Designing an Artificial Pathway for the Biosynthesis of a Novel Phenazine *N*-oxide in *Pseudomonas chlororaphis* HT66

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The supporting information including:

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Gene	DNA sequences
phzS	CGACACCGCTGCGCCGGCGTTTCATGGCGGATAACCGCAAGCGGTTATTCGCCCTACGC GGCCTTGGAGCCCATCTAACCGCACGCGGTCATGCGTACCGCGGCCTCGGAGCCGGTT CGTAGGGCGAATGACGCCACCGGCGTTATCCGCCGCTGCGCCGACGTTTCATCGCGGT AAACGGTCATCCATCCCAGCCGAACCCCCATCGATTCGAACACTCGAGAAAAGGAAGC ACCC **ATG**AGCGAACCCATCGATATCCTCATCGCCGGCGCCGGCATCGGCGGCCT CAGTTGCGCCCTGGCCCTGCACCAGGCCGGCATCGGCAAGGTCACGCTGC TGGAAAGCAGCAGCGAGATACGCCCCCTTGGCGTCGGCATCAATATCCAG CCGGCGGCGGTCGAGGCCCTTGCCGAACTGGGCCTCGGCCCGGCGCTGGC GGCCACCGCCATCCCCACCCACGAGCTGCGCTACATCGACCAGAGCGGCG CCACGGTATGGTCCGAGCCGCGCGGGGTGGAAGCCGGCAACGCCTATCCG CAGTACTCGATCCATCGCGGCGAACTGCAGATGATCCTGCTCGCCGCGGT GCGCGAGCGCCTCGGCCAACAGGCGGTACGCACCGGTCTCGGCGTGGAGC GTATCGAGGAGCGCGACGGCCGCGTGCTGATCGGCGCCCGCGACGGACAC GGCAAGCCCCAGGCGCTCGGTGCCGATGTGCTGGTCGGCGCCGACGGTAT CCATTCGGCGGTCCGCGCGCACCTGCATCCCGACCAGAGGCCGCTGTCCC ACGGTGGGATCACCATGTGGCGCGGCGTCACCGAGTTCGACCGCTTCCTC GACGGCAAGACCATGATCGTCGCCAACGACGAGCACTGGTCGCGCCTGGT CGCCTATCCGATCTCGGCGCGTCACGCGGCCGAAGGCAAGTCGCTGGTGA ACTGGGTGTGCATGGTGCCGAGCGCCGCCGTCGGCCAGCTCGACAACGAG GCCGACTGGAACCGCGACGGGCGCCTGGAGGACGTGCTGCCGTTCTTCGC CGACTGGGACCTGGGCTGGTTCGACATCCGCGACCTGCTGACCCGCAACC AGTTGATCCTGCAGTACCCGATGGTAGACCGCGATCCGCTGCCGCACTGG GGCCGGGGACGCATCACCCTGCTCGGCGACGCCGCCCACCTGATGTATCC GATGGGCGCCAACGGCGCTTCGCAAGCAATCCTCGACGGCATCGAGCTGG CCGCCGCGCTGGCGCGCAACGCCGACGTGGCCGCAGCCCTGCGCGAATAC GAAGAAGCGCGGCGGCCGACCGCCAACAAGATCATCCTGGCCAACCGAG AACGGGAAAAAGAGGAATGGGCCGCGGCTTCGCGACCGAAGACCGAGAA GAGCGCGGCGCTGGAAGCGATCACCGGCAGCTACCGCAACCAGGTGGAA CGGCCACGC**TAG**
phzO	GTACCGAGATAAACATGCTTTGAAGTGCCTGGCTGCTCCAACTTCGAACTC ATTGCGCGAACTTCAACACTTATGACATCCGGTCAACATGAGAAGAGTCC ATATGCGAAAGAACGCGTATTCGAAATACCAAACAGAGAGTCCGGATCAC CAAAGTGTGTAACGACATTAATTCCTATCTGAATCTTATAGTTGCTCTAGA ACGTTGTCCTTGACCCAGCGATAGACATCGGGCCAGAGACGACACAAACA AAGTTAGACATTACTGAGGCTGCTACC**ATG**CTAGATCTTCAAAACAAGCG TAAATATCTGAAAAGTGCAGAATCCTTCAAAGCTTCACTGCGTGATGACC GCACTGTTATTTATCAAGGCCAAGTTGTTGAGGATGTGACTACACACTTCT

