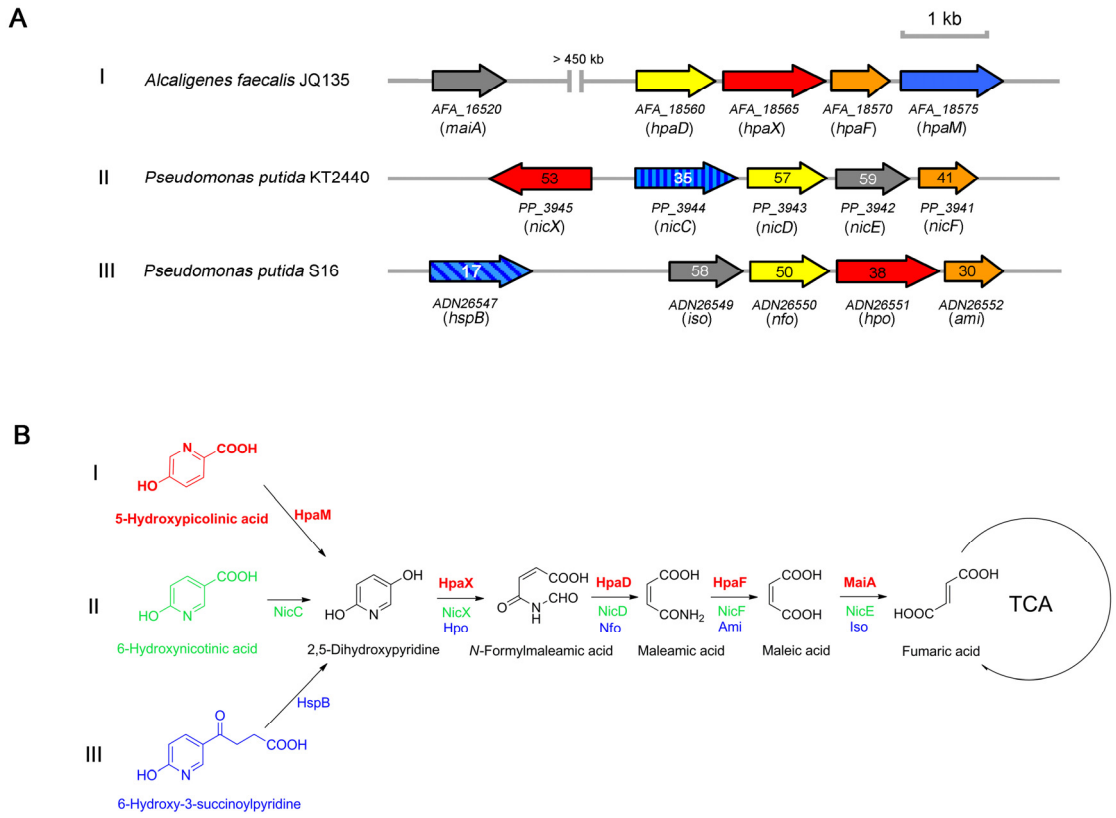


Fig. S3 The degradation gene clusters and pathways for 5HPA, 6HNA, and HSP .

(A) Organization of the *hpa* cluster for 5HPA degradation in *A. faecalis* JQ135 (I), the *nic* cluster for nicotinic acid degradation in *P. putida* KT2440 (II), and the *nic2* cluster for nicotine degradation in *P. putida* S16 (III). Numbers within arrows indicate the protein sequence identity with the orthologous from strain JQ135. (B) The degradation pathways for 5HPA (I), 6HNA (II), and HSP (III). HpaM: 5-hydroxypicolinic acid 2-monooxygenase; HspB: 6-hydroxy-3-succinoylpyridine 3-monooxygenase; NicC: 6-hydroxynicotinate 3-monooxygenase; HpaX, Hpo, and NicX: 2,5-dihydroxypyridine 5,6-dioxygenase; HpaD, Nfo, and NicD: *N*-formylmaleamate deformylase. HpaF, Ami, and NicF: maleamate amidohydrolase; MaiA, Iso, and NicE: maleic acid *cis-trans* isomerase. TCA, tricarboxylic acid cycle.

