

Supporting Information

Assessing the Biosynthetic Inventory of the Biocontrol Fungus

Trichoderma afroharzianum T22

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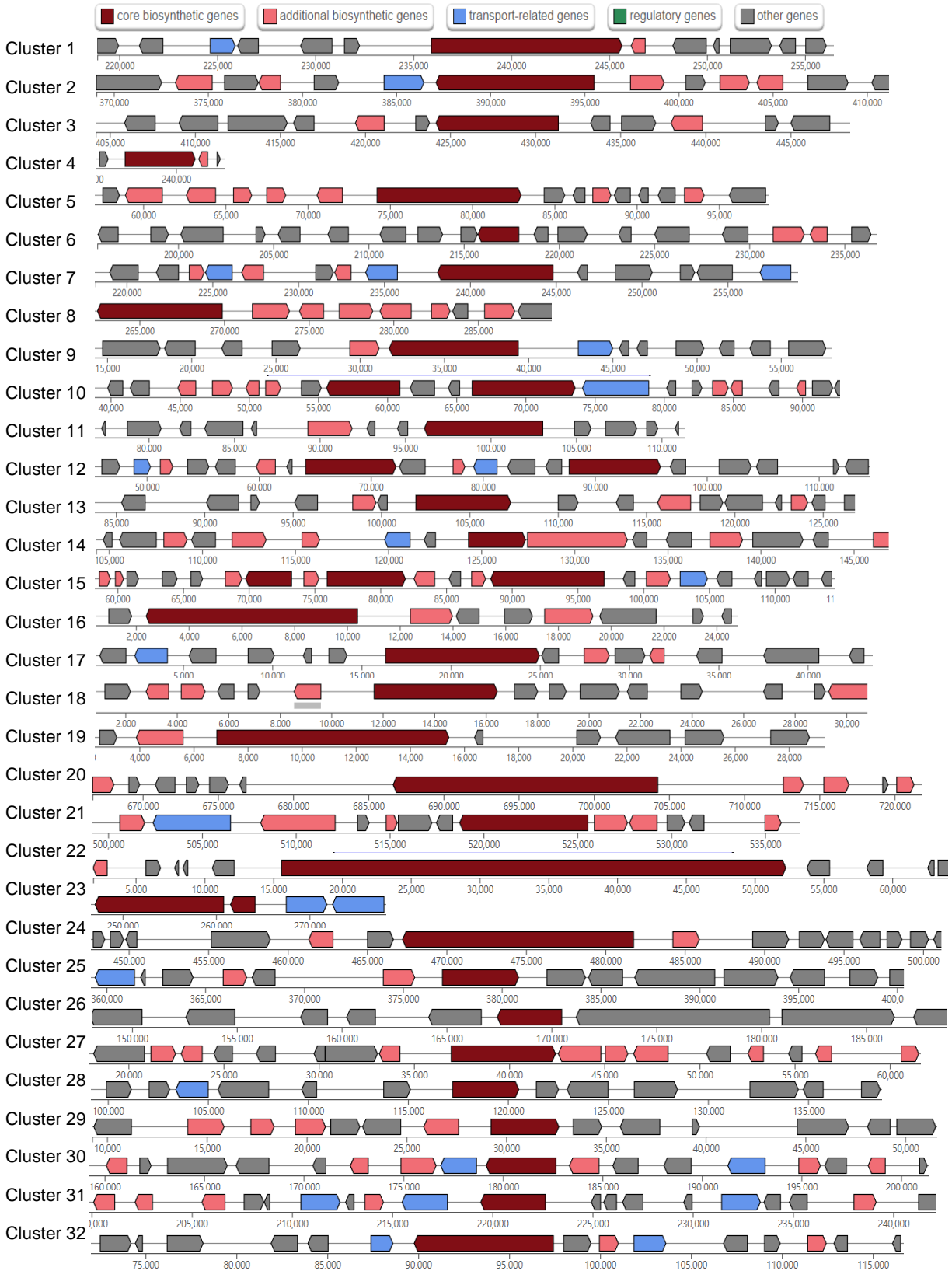
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Table S1. Primers used in this study.

