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Supporting Information

Unveiling the Biosynthetic Pathway of the Ribosomally Synthesized and Post-translationally Modified Peptide Ustiloxin B in Filamentous Fungi

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Supporting Information

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General.

All reagents commercially supplied were used as received. Optical rotations were recorded on JASCO P-2200 digital polarimeter. ^1H - and ^{13}C -NMR spectra were recorded on Bruker AMX-500 spectrometer or Bruker DRX-500. NMR spectra were recorded in D_2O (99.9 atom % enriched, CIL). ^1H chemical shifts were reported in δ value relative to water: δ_{H} 4.79 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (Hz), and integration. Mass spectra were obtained with a Xevo G2-S QToF (Waters) or JEOL JMS-T100LP. Column chromatography was carried out on C18 silica gel. (Wako 30~50 μm).

Oligonucleotides for polymerase chain reaction (PCR) were purchased from Hokkaido System Science Co., Ltd. Cell disruption was dealt with a ultrasonic disrupter UR-200P (TOMY SEIKO, Tokyo, Japan). Analysis of the samples during protein purification was performed using SDS-polyacrylamide gel electrophoresis, and the proteins were visualized by using coomassie brilliant blue staining. Protein concentration was determined by the bradford method with bovine serum albumin as a standard.

Strain and Culture Conditions.

Escherichia coli HST08 and *E. coli* DH5 α were used for cloning, following standard recombinant DNA techniques. *E. coli* BL21-Gold(DE3) was used for protein expression. *Aspergillus flavus* deletion mutants¹ were used for isolation of ustiloxins. *Aspergillus oryzae* NSAR1, a quadruple auxotrophic mutant (*niaD*⁻, *sC*⁻, Δ *argB*, *adeA*⁻), was used for the fungal expression.

Isolation of ustiloxin derivatives from *A. flavus* mutants

Mycelia of *A. flavus* deletion mutants¹ were inoculated into a solid medium containing cracked maize (30 g) and uracil (30 mg) in 500 mL Erlenmeyer flasks for 1 week. The fungal mycelia were then extracted with 70% aqueous acetone (200 mL) for overnight at room temperature. After vaporizing the acetone, the water layer was washed with ethyl acetate and butanol and then subjected to C18 column chromatography eluted with stepwise gradient of CH_3CN (0%, 5%, and 50%) with 0.1% TFA. The fraction containing ustiloxins was separated by HPLC (Shimazu Class VP system) equipped with Wakopak Navi C18-5 column (10 x 250 mm, A linear gradient from 5 to 20% of CH_3CN with 0.1% TFA in water with 0.1% TFA over 10 min, and 20% CH_3CN with 0.1% TFA for 10 min; flow rate of 3 mL/min). Further HPLC purification (Shimazu Class VP system) equipped with Wakopak Navi C18-5 column (10 x 250 mm): 5 to 10% CH_3CN for 5 min and 10% CH_3CN for 10 min, flow rate 3 mL/min) gave compounds as followed:

N-desmethylostiloxin F (2)

$[\alpha]_{\text{D}}^{25}$ -78.8 (c 0.65, H_2O), HR-ESIMS analysis (negative); calcd. for $\text{C}_{20}\text{H}_{27}\text{N}_4\text{O}_8$ $[\text{M}-\text{H}]^-$: 451.1834, found: 451.1829. The NMR data are shown on Table S4.

ustiloxin F (3)

HR-ESIMS analysis (positive); calcd. for $\text{C}_{21}\text{H}_{31}\text{N}_4\text{O}_8$ $[\text{M}+\text{H}]^+$: 467.2136, found: 467.2180. The NMR spectrum is shown on Page S20.

S-deoxyustiloxin H (4)

HR-ESIMS analysis analysis (negative); calcd for C₂₄H₃₄N₅O₁₀S [M-H]⁻: 584.2032, found: 584.2030. The NMR data are shown on Table S4.

ustiloxin H (5)

[α]_D²⁵ -39.9 (c 0.34, H₂O), HR-ESIMS analysis analysis (positive); calcd. for C₂₄H₃₅N₅O₁₁S [M+H]⁺: 602.2127, found: 602.2122. The NMR data are shown on Table S4.

ustiloxin C (6)

HR-ESIMS analysis (positive); calcd. for C₂₃H₃₅N₄O₁₀S [M+H]⁺: 559.2068, found: 559.2108. The NMR spectrum is shown on Page S21.

