

**High efficacy of the Volatile Organic Compounds of *Streptomyces yanglinensis* 3-10 in Suppression of *Aspergillus* Contamination on Peanut Kernels**

Ang Lyu<sup>1,2,3</sup>, Long Yang<sup>1,2</sup>, Jing Zhang<sup>1,2</sup>, Mingde Wu<sup>1,2</sup>, and Guoqing Li<sup>1,2\*</sup>

<sup>1</sup>State Key Laboratory of Agricultural Microbiology and Key Laboratory of Plant Pathology of Hubei Province, <sup>2</sup>Huazhong Agricultural University, Wuhan 430070, China

<sup>3</sup>School of life science and technology, Hubei Engineering University, Xiaogan 432000, China

\*Correspondence: Dr. Guoqing Li, E-mail: guoqingli@mail.hzau.edu.cn

## **I. Supplementary Tables**

**Supplementary Table S1.** List of the microbial isolates used in this study.

Test fungus and isolates	Host and/or origin*
Actinomycetes	
<i>Streptomyces yanglinensis</i> 3-10	Rice ( <i>Oryza sativa</i> ), HZAU
Fungi	
<i>Alteraria alternata</i> TBS-3	Tobacco ( <i>Nicotiana tabacum</i> ), HZAU
<i>Aspergillus flavus</i> NRRL3357	Peanut ( <i>Arachis hypogaea</i> ), NRRL
<i>Aspergillus parasiticus</i> MO527	Rice Rice ( <i>Oryza sativa</i> ), CGMCC
<i>Botrytis cinerea</i> RoseBC-3	Rose ( <i>Rosa chinensis</i> ), HZAU
<i>Bipolaris maydis</i> MB-1	Maize ( <i>Zea mays</i> ), HZAU
<i>Curvularia lunata</i> CFW-4	Rice ( <i>Oryza sativa</i> ), HZAU
<i>Colletotrichum siamense</i> Nj-2	Strawberry ( <i>Fragaria</i> × <i>ananassa</i> ), HAAS
<i>Drechslera graminea</i> BGW014	Barley ( <i>Hordeum vulgare</i> ), HZAU
<i>Fusarium moniliforme</i> WF-3	Rice ( <i>Oryza sativa</i> ) , HZAU
<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> FO-25	Cotton ( <i>Gossypium hirsutum</i> ), HAAS
<i>Gaeumanomyces graminis</i> var. <i>tritici</i>	Wheat ( <i>Triticum aestivum</i> L.), HnAAS
<i>Monilia fructigena</i> BT15-56	Peach ( <i>Amygdalus persica</i> ), HZAU
<i>Mucor hiemalis</i> WHT-1	Tomato ( <i>Lycopersicon esculentum</i> ), HZAU
<i>Pestalotia theae</i> DMZ21	Rice ( <i>Oryza sativa</i> ), HZAU
<i>Pyricularia oryzae</i> PB-1	Rice ( <i>Oryza sativa</i> ), HZAU
<i>Rhizoctonia solani</i> WH-1	Rice ( <i>Oryza sativa</i> ), HZAU
<i>Rhizopus stolonifer</i> WHS-1	Strawberry ( <i>Fragaria</i> × <i>ananassa</i> ), HZAU
<i>Sclerotinia minor</i> Lc-45	Lettuce ( <i>Lactuca sativa</i> ), HZAU
<i>Sclerotinia sclerotiorum</i> Ss-1	Oilseed rape ( <i>Brassica napus</i> ), HZAU
<i>Sclerotium rolfsii</i> B-1	Plumepoppy ( <i>Macleaya cordata</i> ), HZAU
Oomycetes	
<i>Pythium ultimum</i> WLOW2F	Watermelon ( <i>Citrullus lanatus</i> ), WAAST
<i>Pythium aphanidermatum</i> Py-xg	Watermelon ( <i>Citrullus lanatus</i> ), WAAST

\*NRRL = Agricultural Research Service Culture Collection, United States Department of Agriculture; CGMCC = China General Microbiological Culture Collection Center, Beijing, China; HbAAS = Hubei Academy of Agricultural Sciences, Wuhan, China; HnAAS = Henan Academy of Agricultural Sciences, Zhengzhou, China; HZAU = Huazhong Agricultural University, Wuhan, China; WAAST = Wuhan Academy of Agricultural Science and Technology, Wuhan, China.

**Supplementary Table S2.** List of the oligonucleotide primers for RT- PCR amplification in this study.

Gene	GenBank Acc. No.	Sequence (5'-3')	Thermal program in RT-PCR	Size of the RT-PCR product
2-MIB synthase gene	MK861971	Forward: TGGTTGTGGATC TCGACACTG Reverse: ACCTCGTCACGG CCTACTAC	Initial denaturation: 95°C for 5 min; 32 cycles: 95°C for 30s, 56°C for 30s, 72°C for 1 min 15s;	1107 bp
Methyltransfer-ase gene	MK861972	Forward: AGAAACGGTTCT TCGACCCC Reverse: CTGAGGAGAACC CGGTCAAC	Initial denaturation: 95°C for 5 min; 32 cycles: 95°C for 30s, 56°C for 30s, 72°C for 1 min;	876 bp
Geosmin synthase gene	MK861973	Forward: CTCGTGCCAGGT GAGGATGCC Reverse: CGCGTTCTACGT GCCGTACC	Initial denaturation: 95°C for 5 min; 32 cycles: 95°C for 30s, 56°C for 30s, 72°C for 2 min 15s;	2277 bp
Aryl-alcohol dehydrogenase gene	MK861974	Forward: GATGTGCCGGAC CGACC Reverse: GATGAGCTGGTC GAAGGGGAA	Initial denaturation: 95°C for 5 min; 32 cycles: 95°C for 30s, 56°C for 30s, 72°C for 1min; final	1066 bp
(+)-β-caryophyllene synthase gene	MK861975	Forward: CAGATCACCTTA CCGGCGTT Reverse: TCGATCAGTTCC CTTTCCGC	Initial denaturation: 95°C for 5 min; 32 cycles: 95°C for 30s, 55°C for 30s, 72°C for 1 min;	975 bp
gyrB gene	MK861976	Forward: GAGGTCGTGCTG ACCGTGCTGCAC GCGGGCGGCAA GTTCCGGC Reverse: GTTGATGTGCTG GCCGTCGACGTC GGCGTCCGCCAT	Initial denaturation: 95°C for 5 min; 32 cycles: 95°C for 30s, 65°C for 30s, 72°C for 1 min 30s; final extension: 72°C for 10 min.	1284 bp

**Supplementary Table S3.** List of the oligonucleotide primers for qRT- PCR analysis in this study.

Gene name	Gene functions	Primers used in the qRT-PCR
aflR	Pathway regulator	F:AGCACTACAAACACTGACCCAC R:CCAGCACCTTGAGAACGATAA
aflCa	Noranthrone monooxygenase, Norsolorinate-anthrone to norsolorinate	F: GCACCAATGGAGCCGTAT R: GCGGTGTTTCGTAGCGTTC
aflA	FAS alpha subunit, Acetate to polyketide	F: CGTGAGGTCAAGGCATTCCT R: GACTTGCCCCCCTTCTGT
aflS	Transcription enhancer, Pathway regulator	F: CCGAAGATTCCGCTTGGA R: TGAAGACATGCAGCAAAAGGA
aflO	O-methyltransferase B, dihydrodemethylsterigmatocystin to dihydrosterigmatocystin	F: TGCTGTGGCATCCATTCAAA R: GGACTGCGTCTTCCAAAAGG
aflD	NOR reductase, norsolorinic acid to averantin	F: ACTGCGACTCGAAACTGATG R: TGCTCCTCCCACAATGTC
aflP	O-methyltransferase A, sterigmatocystin to O-methylsterigmatocystin	F: TGTGTCGAGTGATGTGGGACTAG R: GCCACCCAGCTCAACCTACA
aflF	NOR dehydrogenase, norsolorinic acid to averantin	F: AAGATGCTGGGCACGTTTG R: CATGGGTGAGGACGAATTGG
aflQ	Oxydoreductase, O-methylsterigmatocystin to AFB <sub>1</sub> and AFG <sub>1</sub> , dihydro-Omethylsterigmatocystin to AFB <sub>2</sub> and AFG <sub>2</sub>	F: TTGCTGGGCTTGTGGATTC R: GAGGAGGACGCGTGTCTTTG
aflX	Monooxygenase/oxidase, VA to DMST	F: ACCGCGTTGCACATCGT R: TGGGTGTCCACAACCTTCGT
AccC	Acetyl-CoA carbosylase, Acetyl-CoA to Malonyl-CoA	F: ATGGTAAGACCTGCCTGCTA R: AGCGAGGATACCGAGGAT
β-tubulin	Endogenous control, Reference gene	F: AGCAGGCGAAGAAGGAGG R: ACGCCACGCATTTGATCTTC

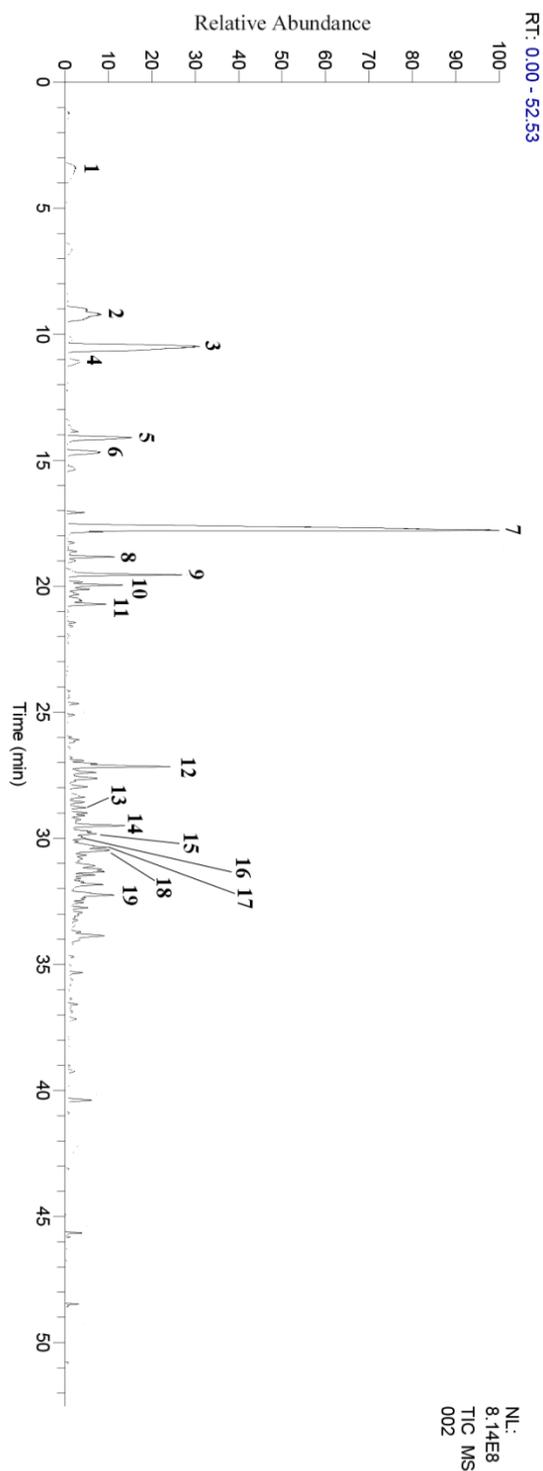
**Reference:** Gong, A. D., Wu, N. N., Kong, X. W., Zhang, Y. M., Hu, M. J., Gong, S. J., Dong, F. Y., Wang, J. H., Zhao, Z. Y., and Liao, Y. C. (2019) Inhibitory effect of volatiles emitted from *Alcaligenes faecalis* N1-4 on *Aspergillus flavus* and aflatoxins in storage. *Front. Microbiol.* 10:1419. doi: 10.3389/fmicb.2019.01419

**Supplementary Table S4.** Effects of the volatile organic compounds (VOCs) from the autoclaved wheat grains (AWG) cultures of *Streptomyces yanglinensis* 3-10 and soil amendment with the AWG cultures of this strain on peanut seedlings growth.