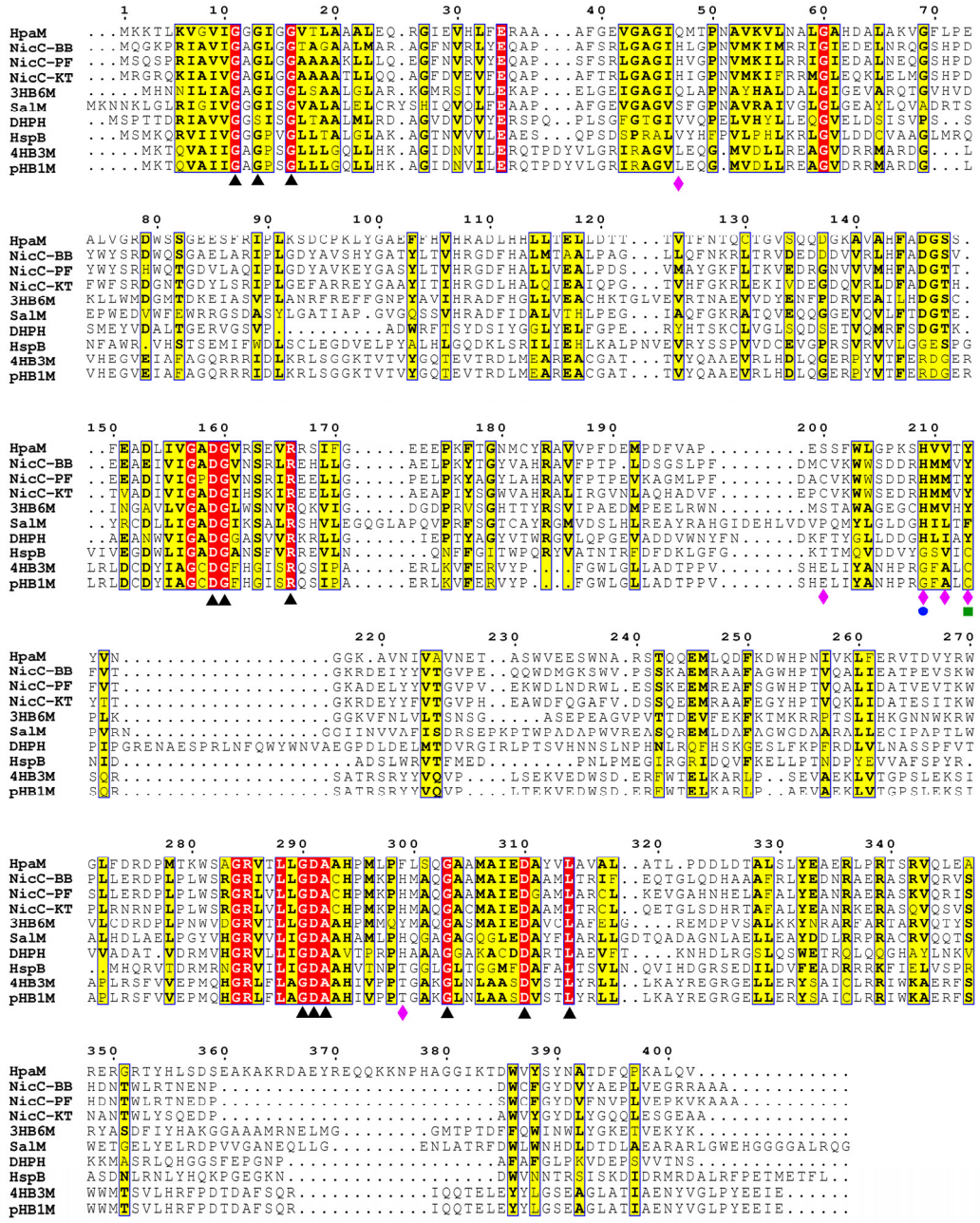


Fig. S4 Sequence alignment of HpaM with related flavin-dependent monooxygenases searched against the UniProtKB/SwissProt database. Identical residues are highlighted in red. Conserved residues are highlighted in yellow. The motifs for FAD binding domains were indicated by solid triangles (▲), including GXGXXG, DGX_5R, and $GDAX_{10}GX_6DX_3L$. The putative 6HNA binding sites in NicC of *P. putida* KT2440,