Primer	Sequence (5' to 3')	Correponding plasmid
ZS-POgpdA-F	tttgctccaggaatacatgtgagcttactg	General fungal plasmids
ZS-POgpdA-R	TTTTGCGATTGTTTGAAGTGTTCTGTATGC	General fungal plasmids
PE-gpdA-F	gattcgtccagggcttcccaag	General fungal plasmids
PE-gpdA-R	GATTGCGGTTTACTAGAAGTTGTTAGATC	General fungal plasmids
ZS-coxAp-F	AATGCCTGATCCAGCCCCCAG	General fungal plasmids
ZS-coxAp-R	TGTCCTGGTGGGTGGGTTGC	General fungal plasmids
4.1A-gpda-F	ATTACCCCGCCACATAGACACATCTAAACA ATGGCTTCCTTAGCACGAGC	ThT-22_4.1A-pYTR
4.1A-riboB-R	GTATCATCGAAAGGGAGTCATCCAATACGC GTTTCTTTGTTCAAATTACAGGCGCATCAG	ThT-22_4.1A-pYTR
4.1B-gpda-F	ATTACCCCGCCACATAGACACATCTAAACA TTAATTAATGCCGTCGCTGTCG	ThT-22_4.1B-pYTR
4.1B-ribo-R	TCATCGAAAGGGAGTCATCCAATACGCGTT TCTGTTGGGTACTTTATTGTTATAACCAGG	ThT-22_4.1B-pYTR
4.1D-gpda-F	ACTAACCATTACCCCGCCACATAGACACAT CTAAACAATGCAGTACGCATCAATAGCAGC	ThT-22_4.1D-pYTU
4.1D-pyr-R	GGAGGACATACCCGTAATTTTCTGACGCGT TATACTCATGTACCTCAAGTCTAGGTAG	ThT-22_4.1D-pYTU
4.1A-gpda-F	ATTACCCCGCCACATAGACACATCTAAACA ATGGCTTCCTTAGCACGAGC	ThT-22_4.1AB-pYTR
4.1A-PO-R	cagtaagctcacatgtattcctggagcaaaTTCTTTGTTCAA ATTACAGGCGCATCAG	ThT-22_4.1AB-pYTR
4.1B-PO-F	GCATACAGAACACTTCAAACAATCGCAAAA ATGCCGTCGCTGTCG	ThT-22_4.1AB-pYTR
4.1B-ribo-R	TCATCGAAAGGGAGTCATCCAATACGCGTT TCTGTTGGGTACTTTATTGTTATAACCAGG	ThT-22_4.1AB-pYTR

Table S2. Annotation of cluster 5 (eujavanicol A).

Gene	JGI Protein ID	size (aa)	Proposed function	Homologous proteins (accession number/identity/similarity, %)
EujA	489590	592	BBE-like enzyme	A0A1V6PBT1.1 46/61
EujB	521911	331	SDR	A0A0C6DRT7.1 44/60
EujC	489593	383	trans-ER	A0A1V6PAP3.1 50/65
EujD	489595	490	P450	Q6F5E4.1 41/60
EujE	241969	2918	HRPKS	A0A1V6PAF7.1 46/61