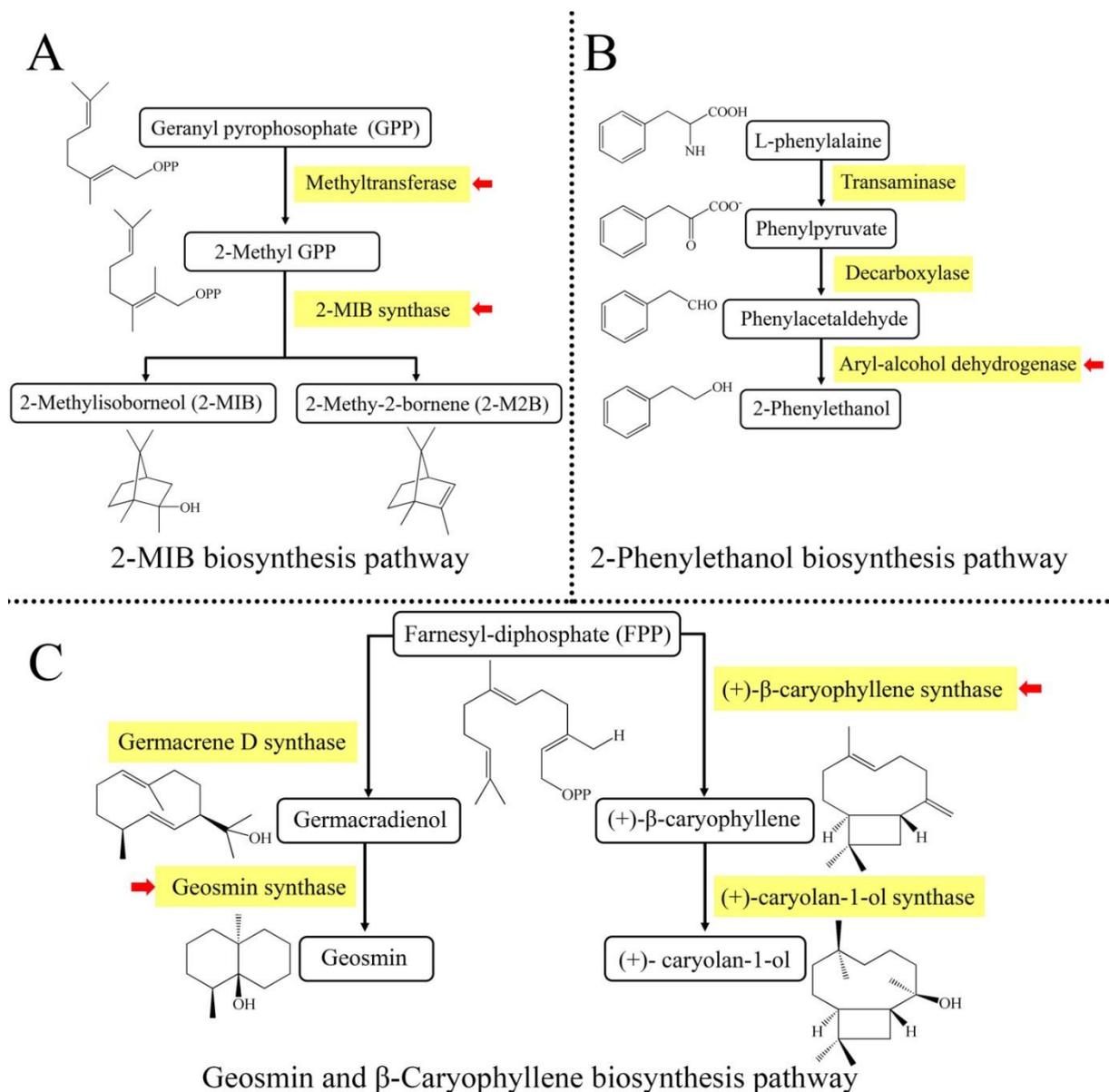
Character	Fumigation with the VOCs			Soil amendment with live culture of <i>S. yanglinensis</i> 3-10	
	AWG culture of <i>S. yanglinensis</i> 3-10 at 12 g/L <i>n</i> =16	AWG culture of <i>S. yanglinensis</i> 3-10 at 34 g/L <i>n</i> =16	Fresh AWG <i>n</i> =16	AWG culture of <i>S. yanglinensis</i> 3-10 at 5% (w/w) <i>n</i> =16	Fresh AWG <i>n</i> =16
Height (cm)	14.1 ± 1.9	11.8 ± 2.2*	14.9 ± 1.70	16.1 ± 4.6	16.7 ± 3.2
TDW (g per seedling)	0.39 ± 0.09	0.38 ± 0.10	0.41 ± 0.11	1.02 ± 0.25	1.05 ± 0.26

\*Significantly different ( $P < 0.05$ ) in comparison with that in the control treatment according to Student's *T* test. TDW =Total dry weight

## **II. Supplementary Figures**



**Supplementary Figure S1.** A SPME-GC-MS chromatogram showing 19 volatile organic compounds (VOCs) emitted from a 10-day-old AWG culture of *Streptomyces yanglinensis* 3-10 (AWG = autoclaved wheat grains). Chemical identity of the labeled VOCs was listed in Table 1. **Peak 1**, methyl 2-methylbutyrate (M2M); **Peak 3** = 2-methyl-2-bornene (2-M2B); **Peak 6**, 2-Phenylethanol (2-PE); **Peak 7**, 2-methylisoborneol (2-MIB); **Peak 12**, *trans*-1,10-dimethyl-*trans*-9-decalinol (geosmin); **Peak 13**, (+)- $\beta$ -caryophyllene



**Supplementary Figure S2.** Biosynthesis pathways of selected VOCs from *S. yanglinensis* 3-10.

The red arrows indicate the genes used for detection of expression by RT-PCR.

References for the biosynthesis pathways: **A**, from Komatsu et al. 2008. Proceedings of the National Academy of Sciences of the United States of America, 105: 7422-7427; **B**: from Zhang et al. 2014, BMC Biotechnology 14: 30; **C**, from Jiang et al. 2007. Nature Chemical Biology 3: 711-715; and Nakano et al. 2011. The Journal of Biological Chemistry 286: 27980-27987.

*S. globisporus* TFH56 (WP\_044368733) .....MPPFCAGGCFEGLAKTRAAAWWABSEGLL 30  
*S. fulvissimus* DSM 40593 (AGK80153) MSQITLFAFHMPPFSTGONPGLKTRAAAWWABAEGILL 40  
*S. cavourensis* TJ430 (WP\_093751234) .....MPPFSTGONPGLDKTRAAAWWABAEGILL 30  
*Streptomyces* sp. SM18 (WP\_103489563) .....MPPFSTGONPGMENTAAAWWABENDIRL 30  
*Streptomyces* sp. PgraA7 (SNB61248) MSQITLFAFHMPPFCAGGCFEGLAKTRAAAWWABSEGLL 40  
*S. globisporus* C-1027(WP\_029182233) .....MPPFCAGGCFEGLPKTRAAAWWABSEGLL 30  
*S. yanglinensis* 3-10 (MK861975) .....MPPFCAGGCFEGLPKTRAAAWWABSEGLL 30  
mpf s gc pg t aaw wae l l

*S. globisporus* TFH56 (WP\_044368733) SVEARRKMRTRPELWISLIFFASQCHLDLFCQWLFWAF 70  
*S. fulvissimus* DSM 40593 (AGK80153) SVEARRKMRTRPELWISLIFFASQCHLDLFCQWLFWAF 80  
*S. cavourensis* TJ430 (WP\_093751234) SVEARRKMRTRPELWISLIFFASQCHLDLFCQWLFWAF 70  
*Streptomyces* sp. SM18 (WP\_103489563) SEVARRKMRTRPELWISLIFFASQCHLDLFCQWLFWAF 70  
*Streptomyces* sp. PgraA7 (SNB61248) SVEARRKMRTRPELWISLIFFASQCHLDLFCQWLFWAF 80  
*S. globisporus* C-1027(WP\_029182233) SVEARRKMRTRPELWISLIFFASQCHLDLFCQWLFWAF 70  
*S. yanglinensis* 3-10 (MK861975) SVEARRKMRTRPELWISLIFFASQCHLDLFCQWLFWAF 70  
s ar km rtrpelwisliiff asq hldlfcqwlwaf