Genomic DNA preparation.

Genomic DNA was extracted according to the following method; the mycelia of *A. flavus* CA14 was collected by filtration, washed with water, and dried using paper towel. The dried mycelia was frozen in liquid nitrogen and crushed by SK-Mill (Tokken). To the frozen powder was added extraction buffer (400 mM of Tris-HCl (pH 8.0), 500 mM of NaCl, 20 mM of ethylenediaminetetraacetic acid (EDTA) and 1% of sodium dodecyl sulfate) and the suspension was kept at room temperature for 5 min. To the suspension was added phenol:chloroform solution and the mixture was vortexed for 2 sec. After incubation at 65°C for 60 min, the reaction mixture was centrifuged at 12000 rpm for 5 min. The supernatant was then treated with RNase at 37°C for 90 min. To the reaction mixture was then added phenol:chloroform solution. After being vortexed for 2 sec, the mixture was centrifuged at 12000 rpm for 5 min. The supernatant was transferred to a new centrifuge tube and re-extracted twice with phenol:chloroform solution followed by chloroform. To the final supernatant was added cold-isopropanol and CH₃COONa solution and genomic DNA was recovered by centrifugation at 12000 rpm for 10 min. The pellet was then washed with 70% ethanol solution and dried for 15 min. Finally, the isolated DNA was resuspended in TE buffer (10 mM of Tris-HCl (pH 8.0) and 1mM of EDTA) and stored at -20°C for further use.

Preparation of expression plasmids.

The *ustA*, *ustQ*, *ustYa*, *ustYb*, *ustM*, and *ustT* were amplified from genomic DNA of *A. flavus* with primer set as shown on Table S1. PCR reactions were performed with the KOD-Plus-Neo (TOYOBO). Each PCR product was inserted into appropriate restriction site (site 1 and/or site 2) of pUARA2, pUSA2, or pAdeA2 using In-Fusion Advantage PCR cloning kit (Clontech Laboratories) to construct expression plasmids. The constructed plasmids are summarized in Table S6.

Transformation of *Aspergillus oryzae*.

Transformation of *A. oryzae* NSAR1 or transformant (1.0 × 10⁸ cells) were performed by the protoplast-polyethylene glycol method reported previously.² To construct transformants harboring ustiloxin B synthase genes, pUSA2-*ustA/Q*, pUARA2-*ustYa/Yb*, pUARA2-*ustYa*, pUARA2-*ustYb*, pAdeA2-*ustYb*, pAdeA2-*ustYb/M*, pUARA2-*ustT* were used for the transformation. The following transformants were obtained: AO-*ustAQYaYb* (co-transformation), AO-*ustAQYa*, AO-*ustAQYb*, AO-*ustAQYaYb* (stepwise incorporation), AO-*ustAQYaYbM*, AO-*ustAQYaYbMT*.

Extraction of metabolites.

Mycelia of *A. oryzae* transformants were inoculated into a solid medium containing polished rice (20 g) and adenine (10 mg). Each culture was incubated at 30°C for 7 days followed by extraction with 70% acetone/H₂O overnight. After centrifuge, 300 µL aqueous acetone supernatant was evaporated out of acetone and washed with an equal volume of ethyl acetate to give the analytical sample.

Analysis of the metabolites.

An 5 µL aliquot of each water extract was analyzed by UPLC (Waters ACQUITY™ UPLC H-Class system) equipped with a ACQUITY™ UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 µm particle size) at the following conditions: Flow rate; 0.7 mL/min, Solvent system: A linear gradient from 5 to 50% of CH₃CN with 0.1% TFA in water with 0.1% TFA over 2 min.