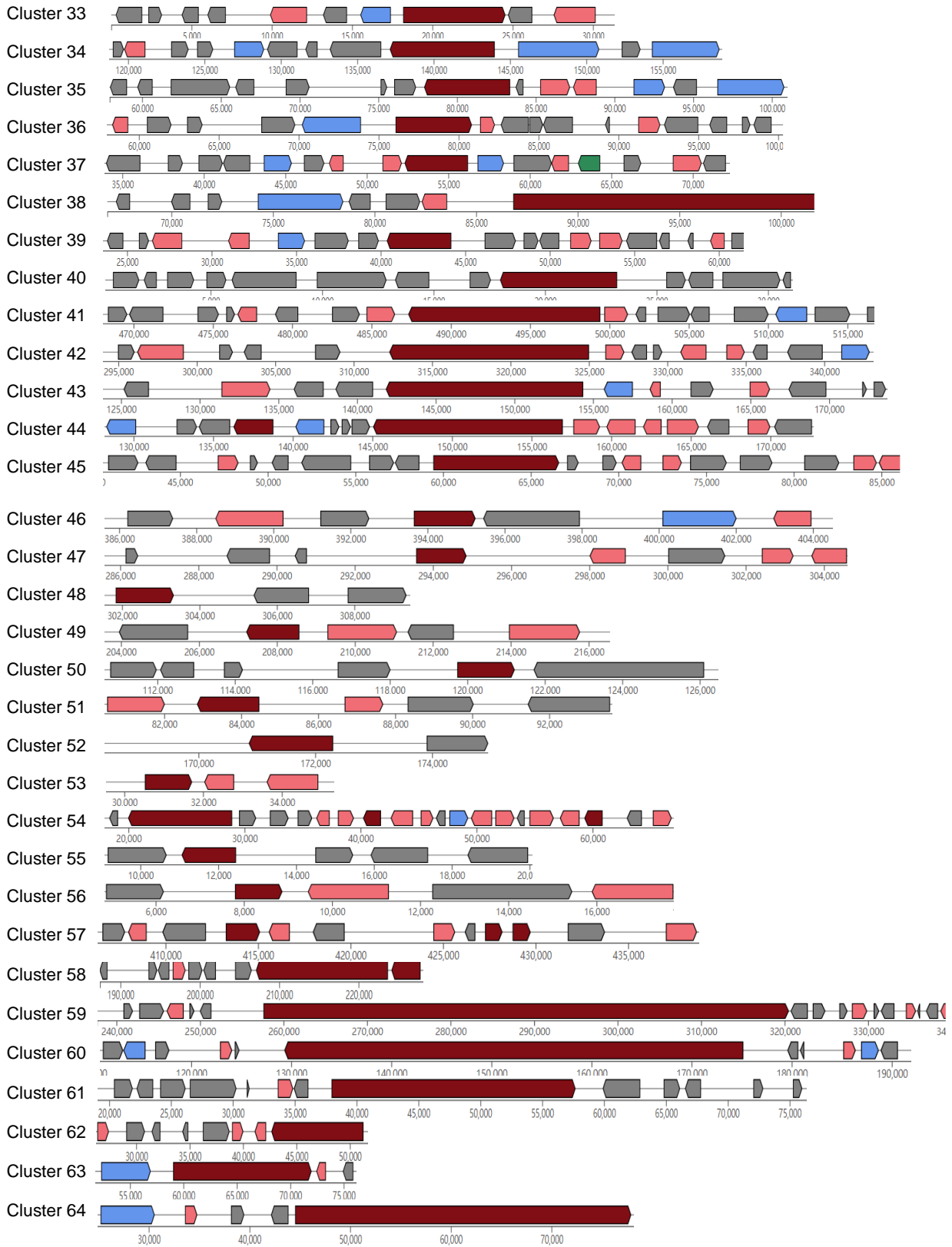


Figure S1. AntiSMASH scheme of BGCs from ThT22.

"20220223 ZS WH 4.3ABCDE (eujavanicol A)" 1 1 C::BIOSYN.NMR
default proton parameters

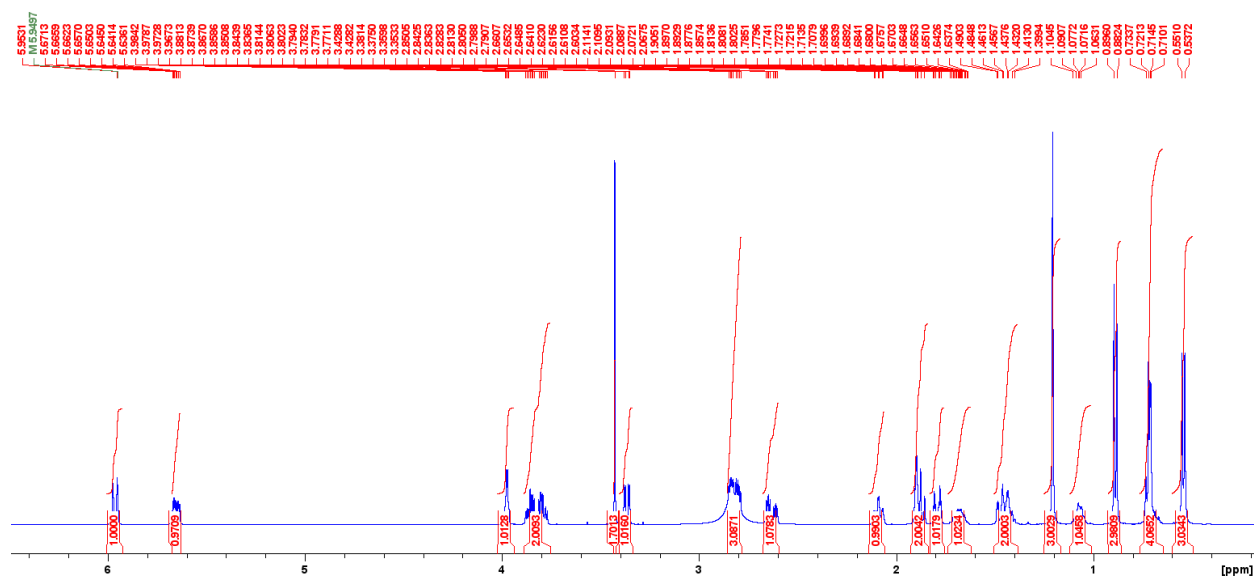


Figure S2. ^1H NMR of eujavanicol A in CDCl_3 , 500 MHz.

"20220223 ZS WH 4.3ABCDE (eujavanicol A)" 2 1 C::BIOSYN.NMR
default carbon parameters

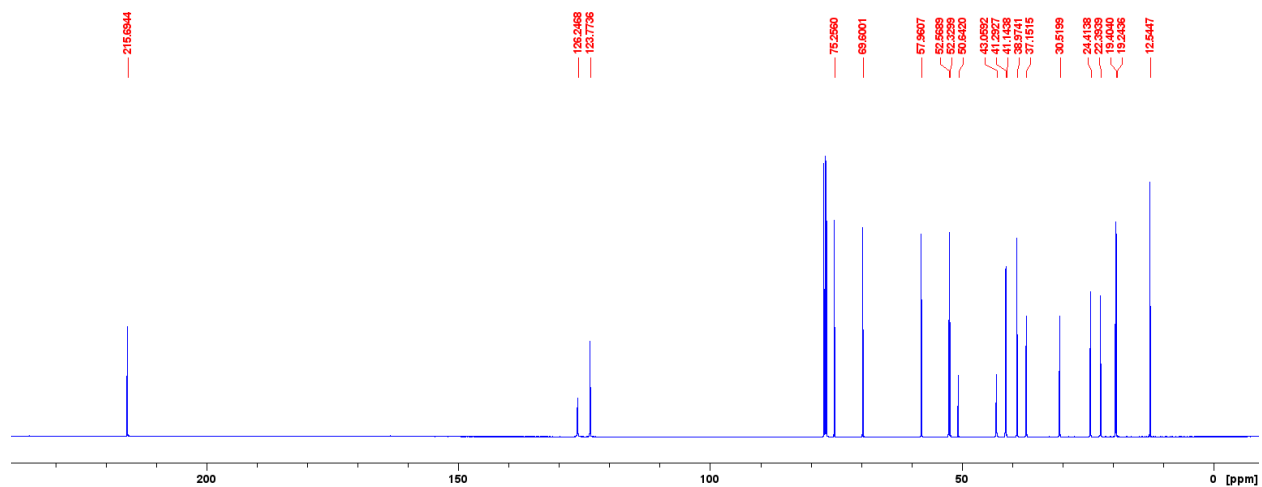


Figure S3. ^{13}C NMR of eujavanicol A in CDCl_3 , 125 MHz.