*S. globisporus* TFH56 (WP\_044368733) LVDDEFDDIPAGRNEIMCEBAIRLVDVLDGAEENGEMBR 110  
*S. fulvissimus* DSM 40593 (AGK80153) LVDDEFDDIPAGRNEIMCEBAIRLVDVLDGAEENGEMBR 120  
*S. cavourensis* TJ430 (WP\_093751234) LVDDEFDDIPAGRNEIMCEBAIRLVDVLDGAEENGEMBR 110  
*Streptomyces* sp. SM18 (WP\_103489563) LVDDEFDDIPAGRNEIMCEBAIRLVDVLDGAEENGEMBR 110  
*Streptomyces* sp. PgraA7 (SNB61248) LVDDEFDDIPAGRNEIMCEBAIRLVDVLDGAEENGEMBR 120  
*S. globisporus* C-1027(WP\_029182233) LVDDEFDDIPAGRNEIMCEBAIRLVDVLDGAEENGEMBR 110  
*S. yanglinensis* 3-10 (MK861975) LVDDEFDDIPAGRNEIMCEBAIRLVDVLDGAEENGEMBR 110  
lvddefdd ipagr n eimceba irlv dv ldgae engembr

meta (Mg<sup>2+</sup>) binding motif  
*S. globisporus* TFH56 (WP\_044368733) ILGLRTRTCPERSEQWNRQFRRTAAWLWYYAAEVERA 150  
*S. fulvissimus* DSM 40593 (AGK80153) ILGLRTRTCPERSEQWNRQFRRTAAWLWYYAAEVERA 160  
*S. cavourensis* TJ430 (WP\_093751234) ILGLRTRTCPERSEQWNRQFRRTAAWLWYYAAEVERA 150  
*Streptomyces* sp. SM18 (WP\_103489563) ILGLRTRTCPERSEQWNRQFRRTAAWLWYYAAEVERA 150  
*Streptomyces* sp. PgraA7 (SNB61248) ILGLRTRTCPERSEQWNRQFRRTAAWLWYYAAEVERA 160  
*S. globisporus* C-1027(WP\_029182233) ILGLRTRTCPERSEQWNRQFRRTAAWLWYYAAEVERA 150  
*S. yanglinensis* 3-10 (MK861975) ILGLRTRTCPERSEQWNRQFRRTAAWLWYYAAEVERA 150  
l glr rtc rse qw nrqfr rtdaawl wyyaaevera

*S. globisporus* TFH56 (WP\_044368733) AGQVFSRSEFAHRRDSVAMQPFLDHEITAEIDLHSAR 190  
*S. fulvissimus* DSM 40593 (AGK80153) AGQVFSRSEFVHRRDSVAMQPFLDHEITAEIDLHSAR 200  
*S. cavourensis* TJ430 (WP\_093751234) AGQVFSRSEFVHRRDSVAMQPFLDHEITAEIDLHSAR 190  
*Streptomyces* sp. SM18 (WP\_103489563) AGQVFCRAIFTRHRRDSVAMQPFLDHEITAEIDLHSAR 190  
*Streptomyces* sp. PgraA7 (SNB61248) AGQVFSRSEFAHRRDSVAMQPFLDHEITAEIDLHSAR 200  
*S. globisporus* C-1027(WP\_029182233) AGQVFSRSEFAHRRDSVAMQPFLDHEITAEIDLHSAR 190  
*S. yanglinensis* 3-10 (MK861975) AGQVFSRSEFAHRRDSVAMQPFLDHEITAEIDLHSAR 190  
agqvp r s e f a h r r d s v a m q p f l d h e i t a e i d l h s a r

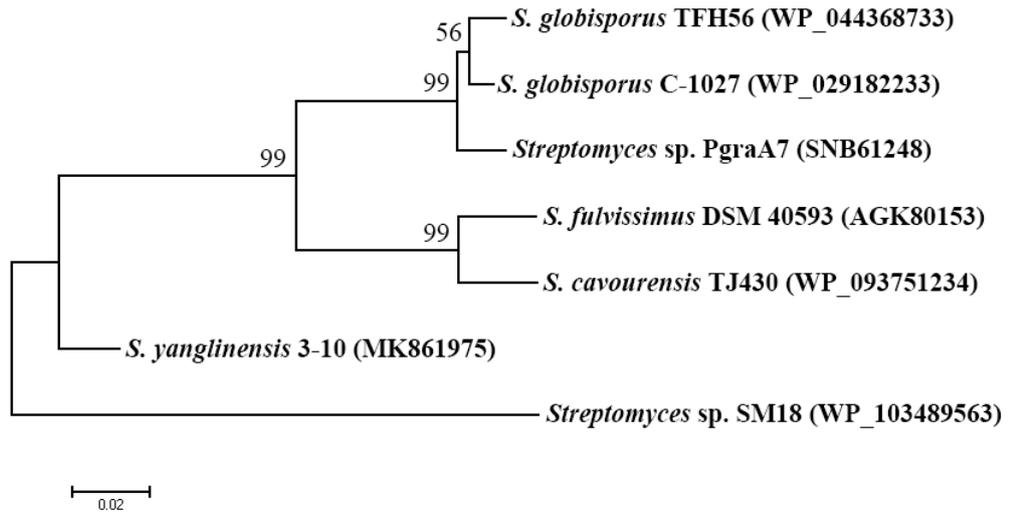
*S. globisporus* TFH56 (WP\_044368733) SLPAYIALRNAVTDHSGLCNDICSFKEEAAGYEHNARVL 230  
*S. fulvissimus* DSM 40593 (AGK80153) SLPAYIALRNAVTDHSGLCNDICSFKEEAAGYEHNARVL 240  
*S. cavourensis* TJ430 (WP\_093751234) SLPAYIALRNAVTDHSGLCNDICSFKEEAAGYEHNARVL 230  
*Streptomyces* sp. SM18 (WP\_103489563) SLPAYIALRNAVTDHSGLCNDICSFKEEAAGYEHNARVL 230  
*Streptomyces* sp. PgraA7 (SNB61248) SLPAYIALRNAVTDHSGLCNDICSFKEEAAGYEHNARVL 240  
*S. globisporus* C-1027(WP\_029182233) SLPAYIALRNAVTDHSGLCNDICSFKEEAAGYEHNARVL 230  
*S. yanglinensis* 3-10 (MK861975) SLPAYIALRNAVTDHSGLCNDICSFKEEAAGYEHNARVL 230  
slpayialrnavtdhsglcndicssfkeeaagye hnarvl

*S. globisporus* TFH56 (WP\_044368733) IQRDRGGLQCAVDEAGIQLARIAERVRABKELICEIDA 270  
*S. fulvissimus* DSM 40593 (AGK80153) IQRDRRSTLQCAVDEAGIQLARIAERVRABKELICEIDA 280  
*S. cavourensis* TJ430 (WP\_093751234) IQRDRGSLQCAVDEAGIQLARIAERVRABKELICEIDA 270  
*Streptomyces* sp. SM18 (WP\_103489563) IQRDRRSTLQCAVDEAGIQLARIAERVRABKELICEIDA 270  
*Streptomyces* sp. PgraA7 (SNB61248) IQRDRGGLQCAVDEAGIQLARIAERVRABKELICEIDA 280  
*S. globisporus* C-1027(WP\_029182233) IQRDRGGLQCAVDEAGIQLARIAERVRABKELICEIDA 270  
*S. yanglinensis* 3-10 (MK861975) IQRDRGGLQCAVDEAGIQLARIAERVRABKELICEIDA 270  
iqrdr gglqca vdeagiqlariaerv rabkeliceida

*S. globisporus* TFH56 (WP\_044368733) RGITGPTRAALERCVDYRGLVRGDFDYHARAERYTRPDL 310  
*S. fulvissimus* DSM 40593 (AGK80153) RGISASTRAALERCVDYRGLVRGDFDYHARAERYTRPDL 320  
*S. cavourensis* TJ430 (WP\_093751234) RGISASTRAALERCVDYRGLVRGDFDYHARAERYTRPDL 310  
*Streptomyces* sp. SM18 (WP\_103489563) RGITGPTRAALERCVDYRGLVRGDFDYHARAERYTRPDL 310  
*Streptomyces* sp. PgraA7 (SNB61248) RGITGPTRAALERCVDYRGLVRGDFDYHARAERYTRPDL 320  
*S. globisporus* C-1027(WP\_029182233) RGITGPTRAALERCVDYRGLVRGDFDYHARAERYTRPDL 310  
*S. yanglinensis* 3-10 (MK861975) RGITGPTRAALERCVDYRGLVRGDFDYHARAERYTRPDL 310  
gi r aalercv dyr glvrgdfdyharaerytrpdl

*S. globisporus* TFH56 (WP\_044368733) VELDERSLSQFA 324  
*S. fulvissimus* DSM 40593 (AGK80153) VELDARNSSQFA 334  
*S. cavourensis* TJ430 (WP\_093751234) VEREARNSSQFA 324  
*Streptomyces* sp. SM18 (WP\_103489563) VEIDRRGALSPFA 324  
*Streptomyces* sp. PgraA7 (SNB61248) VELDERSLSQFA 334  
*S. globisporus* C-1027(WP\_029182233) VELDERSLSQFA 324  
*S. yanglinensis* 3-10 (MK861975) VELDERSLSQFA 324  
ve r s fa

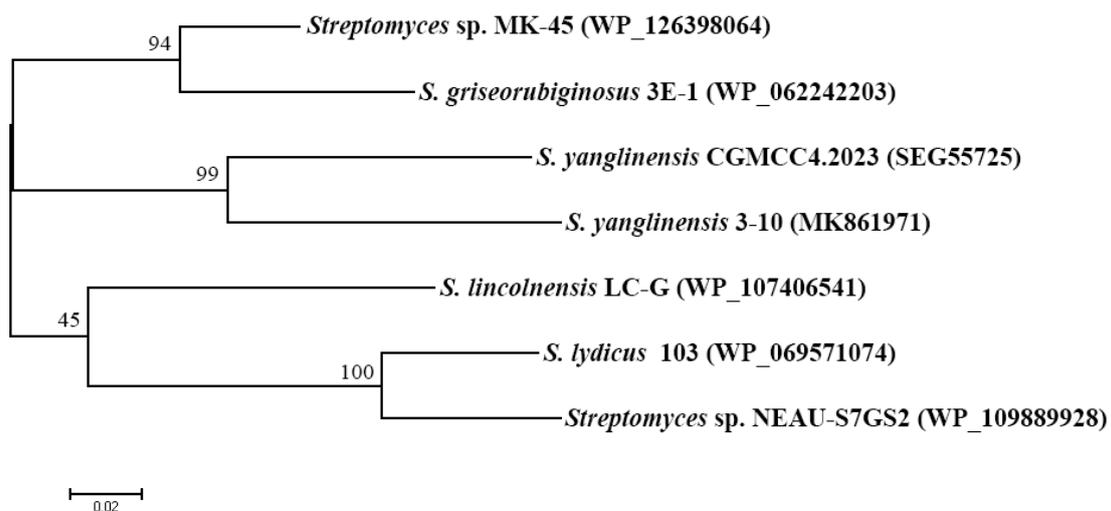
Supplementary Figure S3. (+)-beta-Caryophyllene synthase AA sequence alignment



**Supplementary Figure S4.** A phylogenetic tree based on AA sequence of (+)-beta-caryophyllene synthase using the maximum likelihood method. The numbers at the nodes in that tree indicate the bootstrap percentages ( $n = 1,000$ ). The bar indicates the average nucleotide change per site.