Isolation of *N*-desmethylostinol (2) from *A. oryzae* transformant AO-*ustAQYaYb*

Mycelia of AO-*ustAQYaYb* was inoculated into a solid medium containing polished rice and adenine (10 mg) in 500 mL Erlenmeyer flasks for a week. The fungal mycelia were then extracted with 70% aqueous acetone (200 mL) for overnight at room temperature. After vaporizing the acetone, the water layer was washed with AcOEt and butanol and then subjected to C18 column chromatography. The fraction containing **2** was separated by HPLC (Shimadzu Class VP system) equipped with Wakopak Navi C18-5 column (10 x 250 mm, a linear gradient from 5 to 20% of CH₃CN with 0.1% TFA in water with 0.1% TFA over 10 min, and 20% CH₃CN with 0.1% TFA for 10 min; flow rate of 3 mL/min). Further HPLC purification (Shimadzu Class VP system) equipped with Wakopak Navi C18-5 column (10 x 250 mm): 5 to 10% CH₃CN for 5 min and 10% CH₃CN for 10 min, flow rate 3 mL/min) gave **2** (Yield: 3.5 mg/500 g rice).

mRNA preparation and cDNA synthesis of *A. flavus* strain CA14

mRNA preparation and cDNA synthesis of *A. flavus* strain CA14 was cultured in V8 juice media (100 mL in 500 mL Erlenmeyer flask) for 2 days at 30°C (200 rpm). Total RNA was extracted from each dried mycelia using TRIzol® Reagent (Invitrogen) according to the manufacturer's instructions. The total RNA treated with DNase I (Life Technologies) was used for reverse transcription. Complementary DNA (cDNA) was synthesized with PrimeScript™ II 1st strand cDNA synthesis kit (Takara) using the oligo dT primer according to the manufacturer's instructions.

Overexpression of UstF1, UstF2, and UstD in *E. coli*

The *ustF1*, *ustF2* and *ustD* genes were PCR amplified from the cDNA of *A. flavus* CA14 using primers (table). The PCR products were directly inserted into the *Bam*HI or *Kpn*I-digested pMALc4E to generate pMALc4E-*ustF1*, pMALc4E-*ustF2*, pMALc4E-*ustD*. These plasmids were separately introduced into *E. coli* BL21-Gold (DE3) for overexpression. The transformants were grown at 37°C at an OD₆₀₀ of ~0.6 in a 500 mL flask. After the culture was cooled at 4°C, isopropyl β-D-thiogalactopyranoside (0.1 mM) was added to it. After incubation at 16°C for 18 h, the cells were harvested by centrifugation at 4000 rpm. The harvested cells were resuspended in disruption buffer (20 mM Tris-HCl (pH 7.4), 200 mM NaCl, EDTA 1 mM, DTT 1 mM) and disrupted by sonication. After centrifugation, the supernatant was applied to an amylose column to purify the expressed protein. The concentration of purified proteins was determined by Bradford method.

UstF1 and UstF2 Assays.

Typical conditions are as follows; a reaction mixture (40 μ L of Tris-HCl buffer (pH 8.0)) containing 4 μ M of the substrate, 2 mM NADPH, 5% glycerol, 0.6 μ M of UstF1 or UstF2) was incubated at 30°C. The reaction was quenched by the addition of methanol (40 μ L) and the resultant mixture was vortexed and centrifuged at 12,000 x g. The supernatant was directly analyzed UPLC (Waters ACQUITY™ UPLC H-Class system) equipped with a ACQUITY™ UPLC BEH C18 column (50 mm $\times$ 2.1 mm, 1.7 μ m particle size) at the following conditions: Flow rate; 0.7 mL/min, Solvent system: A linear gradient from 5 to 50% of CH₃CN with 0.1% TFA in water with 0.1% TFA over 2 min. An ACQUITY QDa MS was used for detection.

Large scale reaction with UstF2

Large scale reaction of **5** (2.0 mg) with UstF2 yielded **7** (1.4 mg, 72.6%) as a isomeric mixture (*E/Z* = 2/1). $[\alpha]_D^{27}$ 21.6 (c 0.45, H₂O). HR-ESIMS analysis (positive); calcd. for C₂₃H₃₄N₅O₁₀S [M+H]⁺ 572.2021, found: 572.1983.