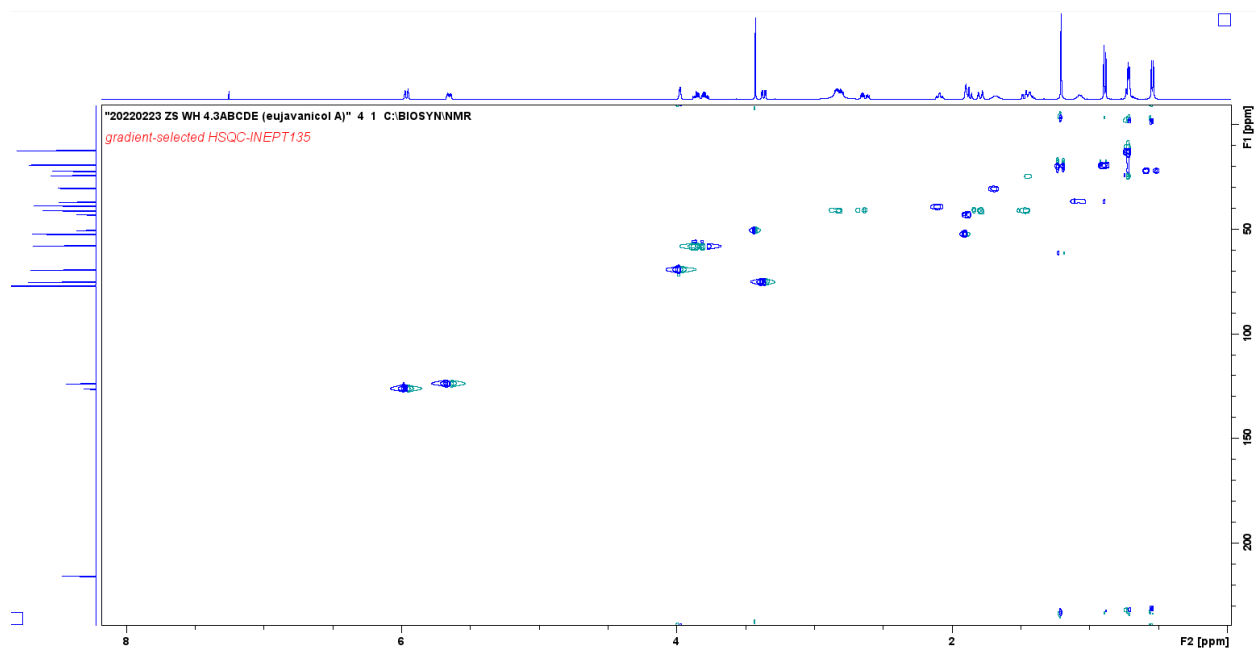


Figure S4. ^1H - ^{13}C HSQC of eujavanicol A in CDCl_3 , 500 MHz.

