Supplementary Materials: Putative Nonribosomal Peptide Synthetase and Cytochrome P450 Genes Responsible for Tentoxin Biosynthesis in *Alternaria alternata* ZJ33

You-Hai Li, Wen-Jin Han, Xi-Wu Gui, Tao Wei, Shuang-Yan Tang and Jian-Ming Jin

GTGTAGCATTTGGTGCACTTCGTATTCCAATACCGACCGAGAATTCCGATCGGGAACGATAGGCACGTCAATGGCTTCTTT

TAGTTGGGTTACCGACCCGGAAGACCACAACAAGCTAGTGCCATTTGGCACAATTGGCGAACTTCTGATAGAGGGACCAAT

TCTGGCACGCGGGTACCTCAACGACATTTCGAAAACTGAGGCTGTTTTCGTTGATGGCCCTCTCTGGCTGAGGCAAGGCAA

CCGCAACGATGAATCCACTCGACGACAGGGAAGACTATA

Figure S1. >NRPS gene fragment 1 from *A. alternata* ZJ33.

GATCAACGCCTACGGGCCCACTGAATGTTGTGTCTCCTGTGTCGCAAGTCCCGACATGAAGGGACTCGATCCGGAACCCAT

TGGGAAGCCGATtGCGTCaATTGGCTGGGTGACTAATCCTAACGATCACAATAGACTAGCACCGCTAGGCGCTGTTGGAGA

GCTGTTGGTTGAGGGACCAAACCTAGCCCGCGGCTACCTGGACGATGCGAAGAAGACAGAGACAGCTTTTGTCCATGATCC

TCTCTGGCTGCTTCGGGGCTGTGAAGGCTACAGTGGGCGGCGAGGTAGACTGTACAAGACAGGCGACCTGGTCTACCACA

Figure S2. >NRPS gene fragment 2 from *A. alternata* ZJ33.