UstD Assays.

Typical conditions are as follows; a reaction mixture (50 μ L of Tris-HCl buffer (pH 7.4)) containing 100 μ M of the substrate, 0.4 μ M UstD, 400 μ M aspartic acid was incubated at 30°C. The reaction was quenched by the addition of methanol (50 μ L) and the resultant mixture was vortexed and centrifuged at 12,000 x g. The supernatant was directly analyzed UPLC (Waters ACQUITY™ UPLC H-Class system) equipped with a ACQUITY™ UPLC BEH C18 column (50 mm $\times$ 2.1 mm, 1.7 μ m particle size) at the following conditions: Flow rate; 0.7 mL/min, Solvent system: a linear gradient from 5 to 50% of CH₃CN with 0.1% TFA in water with 0.1% TFA over 2 min. An ACQUITY QDa MS was used for detection.

Transformation of oxime into geminal diol in acidic condition.

To a solution of **7** (0.9 mg, 1.5 μ mol) in water (10 mL) was added TFA (20 μ L, 0.26 mmol) and the mixture was stirred at room temperature for overnight. The reaction mixture was directly subjected to 40C18 open column (Wakosil, 30~50 μ m, MeCN/H₂O 5/95). The fraction containing **9** was concentrated in vacuo and further purified by HPLC (Shimazu Class VP system) equipped with Wakopak Navi C18-5 column (10 x 250 mm, A linear gradient from 5 to 20% of CH₃CN with 0.1% TFA in water with 0.1% TFA over 10 min, and 20% CH₃CN with 0.1% TFA for 10 min; flow rate of 3 mL/min; detection at 290 nm) to afford **9** (0.365 mg, 40%).

$[\alpha]_D^{26}$ -7.2 (c 0.16, H₂O). HR-ESIMS analysis (positive); calcd. for C₂₃H₃₅N₄O₁₁S [M+H]⁺: 575.2018, found: 575.2004.

Reduction of **9 to give ustiloxin C (**6**)**

6

Figure S1. UV-visible spectra of ustiloxin derivatives.

Figure S2. LC-MS profiles of extracts from transformants: (A) AO-*ustAQYaYb*, (B) AO-*ustAQYaYbM*, (C) AO-*ustAQYaYbMT*.

Figure S3. (A) SDS-PAGE analysis of UstF1, UstF2, and UstD. (B) UV spectra of (1) UstF1 and (2) UstF2.

(A) SDS-PAGE analysis of recombinant proteins

(B) UV spectra of recombinat proteins

Figure S4. MS spectra of (A) non-labeled **7** and (B) deuterium-labeled **7** after D₂O treatment.

(A) non-labeled **7**

(B) deuterium-labeled **7**

Figure S5. Time course analysis of the UstF2 reaction mixture.

Figure S6. LC-MS profiles of the UstD reaction products after dansyl derivatization. (A) Treatment of alanine with dansyl chloride. (B) Treatment of reaction mixture of ustD (in the absence of **9**) with dansyl chloride. (C) Treatment of aspartic acid with dansyl chloride.

(A) alanine after dansylation

(B) reaction mixture after dansylation

(C) aspartic acid after dansylation

Scheme S1. Proposed reaction mechanism of (A) UstF2 catalyzing oxime formation and (B) CrmH catalyzing oxime formation.

(A) UstF2 catalyzing oxime formation

(B) CrmH catalyzing oxime formation

Scheme S2. Proposed reaction mechanism of (A) UstD and (B) aspartate β -decarboxylase catalyzing reaction.

(A) UstD catalyzing reaction

(B) aspartate β -decarboxylase catalyzing reaction

Table S1. Oligonucleotides used for construction of *A. oryzae* expression plasmids.