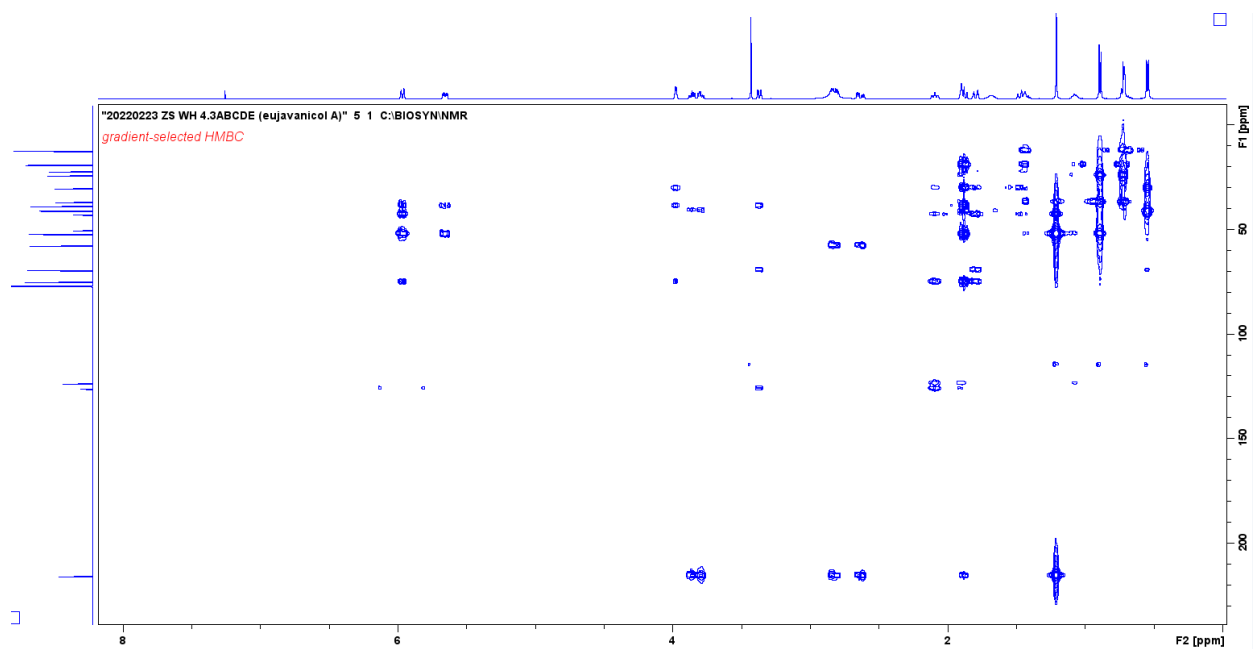


Figure S5. ^1H - ^{13}C HMBC of eujavanicol A in CDCl_3 , 500 MHz.

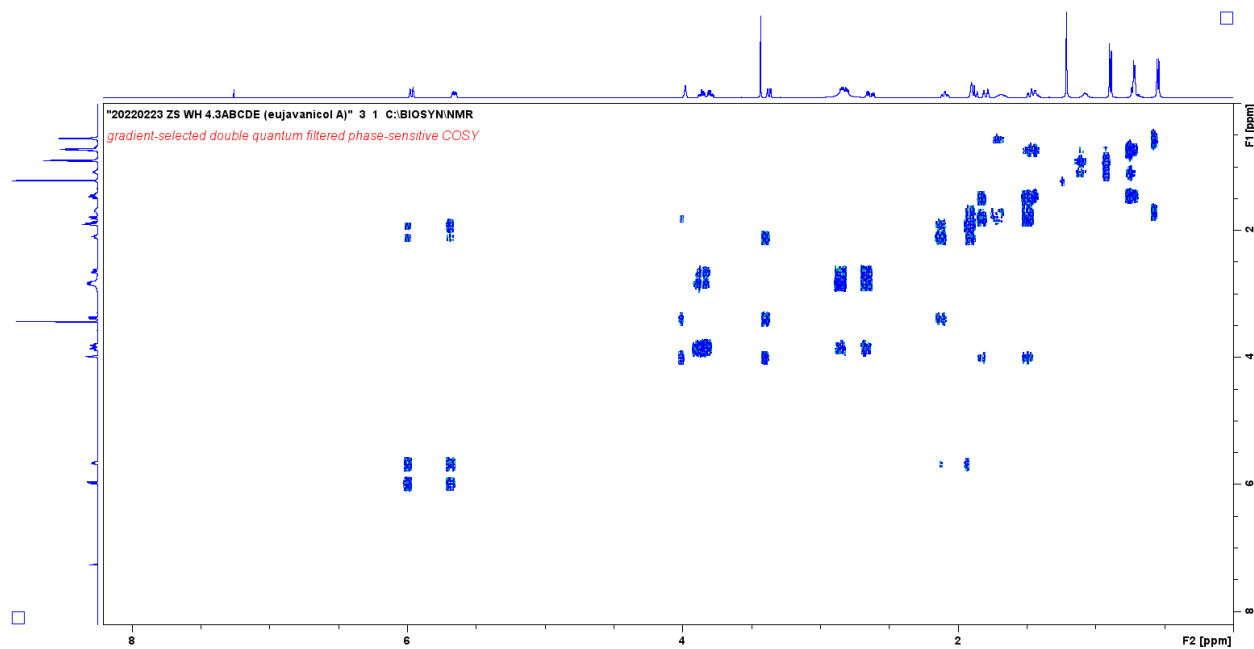


Figure S6. ^1H COSY of eujavanicol A in CDCl_3 , 500 MHz.

