Supplementary Information

A terpene cyclase from *Aspergillus ustus* is involved in the biosynthesis of geosmin precursor germacradienol

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Supplementary Tables

Strain	Genotype	Created with	Reference					
E. coli DH5α	F- endA1 glnV44 thi-1 recA1 gyrA96 deoR nupG purB20 φ 80d <i>lacZ</i> ΔM15 Δ (<i>lacZYA-argF</i>)U169, hsdR17($r_{K}m_{K}^{+}$), λ^{-}	-	2					
E. coli BL21 (DE3)	F ⁻ ompT hsdS _B (r _B -, m _B -) galdcmrne131	-	Merck, KGaA, Darmstadt, Germany					
S. cerevisiae HOD114-2B	MATα ura3-52 his3∆1 Ieu2-3112	-	4					
A. ustus 3.3904	wildtype	-	CGMCCC					
A. nidulans:								
LO8030	<i>pyroA4, riboB2,</i> <i>AfpyrG89, nkuA::argB,</i> deletion of secondary metabolite clusters: (AN7804-AN7825)Δ, (AN2545-AN2549)Δ, (AN1039-AN1029)Δ, (AN10023-AN10021)Δ, (AN8512- AN8520)Δ, (AN8379-AN8384)Δ, (AN9246-AN9259)Δ, (AN7906-AN7915)Δ, (AN6000-AN6002)Δ.	-	1					
MP02	wA-PKS::gpdA(p)-gdlS 3'UTR-Afribo in LO8030	pMP008	This study					
BK06	wA-PKS::gpdA(p) + 500bp 3'UTR-Afribo in LO8030	-	5					

Table S1: Strains used in this study

CGMCCC: China General Microbiological Culture Collection Center (Beijing, China)

Plasmid	Genotype	Description	Reference
pJN017	URA3, wA flanking, AfRiboB, Amp, apdA(p)	standard-vector for heterologous expression in <i>A. nidulans</i> LO8030	3
pMP008	URA3, wA flanking, AfRiboB, Amp, gpdA(p), gdIS	Heterlogous expression of <i>gdlS</i> in <i>A. nidulans</i> LO8030	This study
pET-28a(+)	Kan , T7(p), 6xHis, MCS, T7 Terminator, lacl	Expression vector for <i>E.</i> <i>coli</i> with both N- and C- terminal His6-tag	Merck, Novagen, Darmstadt, Germany
pMP014	Kan , T7(p), 6xHis, gdlS, T7 Terminator, lacl	Overexpression of GdlS protein in <i>E. coli</i> BL21 (DE3)	This study

 Table S2: Plasmids used in this study

Primers	Sequence (5'-3')	Description			
MP3.1_TSfor	TACCCCGCTTGAGCAGACATCACCGGCATGTTCTCT	Cloning gd/S			
MP3.1_TSrev	CCATATTTTAATCCCATGTGGGCCTATCTAGTAAGA	- in pJN017 = pMP008			
	GATAAAACCCGAGATACAG				
Sst81	GCGAGCCTTCCATAGTTACG	Verification of MP02			
MP3.1gene_midrev	CGAGAGCCAGAAATCGTCC				
MP3.1gene_midfor	GGACGTGCTGTATCTCGGG				
JN015ctrl_01	GCACTCTGGAAACGAACTCC				
MDTSforBomUl	GGATCCCTATCTAGTAAGAGATAAAACCCGAGATAC	Cloning of CDS			
	AG	_ <i>gdlS</i> in pET- 28a(+) =pMP014			
MPTSrevNdel	CATATGATGTTCTCTAATAAAACGAAACAGGACG				

 Table S3: Oligonucleotide primers used in this study

Supplementary Figures



Fig. S1 Phylogenetic analysis of the C-terminus (amino acid residues 374–726) of the germacradienol/geosmin synthase SCO6073 from *Streptomyces coelicolor* and selected homologues from bacteria and fungi. The protein sequences were downloaded from NCBI database.

