Supporting Information

Nosiheptide Biosynthesis Featuring a Unique Indole Side Ring Formation on a Characteristic Thiopeptide Framework

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Table S1. Bacterial strains and plasmids used in this study

Strain/Plasmid/Primer	Characteristic(s)	Reference
E. coli		
DH5α	Host for general cloning	Invitrogen
LE392	Host for constructing the genomic library	Promega
ET12567	Donor strain for conjugation between *E.coli* and *Streptomyces*	(*1*)
Streptomyces		
S. actuosus ATCC 25421	Wild type strain, nosiheptide producing	ATCC
SL4001	*ΔnosG* mutant, nosiheptide non-producing	This study
SL4002	*ΔnosM* mutant, nosiheptide non-producing	This study
SL4003	SL4002 derivative that contains pSL4020 for expressing *nosM* in trans, nosiheptide producing	This study
SL4004	*ΔnosH* mutant, nosiheptide non-producing	This study
SL4005	*ΔnosL* mutant, nosiheptide non-producing	This study
SL4006	*ΔnosN* mutant, nosiheptide non-producing	This study
SL4007	*ΔnosP* mutant, nosiheptide non-producing	This study
Plasmids		
pMD19-T	*E. coli* subcloning vector	TaKaRa
pOJ446	*E.coli-Streptomyces* shuttle vector for construction of the genomic library	NRRL 14791

pOJ260	*E.coli-Streptomyces* shuttle vector for gene inactivation, nonreplicating in *Streptomyces*	NRRL14785
pKC1139	*E.coli-Streptomyces* shuttle vector for gene inactivation, temperature sensitive replication in *Streptomyces*	(*2*)
pSET152	*E.coli-Streptomyces* shuttle vector for heterologous complementation, site-specific integration in *Streptomyces*	(*2*)
pSL4001	pOJ446-based *S. actuosus* genomic library cosmid	This study
pSL4002	pMD19-T derivative containing a 0.7 kb PCR product from *S. actuosus* genomic DNA	This study
pSL4003	pOJ260 derivative containing a 0.7 kb fragment, construct for *nosG* inactivation	This study
pSL4004	pMD19-T derivative containing a 2.2 kb PCR product from pSL4001	This study
pSL4005	pMD19-T derivative containing a 2.1 kb PCR product from pSL4001	This study
pSL4006	pKC1139 derivative containing a 4.3 kb fragment, construct for *nosM* in-frame inactivation	This study
pSL4007	pMD19-T derivative containing a 1.8 kb PCR product from pSL4001	This study

pSL4008	pMD19-T derivative containing a 2.0 kb PCR product from pSL4001	This study
pSL4009	pKC1139 derivative containing a 3.8 kb fragment, construct for *nosH* in-frame inactivation	This study
pSL4010	pMD19-T derivative containing a 2.0 kb PCR product from pSL4001	This study
pSL4011	pMD19-T derivative containing a 1.9 kb PCR product from pSL4001	This study
pSL4012	pKC1139 derivative containing a 3.9 kb fragment, construct for *nosL* in-frame inactivation	This study
pSL4013	pMD19-T derivative containing a 1.9 kb PCR product from pSL4001	This study
pSL4014	pMD19-T derivative containing a 1.8 kb PCR product from pSL4001	This study
pSL4015	pKC1139 derivative containing a 3.7 kb fragment, construct for *nosN* in-frame inactivation	This study
pSL4016	pMD19-T derivative containing a 1.9 kb PCR product from pSL4001	This study
pSL4017	pMD19-T derivative containing a 2.0 kb PCR product from pSL4001	This study
pSL4018	pKC1139 derivative containing a 3.9 kb fragment, construct for *nosP* in-frame inactivation	This study

pSL4019	pMD19-T derivative containing a 0.5 kb PCR product from pSL4001	This study
pSL4020	pSET152 derivative containing a 0.9 kb fragment, construct for △*nosM* complementation in trans	This study

Table S2. Primers used in this study for in-frame deletion of *nosM*, *nosH*, *nosL*, *nosN* and *nosP* in *S. actuosus*.