His47, Cys202, His211, Met213, Tyr215, His302 (NicC numbering) were indicated by magenta diamond (◆). The putative general base His209 and Tyr213 (HpaM numbering) is indicated with blue circle (●) and green rectangle (■), respectively. The enzyme names, UniProtKB/SwissProt accession numbers, and sequences identities used in the alignment were list below. **NicC-BB**, 6-hydroxynicotinate 3-monooxygenase from *Bordetella bronchiseptica* RB50 (sequence ID: A0A0H3LKL4; 34.2% identity); **NicC-PF**, 6-hydroxynicotinate 3-monooxygenase from *Pseudomonas fluorescens* TN5 (sequence ID: P86491; 33.1% identity); **NicC-KT**, 6-hydroxynicotinate 3-monooxygenase from *Pseudomonas putida* KT2440 (sequence ID: Q88FY2; 31.4% identity); **3HB6M**, 3-hydroxybenzoate 6-monooxygenase (sequence ID: Q9F131; 30.7% identity); **SalM**, salicylate 1-monooxygenase (sequence ID: P23262; 27.9% identity); **DHPH**, 2,6-dihydroxypyridine 3-monooxygenase (sequence ID: Q93NG3; 18.9% identity); **HspB**, 6-hydroxy-3-succinoylpyridine 3-monooxygenase (sequence ID: F8G0M4; 16.9% identity); **4HB3M**, 4-Hydroxybenzoate 3-monooxygenase (sequence ID: P20586; 16.9% identity); ***p*HB1M**, p-Hydroxybenzoate monooxygenase (sequence ID: P00438; 16.6% identity).

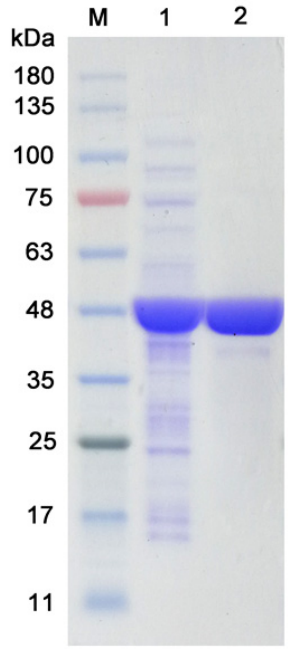


Fig. S5 SDS-PAGE analysis of protein markers (lane M), cell extracts of induced *E. coli* BL21(DE3) containing pET-*hpaM* (lane 1), and the purified HpaM (lane 2).