	CTACGGCTGGAGGCATATCGCAAGTTGCAGAAATCTACGAAGAACAATTC AGCGGTGAACACGACGACATTCTGACTTACGTACGCCCCGACGGTTACCT GGCCTCTTCTGCCTATATGCCCCCTAGAAACAAAGAAGACTTGGCGTCGCG ACGCCGCGCAATCACGTACGTCTCGCAAAAAACCTGGGGCACCCACTGCC GCAACCTGGACATGATCGCCAGCTTCACCGTCGGCATGATGGGATATCGG CCGACATTCAGGAAAAAATGCCCTGAGTACGCAGAAAACATTACCGAATA CCATGACTACGCCGAGCGCAACAGCCTGTATTTGTCTGAGGCCATTGTTGA TCCACAGGGCTATCGGGCACGTACCCACGGCACCGACCTCAACCTGCCGC CGCCCGATCGTGCCGTGATGAGGATCAACAAGCAGAACGCCGAGGGCATC TGGATCAGCGGCGTCAAAGGCGTGGGCACGGTAGCACCGCAGTCCAATGA AATATTTGTTGGCAGCTTGTTCCCCGCAGCGCCCAACGAGTCATTCTGGGC TTACGTCCCTGCCGATGCGCCGGGGGTGAAGATTTTTGCCGAGAGATTGT CTCCCAGCCTCACGCCAGCGCCTATGACCACCCGCTCATATCCAAAGGTGA AGAAGCCGAGGCGATGGTGGTATTCGATAACGTGTTCATTCCACGCTGGC GAATCATGGCGGCGAACGTGCCGGAACTGGCCAACGCCGGCTTCTTCAGC CTGTGGACCTCATACAGCCATTGGTACACGCTCGTGCGCCTGGAAACCAA GGCTGACCTGTATGCCGGACTGGCCAAGGTGATCATGGAAGTCCTGGGCC TTGAGGGGATTGCGGTGGTTC
	GCCAGCGGGTCAGCGAAATAGTGCAGCTTGCGGAAATACTCAAAGGCATG TGCATCGCCTCCATCGAAACGGCCGAGATGTCCGAAGGCGACATATTGCT GCCTGGCCCCAACGCACTGGCCGCCGGAAGGATTTTTGCCATGGAGAAAT TGCCTCGGGTGCTGCATTTGCTCAGAGAGCTGTGCGGACAGGGCTTGATC CTCAGGTTCAACGAGAAAGACTTGGCCACCGACGCCGCCTTTGGCCAGAA GTTCTCCTGGTTTCTTGACACGCAAAGCGTGGGCGCCAGAGAGAAGAACC TGCTGATGAATCTGGTGTGGGACGTGGCTGCCAGTGAGCACTCCACACGT GCATTGGTGTTTGAAGAACAGCACGCACTCAGCGAGCCCCTGCTGCGCGA TAGCCTGGTGCTGGACTACGACTACCGCAAAAGCACAAGCCTGATACGCC GTATGGTGGGGCTCAACGCCAAA**TAG**
naphzNO1	**GTG**ACCAACGCGAAGAACACCGATCTCGACGCGATCGTCGTCGGTGCCGG GTTCGCCGGCATCTACGCGCTGCACAAGCTCCGCAACGAACTGGGTCTGTC CGTCCGGGCCTTCGACAAGGCGGGCGGGGTCGGGGGCACCTGGTACTGGA ACCGCTACCCCGGCGCCATGTCCGACAGCGAGGGCTTCATCTACCAGTACT CCTTCGACCGCGACCTGCTGCGGGAGTGGACCTGGAAGAAGCGCTACCTG TCCCAGGCGGAGATCCTGGGCTACCTGGAGGCGGTCGTGGAGCGGCACGA CCTCGCCAGGGACATCCAGCTCAACACCGGCGTCGAGACGCTGGTCTACG ACGAGGCCGCCGCCCTGTGGACCGCGACCACCAGTGACGGCCAGACCCTC ACCGCCCGCTACGTGGTGACCGCCCTCGGACCGCTGTCCACCTCCCACTTC CCCGACTTCAAGGGCCGCGACAGCTTCCGGGGCCGCCTGGTCCACACCGG CTCCTGGCCCGACGACCTCGACATCGAGGGCAAGAGGGTCGGCGTCATCG GCACCGGCTCCACCGGAACCCAGTTCATCTGCGCGGCCTCGAAGGTGGCC GGGCAGCTCACCGTGTTCCAGCGGACCCCCCAGTACAACGTGCCCTCGGG CAACGCCGAGGTGGACGAGGCCTACTTCACCGACCTGCGCGGCCGCTACG ACCAGGTCTGGGAGCAGGCCAAGAAGTCCCGCGTGGCGTGCGGCTTCGAG GAGAGCGAGATCGCCGCGATGAGCGTCTCCGAGGAGGAGCGCCGACGCG