<i>Streptomyces</i> sp. MK-45 (WP_126398064)	.....MSTSSPT...SLGFPNLSLRL	20
<i>S. lincolnensis</i> LC-G (WP_107406541)	.....MRTSR	5
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	.....MTATSSR...SLGFPDIA SRLR	20
<i>S. lydicus</i> 103 (WP_069571074)	.....MSTFPHTAGFTLPGFPRIAFAFR	24
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	.....MSTFPNTAAAFSLGFPFNLA RTLR	24
<i>S. yanglinensis</i> CGMCC (SEG55725)	.MEQDPATEFAAAAEPDFGAGTGPARVWVPGFAGI PFPAP	39
<i>S. yanglinensis</i> 3-10 (MK861971)	VTAADRVEHFARTSPGTRFPFARPARLFLGFP SLRPSVA	40
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	.ARRGGIIFGLRYRQVDFDFKAFEDRRLDPWARRLDL	59
<i>S. lincolnensis</i> LC-G (WP_107406541)	ARR.GGFIIFGIRHFAVEFDFKVFEDRRLDPWARGLDL	44
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	TFPRGGIIFGLRYRQVDFDFKAFEDRRLDPWARRLDL	60
<i>S. lydicus</i> 103 (WP_069571074)	NRR.GGAVPGLRYRFAFDFDFKVFEDRRLDPWARRLDL	63
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	TRPPGGAVPGLRYRFAFDFDFKAFEDRRLDPWARGLDL	64
<i>S. yanglinensis</i> CGMCC (SEG55725)	ARR.TGAVPGLRYRQVDFDFKVFEDRRLDPWAHLDL	78
<i>S. yanglinensis</i> 3-10 (MK861971)	ARR.TGAIIFGLTYRAVDFDFDFKVFEDRRLDPWARRLDL	79
	g pg r p dp k e d rle wa ldl	
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	FEARWSDGDFSGQFGRAVVLQHEAADLRRLTRAGL LLA	99
<i>S. lincolnensis</i> LC-G (WP_107406541)	FEFWSGDFSGQFGRAVVLQHECAADLRRLTRAGL LLA	84
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	FEFWSGDFSGQFGRAVVLQHEAADLRRLTRAGL LLA	100
<i>S. lydicus</i> 103 (WP_069571074)	FHSFWSGDFSFQFGRAVVLQHECAADLRRLTRAGL LLA	103
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	FEAQRGDFHCFQFGRAVVLQHECAADLRRLTRAGL LLA	104
<i>S. yanglinensis</i> CGMCC (SEG55725)	FEFWSGDFSGQFGRAVVLQHEAADLRRLTRAGL LLA	118
<i>S. yanglinensis</i> 3-10 (MK861971)	FETWIGDFAGCFQFGRAVVLQHECAADLRRLTRAGL LLA	119
	fp w gdf fqfgravvlqhp aadl rlt ag llla	
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	ENIVDSYCYEEDERGGARRGLGGSLIMAGSALDFYEGIF	139
<i>S. lincolnensis</i> LC-G (WP_107406541)	ENIVDSYCYEEDERGGARRGLGGSLIMAGSALDFYEGIF	124
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	ENIVDSYCYEEDERGGARRGLGGSLIMAGSALDFYEGIF	140
<i>S. lydicus</i> 103 (WP_069571074)	ENIVDNCYCEEDERGGARRGLGGSLIMAGSALDFYEGIF	143
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	ENIVDNCYCEEDERGGARRGLGGSLIMAGSALDFYEGIF	144
<i>S. yanglinensis</i> CGMCC (SEG55725)	ENIVDSYCYEEDERGGARRGLGGSLIMAGSALDFYEGIF	158
<i>S. yanglinensis</i> 3-10 (MK861971)	ENIVDNCYCEEDERGGARRGLGGSLIMAGSALDFYEGIF	159
	en iv ds ycyee d er g g arr gl g g sl im ag s al df y eg if	
	metal (Mg <sup>2+</sup> )-binding motif	
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	ETEEWRREGVADGFLRSYHVALDYAATATPSCDRFVH	179
<i>S. lincolnensis</i> LC-G (WP_107406541)	APEAGWRREGVADGFLRSYHVALDYAATATPSCDRFVH	164
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	ETEEWRREGVADGFLRSYHVALDYAATATPSCDRFVH	180
<i>S. lydicus</i> 103 (WP_069571074)	ETEEWRREGVADGFLRSYHVALDYAATATPSCDRFVH	183
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	ETEEWRREGVADGFLRSYHVALDYAATATPSCDRFVQ	184
<i>S. yanglinensis</i> CGMCC (SEG55725)	ETEEWRREGVADGFLRSYHVALDYAATATPSCDRFVH	198
<i>S. yanglinensis</i> 3-10 (MK861971)	GSEELWRREGVADGFLRSYHVALDYAATATPSCDRFVH	199
	e wr g adgflrsyh val dya atpsq rfv	
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	DIARLHLGYLAEAAWPEIRHFRVWEYLVMRQFNNFRPCL	219
<i>S. lincolnensis</i> LC-G (WP_107406541)	DIARLHLGYLAEAAWSEIRYTRVWEYLVMRQFNNFRPCL	204
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	DIARLHLGYLAEAAWPEIRHFRVWEYLVMRQFNNFRPCL	220
<i>S. lydicus</i> 103 (WP_069571074)	DIARLHLGYLAEAAWPEIRHFRVWEYLVMRQFNNFRPCL	223
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	DIARLHLGYLAEAAWPEIRHFRVWEYLVMRQFNNFRPCL	224
<i>S. yanglinensis</i> CGMCC (SEG55725)	DIARLHLGYLAEAAWPEIRYTRVWEYLVMRQFNNFRPCL	238
<i>S. yanglinensis</i> 3-10 (MK861971)	DIARLHLGYLAEAAWPEIRHFRVWEYLVMRQFNNFRPCL	239
	diarlhlgylaeaa w e ir h fr v we yl vm r q f nn fr p cl	
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	SIVDADGYELFEAVYRPEIQRTALANATTVNDLYS	259
<i>S. lincolnensis</i> LC-G (WP_107406541)	SIVDADGYELFEAVYRPEIQRTALANATTVNDLYS	244
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	SIVDADGYELFEALYRPEIQRTALANATTVNDLYS	260
<i>S. lydicus</i> 103 (WP_069571074)	SIVDADGYELFEALYRPEIQRTALANATTVNDLYS	263
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	SIVDADGYELFEALYRPEIQRTALANATTVNDLYS	264
<i>S. yanglinensis</i> CGMCC (SEG55725)	SIVDADGYELFEAVYRPEIQRTALANATTVNDLYS	278
<i>S. yanglinensis</i> 3-10 (MK861971)	SIVDADGYELFEALYRPEIQRTALANATTVNDLYS	279
	sivda dgyel fe av y r pe iq r ta la n att v nd lys	
	metal (Mg <sup>2+</sup> )-binding motif	
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	FTRELDSDHHLNLCQVVAANQKGLKAYLRSVEIHNFI	299
<i>S. lincolnensis</i> LC-G (WP_107406541)	FTRELDSDHHLNLCQVVAANQKGLKAYLRSVEIHNFI	284
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	FTRELDSDHHLNLCQVVAANQKGLKAYLRSVEIHNFI	300
<i>S. lydicus</i> 103 (WP_069571074)	FTRELDSDHHLNLCQVVAANQKGLKAYLRSVEIHNFI	303
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	FTRELDSDHHLNLCQVVAANQKGLKAYLRSVEIHNFI	304
<i>S. yanglinensis</i> CGMCC (SEG55725)	FTRELDSDHHLNLCQVVAANQKGLKAYLRSVEIHNFI	318
<i>S. yanglinensis</i> 3-10 (MK861971)	FTRELDSDHHLNLCQVVAANQKGLKAYLRSVEIHNFI	319
	ft el sd h hlnl c q v va an q k gl k ay l rs ve i h n f i	
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	MESFEEESALISASFLVGRYARCSITLWVGNHWEHTNT	339
<i>S. lincolnensis</i> LC-G (WP_107406541)	MDTFEEEPATLISASFLVGRYARCSIAWVGNHWEHTNT	324
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	MAAFEEESALISASFLVGRYARCSITLWVGNHWEHTNT	340
<i>S. lydicus</i> 103 (WP_069571074)	MEAFEEESALISASFLVGRYARCSIAWVGNHWEHTNT	343
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	MEAFEEESALISASFLVGRYARCSIAWVGNHWEHTNT	344
<i>S. yanglinensis</i> CGMCC (SEG55725)	MDSFEEEPALISASFLVGRYARCSITLWVGNHWEHTNT	358
<i>S. yanglinensis</i> 3-10 (MK861971)	MASFEEEPALISASFLVGRYARCSITLWVGNHWEHTNT	359
	m f e e e s a l i s a s f l v g r y a r c s i t l w v g n h e w h t n t	
<i>Streptomyces</i> sp. MK-45 (WP_126398064)	FRYELPNY	347
<i>S. lincolnensis</i> LC-G (WP_107406541)	FRYELPNY	332
<i>S. griseorubiginosus</i> 3E-1 (WP_062242203)	FRYELPNY	348
<i>S. lydicus</i> 103 (WP_069571074)	DRYCLPNY	351
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889928)	DRYCLPNY	352
<i>S. yanglinensis</i> CGMCC (SEG55725)	FRYELPNY	366
<i>S. yanglinensis</i> 3-10 (MK861971)	FRYELPNY	367
	ry l pny	

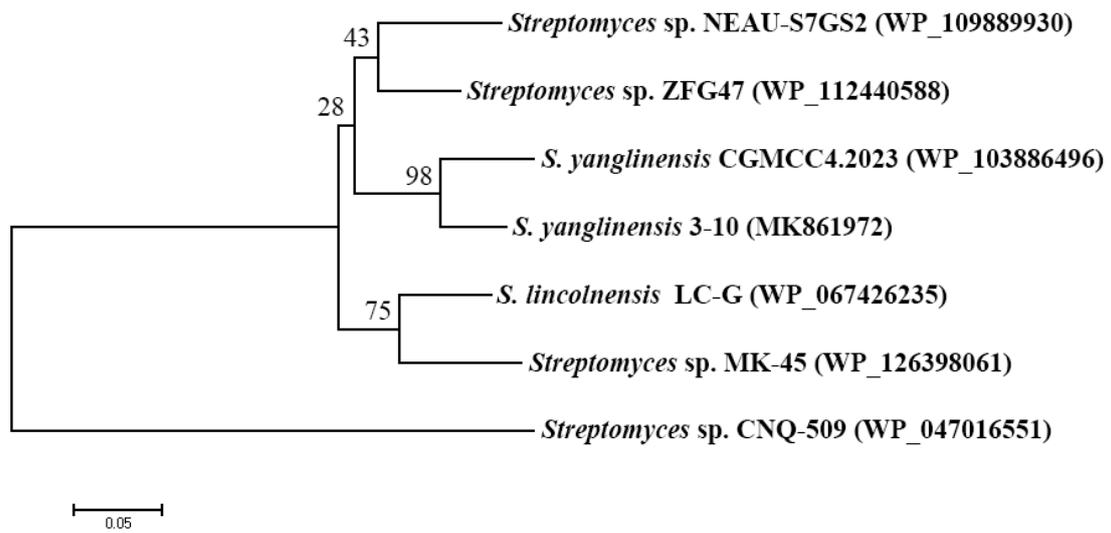
Supplementary Figure S5. Alignment of the amino acid sequences of 2-MIB synthase in *Streptomyces* species



**Supplementary Figure S6.** A phylogenetic tree inferred based on the amino acid sequences of 2-MIB synthase in *Streptomyces* using the maximum likelihood method. The numbers at the nodes in that tree indicate the bootstrap percentages ( $n = 1,000$ ). The bar indicates the average nucleotide change per site.