Gene	**Primers used in this study (5'-3')***
nosM	M-inf-Ls: CGACAAGCTTCAGGGAGCCGTTGAGGACG
	M-inf-La: GTGTCTAGACCACCGGAAACCCCTTCCATGTC
	M-inf-Rs: TCCTTCTAGAGGAGACGGCGCCCTGTACCG
	M-inf-Ra: TCGAGAATTCGGTGGAACGCGCTTTCGG
nosH	H-inf-Ls: CAAGCTTCCTCAACGGCTCCCTGAAGTCC
	H-inf-La: GTCTAGAGCGGGTGAGGGTGTCGGTCAG
	H-inf-Rs: TCTAGAACCGACCTGCTGCCCTTCCTCAC
	H-inf-Ra: CGAATTCCGAAGGTCATCGGGATCAGGC
nosL	L-inf-Ls: CAAGCTTACGCCCCGCAGGAAGAACC
	L-inf-La: TCTAGAATTGCCTGGGAGTTCTGCG

	L-inf-Rs: TCTAGACGTCGACCACTTCGTGAACC
	L-inf-Ra: GAATTCGTGGTCGAGGGCGATGG
nosN	N-inf-Ls: CAAGCTTCCGTGCACCAGGTAGACGG
	N-inf-La: GATCTAGAGAAAGGGATGTGCACATAGATCATC
	N-inf-Rs: TGTCTAGAGAGATGGCCATGTCCCAGC
	N-inf-Ra: CGAATTCGCAGATGCAGGATGTACCCC
nosP	P-inf-Ls: GGACAAGCTTTTCAAGTCCCACCAGAAGGGCC
	P-inf-La: GCGAGAATTCAAGCGGTCCCAGGACATCG
	P-inf-Rs: CGACGAATTCAACGTCACCTGGGACGTC
	P-inf-Ra: GGTTCTAGATTCACCCTGAAGACCACGCTGG

* Restriction sites for HindIII (AAGCTT), XbaI (TCTAGA) and EcoRI (GAATTC) are double-underlined

Inactivation of *nosG*, *nosM*, *nosH*, *nosL*, *nosN* and *nosP* in *S. actuosus*

To inactivate *nosG*, the 0.7 kb ThioF-ThioR PCR product P1 (0.7 kb internal fragment of *nosG*, ref. *3*) was initially cloned into pMD19-T, yielding pSL4002. The 0.7 kb HindIII-XbaI fragment was recovered from pSL4002 and then cloned into the same sites of pOJ260, giving the recombinant plasmid pSL4003.

To inactivate *nosM*, a 2.2 kb fragment amplified by PCR using the primers M-inf-Ls/M-inf-La and a 2.1 kb fragment amplified by using the primers M-inf-Rs/M-inf-Ra were initially cloned into pMD19-T, giving pSL4004 and pSL4005, respectively. After sequencing to confirm the fidelity,

the 2.2 kb HindIII/XbaI and 2.1 kb XbaI/EcoRI fragments were recovered and co-ligated into the HindIII/EcoRI site of pKC1139, yielding the recombinant plasmid pSL4006, in which a 144 bp in-frame coding region (corresponding to AA_3-AA_{50} of the deduced product NosM) of *nosM* was deleted.

To inactivate *nosH*, a 1.8 kb fragment amplified by PCR using the primers H-inf-Ls/H-inf-La and a 2.0 kb fragment amplified by using the primers H-inf-Rs/H-inf-Ra were initially cloned into pMD19-T, giving pSL4007 and pSL4008, respectively. After sequencing to confirm the fidelity, the 1.8 kb HindIII/XbaI and 2.0 kb XbaI/EcoRI fragments were recovered and co-ligated into the HindIII/EcoRI site of pKC1139, yielding the recombinant plasmid pSL4009, in which a 1761 bp in-frame coding region (corresponding to AA_{26}-AA_{612} of the deduced product NosH) of *nosH* was deleted.

To inactivate *nosL*, a 2.0 kb fragment amplified by PCR using the primers L-inf-Ls/L-inf-La and a 1.9 kb fragment amplified by using the primers L-inf-Rs/L-inf-Ra were initially cloned into pMD19-T, giving pSL4010 and pSL4011, respectively. After sequencing to confirm the fidelity, the 2.0 kb HindIII/XbaI and 1.9 kb XbaI/EcoRI fragments were recovered and co-ligated into the HindIII/EcoRI site of pKC1139, yielding the recombinant plasmid pSL4012, in which a 1131 bp in-frame coding region (corresponding to AA_8-AA_{385} of the deduced product NosL) of *nosL* was deleted.