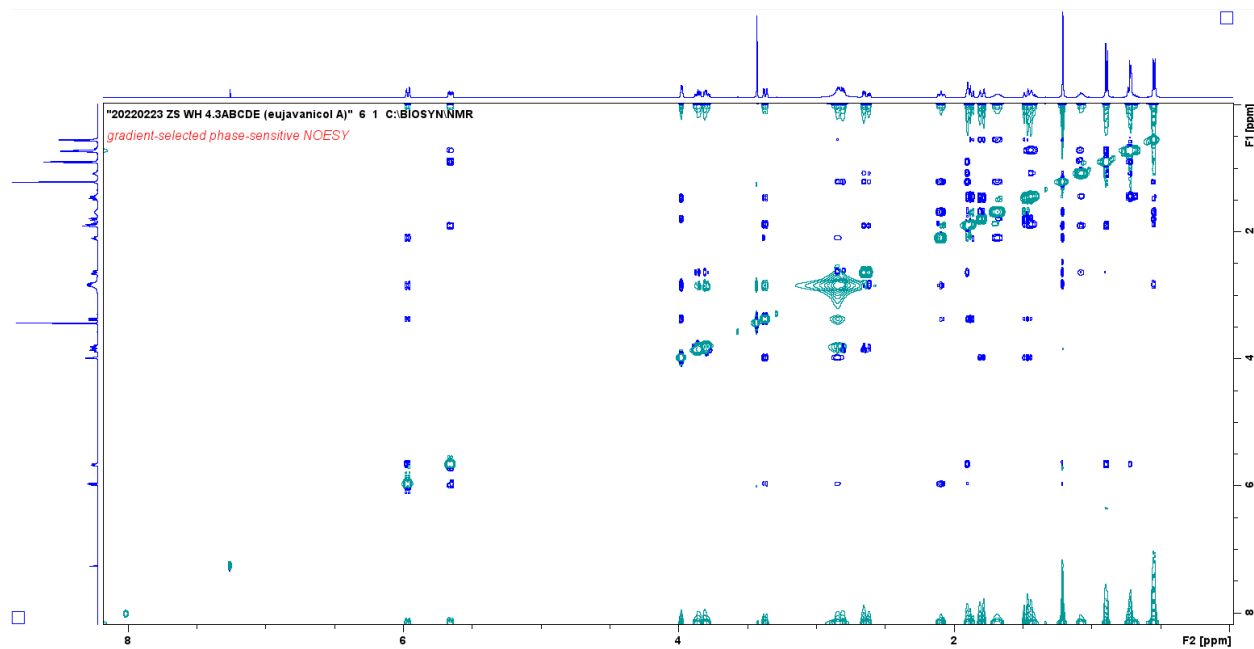


Figure S7. ^1H NOESY of eujavanicol A in CDCl_3 , 500 MHz.