ATGCCGGGATCGCTACTGTTTGACGTCAACAAACTCGATGTCGAGAAGATATGGGAGAGAAACCGCGGACCTTTGCGAGC

GGTCAATCGCTGCGTTCACAGCCTATACGAAGAGCAAGCTATAGCTCGACCGAACGCATGCGCCATTCACGCATGGGACG

GCAACATGACCTACGAGCAATTGAATCAGCATTCAACACGATTGGCAAGCTATCTTGTCACTCAAGGCATTGGAACCGAG

GTGATGGTACCACTGTGCTTTGAGAAGTCGATATGGGCGATCGTAGCTATGTTAGCTGTTCTCAAGGCTGGTGCCGCGTT

CGTGCCACTAGACCCAATGCATCCAAGAGCCCGCCACGAAGAGATATTCAAGCAGACCAATGCGAAGTTGGTCCTTACTT

CCGTACAGCACGCCGCTCTCTGGCCAAATTCGGGTCTGCAGTTTCTGGCAATCGACAAAACCTTTGTTGATCAATTGCCA

TGGGAAACCAAGATCCGGTCCAAAGTCAAGCCAATAGATGCTGTCTATGTTATGTTCACATCTGGTAGTACTGGTGTTCC

GAAAGGCGTTGTCTTGGAGCATAGAGCAATCGCAACAAGCTGCCTTGCCCATGGAATGGAAATGAAACTTGGATCTGATA

GCCGAGCACTCCAGTTCGCGGCTTATACGTTCGATATTTGTATCGCTGAGATTTTTACGACTTTGATATTCGGCGGCTGT

GTCTGTGTCCCTTCGGAAGACGATCGACGTAACGCCTTATCCGAAGTAATCAACAACAATAACATCAACTGGGCTCAACT

CACGCCGACTGTTGCTCGTCTACTAGATCCCAGCACGGTCCCTTCTCTGAGAGTGCTCGTCCTTGGAGGAGAACGAGTGG

ACGAGGCCGACTGGAAGAGATGGGGTGACGATATCGTGAAGGTGAACGTTTACGGCCCGACGGAGTGTAGCATTTGGTGC

ACTTCGTATTCCAATACCGACCGAGAATTCCGATCGGGAACGATAGGCACGTCAATGGCTTCTTTTAGTTGGGTTACCGA

CCCGGAAGACCACAACAAGCTAGTGCCATTTGGCACAATTGGCGAACTTCTGATAGAGGGACCAATTCTGGCACGCGGGT

ACCTCAACGACATTTCGAAAACTGAGGCTGTTTTCGTTGATGGCCCTCTCTGGCTGAGGCAAGGCAACCGCAACGATGAA

TCCACTCGACGACAGGGAAGACTATACAAGACTGGTGATCTAGTCTACTATGATGCAGACGGTAATCTGGTTTACGCGGG

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CAGGCATCAAACAAGTTGCGGCTGAAGTCATCCTACCATCAGGTGACCAAGCAAAAGCGATGGTGGCAGCATTCGTGCAG

CTAAGCGAAGAGCCACGCCATGCACTCGTCCAACAGACTTCTAACGGTGACTTGGAAGTGCGAGTCATCTTCCCAACCTA

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CAACATCCGCTAAAGTGGACCGGCAGAAGCTGCGCAAGATTGGCGCCTCCTTTTCTGCACAACAATTGGCCCAGCTTCGA

ACGTATAGCGATGACCCAAAGCGACAACCAGAGACTGAGAAAGAGCAGATCTTGCATCATCTCTGGGCACAGGTCCTAAG

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AGGTCGGATGTGGTTCTTAGAAGAACTTCACCCTGGTCTTACCTGGTACCTCATGCCGGTTGTTGTGCGTATGAGAGGCC

CGTTGGAGCTGACAGCGCTCCAATCTGCGCTGAATGCAATTGAGAGCCGCCATGAGACCCTCCGGACGACTTTTGAAACC

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GAGTTGGGCGCGTTCTATTCAGCCTCAATACGAGGCCACGACCCTCTTGTTCAAGTCCAACCTCTGCCTATTCAGTATCG

AGACTTCTCTGTGTGGCAGAGGCAACAAGCCCAGATCGACAAACACCGGAGTCAGCTCAATTATTGGTTCAATGTGCTTA

ATACCAGCAGACCCGCTGAGCTGCTTTGCGACAAAGCTCGACCAGCTGCTTTATCTGGGGAAGCTTCCAAGCAGACGATC

CAGATCGACGGCCCGCTATACATTCAGCTGCTGCAGTTCTGCAAAGCGAAAGGAGTCACAAAGTTCATGGTGCTATTTGC

GGCTTTTAGAGCTACACACTTCCGCCTCACTGGCCAGAACGATGCCACCATTGGCACTGTGAACGCCAATCGGGATCGAT

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AACTGAAACTTGAGGGCCTGGAAACAGAGAGCCTGGATAATGCACCGAAGTCAAGATTTGATCTTGAATTCCACTTTTTC

CAGCAAGAAGACAGCCTGAAGGGTGAAGTTGTCTATTCCACCGACATTTATTCTCCAGAGGCGATCGACAACATGCTGTC

GATCTTCCAAATCGTTCTCGAGGGGTGCCTCCAAGAGCCGAAAGCTGCGATTGCGTCCTTGTCATTGTTATGCGACGTCG

AATTATCGAAGCTCAATAGCATGGGTCTGATCCAGGTTGAAAAGACGGATTATCCACGAGAGTCGAGTGTAGTCGATCTG

TTTCGCCAACAAACTTCCCTCTGTCCGTCCAGGATTGCTGTGAAGGACGCATCAGTAACCATGACTTATACTCAGCTGGA

CAAGGAATCTGACATTCTTGCTCAGTGGTTAGCCAAACAATCGCTCGCTCCGGAGACACTGGTCAGCGTATTAGCAGGGC

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CCGCTTGAAGACGGGCTTAGGATTGTGGTTAGGGGTAACGAACACATCATAACGCCAGAGAGTGCGGAGAGAATCAACAA
ACTGCTCTGTGAGACGATCGGGGCGCTGTCTTCTTCGCTCCAGTAA

Figure S3. >Seq1 (organism = *Alternaria alternata*) tentoxin synthase (TES) gene of *A. alternata* ZJ33, complete cds.