Primers

Insert	Restriction site	Sequence 5'-3'	Size vector
<i>ustA</i>	<i>KpnI</i>	F: AATTCGAGCTCGGTACATGAAGCTTATTCTTACTCTACTCG R: TTAATGCCCTCCATGGCGCTTGTC	770 bp pUSA2
<i>ustQ</i>	<i>NheI</i>	F: ATGGCCGTGGAATATTTCCAGGAAAACTA R: ACGACTACCCGGGTCACTAATACGTATAACATA	1350 bp pUSA2
<i>ustYa</i>	<i>NheI</i>	F: ATCGATTTGAGCTAGATGGCAGAGCGCTCATCT R: TAGTGCGGCCGCTAGTTAATGAATCCCATACGTCG	946 bp pUARA2
<i>ustYb</i>	<i>KpnI</i>	F: CGGAATTCGAGCTCGATGTCGGATCTCTACGCAC R: ACTACAGATCCCCGGCTAATTATGCTTAGCATCCAC	974 bp pUARA2
<i>ustM</i>	<i>NheI</i>	F: ATCGATTTGAGCTAGATGGAAACAATCCTATCCAG R: TAGTGCGGCCGCTAGTCAAGCGCCCAGCTCCAG	891 bp pAdeA2
<i>ustT</i>	<i>KpnI</i>	F: CGGAATTCGAGCTCGATGTCGGGCACCTCTCCA R: ACTACAGATCCCCGGTCAATACGACCTTGCTGCA	1791 bp pUARA2

Table S2. Summary of expression plasmids for *A. oryzae* transformation

Plasmid name	Original vector	Gene 1 (Restriction site)	Gene 2 (Restriction site)
pUSA2- <i>ustA/Q</i>	pUSA2	<i>ustA</i> (<i>KpnI</i> site)	<i>ustQ</i> (<i>SpeI</i> site)
pUARA2- <i>ustYa/Yb</i>	pUARA2	<i>ustYa</i> (<i>NheI</i>)	<i>ustYb</i> (<i>KpnI</i>)
pUARA2- <i>ustYa</i>	pUARA2	<i>ustYa</i> (<i>NheI</i>)	
pUARA2- <i>ustYb</i>	pUARA2		<i>ustYb</i> (<i>KpnI</i>)
pAdeA2- <i>ustYb</i>	pAdeA2		<i>ustYb</i> (<i>KpnI</i>)
pAdeA2- <i>ustYb/M</i>	pAdeA2	<i>ustM</i> (<i>NheI</i>)	<i>ustYb</i> (<i>KpnI</i>)
pUARA2- <i>ustT</i>	pUARA2	<i>ustT</i> (<i>kpnI</i>)	

Table S3. Oligonucleotides used for construction of *E. coli* expression plasmids

Insert	Restriction site	Sequence 5'-3'	Size vector
<i>ustF1</i>	<i>Bam</i> HI	F: ACCGGAATTCGGATCCATGACTGTTTCTCAGGTTCG R: GCTTGACGGTGTAGAATTAATCCTAGGAGATCTCAGCT	1515 bp pMalc4E
<i>ustF2</i>	<i>Kpn</i> I	F: GATGACGATGACAAGATGGCCAATCCTCAAACCA R: AGGATCCGAATTCCGCTACTTGTTAACTTGAGCCC	1428 bp pMalc4E
<i>ustD</i>	<i>Kpn</i> I	F: GATGACGATGACAAGATGAAATCGGTAGCAACCTC R: AGGATCCGAATTCCGCTAGGTATCCCGCGTGAC	1320 bp pUARA2