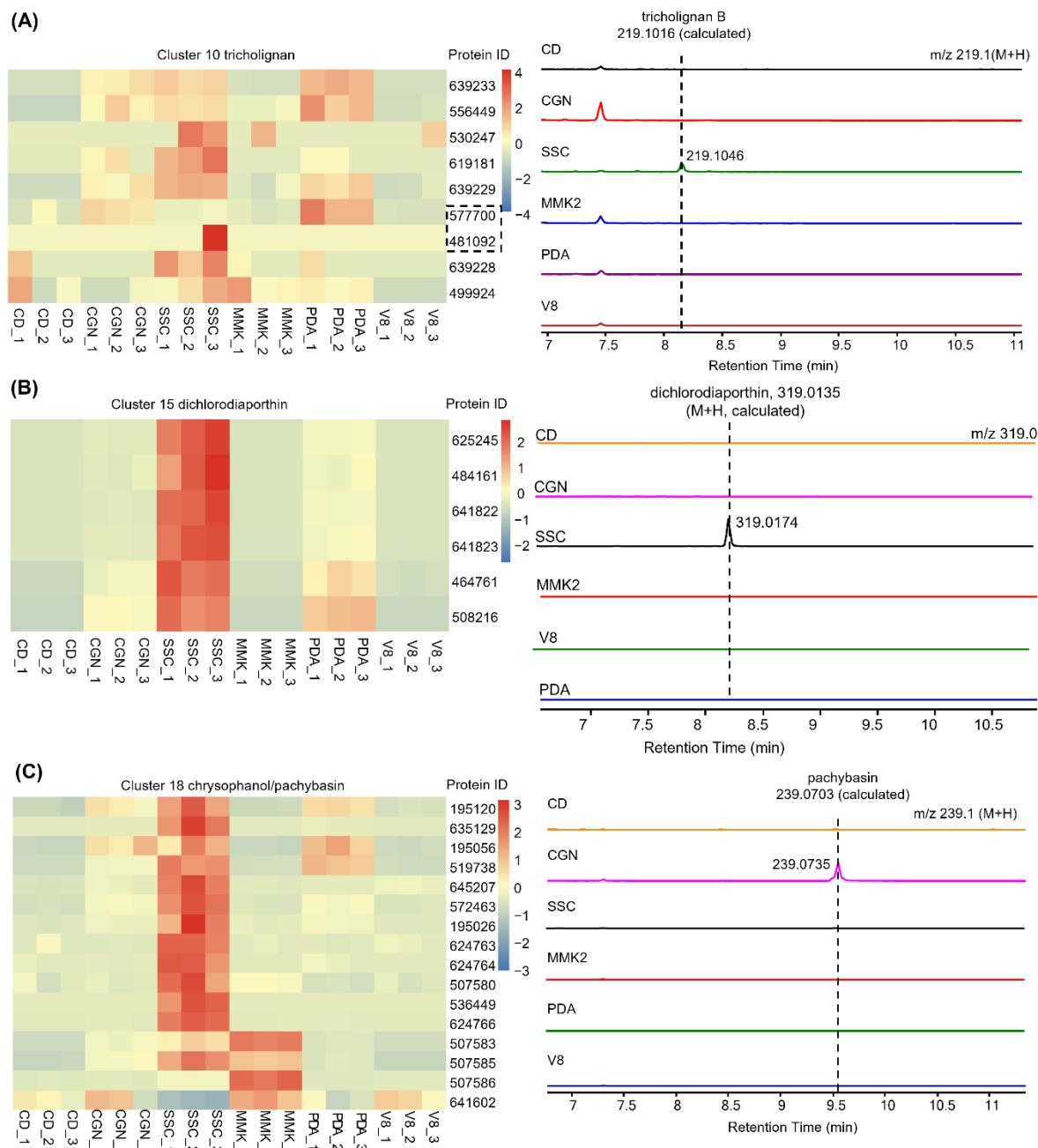


Figure S8. Transcription and metabolite profiles of PKS BGCs. **(A)**, cluster 10-tricholignan. Despite that the two genes in dashed box are not co-upregulated with other genes in the BGC on the SSC medium, these two genes are involved in the biosynthesis.¹ **(B)**, cluster 15-dichlorodiaporthin. **(C)**, cluster 18-chrysolphanol/pachybasin. Left panel, the transcription profile of the BGC and genes nearby on different media. Three independent transcription profiles are shown for each medium. Colors in the heatmaps are scaled based on the RPKM number relative to that of CD. Right panel, detection of the metabolite encoded by the BGC via LC-MS. One representative metabolic profile of three independent experiments is shown for each medium. Traces are shown as extracted ion chromatograms.

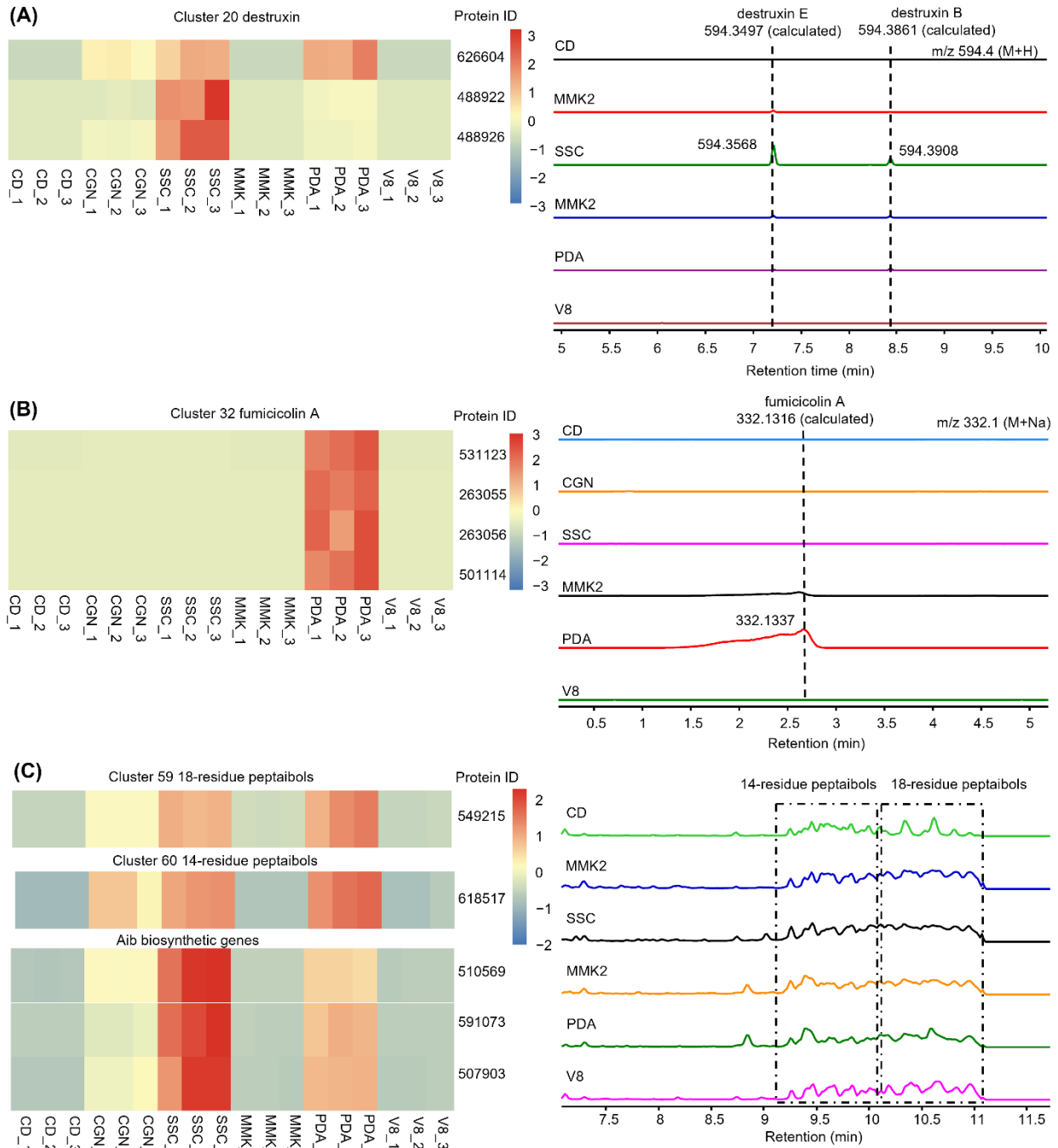


Figure S9. Transcription and metabolite profiles of NRPS BGCs. **(A)**, cluster 20-destruxin. **(B)**, cluster 32-fumicolin A. **(C)**, cluster 59,60-peptaibol. Left panel, the transcription profile of the BGC and genes nearby on different media. Three independent transcription profiles are shown for each medium. Colors in the heatmaps are scaled based on the RPKM number relative to that of CD. Right panel, detection of the metabolite encoded by the BGC via LC-MS. One representative metabolic profile of three independent experiments is shown for each medium. Traces are shown as extracted ion chromatograms except for **(C)** where total ion chromatograms are shown.