	TCTTCCAGGAGAACTGGGACCGGGGCAACGGCTTCCGCTTCATGTTCGGC ACCTTCTCCGACATCATCTTCGACCCCGAGGCCAACGAGGCGGCGGCCGA CTTCATCCGGTCCAAGATCCGGGAGATCGTCAAGGACCCGGAGACCGCCC GCAAGCTCCAGCCGACCGACTACTACGCCAAGCGCCCGGTCTGCAACGAG GACTACTACGAGTCCTACAACCGGGACAACGTCAGCCTGGTGAGCCTCAA GGAGACCCCGATCCGGGAGTTCACGCCCACGGGGATCGTGACCGAGGACG GGGTGGAGCACGAGCTGGACATCGTCGTCTTCGCGACCGGTTTCGAGGCC GTCGAGGGCAGCTACCGGCAGATGGAGATCCGCGGCCGGGGCGGCGTGA CCATCGAGGAGCACTGGGGGGACACGCCCGCCAGCTACCTCGGGGTCAAC GTCTCGGGCTTCCCCAACATGTTCATGGTCTACGGCCCCAACAGCGTCTTC AGCAACCTGCCCACGGCCATCGAGACCCAGGTCGAGTGGATCACCGACCT GGTCCGGATGATGGAGGAGCGCGACCTGACCTCCATCGAGCCGACCCCGG AGGCGGAGGAGGGCTGGACCGAGCTGTGCACGCAGATCGCCGACCACTCC CTGTTCCCCAAGGTCAACTCCTGGATCTTCGGGGCGAACATCCCGGGCAA GAAGAAGCGGGTCCTGTTCTACTTCGCGGGGCTCGGCAACTACCGCCAGA AGCTCGGTGACGTGGCCGCGGCCGACTACGAGGGCTTCATGCTCAAGGGC AACCCCTCGGTGGTGACCGCC**TGA**
P_{PHZ}	TACGAATCCCATCCCAACTGCTGCCCTAACTCCATTTTGAGCACCACTAAA GTTGAAAACAGGCCGTTAGACTAGACCAACCTGAACCCCTGTCAACAAGC AAAACCACAGAGTCATAGACCGCTTCGTACTAGCACTAGATTTCCTGCATC CGCCCCAACTAACTGGCCATAGGACATTGCATCCTGACGATGTTAGTAAG CCATTAGTGCCTCCCAGCCGACGAATGAACCTGCCTCTTCACAATAATGCA ACAGTTCACCCGGTGTGACTGCAGCCAGCTCGTTCACGCCCTAATCACTAC AAGATCTGGTAGTTCCAGCCCCAAGAAATGCAGGTGTATAAACAACACCC GTTCAGCACCGCCACGAAGGAACAATAGTTAAAAACTGTAAATTATCTAC CCGCACCAAATTAACTAACCATTTACTTTCAAAAAATACCAGCCCAACTAA GGAGGATGCCGCC

*The red capital letter indicates promoter of the gene *phzS*.

Table S2. ^{1}H (MeOH) and ^{13}C (MeOH) spectra data for 1-hydroxyphenazine *N*'10-oxide recorded by 600 MHz NMR spectrometer.

Position	δ_H (J in Hz)	δ_C	HMBC	HSQC (ppm)	COSY
1		153.5	H-3,4		
2	8.7/1.0	120.2	H-4	7.66, dd	H-3
3	8.7/7.8	134.0		7.78, dd	H-4,2
4	7.8/1.0	113.3	H-2	7.07, dd	H-3
4a		147.9	H-3		
5a		146.6	H-9,7		
6	8.9	130.8	H-8,9a	8.16, d	H-7
7		133.5	H-9	7.95, m	H-8,6
8		132.5	H-6	7.88, m	H-9,7
9	8.9	119.1	H-7	8.62, d	H-8
9a		134.2	H-6,8		
10a		126.4	H-4,2		

Table S3. Primers used in this study.