		1			10			2	0			30			40			5	0			
Gdls KAF8801858. RCJ34029.1 Q9X839.3	1	MFS.MA	RV	TK PT N MT	DD FS QP	WIS LL EL QL	LEG	.QH .Q .E	VRV ISC FYT FYL	PYL PFP PWP PHP	PRL VSY ARL ARL	FPS HPN NPN NPH	WKA GDI LEA LDE	SLH IAA ARV ARA	PEYASDHSK	ERA KWF AWA TWA	RDE EAE YEM REM	V L N S Y Y G I L G M L	FT	IRR EEE	AQ1	VDDD .EA ISVI .SGV
Gdls KAF8801858. RCJ34029.1 Q9X839.3	1	O RRF WDE WEQ	SK RL HT SD	LQI HQI FDS LEJ	AEI KV(HD)	GVP RLV ALI GLI	AAV TCC CSY CAY	TH TH	ADS RVI PDA PDC	P F D N D N P G A D G P	RIC RIR AIS	90 IVA IVC LVT LIT	KYF DFM DWY DWY	AWY NVL VWV VWV	10 FIM FHM FF FF	** ODI DDL DD.	* CDF .HF .HF	GSI LST LEI LEK	QG SE YK YK	QPQ AVT RTQ RSQ	AMI FSI DMI DRI	KEYR EVIM AGAK LAGK
Gdls KAF8801858. RCJ34029.1 Q9X839.3	12	e AS NAL EYL AHL	AS DR DR	YIP PHI LPI	HQI YR FMI		SGP SGP TDT AAG	1 NLI .PI	40 LSK PAV SVP PEP	YST EPE TNP RNP	ELQ ASK VER VEA	ISO KAL LIR GLA GLA	ECW NFW DLW DLW	AEV ORC SRT TRT	16 GYH IVD AFT VPA	MRS MAP KSV MSA	VCD GIQ DWR DWR	RGI ARF LRF RRF	7 0 CEEEAV	VSS NVS STK ATE	DAN ATV NLI HLI	LDY KSI EES NES
Gdls KAF8801858. RCJ34029.1 Q9X839.3	18	INA ETE LWE MWE	VD RK LA	DAN FR. NI.	19 NALI	GKI NQI NEC	SSIP SLP RVA SRVA	2 DF NP	O O E E Y A A Y V E Y	WRR IAF IEM IEM	* REDY RED REK REK	210 AAG NSS VGG VGG	VYP CRN APW APW	TIA ALD SAD SAG	22 TIP LLE LVE LVE	9 FAL YSM HAV YAT	GVD EIE FIE .AE	ISP LPN IPA VPA	ED ED EV EI AV	A A N R N D A S T A G T	PRIRPI	ODI TVL RIL RVL
Gdls KAF8801858. RCJ34029.1 Q9X839.3	24	WKH SNC KDT MET	TS	YLV DF1 DGV	25 (HI) (AL) (HL) (HL)	** ND ND ND ND	* (ISI (FSY (FSY (FSY)		60 LL V V V V V V V	KDG SRG DEG	QIE DIC ENA ELS	27 NIV HSV NCV	PVL PII LVL LVL	MLN MKI ERF ETF	2 KGI YKL LNV FGC	SIN DLQ STQ TTQ	EAI GAV EAA EAA	KQS DHI NLT DLV	29 YK AD NE ND	PAE MCR LLT VLT	ENI ESI SRI SRI	RGI IESF YQF HQF
Gdls KAF8801858. RCJ34029.1 Q9X839.3	3	DQA Q.A DNI EHI	AA AK AV	ALI TEI TEV	SEI PSI SPI PAT	FDE NGTE LFE VAL	RND	DV.	VAR	V VNV AAV	320 KAF ALY LLY GAY	ARG IGG IKG TKG	CMD IED LOD	33 IAA WMS WQS WQS	GLI GNI GGH	HWSS EWS EWH	3 YSG LLS MRS	40 ERY ERY SRY	FK. FP	ASE PGS KGE	VD MKI RPI	S O TENV EGVV
Gdls KAF8801858. RCJ34029.1 Q9X839.3	1	FHF TLL	WT PR	360 TTS QTI	NN SNN S	SPKR	37 (APA	Q REI	DTG	YIL	380 PST	I I Y	T L P	39 AFA	o VGS	FLA	4 LVY	o o G G P	AV	SRV	41 LSI	LTR