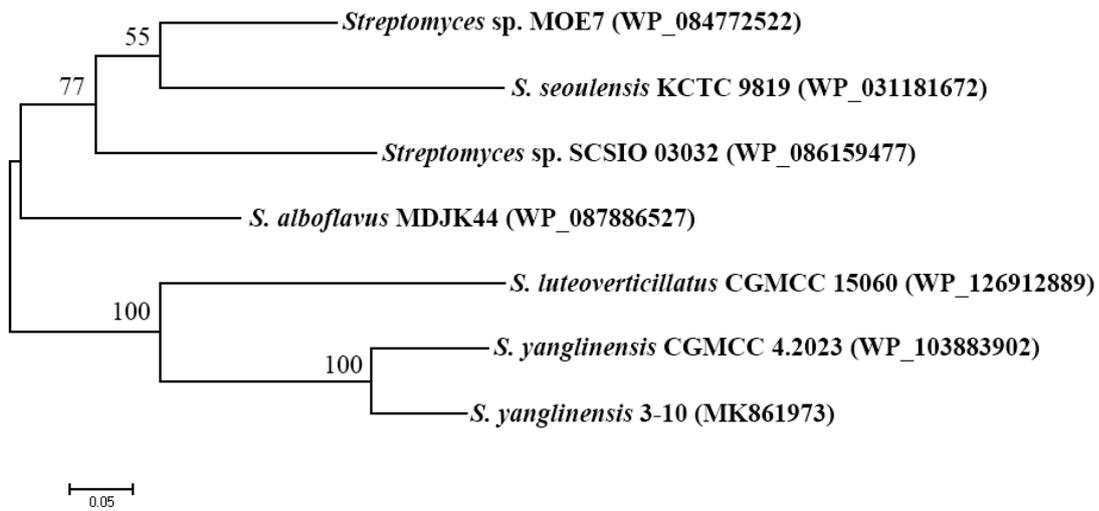
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889930)	..MTITHADIAARTFVETQSTYQSRVADYWNFEENPVNLEL	38
<i>S. lincolnensis</i> LC-G (WP_067426235)	..MTITADAVITTAAPVETQSTYQNRVADYWNFEENPVNLEL	38
<i>Streptomyces</i> sp. MK-45 (WP_126398061)	MTIAFEAGTSTAVPVESQSTYQTRVADYWNFEENPVNLEL	40
<i>Streptomyces</i> sp. ZFG47 (WP_112440588)	..MTIAHAGTTTAPVETQSTYQNRVADYWNFEENPVNLEL	38
<i>Streptomyces</i> sp. CNQ-509 (WP_047016551)	...MAAADTNVLTSE...YQHSIADYWNFERDPVNLEL	33
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103886496)	..MTIQHFGTSTAPVETQSTYQTRVADYWNFEENPVNLEL	38
<i>S. yanglinensis</i> 3-10 (MK861972)	..LITTHRGTTTAPVEAQTSTYQTRVADYWNFEENPVNLEL	38
	p yq adywn e pvn l l	
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889930)	GKIDDLYHHHYGIGEA DRSVLNEPDPGRRRLRVIGELHRL	78
<i>S. lincolnensis</i> LC-G (WP_067426235)	GKLDLHYHHHYGIGDVLVLDYEDFVRRRERITZELHRL	78
<i>Streptomyces</i> sp. MK-45 (WP_126398061)	GKIDDLYHHHYGIGD DRSVLDEPDPVRRRERITZELHRL	80
<i>Streptomyces</i> sp. ZFG47 (WP_112440588)	GKIDDLYHHHYGVGDVLDVLDDETRARRRERITZELHRL	78
<i>Streptomyces</i> sp. CNQ-509 (WP_047016551)	GDSVGTYYHHHYGIGEVLDQSVLGGPEE.TRGERIIPEMHRL	72
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103886496)	GKIDDLYHHHYGIGEA DWSVLDDETPALRRRERITZELHRL	78
<i>S. yanglinensis</i> 3-10 (MK861972)	GKIDDLYHHHYGIGEA DRSVLDEPDPALRRRERITZELHRL	78
	g yhhhyg g d vl r r e hrl	
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889930)	BHAQAELLASHLGDLSPTRVFDAGCGRGGESVVAHLRFG	118
<i>S. lincolnensis</i> LC-G (WP_067426235)	BHAQAELLASRLGPLSPGDFVFDAGCGRGGESVVAHLRFG	118
<i>Streptomyces</i> sp. MK-45 (WP_126398061)	BHAQAELLADRLGPLTPDDRVDAGCGRGGESVVAHLRFG	120
<i>Streptomyces</i> sp. ZFG47 (WP_112440588)	BHAQAELLAGHLGPLTPADRVDAGCGRGGESVVAHLRFG	118
<i>Streptomyces</i> sp. CNQ-509 (WP_047016551)	ETAQATLLLDHLGEPDRGARLLDAGSGRGGTSFVAHERFG	112
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103886496)	BHAQAELLASHLGPLTSA DRFVFDAGCGRGGESVVAHLRFG	118
<i>S. yanglinensis</i> 3-10 (MK861972)	BHAQAELLASRLGPLTPADRVDAGCGRGGESVVAHLRFG	118
	e aqa ll lg r dag grgg s a r g	
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889930)	CHADGATISAKCADFANQCARRRGIDDKVRRHHRNMLDTG	158
<i>S. lincolnensis</i> LC-G (WP_067426235)	GERDGVITISAKCAFANQCARRRGIDDLVRRHHRNMLDTG	158
<i>Streptomyces</i> sp. MK-45 (WP_126398061)	GERDGVITISAKCAFANQCARRRGIDGVRRHHRNMLDTG	160
<i>Streptomyces</i> sp. ZFG47 (WP_112440588)	CHADGVTISAKCADFANQCARRRGIDGVRRHHRNMLDTG	158
<i>Streptomyces</i> sp. CNQ-509 (WP_047016551)	CRVDCISTISQSCVVFANDCVQRRGLGSAVRRHHRNMLDTG	152
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103886496)	GSADGVITISAKCADFANQCARRRGIDGVRRHHRNMLDTG	158
<i>S. yanglinensis</i> 3-10 (MK861972)	GSADGVITISAKCADFANQCARRRGIDGVRRHHRNMLDTG	158
	c dg s q fan q rg vr h nml dtg	
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889930)	FTEGAYAA SWNNESTMYVELDILFAEHARLIRGGRYVVI	198
<i>S. lincolnensis</i> LC-G (WP_067426235)	LPSGAFAA SWNNESTMYVELDILFAEHARLIRGGRYAVI	198
<i>Streptomyces</i> sp. MK-45 (WP_126398061)	LPSGAFAA SWNNESTMYVELDILFAEHARLIRGGRYVVI	200
<i>Streptomyces</i> sp. ZFG47 (WP_112440588)	FTTGAYAA SWNNESTMYVELDILFAEHARLIRGGRYVVI	198
<i>Streptomyces</i> sp. CNQ-509 (WP_047016551)	FETGAFRGIWNNESTMYVELAQLFKEHARLIRGGRYVVI	192
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103886496)	FSTGGYAA SWNNESTMYVELDILFAEHARLIRGGRYVVI	198
<i>S. yanglinensis</i> 3-10 (MK861972)	FRTGGYTA SWNNESTMYVELDILFAEHARLIRGGRYVVI	198
	g wnnestmyvel lf eharll rgry i	
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889930)	TGCYNDYGRASREVS IINAHYICDIHPRS YFRAMA RNR	238
<i>S. lincolnensis</i> LC-G (WP_067426235)	TGCYNDYGRASREVS IINAHYICDIHPRS YFRAMA RNR	238
<i>Streptomyces</i> sp. MK-45 (WP_126398061)	TGCYNDYGRASREVS IINAHYICDIHPRS YFRAMA RNR	240
<i>Streptomyces</i> sp. ZFG47 (WP_112440588)	TGCYNDYGRASREVS IINAHYICDIHPRS YFRAMA RNR	238
<i>Streptomyces</i> sp. CNQ-509 (WP_047016551)	TGCYNDYGLRSRAVSC IINAHYICNVHPRS YFRELIVANR	232
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103886496)	TGCYNDYGRASREVS IINAHYICDIHPRS YFRAMA RNR	238
<i>S. yanglinensis</i> 3-10 (MK861972)	TGCYNDYGRASREVS IINAHYICDIHPRS YFRAMA RNR	238
	tgcynd yg sr vs inahyic hprs yf nr	
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889930)	LVPVHVEDL TEAALPYWELRQADHLV TGEEDTFLTAYRN	278
<i>S. lincolnensis</i> LC-G (WP_067426235)	LVPVHVEDL TAAAIPYWELRQADHLV TGEEDTFLTAYRN	278
<i>Streptomyces</i> sp. MK-45 (WP_126398061)	LVPVHVQDLTSAIPYWELRQADHLV TGEEDTFLSAYRN	280
<i>Streptomyces</i> sp. ZFG47 (WP_112440588)	LVPVHVEDL TEATIPYWELRSQADHLV TGEEDTFLTAYRN	278
<i>Streptomyces</i> sp. CNQ-509 (WP_047016551)	LVEA QVLDL TEATIPYWELRAKSS.VATGDEVELTAYRS	271
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103886496)	LVPVHVEDL TEAALPYWELRQDAEHLV TGEDEFFITAYRN	278
<i>S. yanglinensis</i> 3-10 (MK861972)	LVPVHVEDL TATAIPYWELRQAEHLV TGEDEFFITAYRN	278
	lvp v dlt pywelr tg e f ayr	
<i>Streptomyces</i> sp. NEAU-S7GS2 (WP_109889930)	GSFQYLLI EADR	290
<i>S. lincolnensis</i> LC-G (WP_067426235)	GSFQYLLI EADR	290
<i>Streptomyces</i> sp. MK-45 (WP_126398061)	GSFQYLLI EADR	292
<i>Streptomyces</i> sp. ZFG47 (WP_112440588)	GSFQYLLI EADR	290
<i>Streptomyces</i> sp. CNQ-509 (WP_047016551)	GSFQYLLI EADR	283
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103886496)	GSFQYLLI EADR	290
<i>S. yanglinensis</i> 3-10 (MK861972)	GSFQYLLI EADR	290
	gsfqyl i a r	

Supplementary Figure S7. Methyltransferase AA sequence alignment



**Supplementary Figure S8.** A phylogenetic tree based on AA sequence of methyltransferase using the maximum likelihood method. The numbers at the nodes in that tree indicate the bootstrap percentages ( $n = 1,000$ ). The bar indicates the average nucleotide change per site.