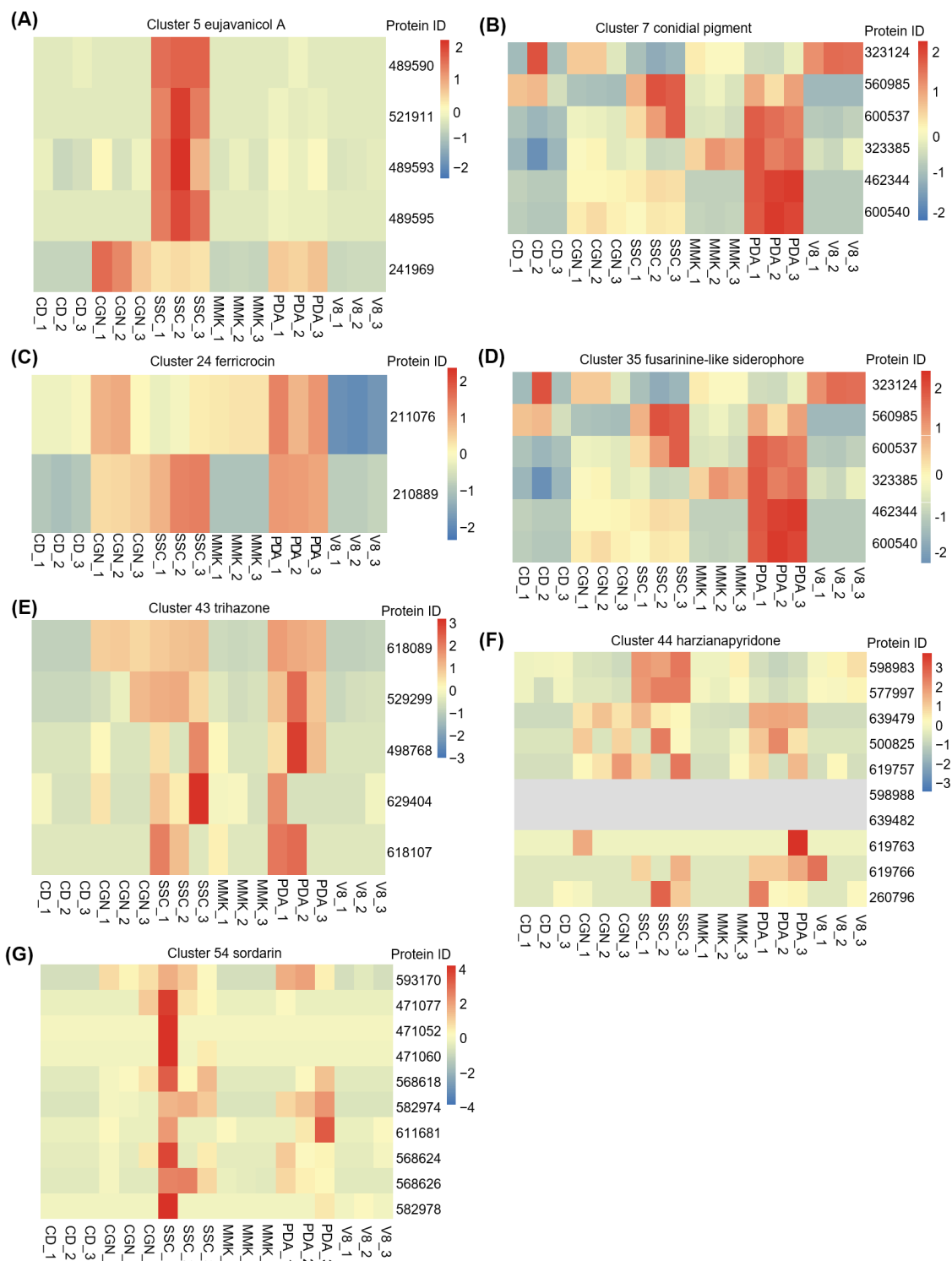


Figure S10. Transcription profiles of known BGCs whose corresponding metabolites were not detected on any of the six media. (A), eujavanicol A; (B), conidial pigment; (C), ferricrocin; (D), fusarinine-like siderophore; (E), trihazone; (F), harzianopyridone; (G), sordarin. Three independent transcription profiles are shown for each medium. Colors in the heatmaps are scaled based on the RPKM number relative to that of CD.

References:

- (1) Chen, M.; Liu, Q.; Gao, S.-S.; Young, A. E.; Jacobsen, S. E.; Tang, Y. Genome Mining and Biosynthesis of a Polyketide from a Biofertilizer Fungus That Can Facilitate Reductive Iron Assimilation in Plant. *Proc. Natl. Acad. Sci.* 2019, 116 (12), 5499–5504.