Fig. S2 Multiple sequence alignments of GdIS. The fungal terpene synthase KAF8801858.1 is from *Cortinarius glaucopus*. The N-terminal sequences of the bacterial germacradienol/geosmin synthases are from RCJ34029.1 (*Nostoc punctiforme*) and Q9X839.3 (*Streptomyces coelicolor*). Amino acid residues marked with * are responsible for catalytic activity of terpene synthases.



Fig. S3 Verification of plasmid pMP008 for heterologous expression of *gdlS* in *A. nidulans* LO8030 and correct integration in *wA* locus. The GeneRuler DNA Ladder mix by Thermo Fisher was used as size standard for DNA fragments. Verification of transformants *via* PCR was performed with primers binding outside of the integration construct in genomic DNA and inside of integrated gene. Verification of pMP008 was carried out by digestion with restriction enzyme HindIII. Gel image of pMP008 digestion was mirrored and cut. The electrophoretic gel of the verification of putative transformants was assembled out of two gels and rearranged at the dashed lines.



Fig. S4 Verification of plasmid pMP014 for GdlS overproduction in *E. coli* BL21 (DE3). GeneRuler DNA Ladder mix by Thermo Fisher served as size standard for DNA fragments. The verification of the plasmid was done *via* restriction with BamHI, NdeI and PvuII and by sequencing. The gel image for the digestion of pMP014 was cut and rearranged at the dashed lines.



Fig. S5 LC-MS analysis of the wildtype *A. ustus* 3.3904. Illustrated are UV absorptions at 190 – 400 nm, EICs with the main fragment ion of germacradienol at m/z 205.195 and the [M+H]⁺ ion of geosmin at m/z 183.174.



Fig. S6 LC-MS analysis of feeding *A. ustus* 3.3904 with germacradienol. UV absorption at 190 – 400 nm is illustrated in A. EIC of the main fragment of germacradienol is shown in B.



Fig. S7 ¹H NMR spectrum of germacradienol in CDCl₃ (400 MHz).



Fig. S8 ¹³C NMR spectrum of germacradienol in CDCI₃ (100 MHz).



Fig. S9 HSQC spectrum of germacradienol in CDCl₃.



Fig. S10 ¹H-¹H COSY of germacradienol in CDCl₃.



Fig. S11 HMBC spectrum of germacradienol in $CDCI_3$.

Raw data



Fig. S12 SDS-PAGE of purified and recombinant GdlS in three different dilutions (1.1 μ g, 0.6 μ g, 0.3 μ g).



Fig. S13 Verification of plasmid pMP008 for heterologous expression of *gdlS* in *A. nidulans* LO8030. Plasmids isolated from 24 *E. coli* colonies were tested.



Fig. S14 Control of correct integration of *gpdA:gdlS* in *wA* locus in *A. nidulans* LO8030. Lane 4 and 5 represent the negative control.



Fig. S15 Control of correct integration of *gpdA:gdlS* in *wA* locus in *A. nidulans* LO8030. Lane 13 represents the marker, lane 14 corresponds to the amplification of the upstream fragment A in the transformant strain MP02, lane 10 exhibits amplification of the downstream fragment B in the transformant strain MP02.



Fig. S16 Verification of plasmid pMP014. The upper image exhibit digestion with BamHI and Ndel with plasmids isolated from 16 different *E. coli* colonies, whereas digestion with PvuII in 14 distinct plasmids isolated from *E. coli* is shown in the lower image.

References

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