ATGGAGCCTGAGCCTCTAGACCCAAGGGCAATCGGTTTTATGCGTCGAAACCTTGCAATGCTGCGACAAACCCCAATGTC
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TCCATGCGACATGAGTGGCGTAGTTGCTCACGCGCACCCAGGGCCTGGAGGAAACCTACACTTATGTCGACATCAAATTT
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GCCGCTTCGAGAGGAGATTCGAAATACTTTGAACAGCGACGGATCGATTCCAGTCAGTGCGATGAAGGAGTTCGGGAAAA
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ACTGAGTTCCGATGGGGCTATGACAACCATGTCTGTCCTGGCAGATTCTATGCGCATAATCTGCTCAAAATTATCTTTGC
TAGAATAGTCGAAAATTACGATATCAAGATGCCAGGGAATGTTGAGGGAATAGAGGCTAGGTATCCAATGGTCGAGCACG
GGAATGTTGTTATGGAACCAAGAGACAAACCTCTTCTACTCAGAAGGGTTAAGAGCCATCAGGGTCACGTAGATTGA

Figure S4. >Seq2 (organism = *Alternaria alternata*) cytochrome P450 (TES1) gene of *A. alternata* ZJ33, complete cds.

GAAGTAGATGAAGCACATGTGCATAGTAAGCAAAGCTGCACGCCTTCCAACCCTCCATTTTCCCCCACATAGCATCATGT
GCTCTCTCAACAAGATTTCCTCCGTATCGAGCTAGTCTTCGCATGCATAAGCATCTGTCTGTACACGTCAATCTACGTGA
CCCTGATGGCTCTTAACCCTTCTGAGTAGAAGAGGTTTGTCTCTTGGTTCCATAACAACATTCCCGTGCTCGACCATTGG
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TCACTGCTAGCGTATGCAAAATGGCAATCAAAGTCTGCATCGATGCATTGATCGTTCCGAGTATAAGAAGAACTTGGGCA
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GACGTTTTACCTCTGGCAGTCGGGGAGCTAGGAAGGGTCTGAGCCACACCCGCGTGACTTTGATTGCTCTGGTGGCCTCA
CCCAGATGGACGGTAAAGTTCTTTGTACATTCGAGATAACCCGGGTCGTTGGCAACATCGCCTCCGATGAAGAGGTGGGC
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CCCTAAACTAGGAGCGAGTTAATAGAATGAAATGTATCTGCTTATGTCGAAATACCTTCTGAGAGCCATCCTTGTAGAGT
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GCAGCATTGCAAGGTTTCGACGCATAAAACCGATTGCCCTTGGGTCTAGAGGCTCAGGCTCCAT**CTTTGGAGTGTATCAG**
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CGAGATGGAGAAGCCCCCGTTATCTATTGGTTCGGATTCGTTTATCTGCGGTTCATCGTAACATCCAAGATATACTAGGC
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CCTCCGCGTGTCCACAATGCAAGCGTATCATGTTGTAAGAAAAAGTACGTGCGATGTCCGACTTGATCTATAGGGCCATA
GGCTACTTTCTCGGTGGAACGCGTAACACTCGCTGACACGAGATGGTCCGAATAGTAAGGGTGCTTCATGTATCCCAAGC
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TCTTCTGACATAAATATGCTCGAAAAATAGTCCTGAATCTCAAATTACAGCTTTTCATACACTGAGATCAGCTGACTCAT
CATCTTCCCATCTGAATTCTGTTCCACGGAAACTTCATTCAACAAGAGCCGACATGCCGGGATCGCTACTGTTTGACGTC
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Figure S5. DNA sequences between *TES* and *TES1* (**red**).