Table S4. NMR spectral data of ustiloxins

	<i>N</i> -desmethylostiloxin F (2)		<i>S</i> -deoxyustiloxin H (4)		ustiloxin H (5)	
	δ_C	δ_H (multiplicity, <i>J</i> in Hz)	δ_C	δ_H (multiplicity, <i>J</i> in Hz)	δ_C	δ_H (multiplicity, <i>J</i> in Hz)
2	88.3	-	86.3	-	89.7	-
3	61.7	4.66 (s)	59.2	4.71 (s)	62.3	4.73 (s)
5	175.1	-	172.1	-	174.5	-
6	52.3	4.33 (q, 7.0)	49.6	4.43 (q, 7.0)	52.1	4.48 (q, 7.0)
8	169.9	-	166.2	-	168.3	-
9	62.5	3.90 (d, 9.5)	64.6	4.65 (d, 7.0)	68.7	4.20 (d, 10.3)
10	74.9	4.81 (d, 9.5)	73.8	4.98 (brs)	75.80	4.99 (d, 10.3)
11	133.3	-	129.1	-	130.6	-
12	125.5	7.26 (dd, 8.5, 2.0)	129.3	-	139.1	-
13	121.1	7.06 (d, 8.5)	119.2	7.14 (s)	116.0	7.63 s
14	153.3	-	151.2	-	154.6	-
15	144.7	-	141.4	-	148.5	-
16	126.5	7.41 brs	126.0	7.27 (brs)	126.4	7.43 brs
17	173.5	-	170.3	-	172.4	-
19 a	44.0	4.08 (d, 17.8)	43.3	3.88 (d, 17.0)	46.2	3.84 (d, 17.3)
19 b	-	4.00 (d, 17.8)	-	3.83 (d, 17.0)	-	3.78 (d, 17.3)
20	175.7	-	175.6	-	178.9	-
21	24.7	1.66 s	21.4	1.65 s	24.2	1.73 s
22 a	32.6	2.04 (dq 14.0, 7.5)	30.9	2.05 (dq 14.0, 5.5)	33.6	2.13 (dq, 13.5, 7.3)
22 b	-	1.72 (dq, 14.0, 7.0)	-	1.73 (dq, 14.0, 5.5)	-	1.70 (dq, 13.5, 7.3)
23	10.5	0.91 (t, 7.5)	7.7	0.98 (t, 7.5)	10.3	0.96 (t, 7.3)
24	18.1	1.18 (d, 7.5)	15.1	1.23 (d, 7.5)	17.8	1.18 (d, 7.0)
<i>N</i> -C	-	-	32.1	2.78 s	34.2	2.76 s
H ₃						
2' a	-	-	35.2	3.61 (dd, 15.0, 6.5)	59.8	3.71 (dd, 13.7, 7.5)
2' b	-	-	-	3.43 (dd, 15.0, 6.0)	-	3.16 (dd, 13.7, 7.0)
3'	-	-	53.6	3.95 (t, 6.0)	53.8	4.27 (t, 7.3)
4'	-	-	172.7	-	174.2	-

	7 (E/Z mixture)		9	
	δ_C	δ_H (multiplicity, <i>J</i> in Hz)	δ_C	δ_H (multiplicity, <i>J</i> in Hz)
2	89.1	-	89.5	-
3	62.2	4.73 (s)	62.0	4.70 (s)
5	174.5	-	174.6	-
6	51.9	4.42 (q, 7.0)	52.1	4.44 (q, 7.0)
8	169.8	-	168.2	-
9	69.6	4.04 (d, 11.0)	68.6	4.19 (d, 10.0)
10	76.3	4.92 (d, 11.0)	75.8	4.96 (d, 10.0)
11	130.9	-	130.4	-
12	136.8 (Major)	-	138.9	-
	137.6 (Minor)	-	-	-
13	117.2 (Major)	7.42, s	116.5	7.60, s
	116.6 (Minor)	7.53, s	-	-
14	154.3	-	154.7	-
15	148.4	-	148.1	-
16	126.6	7.35, brs	126.9	7.42, brs
17	172.5	-	173.0	-
19 a	46.2	3.82 (d, 17.0)	45.6	3.95 (brs)
19 b	-	3.76 (d, 17.0)	-	-
20	178.9	-	176.6	-
21	24.1	1.74, s	24.4	1.73, s
22 a	33.7	2.11 (dq, 14.3, 7.5)	33.4	2.10 (dq, 14.0, 6.5)
22 b	-	1.70 (dq, 14.3, 7.5)	-	1.69 (dq, 14.0, 6.5)
23	10.3	0.99 (t, 7.0)	10.4	0.96 (t, 7.0)
24	17.7	1.18 (d, 7.0)	17.9	1.19 (d, 7.0)
<i>N</i> -CH ₃	34.7	2.66 (s)	34.3	2.75 (s)
2' a	65.2	3.65 (dd, 12.0, 4.3)	*	*
2' b	-	3.56 (dd, 12.0, 6.3)	-	*
3'	146.0 (Major)	7.43 (s)	88.9	5.48 (s)
	144.2 (Minor)	6.96 (s)	-	-

cDNA sequences of (A) *ustF1*, (B) *ustF2*, and (C) *ustD*.