To inactivate *nosN*, a 1.9 kb fragment amplified by PCR using the primers N-inf-Ls/N-inf-La and a 1.8 kb fragment amplified by using the primers N-inf-Rs/N-inf-Ra were initially cloned into pMD19-T, giving pSL4013 and pSL4014, respectively. After sequencing to confirm the fidelity, the 1.9 kb HindIII/XbaI and 1.8 kb XbaI/EcoRI fragments were recovered and co-ligated into the

HindIII/EcoRI site of pKC1139, yielding the recombinant plasmid pSL4015, in which a 1113 bp in-frame coding region (corresponding to AA_{15}-AA_{385} of the deduced product NosN) of *nosN* was deleted.

To inactivate *nosP*, a 1.9 kb fragment amplified by PCR using the primers P-inf-Ls/P-inf-La and a 2.0 kb fragment amplified by using the primers P-inf-Rs/P-inf-Ra were initially cloned into pMD19-T, giving pSL4016 and pSL4017, respectively. After sequencing to confirm the fidelity, the 1.9 kb HindIII/EcoRI and 2.0 kb EcoRI/XbaI fragments were recovered and co-ligated into the HindIII/XbaI site of pKC1139, yielding the recombinant plasmid pSL4018, in which a 912 bp in-frame coding region (corresponding to AA_{10}-AA_{314} of the deduced product NosP) of *nosP* was deleted.

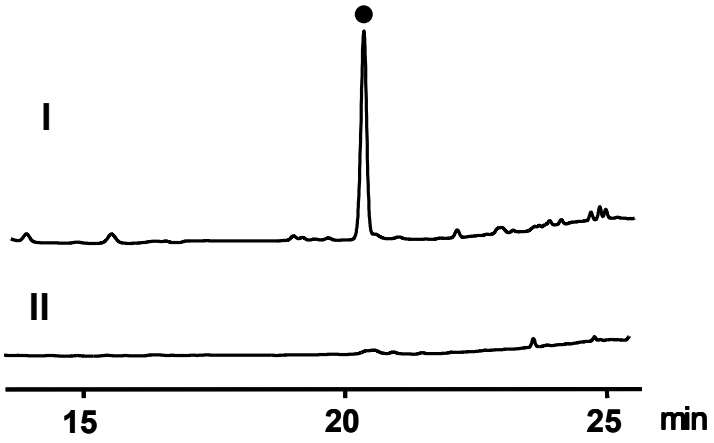
Introduction of pSL4003, pSL4006, pSL4009, pSL4012, pSL4015 or pSL4018 into *S. actuosus* was carried out by *E. coli*-*Streptomyces* conjugation, following the procedure described previously (*4*). For inactivation of *nosG*, the colonies that were apramycin-resistant were identified as the recombinant strain SL4001, whose genotype was further confirmed by Southern hybridization. For inactivation of *nosM*, *nosH*, *nosL*, *nosN* and *nosP*, the colonies that were apramycin-resistant at 37℃ were identified as the integrating mutants, in which a single-crossover homologous recombination event took place. These mutants were further cultured in liquid TSB medium for three rounds without the presence of apramycin. The genotypes of resulting strains that were apramycin-sensitive were confirmed by PCR amplification and sequencing, leading to the identification of the recombinant strain SL4002, SL4004, SL4005, SL4006 and SL4007, respectively.

In trans complementation of *nosM* for the nosiheptide production in *S. actuosus*

To make the *nosM* expression construct, a 0.5 kb PCR product containing *nosM* was amplified by PCR using the primers 5'-TCCAAGCTTATGGTTAGCAGCGCTTTAGC-3' (HindIII site double-underlined) and 5'-TGGTCTAGACCCAGTCGCAGAACGTGCAC-3' (XbaI site double-underlined) and cloned into pMD19-T, yielding pSL4019. The 0.5 kb HindIII/XbaI fragment was recovered from pSL4019 and co-ligated with a 0.4 kb EcoRI/HindIII fragment harboring the promoter *PermE** into the EcoRI/XbaI site of pSET152, yielding pSL4020, in which the 0.5 kb fragment containing *nosM* is under the control of the *PermE** promoter.

Introduction of pSL4020 into SL4002 was carried out by *E. coli-Streptomyces* conjugation, following the procedure described previously (*4*). Colonies that were apramycin resistant were identified as the recombinant strain SL4003.

Figure S1. HPLC analysis of nosiheptide (solid circle) production in the wild type strain (I) and the *nosG* mutant SL4001 (II).



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