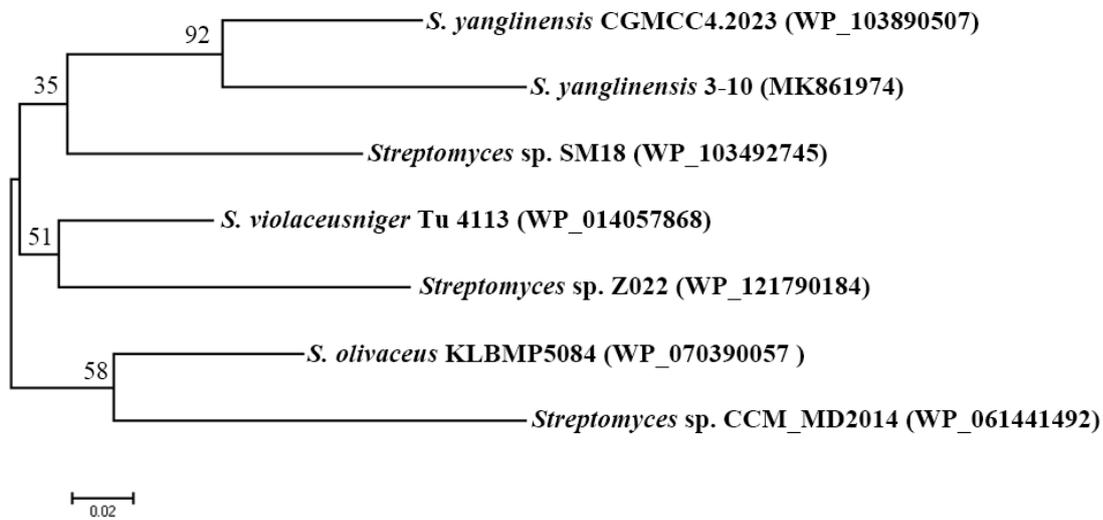




**Supplementary Figure S10.** A phylogenetic tree based on AA sequence of geosmin synthase using the maximum likelihood method. The numbers at the nodes in that tree indicate the bootstrap percentages ( $n = 1,000$ ). The bar indicates the average nucleotide change per site.

<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	MTKRFGAAVLRSESEFRAVEEVLINARPAAGHVLVRIAGC	40
<i>Streptomyces</i> sp. SM18 (WP_103492745)	..MRFGAAVLRADSAFTLEEVVLINAGPAAGHVLVRIAGC	38
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	..MRFAAAVLRSEEFRAVEEVLINAGPAAGHVLVRIAGC	38
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	..MRFGAAVLRSEDEFRAVEEVLINAGPAAGHVLVRIAGC	38
<i>Streptomyces</i> sp. Z022 (WP_121790184)	..MRFQAAVLRSEDEFRAVEEVLINAGPAAGHVLVRIAGC	38
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	..MRFGAAVLRGDRFRFAVEEVLINAGPAAGHVLVRIAGC	38
3-10 (MK861974)	.....RFAVEEVLINAGPAAGHVLVRIAGC	25
	f eev l pa ge lv iaqc	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	GMCRDDLAVRRSAGSPLFVLGHEGSGVVMETGG.PDTG	79
<i>Streptomyces</i> sp. SM18 (WP_103492745)	GMCRDDLAVRRSAGSPLFVLGHEGSGVVMETGG.GDTG	77
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	GMCRDDLAVRRSAGSPLFVLGHEGSGVVAASGG.TGTG	77
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	GMCRDDLAVRRSAGSPLFVLGHEGSGVVMETGG.TGTG	77
<i>Streptomyces</i> sp. Z022 (WP_121790184)	GMCRDDLAVRRSAGSPLFVLGHEGSGVVMHAGGGPDSG	78
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	GMCRDDLAVRRSAGSPLFVLGHEGSGVVMQTTGG.PDTG	77
3-10 (MK861974)	GMCRDDLAVRRSAGSPLFVLGHEGSGVVMQTTGG.PDTG	64
	gmcrtdlavr s g splf vlgheg g v vm et gg . pdt g	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	LGVDHVVLSFDSGCGRNCIGAPAYCDSFASLNLGGRR	119
<i>Streptomyces</i> sp. SM18 (WP_103492745)	LSVDHVVLSFDSGCGRNCIGAPAYCDSFASLNLGGRR	117
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	LGVDHVVLSFDSGCGRNCIGAPAYCDSFASLNLGGRR	117
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	FSVDHVVLSFDSGCGRNCIGAPAYCDSFASLNLGGRR	117
<i>Streptomyces</i> sp. Z022 (WP_121790184)	IGVDHVVLSFDSGCGRNCIGAPAYCDSFASLNLGGRR	118
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	LDVDHVVLSFDSGCGRNCIGAPAYCDSFASLNLGGRR	117
3-10 (MK861974)	LDVDHVVLSFDSGCGRNCIGAPAYCDSFASLNLGGRR	104
	gdhvvlsfds c g r n c i g a p a y c d s f a s l n l g g r	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	TENRFRITDAAGGLAPRWFGQSSFAEYAVVARNAVRVD	159
<i>Streptomyces</i> sp. SM18 (WP_103492745)	EDRAGRITDAAGGLAPRWFGQSSFAEYAVVARNAVRVD	157
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	EDRAGRITDAAGGLAPRWFGQSSFAEYAVVARNAVRVD	157
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	EDRAGRITDAAGGLAPRWFGQSSFAEYAVVARNAVRVD	157
<i>Streptomyces</i> sp. Z022 (WP_121790184)	KEEAFRLITDAAGGLAPRWFGQSSFAEYAVVARNAVRVD	158
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	KENRFRITDAAGGLAPRWFGQSSFAEYAVVARNAVRVD	157
3-10 (MK861974)	RENFRITDAAGGLAPRWFGQSSFAEYAVVARNAVRVD	144
	e a r d g laprwfgqs faeya v arnavrvd	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	SSLFVELLGLPLGCGFLTGAGAVNSFGAGPGDTLIVFGAG	199
<i>Streptomyces</i> sp. SM18 (WP_103492745)	ESLVELLGLPLGCGFLTGAGAVNSFGAGPGDTLIVFGAG	197
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	ESLVELLGLPLGCGFLTGAGAVNSFGVAPGDTLIVFGAG	197
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	ESLVELLGLPLGCGFLTGAGAVNSFGAGPGDTLIVFGAG	197
<i>Streptomyces</i> sp. Z022 (WP_121790184)	FALVELLGLPLGCGFLTGAGAVNSFGAGPGDTLIVFGAG	198
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	ESLVELLGLPLGCGFLTGAGAVNSFGVAPGDTLIVFGAG	197
3-10 (MK861974)	ESLVELLGLPLGCGFLTGAGAVNSFGAGPGDVIVFGAG	184
	lp llgplg c g f l t g a g a v n s f g a g p g d t l i v f g a g	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	AVGLAAVMAATVAGVTVAVDRHFRRLAIAERLGAIVPLFA	239
<i>Streptomyces</i> sp. SM18 (WP_103492745)	AVGLAAVMAATAAGVTVAVDRHFRRLAIAERLGAIVPLFA	237
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	AVGLAAVMAATAAGVTVAVDRHFRRLAIAERLGAIVPLFA	237
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	AVGLAAVMAATAAGVTVAVDRHFRRLAIAERLGAIVPLFA	237
<i>Streptomyces</i> sp. Z022 (WP_121790184)	AVGLAAVMAATAAGVTVAVDRHFRRLAIAERLGAIVPLFA	238
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	AVGLAAVMAATAAGVTVAVDRHFRRLAIAERLGAIVPLFA	237
3-10 (MK861974)	AVGLAAVMAATAAGVTVAVDRHFRRLAIAERLGAIVPLFA	224
	avglaa maa ta a g v t v a v d r h f r r l a i a e r l g a i v p l f a	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	ASAGFERIRKLTGGGAYALDITGSAFLINAIQLRLRF	279
<i>Streptomyces</i> sp. SM18 (WP_103492745)	ASPGFGRIRKLTGGGAYALDITGSAFLINAIQLRLRF	277
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	ASTDEARVRLTGGGAYALDITGSAFLINAIQLRLRF	277
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	TATGSDSIVRLTGGGAYALDITGSAFLINAIQLRLRF	277
<i>Streptomyces</i> sp. Z022 (WP_121790184)	GTAGFERIRKLTGGGAYALDITGSAFLINAIQLRLRF	278
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	DTADFERIRKLTGGGAYALDITGSAFLINAIQLRLRF	277
3-10 (MK861974)	APAGFAARLRLTGGGAYALDITGSAFLINAIQLRLRF	264
	l t g g a y a l d i t t a s a f l i n a i q l r l r f	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	GLGLVARLHTLPEEGLDGRGIRHICEGDVAVPGHLI	319
<i>Streptomyces</i> sp. SM18 (WP_103492745)	GLGLVARLHTLPEEGLDGRGIRHICEGDVAVPGHLI	317
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	GLGLVARLHTLPEEGLDGRGIRHICEGDVAVPGHLI	317
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	GLGLVARLHTLPEEGLDGRGIRHICEGDVAVPGHLI	317
<i>Streptomyces</i> sp. Z022 (WP_121790184)	GLGLVARLHTLPEEGLDGRGIRHICEGDVAVPGHLI	318
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	GLGLVARLHTLPEEGLDGRGIRHICEGDVAVPGHLI	317
3-10 (MK861974)	GLGLVARLHTLPEEGLDGRGIRHICEGDVAVPGHLI	304
	g l g l v a r l h t l p e e g l d g r g i r h i c e g d a v p g h l i	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	FRITGLWQAGRFPPFCLRTYPLADINAEFDCDGRVVK	359
<i>Streptomyces</i> sp. SM18 (WP_103492745)	FRITGLWQAGRFPPFCLRTYPLADINAEFDCDGRVVK	357
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	FRITGLWQAGRFPPFCLRTYPLADINAEFDCDGRVVK	357
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	FRITGLWQAGRFPPFCLRTYPLADINAEFDCDGRVVK	357
<i>Streptomyces</i> sp. Z022 (WP_121790184)	FRITGLWQAGRFPPFCLRTYPLADINAEFDCDGRVVK	358
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	FRITGLWQAGRFPPFCLRTYPLADINAEFDCDGRVVK	357
3-10 (MK861974)	FRITGLWQAGRFPPFCLRTYPLADINAEFDCDGRVVK	344
	p l l w q a r f p f d l r t y p l a d i n a e f d c d g r v v k	
<i>S. violaceusniger</i> Tu 4113 (WP_014057868)	FVLEAGTGR.....	369
<i>Streptomyces</i> sp. SM18 (WP_103492745)	FVLE.....	362
<i>S. olivaceus</i> KLBMP 5084 (WP_070390057)	FVLEEGLDIWSVTPH	372
<i>Streptomyces</i> sp. CCM_MD2014 (WP_061441492)	FVLEEDGRRH.....	367
<i>Streptomyces</i> sp. Z022 (WP_121790184)	FVLEEGRSR.....	368
<i>S. yanglinensis</i> CGMCC4.2023 (WP_103890507)	FVLE.....	362
3-10 (MK861974)	FVLEEGAGR.....	354
	p v l e a g t g r . . . . .	