Accession number BR001206

(A) *ustF1*

ATGACTGTTTCTCAGGTTGCGCCGAGTGGCCGTGATCGGCGCTGGAATCAGCGGAGTTGT
TTCAACAGCCCATCTTGTAGCTGCTGGATTCTGAAGTCACCGTGTTCTGAAAGAAACCAGC
AAACAGGGGGGATTTGGCTGTACGATGAACAGACACCATTGGAATGTTCTTTTCTTCTC
CTGGTCCTTCTTTAGCAGATAAGGTAGAGAAAAACGCCAGATTTCGATAGAGAGAAGCTC
CGACTACAGCATGCTCCTCCAGGACCATGCTACAAAAATCTCACAACAAATGTGTCCACC
CCTCTCATGCGAATAAACTTCGCGCCTGGCCAGAAAAACACACCCGATTTTGTTCACCAC
AGTGTCGTGAATGAATATATCAGGGATATAGCACTCAGTACTGGAGTCGACGAGCGAACA
ATATACGGCGCTCGAGTGGAGCATGTCTATAAAAAATGGTGGGAAGTGGCATGTCAATTGG
TCAGTACTAGACGACAATGGCAGTATTGACGGTCTAGAAGAGAGGGCGGCTGATCTCTACC
TTTGACGCGGTTGTAGTCGCGTCTGGCCATTATCATTACCTCACATTCCGGACATACCTG
GGTTATCCGAAGTAAAGAAAAGATGGCCTTCAAGGGTTATACATTCCAAGCGATATAGAA
CACCGGAAGTCTACAGGGACGAAAATGTTTTGATGATTGGCGGAGGAGTATCATCGATGG
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GTATCAAATTGTATTCCCGTTCCTTCCAGACTATCACGACGATTCAATGCCGCTTCAGGAT
GCTAATAATACTATTCTTGTACCAACGGGACACAAGTGCATAACATTCATCGAGACATCT
TTTATATCCCAGACCCGACTCTGGCTTTTGTCTGGGATACCTTACTTCAATACAACATTTACC
CTCTTTGAATTCCAGGCTATTGCAGTGACAGCCGTCTGGTCCCGAACTGCATGTCTACCAT
CAACCACCGAGATGAGACGGGAGTACCTAGTAAAGCAAAAGCAGACCCGGTGGCGGGCG
CAAATTCCACTCGTTGAAGGATAAAGAAAAGGAATACGTCCGCGACCTTATGGCGTGGAT
AAATGATGGTAGAAACGCACATGGGCTTGTTCCTATTGAAGGTCATACGGCGGCTTGGTT
TGAAGCTATGGATAAACTGTGGGACGAGGCGAGGGCGGCGATGAAAGAAAGAAAGGAG
CAACAAGAGAAGATTATCAAGCGCATTCCATTTTCGGCTGATTGTACTTCGGTCGAACTG
CCACATCTTAATTAG