primes	Sequence 5' → 3'	application
phzS-F1	ccggggatcctctagaCGAAGTATCAGGCAATGGCGTCA	
phzS-R1	TCAGGGCTGCAGGCGGGT	
phzS-F	*ACCCGCCTGCAGCCCTGA*CGACACCGCTGCGCCGGCGT	*phzS* replaced gene *phzH*
phzS-R	CTAGCGTGGCCGTTCCACCTGGTTG	
phzS-F2	*GTGGAACGGCCACGCTAG*TACGAGCCTGAGGGAGCCAC	
phzS-R2	ggccagtgccaagcttCGCCATATGCTGGTCGGC	
phzO-F1	acatgattacgaattaGCCGCTGTTGGGTAAAGG	
phzO-R1	GTTTATCTCGGTACCTCAGGGTTGCAAACGCC	
phzO-F	*GGTACCGAGATAAAC*ATGCTTTGAAGTGC	*phzO* replaced gene *phzH*
phzO-R	CTATTTGGCGTTGAGCCCCACCATA	
phzO-F2	*CTCAACGCCAAATAG*TACGAGCCTGAGGGAGCCACGGCAG	
phzO-R2	cgactctagaggatcaTGGCCGAACCACCCTTGC	
NaphzNO1-F1	ccggggatcctctagaTGCGCGAAGGGTAATGC	
NaphzNO1-R1	GCAAAGACTCCTGAGTTCAAGC	
NaphzNO1-PF	*GAACTCAGGAGTCTTTGC*TACGAATCCCATCCCAACTGC	*NaphzNO1* replaced gene *pykA* (PF, PR were used to clone PHZ promoter.
NaphzNO1-PR	GGCGGCATCCTCCTTAGTTG	
NaphzNO1-F	*ACTAAGGAGGATGCCGCC*GTGACCAACGCGAAGAACACC	
NaphzNO1-R	TCAGGCGGTCACCACCGA	
NaphzNO1-F2	*TCGGTGGTGACCGCCTGA*GCCACCTGACGCAACAATAAAG	
NaphzNO1-R2	ggccagtgccaagcttTGTTGTTGCTGGGTGAGGGTT	
PET-NaphzNO1-F	GGAATTCCATATG GTGACCAACGCGAAGAACACC	NaphzNO1 expression
PET-NaphzNO1-R	CCGCTCGAGTCAGGCGGTCACCACCGA	

*The lowercase indicates that the overlapped fragments were used to construct plasmids by the In-fusion method. The italic capital letter indicates the overlapped fragments between Upstream and downstream homologous arms. The red capital letter indicates enzyme cut site.

Figure S1. The alignment of protein sequence between NaphzNO1 and LaphzNO1.

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LaphzNO1     VIDNDKTHFDAIVIGTGFAGIYMLHKLRNELGLKVRAFDKASGVGGTWYWNKYPGARADT     60
NaphzNO1     VTNAKNTDLDAIVVGAGFAGIYALHKLRNELGLSVRAFDKAGGVGGTWYWNRYPGAMSDS     60
Consensus    v     t  daiv g gfagiy lhklrnelgl vrafdka gvggtwywn ypga  d

LaphzNO1     ESFVYRYSFDKETSEGWDWRNRYVDQPEMLGYLQAVVDRHGLAKDIQLKTGIDSAVFDEI    120
NaphzNO1     EGFIYQYSFDRDLLREWTWKKRYLSQAEILGYLEAVVERHDLARDIQLNTGVETLVYDEA    120
Consensus    e f y ysfd      w w  ry  q e lgyl avv rh la diql tg    v de

LaphzNO1     HHIWTLTTSTGELFTARYLVNAVGVLSKIVIPQIPGRDKFQGQIVHTGAWPADLSLEGKR    180
NaphzNO1     AALWTATTSDGQTLTARYVVTALGPLSTSHFPDFKGRDSFRGRLVHTGSWPDDLDIEGKR    180
Consensus       wt tts g   tary v a g ls    p   grd f g  vhtg wp dl  egkr

LaphzNO1     VGVIGTGSTGVQFICAASKLARHLTVFQRSAQFCVPAGSRQVSEAYVAEYKNNFEQIWDG    240
NaphzNO1     VGVIGTGSTGTQFICAASKVAGQLTVFQRTPQYNVPSGNAEVDEAYFTDLRGRYDQVWEQ    240
Consensus    vgvigtgstg qficaask a  ltvfqr  q  vp g   v eay         q w