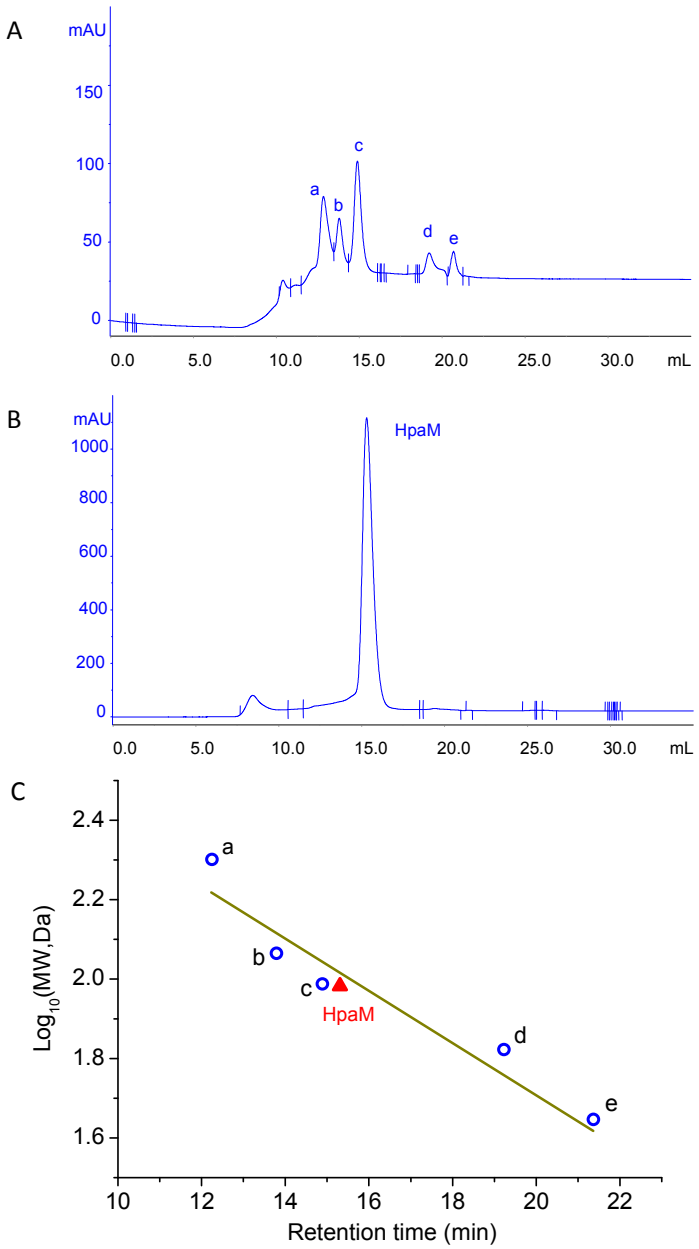


Fig. S6 Gel filtration of HpaM. (A) The peak volume of native molecular mass of standard proteins. (B) The peak volume of native 6×His-tagged HpaM (15.31 mL). The theoretical value of monomer 6×His-tagged HpaM is 45.2 kDa. (C) The calibration line calculated from A and B.

The standard proteins include: myosin (a, 200 kDa, 12.85 mL), *β*-galactosidase (b, 116 kDa, 13.79 mL), phosphorylase b (c, 97 kDa, 14.89 mL), bovine serum albumin (d, 66 kDa, 19.23 mL), ovalbumin (e, 45 kDa, 20.69 mL).

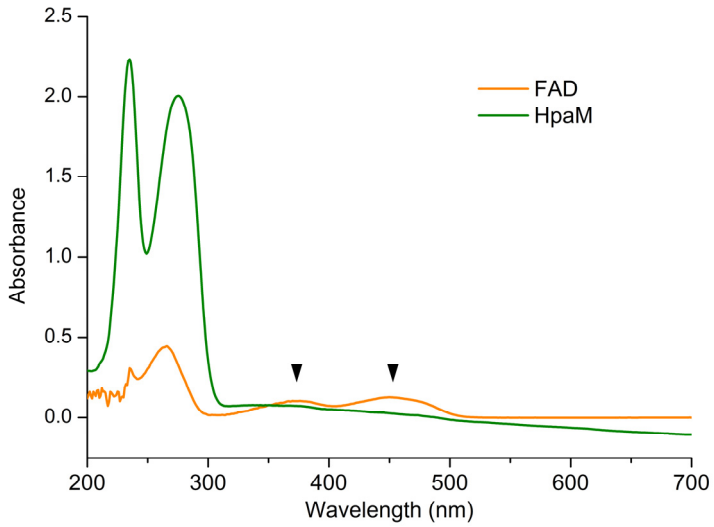


Fig. S7 Absorbance spectra of the purified HpaM and authentic FAD.

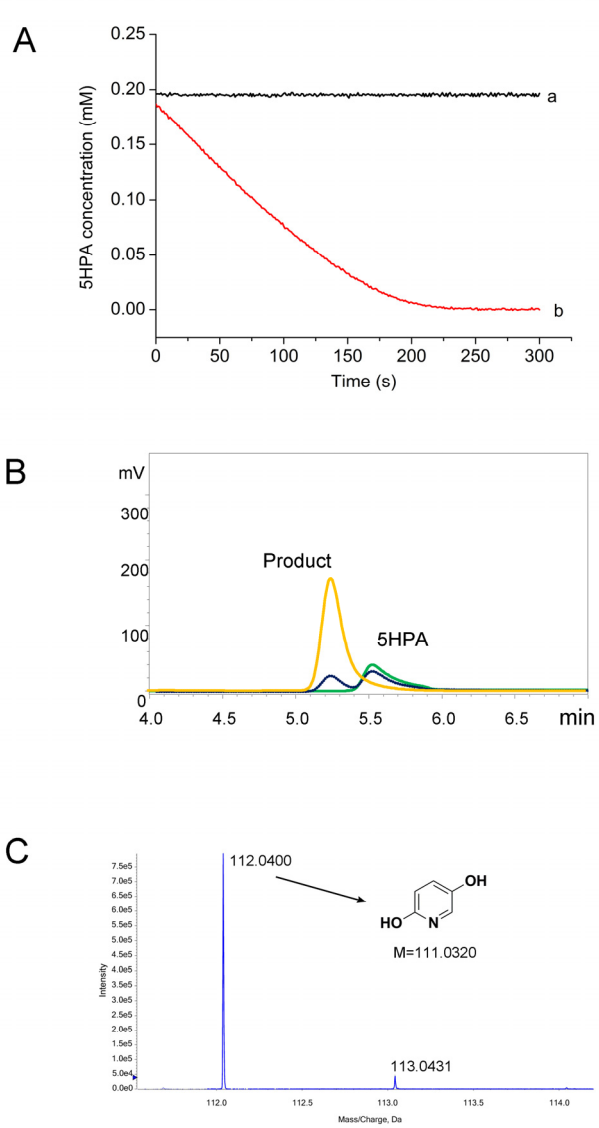
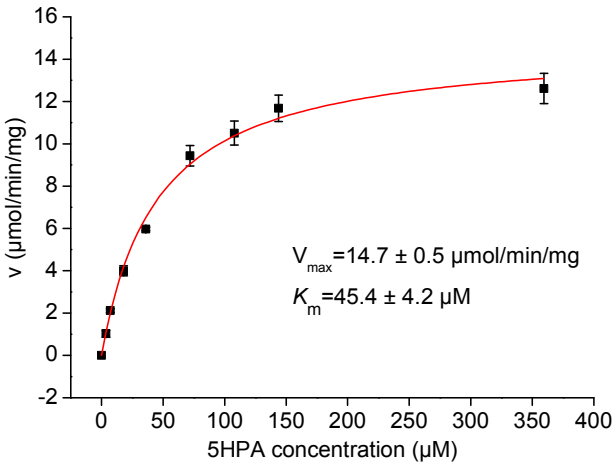