(B) *ustF2*

ATGGCCAATCCTCAAACCACCCGTGTAGCCGTGGTTGGTGCTGGGATTAGCGGTGTTCTG
GCCGCCGGACATCTCCTCGCGACAGGTCTTGAAGTGACCGTTTTCTGAACGCAATGCGGC
CCCCGGTGGAGTCTGGTTATACGATGAACGAACACCGATCGAGCCATCGTATCCGGCCAT
GAAGCCCTCAAAGGCCGACCCGCCAGCAACAAAAGAACAAAGAAACGAGCAGGTTTCATG
CTACAGCATGCCCCGCCTGGGCCGTGCTATTATAACCTCCAGAACAATGTCCCCACGCCA
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TGTAATCCAACGGTTTATTTCAGGATATGTGATTGAGGCCAAGGTGCATGATGTGACACG
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CGGAAAACCTCAGGAACAAGAATATTCTTATGATCGGTGGAGGAGTCTCGTCGATCGACA
TCGCCAACGATATCAGTCCATTCGCCAACACCATTTACCAGAGCACGAGGAACAGTAAAT
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CTGAGACTGAAATGAGATCGGAATACAACGCCAAGGTGGAACGAGTCGGTCTTGAAAA
GGTGTTCATTCGATTCTGGGGACAGAAGAAAACCTACGTGCATGATTTGTAAACGTGGGT
GAATACTAGCAGGGCAGCGCAGGAGCTTGTTCGCGATTAAAGGGTTTAGCCCCGAGATGGT
ACGAGGCAAAGGAGGCACTGAGACAGAAATACAGGGCTCAAGTTAACAAGTAG

(C) *ustD*

ATGAAATCGGTAGCAACCTCATCACTTGATGATGTCGACAAAGATTTCGGTCCCACTGGGC
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GGGACGGTTGTTCTGAAGGAAGCGATTGAAAGTACAAGCAACTTCATGTATTCCCTTCCCT
TTCCCCCGGGCGTCGATGCGAAGAGCATGGAGGCAATCACAGCCTATACGGGGGAACAA
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CCACATGCCTATTTTCGCCTGCTAGGATTATCTCTCAAACCCATGCTAAACAACGATTGTGA
AATCGTCTGCTCAACCCTCTGCCACGAGGCAGCAGCCAGTGCCTGGATCCATCTTTCCAG
AGAACTAGGAATCACCATTAAATGGTGGTCCCCAACCAACCCCCGAACAGCCCCGACG
ACCCGGTCCTCACCACAGACAGCCTCAAACCACTCCTCTCTCCCAAACCCGACTCGTC
ACCTGCAACCACGTCTCCAACGTTCGTCGGAACCATCCATCCCATCCGCGAGATCGCAGA
CGTAGTGCACACTATCCCAGGCTGCATGCTCATCGTCGACGGCGTAGCCTGCGTGCCCCA
TCGTCCCGTGACGTGAAGGAGCTCGACGTGCACTTCTACTGTTTCAGCTGGTACAAGC
TGTTTCGGCCCCCATATGGGCACGCTCTATGCGAGTCGCAAAGCACAGGACCGATACATGA
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GGATGTTTTGAAGCCGAAGAGTAGCGATGGTTTGGTTAGGGTTAGTTTTGTCCATTACAAT
ACTGTCGAGGAAGTTAGGGCGTTTTGCAGTGAGTTGGATGAGATTGTCACGCGGGATAC
CTAG

^1H -NMR spectrum of ustiloxin F (**3**)

¹H-NMR spectrum of ustiloxin C (**6**)

¹H-NMR spectrum of *N*-desmethylostiloxin F (2)

¹³C-NMR spectrum of *N*-desmethylostiloxin F (2)

H-H COSY spectrum of *N*-desmethylostiloxin F (**2**)

HSQC spectrum of *N*-desmethylostiloxin F (**2**)

HMBC spectrum of *N*-desmethylostiloxin F (2)

¹H-NMR spectrum of deoxyustiloxin H (4)

^{13}C -NMR spectrum of deoxyustiloxin H (4)

H-H COSY spectrum of deoxyustiloxin H (4)

HSQC spectrum of deoxyustiloxin H (4)

HMBC spectrum of deoxyustiloxin H (4)

^1H -NMR spectrum of ustiloxin H (**5**)

¹³C-NMR spectrum of ustiloxin H (5)

H-H COSY spectrum of ustiloxin H (**5**)

HSQC spectrum of ustiloxin H (**5**)

HMBC spectrum of ustiloxin H (5)

¹H-NMR spectrum of 7

^{13}C -NMR spectrum of **7**

H-H COSY spectrum of 7

HSQC spectrum of 7

HMBC spectrum of **7**

^1H -NMR spectrum of **9**

¹³C NMR spectrum of **9**

H-H COSY spectrum of **9**

HSQC spectrum of **9**

HMBC spectrum of **9**