LaphzNO1     IRNSRIACGFEESGVSAMSVSEEERQKVFEHHWEIGNGFRFMFGTFSDIAVDPAANQAAS    300
NaphzNO1     AKKSRVACGFEESEIAAMSVSEEERRRVFQENWDRGNGFRFMFGTFSDIIFDPEANEAAA    300
Consensus       sr acgfees   amsvseeer  vf   w  gngfrfmfgtfsdi  dp an aa

LaphzNO1     DFIRAKIRQIVKDPETARKLCPTDLYAKRPVCTNDYYETYNLPNVSLVSLPENPIKELTA    360
NaphzNO1     DFIRSKIREIVKDPETARKLQPTDYYAKRPVCNEDYYESYNRDNVSLVSLKETPIREFTP    360
Consensus    dfir kir ivkdpetarkl ptd yakrpvc  dyye yn  nvslvsl e pi e t

LaphzNO1     NGVLTEDGVEHKLDLLVFATGFETVEGSYNQMEIRGRGGETLQHHWKDAPSSYLGVATAG    420
NaphzNO1     TGIVTEDGVEHELDIVVFATGFEAVEGSYRQMEIRGRGGVTIEEHWGDTPASYLGVNVSG    420
Consensus     g  tedgveh ld  vfatgfe vegsy qmeirgrgg t   hw d p sylgv   g

LaphzNO1     FPNMFMVLGPNSAFSNLPPSIESQVEWIGELIGWAEGESTPVIETTREAEEAWTATCKEI    480
NaphzNO1     FPNMFMVYGPNSVFSNLPTAIETQVEWITDLVRMMEERDLTSIEPTPEAEEGWTELCTQI    480
Consensus    fpnmfmv gpns fsnlp  ie qvewi  l    e      ie t eaee wt  c  i

LaphzNO1     AAYTLFPKVKSWIFGENIDGRSSRVLFYFGGLAGYRAKLREVADADYEGFILHSDPSSMV    540
NaphzNO1     ADHSLFPKVNSWIFGANIPGKKKRVLFYFAGLGNYRQKLGDVAAADYEGFMLKGNPSVVT    540
Consensus    a   lfpkv swifg ni g   rvlfyf gl  yr kl  va adyegf l   ps
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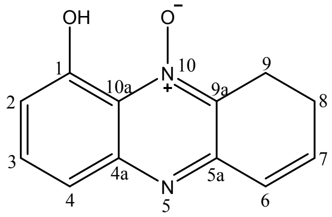
Figure S2. The MS/MS result of 1-hydroxyphenazine *N*’10-oxide.



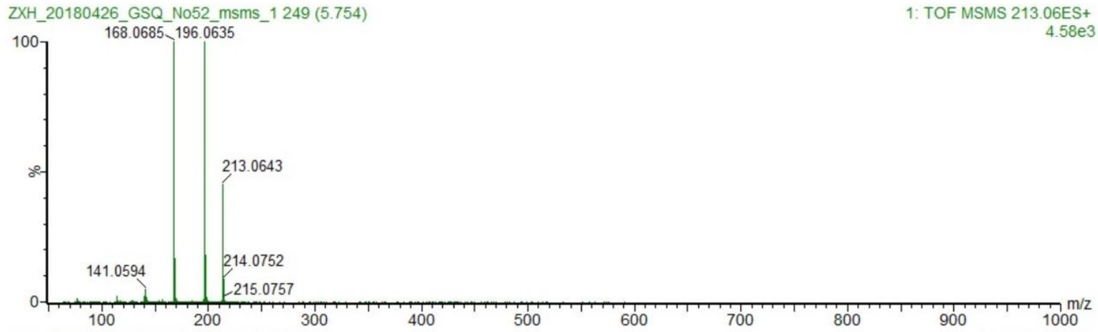


Figure S3. The [1]H NMR spectrum of 1-hydroxyphenazine *N*'10-oxide (MeOH, 600 MHz).