Fig. S8 Degradation of 5HPA by HpaM. (A) Time course of 5HPA rapid degradation. **a**, the reaction mixture contained 0.2 mM FAD, 0.5 mM NADH, 0.2 mM 5HPA and 4 μg purified HpaM in 1 mL 50 mM PBS pH 7.0. **b**, negative control, the reaction mixture same with a but omitted FAD. (B) HPLC analysis of 5HPA degradation and 2,5DHP formation. The green, dotted black, and orange lines indicate the start, middle (50 s), and end (300 s) of the enzymatic reaction, respectively. The samples were monitored at 310 nm. (C) LC/MS spectrum of product 2,5DHP.

A



B

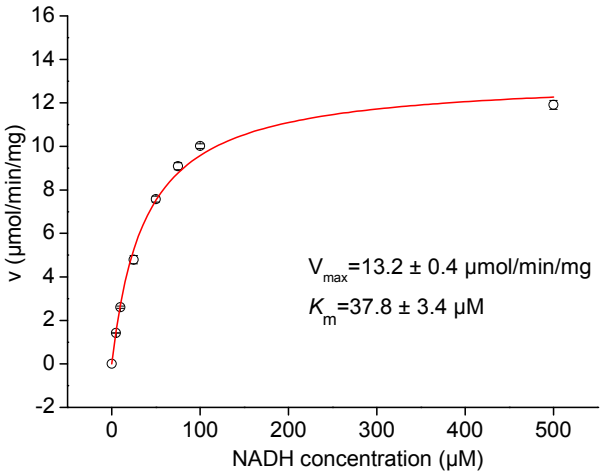


Fig. S9 The kinetic curves of HpaM for 5HPA (A) and NADH (B). Data were shown in means ± S.E.M.