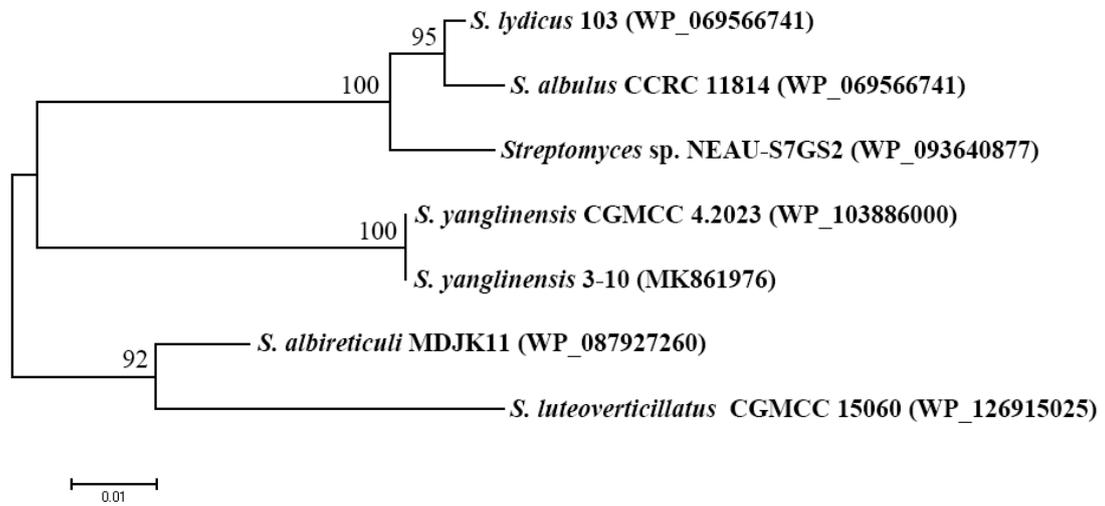
Supplementary Figure S11. Aryl-alcohol dehydrogenase (2-phenylethanol synthase) AA sequence alignment



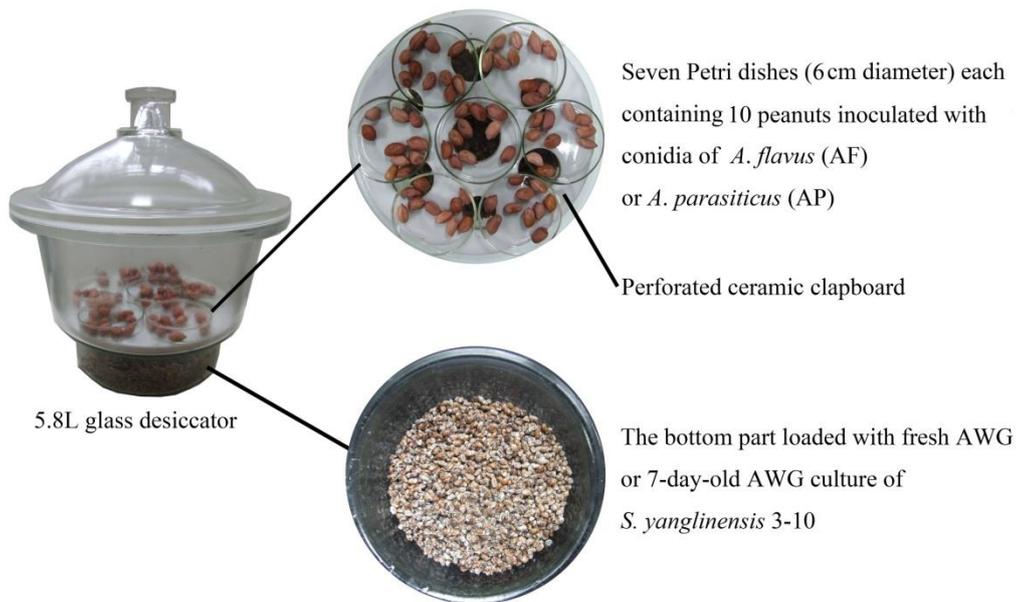
**Supplementary Figure S12.** A phylogenetic tree based on AA sequence of Aryl-alcohol dehydrogenase (2-phenylethanol synthase) using the maximum likelihood method. The numbers at the nodes in that tree indicate the bootstrap percentages ( $n = 1,000$ ). The bar indicates the average nucleotide change per site.