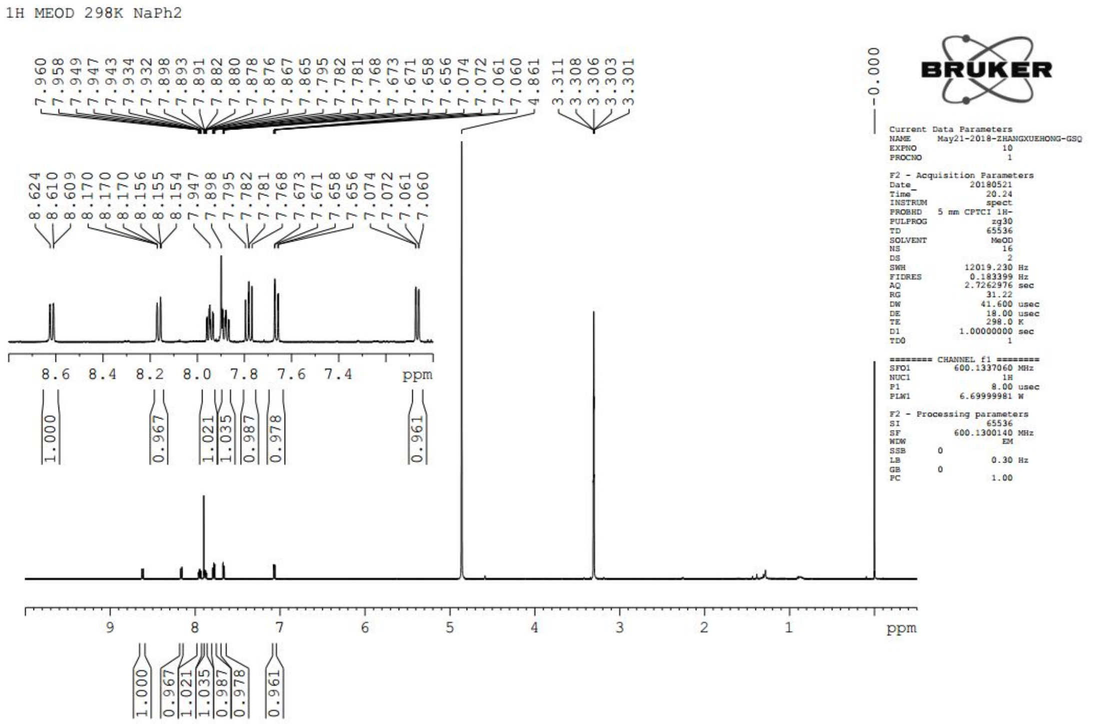
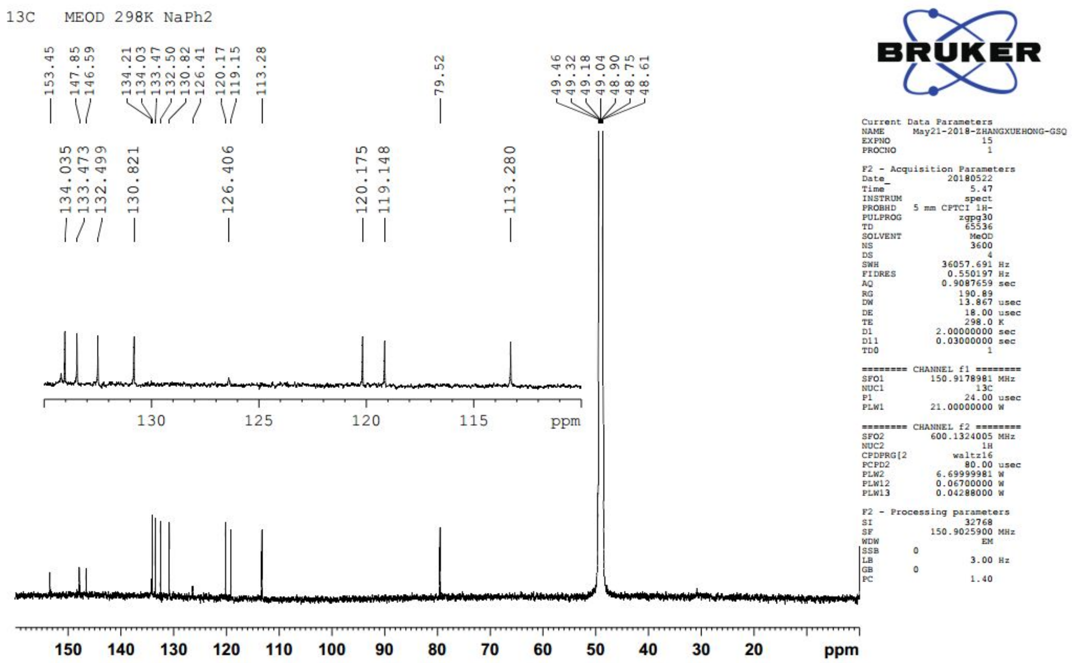


Figure S4. The ^{13}C NMR spectrum of 1-hydroxyphenazine *N*'10-oxide (MeOH, 151 MHz).