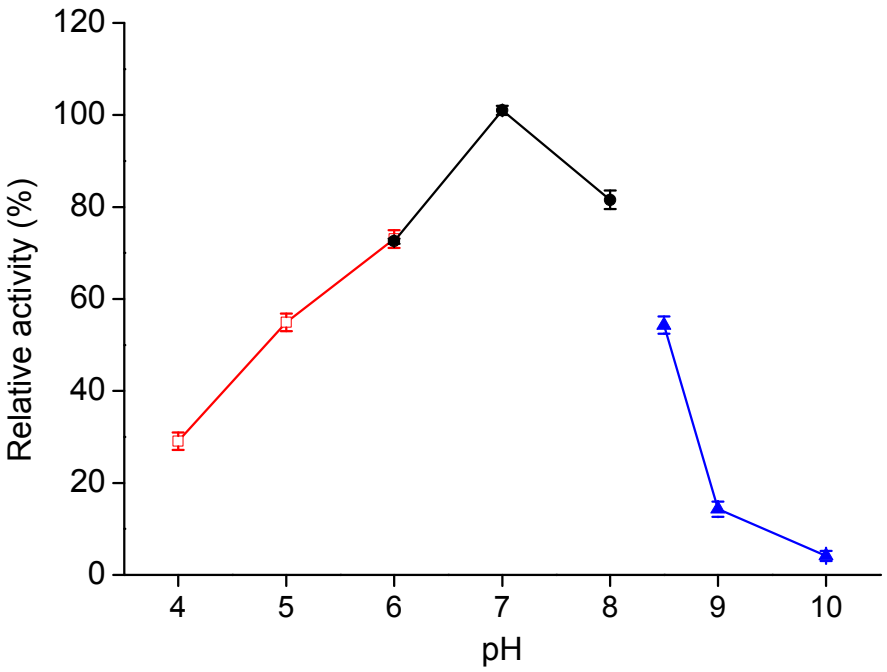


Fig. S10 Effects of pH on HpaM activities. The relative activity was calculated by assuming that the activity observed at 25 ºC and pH 7.0 was 100%. Red, pH 4.0 to 6.0 (citric acid-sodium phosphate). Black, pH 6.0 to 8.0 (KH_2PO_4-K_2HPO_4 buffer). Blue, pH 8.5 to 10.0 (glycine-NaOH).

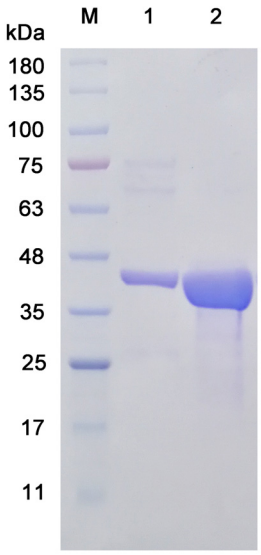


Fig. S11 SDS-PAGE analysis of protein markers (lane M), cell extracts of induced *E. coli* BL21(DE3) containing pET-*hpaX* (lane 1), and the purified HpaX (lane 2).

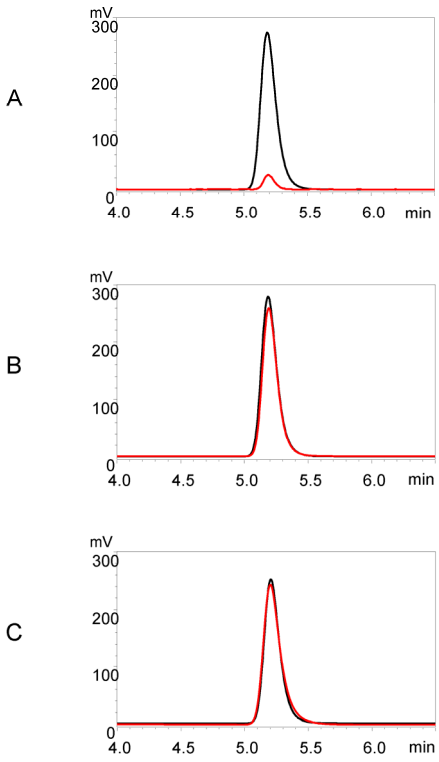


Fig. S12 HPLC analysis of 2,5DHP degradation by purified HpaM. (A) The reaction mixture contained 1.0 mM 2,5DHP, 1 mM Fe^{2+}, and 10 ng purified HpaX protein in 1.0 mL 50 mM PBS buffer (pH 7.0). (B) The reaction was performed without Fe^{2+}. (C) The reaction was performed under anaerobic conditions. The black and red lines indicated the start and end of the enzymatic reactions, respectively.

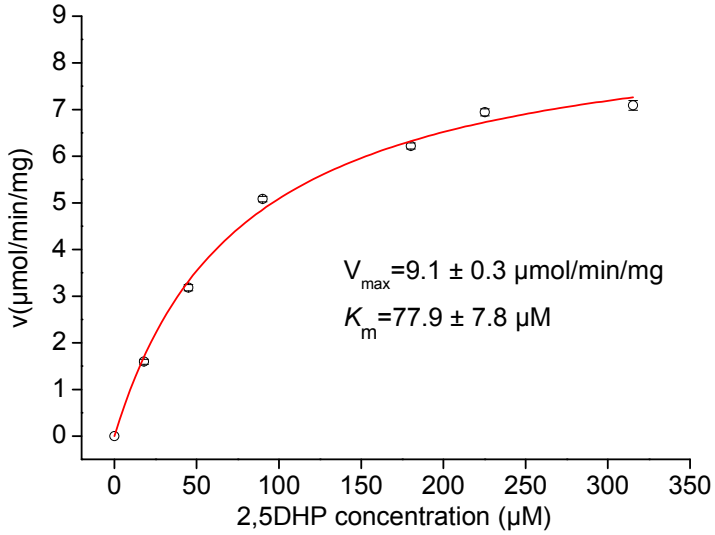


Fig. S13 The kinetic curves of HpaX for 2,5DHP. Data were shown in means ± S.E.M.