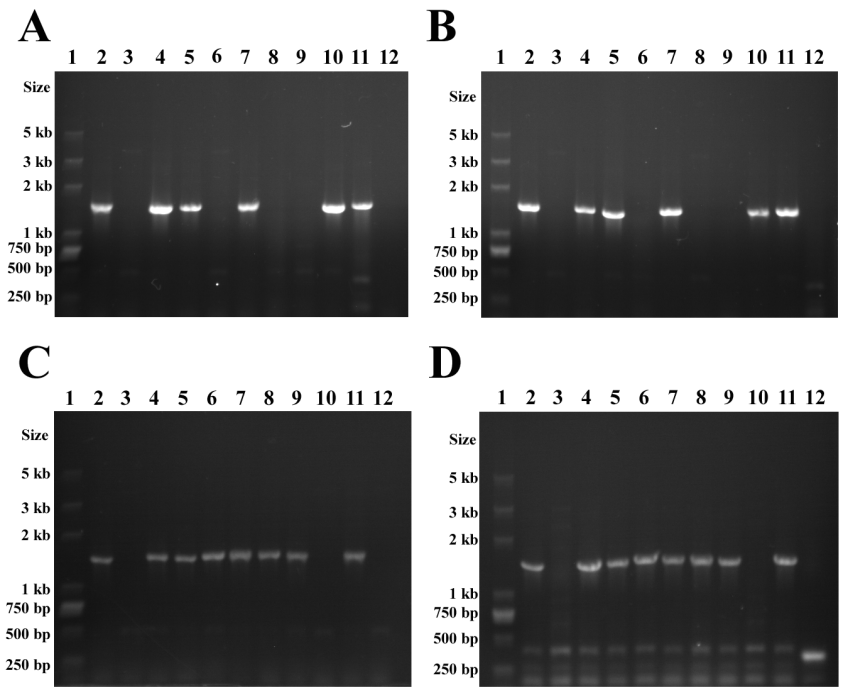

Figure S6. Verification of *TES* or *TES1* disruption by PCR analysis. lanes 1: DNA ladder markers; lanes 2–11: transformants; lane 12: wild-type strain ZJ33; (**A)** verification of *TES* disruption by PCR amplification using primer pairs F1 (TES-5for) + R1 (Hyg-5rev); lane 2, lanes 4–5, lane 7 and lanes 10–11: *TES* null mutants; lane 3, lane 6 and lanes 8–9: ectopic transformants; (**B**) verification of *TES* disruption by PCR amplification using primer pairs F2 (Hyg-3for) + R2 (TES-3rev); lane 2, lanes 4–5, lane 7 and lanes 10–11: *TES* null mutants; lane 3, lane 6 and lanes 8–9: ectopic transformants; (**C**) verification of *TES1* disruption by PCR amplification using primer pairs F3 (TES1-5for) + R1 (Hyg-5rev); lane 2, lanes 4–9 and lane 11: *TES1* null mutants; lanes 3 and 10: ectopic transformants; (**D**) verification of *TES1* disruption by PCR amplification using primer pairs F2 (Hyg-3for) + R3 (TES1-3rev); lane 2, lanes 4–9 and lane 11: *TES1* null mutants; lanes 3 and 10: ectopic transformants.

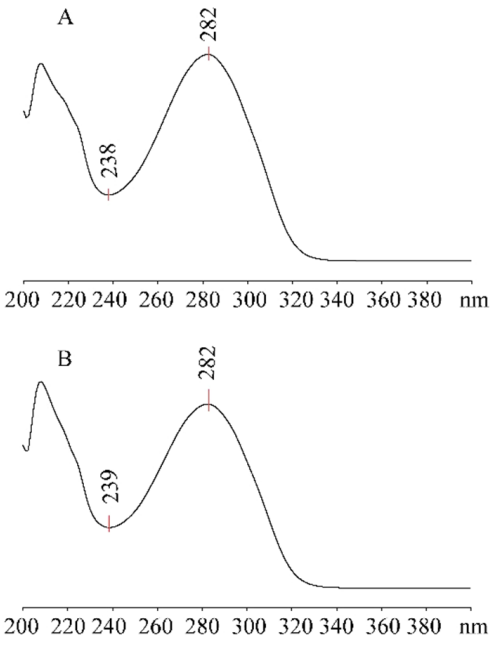

Figure S7. Absorbance spectrum of standard tentoxin and tentoxin from *A. alternata* ZJ33. (**A**) Standard tentoxin (Peak 1); (**B**) Tentoxin (Peak 1) from *A. alternata* ZJ33.