*The peak at ~78 ppm in ^{13}C-NMR was impurity signal interference.

Figure S5. The COSY spectrum of 1-hydroxyphenazine *N*'10-oxide (MeOH, 600 MHz).

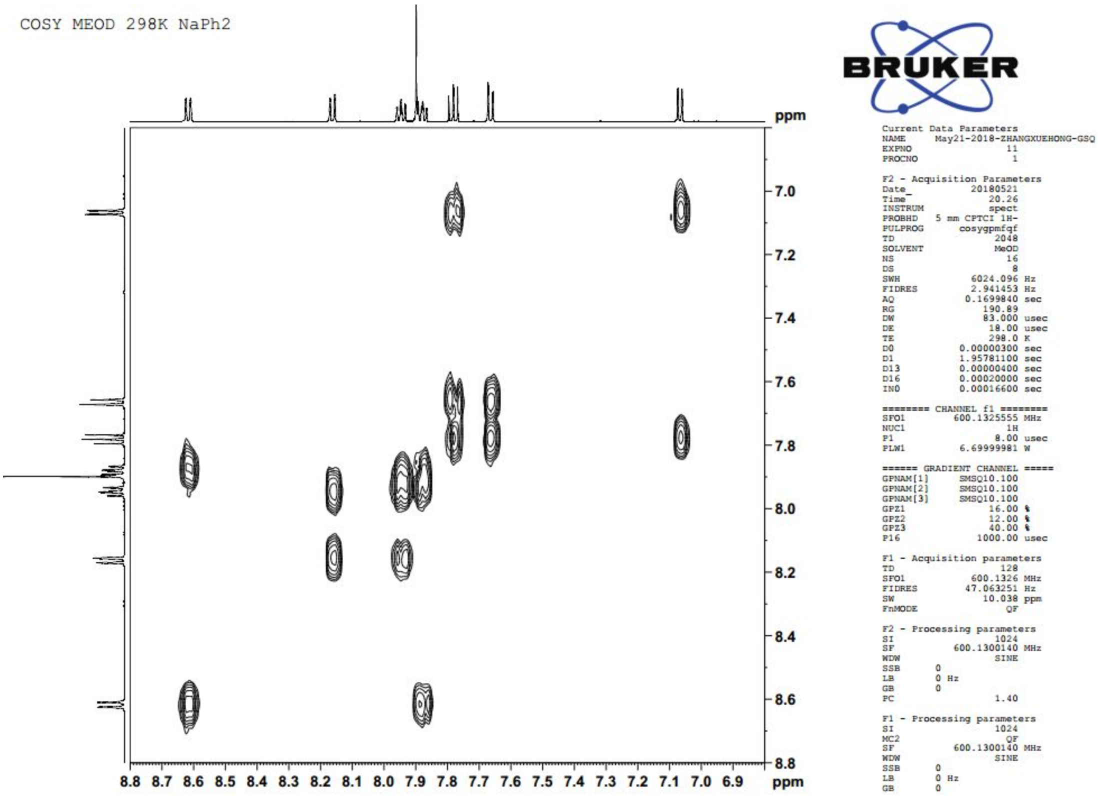
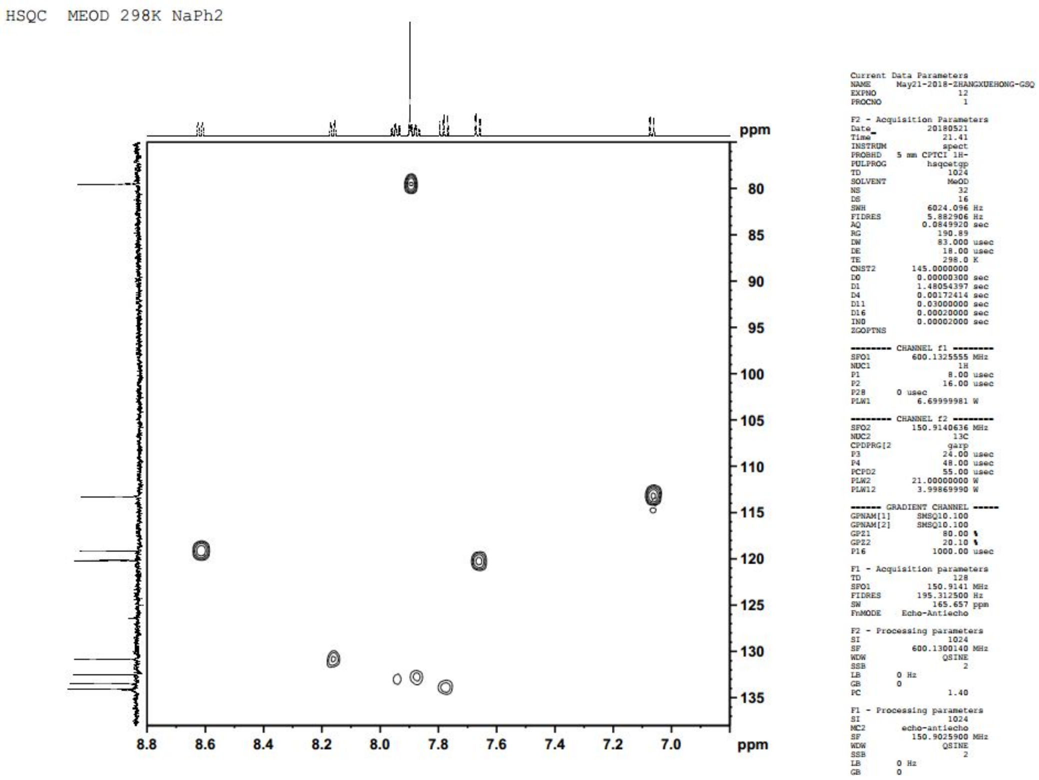