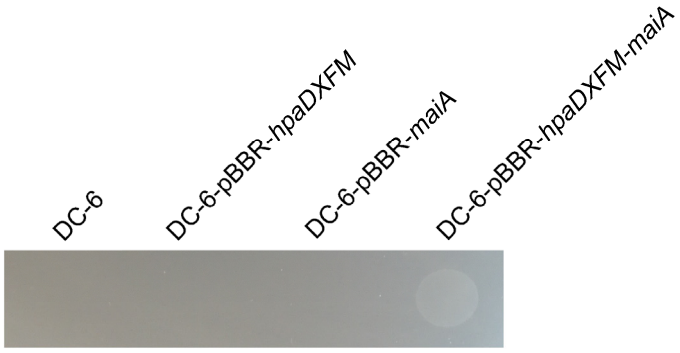
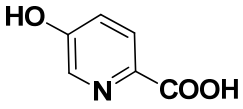
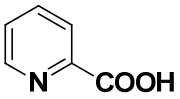
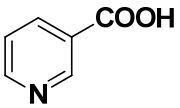
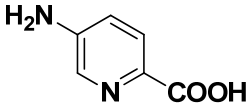
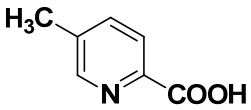
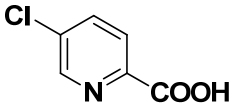
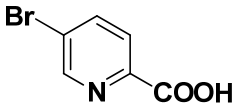
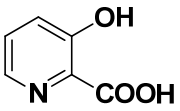
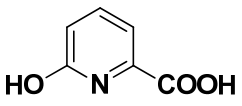
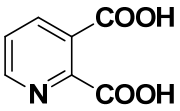
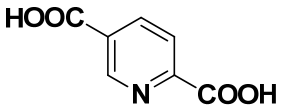
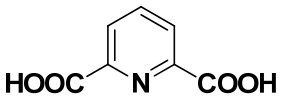
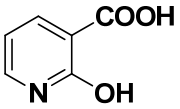
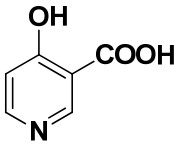
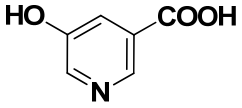
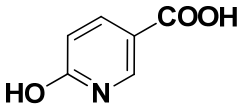
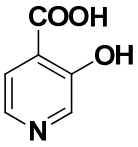
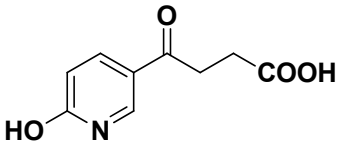


Fig. S14 Growth phenotype of the wild type *Sphingomonas wittichii* DC-6 and recombinant DC-6-pBBR-*hpaDXFM*, DC-6-pBBR-*maiA* and DC-6-pBBR-*hpaDXFM-maiA* on MSM plate with 1.0 mM 5HPA as sole carbon source.

Table S1 Substrate specificity of HpaM of *A. faecalis* JQ135.

Substrate		Activity
5-Hydroxypicolinic acid (5HPA)		100%
Picolinic acid (PA)		No activity
Nicotinic acid (NA)		No activity
5-Aminopicolinic acid		No activity
5-Methylpicolinic acid		No activity
5-Chloropicolinic acid		No activity
5-Bromoopicolinic acid		No activity
3-Hydroxypicolinic acid		No activity
6-Hydroxypicolinic acid		No activity
2,3-Pyridinedicarboxylic acid		No activity
2,5-Pyridinedicarboxylic acid		No activity

2,6-Pyridinedicarboxylic acid		No activity
2-Hydroxynicotinic acid		No activity
4-Hydroxynicotinic acid		No activity
5-Hydroxynicotinic acid		No activity
6-Hydroxynicotinic acid		No activity
3-Hydroxyisonicotinic		No activity
6-Hydroxy-3-succinoylpyridine (HSP)		No activity