<i>S. albireticuli</i> MDJK11 (WP_087927260)	VEYEGGIDVYVYLNRSRKGELVHPTVIDIEADKERLISV	40
<i>S. lydicus</i> 103 (WP_069566741)	VEYEGGIDVYVYLNRSRKGELVHPTVWVVEADKERLISV	40
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	VEYEGGIDVYVYLNRSRKGELVHPTVWVVEADKERLISV	40
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	VEYEGGIDVYVYLNRSRKGELVHPTVWVVEADKERLISV	40
<i>S. albulus</i> CCRC 11814 (EOT04707)	VEYEGGIDVYVYLNRSRKGELVHPTVWVVEADKERLISV	40
<i>S. yanglinensis</i> CGMCC (WP_103886000)	VEYEGGIDVYVYLNRSRKGELVHPTVWVVEADKERLISV	40
<i>S. yanglinensis</i> 3-10 (MK861976)	VEYEGGIDVYVYLNRSRKGELVHPTVWVVEADKERLISV	40
	y yeggi d v ylnsrkge l hpt v i d i e a d k e r l i s v	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	EITMQWNNQYSEGVYSFANIHTHEGGTHEEGFRAALTGL	80
<i>S. lydicus</i> 103 (WP_069566741)	DLIMQWNNQYSEGVYSFANIHTHEGGTHEEGFRAALTGL	80
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	DLIMQWNNQYSEGVYSFANIHTHEGGTHEEGFRAALTGL	80
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	EVSIMQWNNQYSEGVYSFANIHTHEGGTHEEGFRAALTGL	80
<i>S. albulus</i> CCRC 11814 (EOT04707)	DLIMQWNNQYSEGVYSFANIHTHEGGTHEEGFRAALTGL	80
<i>S. yanglinensis</i> CGMCC (WP_103886000)	EVSIMQWNNQYSEGVYSFANIHTHEGGTHEEGFRAALTGL	80
<i>S. yanglinensis</i> 3-10 (MK861976)	EVSIMQWNNQYSEGVYSFANIHTHEGGTHEEGFRAALTGL	80
	m q w n q y e g v y s f a n i h t h e g g t h e e g f r a a l t g l	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	NRRYARPKLLREKDDNLTGDIRREGLTAAISVKLEPQF	120
<i>S. lydicus</i> 103 (WP_069566741)	NRRYARPKLLREKDDNLTGDIRREGLTAAISVKLEPQF	120
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	NRRYARPKLLREKDDNLTGDIRREGLTAAISVKLEPQF	120
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	NRRYARPKLLREKDDNLTGDIRREGLTAAISVKLEPQF	120
<i>S. albulus</i> CCRC 11814 (EOT04707)	NRRYARPKLLREKDDNLTGDIRREGLTAAISVKLEPQF	120
<i>S. yanglinensis</i> CGMCC (WP_103886000)	NRRYARPKLLREKDDNLTGDIRREGLTAAISVKLEPQF	120
<i>S. yanglinensis</i> 3-10 (MK861976)	NRRYARPKLLREKDDNLTGDIRREGLTAAISVKLEPQF	120
	n r r y a r p k l l r e k d d n l t g d i r e g l t a a i s v k l e p q f	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	EGQTKTKLGNTERTVQKWHHEHLDWLDRNPNEADII	160
<i>S. lydicus</i> 103 (WP_069566741)	EGQTKTKLGNTERTVQKWHHEHLDWLDRNPNEADII	160
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	EGQTKTKLGNTERTVQKWHHEHLDWLDRNPNEADII	160
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	EGQTKTKLGNTERTVQKWHHEHLDWLDRNPNEADII	160
<i>S. albulus</i> CCRC 11814 (EOT04707)	EGQTKTKLGNTERTVQKWHHEHLDWLDRNPNEADII	160
<i>S. yanglinensis</i> CGMCC (WP_103886000)	EGQTKTKLGNTERTVQKWHHEHLDWLDRNPNEADII	160
<i>S. yanglinensis</i> 3-10 (MK861976)	EGQTKTKLGNTERTVQKWHHEHLDWLDRNPNEADII	160
	e g q t k t k l g n t e r t v q k w h h e h l d w l d r n p n e a d i i	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	RKSIQAATARVAARKARDLTRRKGLEASLPGKLSDCQS	200
<i>S. lydicus</i> 103 (WP_069566741)	RKSIQAATARVAARKARDLTRRKGLEASLPGKLSDCQS	200
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	RKSIQAATARVAARKARDLTRRKGLEASLPGKLSDCQS	200
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	RKSIQAATARVAARKARDLTRRKGLEASLPGKLSDCQS	200
<i>S. albulus</i> CCRC 11814 (EOT04707)	RKSIQAATARVAARKARDLTRRKGLEASLPGKLSDCQS	200
<i>S. yanglinensis</i> CGMCC (WP_103886000)	RKSIQAATARVAARKARDLTRRKGLEASLPGKLSDCQS	200
<i>S. yanglinensis</i> 3-10 (MK861976)	RKSIQAATARVAARKARDLTRRKGLEASLPGKLSDCQS	200
	r k s i q a a t a r v a a r k a r d l t r r k g l e a s l p g k l s d c q s	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	NIEPKCEIFIVEGDSAGGSARSGRNEQYAILPIRGKILN	240
<i>S. lydicus</i> 103 (WP_069566741)	NIEPKCEIFIVEGDSAGGSARSGRNEQYAILPIRGKILN	240
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	NIEPKCEIFIVEGDSAGGSARSGRNEQYAILPIRGKILN	240
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	NIEPKCEIFIVEGDSAGGSARSGRNEQYAILPIRGKILN	240
<i>S. albulus</i> CCRC 11814 (EOT04707)	NIEPKCEIFIVEGDSAGGSARSGRNEQYAILPIRGKILN	240
<i>S. yanglinensis</i> CGMCC (WP_103886000)	NIEPKCEIFIVEGDSAGGSARSGRNEQYAILPIRGKILN	240
<i>S. yanglinensis</i> 3-10 (MK861976)	NIEPKCEIFIVEGDSAGGSARSGRNEQYAILPIRGKILN	240
	n i e p k c e i f i v e g d s a g g s a r s g r n e q y a i l p i r g k i l n	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	VEKARDKILQNEEQALISAFGTGVHEDFDIKLRYHKI	280
<i>S. lydicus</i> 103 (WP_069566741)	VEKARDKILQNEEQALISAFGTGVHEDFDIKLRYHKI	280
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	VEKARDKILQNEEQALISAFGTGVHEDFDIKLRYHKI	280
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	VEKARDKILQNEEQALISAFGTGVHEDFDIKLRYHKI	280
<i>S. albulus</i> CCRC 11814 (EOT04707)	VEKARDKILQNEEQALISAFGTGVHEDFDIKLRYHKI	280
<i>S. yanglinensis</i> CGMCC (WP_103886000)	VEKARDKILQNEEQALISAFGTGVHEDFDIKLRYHKI	280
<i>S. yanglinensis</i> 3-10 (MK861976)	VEKARDKILQNEEQALISAFGTGVHEDFDIKLRYHKI	280
	v e k a r d k i l q n e e q a l i s a f g t g v h e d f d i k l r y h k i	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	ILMADADVGGHINTLLTFLFRFMRPLVEAGHVLSRPP	320
<i>S. lydicus</i> 103 (WP_069566741)	ILMADADVGGHINTLLTFLFRFMRPLVEAGHVLSRPP	320
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	ILMADADVGGHINTLLTFLFRFMRPLVEAGHVLSRPP	320
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	ILMADADVGGHINTLLTFLFRFMRPLVEAGHVLSRPP	320
<i>S. albulus</i> CCRC 11814 (EOT04707)	ILMADADVGGHINTLLTFLFRFMRPLVEAGHVLSRPP	320
<i>S. yanglinensis</i> CGMCC (WP_103886000)	ILMADADVGGHINTLLTFLFRFMRPLVEAGHVLSRPP	320
<i>S. yanglinensis</i> 3-10 (MK861976)	ILMADADVGGHINTLLTFLFRFMRPLVEAGHVLSRPP	320
	i l m a d a d v g g h i n t l l t f l f r f m r p l v e a g h v l s r p p	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	LNRRKWRDDEYAYSBERDALIQIGRENKRRIRBDSIQ	360
<i>S. lydicus</i> 103 (WP_069566741)	LNRRKWRDDEYAYSBERDALVEIGKQGRRIIRBDSIQ	360
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	LNRRKWRDDEYAYSBERDALVEIGKQGRRIIRBDSIQ	360
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	LNRRKWRDDEYAYSBERDALIQIGRENKRRIRBDSIQ	360
<i>S. albulus</i> CCRC 11814 (EOT04707)	LNRRKWRDDEYAYSBERDALVEIGKQGRRIIRBDSIQ	360
<i>S. yanglinensis</i> CGMCC (WP_103886000)	LNRRKWRDDEYAYSBERDALVEIGKQGRRIIRBDSIQ	360
<i>S. yanglinensis</i> 3-10 (MK861976)	LNRRKWRDDEYAYSBERDALVEIGKQGRRIIRBDSIQ	360
	l n r r k w r d d e y a y s b e r d a l i q i g r e n k r r i r b d s i q	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	RFKGLGEMNAEELRTTMDHHRVLGVLLDDAARADDLF	400
<i>S. lydicus</i> 103 (WP_069566741)	RFKGLGEMNAEELRTTMDHHRVLGVLLDDAARADDLF	400
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	RFKGLGEMNAEELRTTMDHHRVLGVLLDDAARADDLF	400
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	RFKGLGEMNAEELRTTMDHHRVLGVLLDDAARADDLF	400
<i>S. albulus</i> CCRC 11814 (EOT04707)	RFKGLGEMNAEELRTTMDHHRVLGVLLDDAARADDLF	400
<i>S. yanglinensis</i> CGMCC (WP_103886000)	RFKGLGEMNAEELRTTMDHHRVLGVLLDDAARADDLF	400
<i>S. yanglinensis</i> 3-10 (MK861976)	RFKGLGEMNAEELRTTMDHHRVLGVLLDDAARADDLF	400
	r f k g l g e m n a e e l r t t m d h h r v l g v l l d d a a r a d d l f	
<i>S. albireticuli</i> MDJK11 (WP_087927260)	SVLMGEDVEARRFIQRNAKDVRFELD	426
<i>S. lydicus</i> 103 (WP_069566741)	SVLMGEDVEARRFIQRNAKDVRFELD	426
<i>Streptomyces</i> sp. NEAU-S7G82 (WP_093640877)	SVLMGEDVEARRFIQRNAKDVRFELD	426
<i>S. luteoverticillatus</i> CGMCC 15060 (WP_126915025)	SVLMGEDVEARRFIQRNAKDVRFELD	426
<i>S. albulus</i> CCRC 11814 (EOT04707)	SVLMGEDVEARRFIQRNAKDVRFELD	426
<i>S. yanglinensis</i> CGMCC (WP_103886000)	SVLMGEDVEARRFIQRNAKDVRFELD	426
<i>S. yanglinensis</i> 3-10 (MK861976)	SVLMGEDVEARRFIQRNAKDVRFELD	426
	s v l m g e d v e a r r f i q r n a k d v r f e l d	

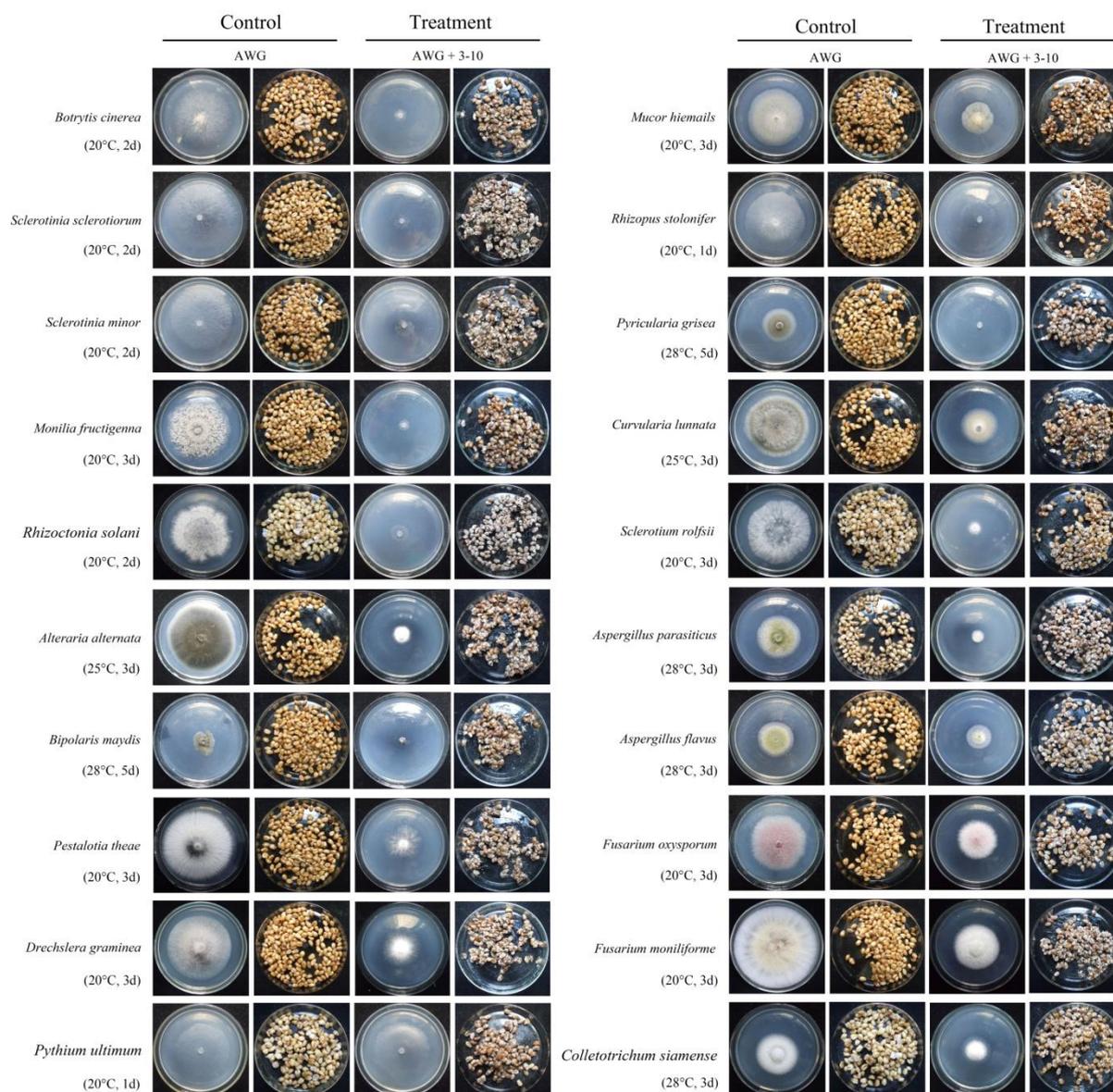
Supplementary Figure S13. gyrB subunit AA sequence alignment



**Supplementary Figure S14.** A phylogenetic tree based on AA sequence of gyrB subunit using the maximum likelihood method. The numbers at the nodes in that tree indicate the bootstrap percentages ( $n = 1,000$ ). The bar indicates the average nucleotide change per site.



**Supplementary Figure S15.** A glass desiccator showing set-up of the bioassay for determination of the efficacy of the volatiles from *Streptomyces yanglinensis* 3-10 in suppression of colonization of peanut kernels by *Aspergillus flavus* and *A. parasiticus*.



**Supplementary Figure S16.** Antifungal spectrum of the VOCs from *Streptomyces yanglinensis* 3-10.

Control = fresh AWG, Treatment = fumigated with the VOCs from the AWG cultures of *S. yanglinensis* 3-10



**Supplementary Figure S17.** Effect of the VOCs from the 7-day-old AWG culture of *Streptomyces yanglinensis* 3-10 (VOCs<sup>3-10AWG</sup>). AWG = autoclaved wheat grains. The seedlings were fumigated for seven days under the VOCs from fresh AWG or the AWG culture of *Streptomyces yanglinensis* 3-10.