Figure S6. The HSQC spectrum of 1-hydroxyphenazine *N*'10-oxide (MeOH, 151 MHz).



*The peak at ~78 ppm in ^{13}C-NMR was impurity signal interference.

Figure S7. The HMBC spectrum of 1-hydroxyphenazine *N*'10-oxide (MeOH, 151 MHz).

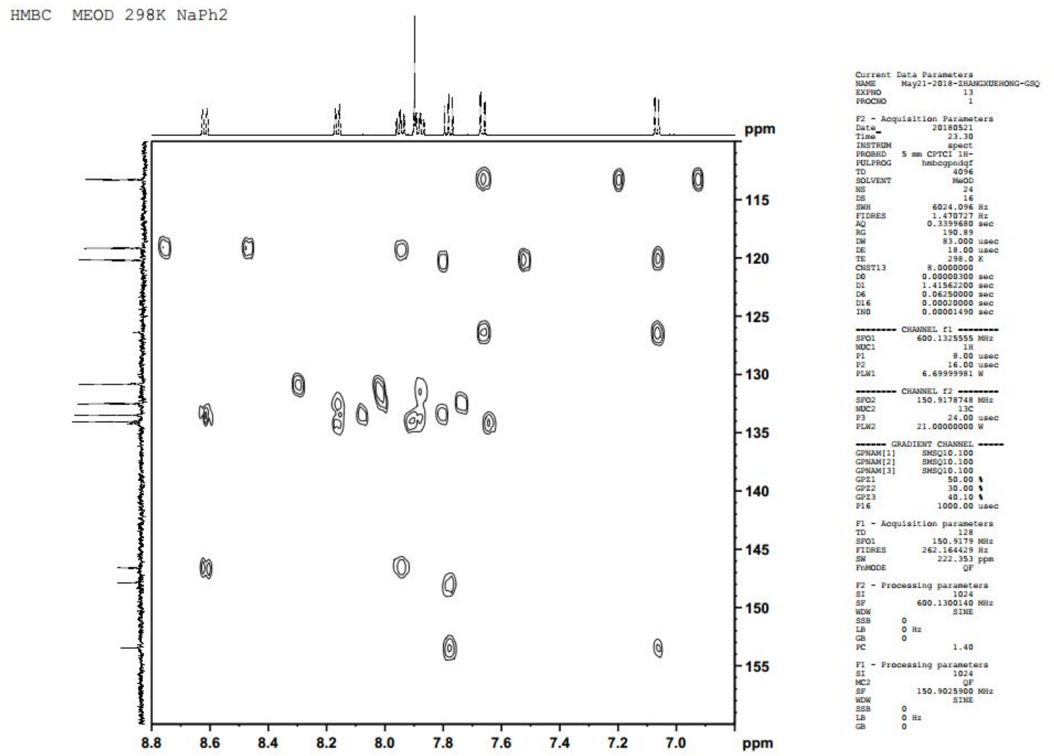


Figure S8. Culture profiles of 1-hydroxyphenazine *N*'10-oxide-producing *P. chlororaphis* strains. (A): HT66-SN; (B): P3-SN. (Data represent the mean ± SD from three independent cultures).