

Supplementary Information

Unveiling the Bacterial Sesquiterpenome of *Streptomyces* sp. CBMAI 2042 Discloses Cyclases with Versatile Performances

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Table S1. Gene clusters encoded in *Streptomyces* sp. CBMAI 2042 genome, antiSMASH output¹

Cluster region	Coordinates (nt location in the contig)	Gene cluster type	Predicted metabolite
1.1	37097-47365	butyrolactone	coelimycin P1 (12% of genes show similarity)
1.2	75718-97140	terpene	geosmin (100% of genes show similarity)
1.3	103490-210790	transAT-pks, pks-like, t1pks, nrps	streptobactin (88% of genes show similarity)
1.4	235445-285210	nrps, melanin	coelichelin (81% of genes show similarity)
1.5	307626-357296	nrps, t1pks	arsono-polyketide (45% of genes show similarity)
1.6	386638-425716	t3pks	herboxidiene (6% of genes show similarity)
1.7	427457-474180	nrps-like,nrps,betalactone	lasalocid (3% of genes show similarity)
1.8	934071-977287	nrps-like	taylorflavin A/taylorflavin B (20% of genes show similarity)
1.9	989348-1008715	terpene	steffimycin D (19% of genes show similarity)
1.10	1467946-1478344	ectoine	ectoine (100% of genes show similarity)
1.11	2499671-2530864	lanthipeptide-class-ii,lanthipeptide-class-iii	–
1.12	2623741-2632942	siderophore	desferrioxamin B (100% of genes show similarity)
1.13	2732105-2775515	nrps-like	bottromycin A2 (39% of genes show similarity)
1.14	3034877-3068884	lap, thiopeptide	–
1.15	3238407-3247366	melanin	grixazone (61% of genes show similarity)
1.16	3566509-3631814	t1pks	lavendiol (32% of genes show similarity)
1.17	4172278-4194944	lanthipeptide-class-i	–
1.18	4450329-4472966	lassopeptide	keywimysin (100% of genes show similarity)
1.19	5222983-5230559	RiPP-like	–
1.20	5394647-5417310	lanthipeptide-class-iii	AmfS (100% of genes show similarity)
1.21	5782649-5800237	terpene	–
1.22	6209563-6224235	siderophore	ficellomycin (3% of genes show similarity)
1.23	6610631-6620483	RiPP-like	–

Table S1. Gene clusters encoded in *Streptomyces* sp. CBMAI 2042 genome - antiSMASH output (cont.)

Cluster region	Coordinates (nt location in the contig)	Gene cluster type	Predicted metabolite
1.24	6857561-6980094	nrps, t3pks, nrps-like	enduracidin (20% of genes show similarity)
1.25	7041436-7052173	butyrolactone	—
1.26	7165964-7190444	lanthipeptide-class-i	SF2575 (4% of genes show similarity)
1.27	7270805-7297029	terpene	hopene (69% of genes show similarity)
1.28	7380243-7428083	nrps, t1pks	SGR PTMs (100% of genes show similarity)
1.29	7437814-7448611	RiPP-like	tetronasin (3% of genes show similarity)
1.30	7493401-7559043	nrps	crochelin A (7% of genes show similarity)
1.31	7600749-7657273	nrps	valinomycin/montanastatin (91% of genes show similarity)
1.32	7664270-7674749	melanin	melanin (100% of genes show similarity)
1.33	7705870-7746922	t3pks	alkylresorcinol (100% of genes show similarity)
1.34	7814067-7839675	terpene	isorenieratene (100% of genes show similarity)
1.35	7855790-7900241	nrps, t1pks, thiopeptide, lap	lactazole (33% of genes show similarity)

Table S2. Plasmids used in cloning and production of recombinant protein

Plasmid	Function	Genes	Source
pET28b(+)	protein production vector	T7 promoter, T7 terminator, <i>KanR2</i> , <i>pBR322</i> , <i>His-Tag</i> , <i>T7-Tag</i> , <i>lacI</i>	Novagen®
pET28b(+):: <i>ts-1</i>	EnzTS-1 protein production vector	pET28b(+) + <i>ts-1</i>	this work
pET28b(+):: <i>ts-2</i>	EnzTS-2 protein production vector	pET28b(+) + <i>ts-2</i>	this work
pET28b(+):: <i>ts-3</i>	EnzTS-3 protein production vector	pET28b(+) + <i>ts-3</i>	this work

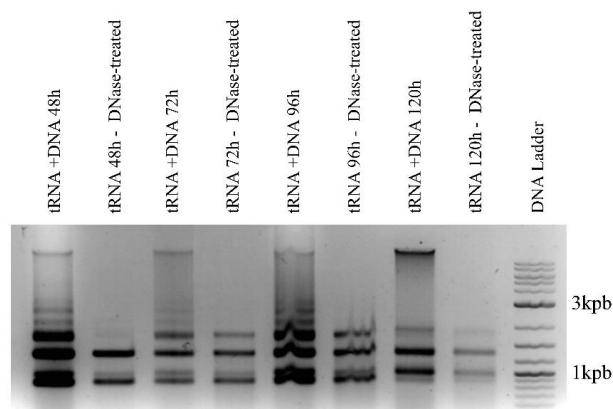


Figure S1. tRNA extraction from *Streptomyces* sp. CBMAI 2042 cultured in TSBY at 48, 72, 96 and 120 h.

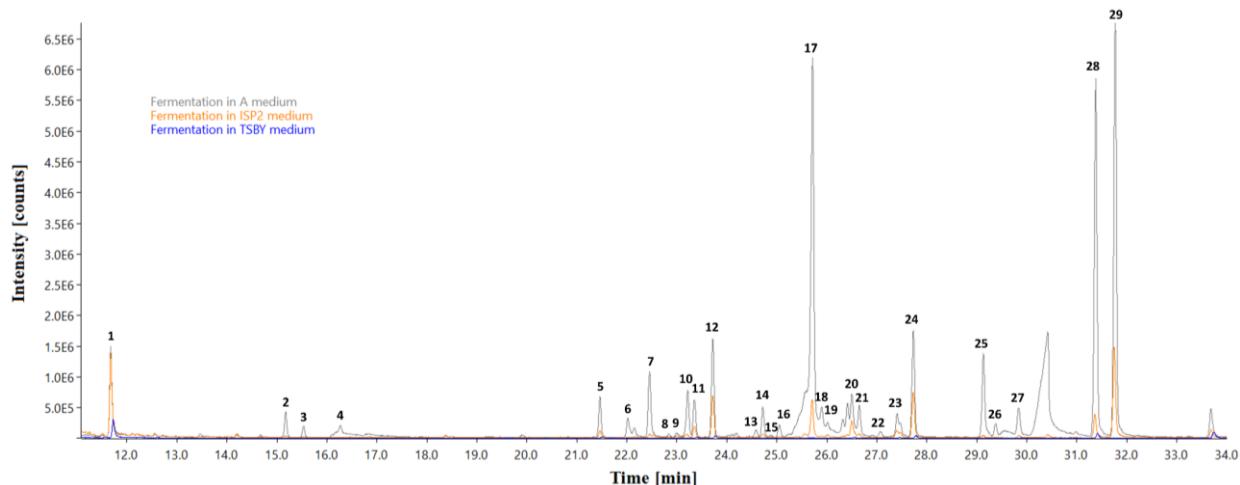


Figure S2. Overlapping of chromatograms referring to *Streptomyces* sp. CBMAI 2042 mycelium extracts in A medium, ISP2 medium and TSBY medium. Compounds numbers were assigned in ascending order of retention time, according to Table 1 (main article).

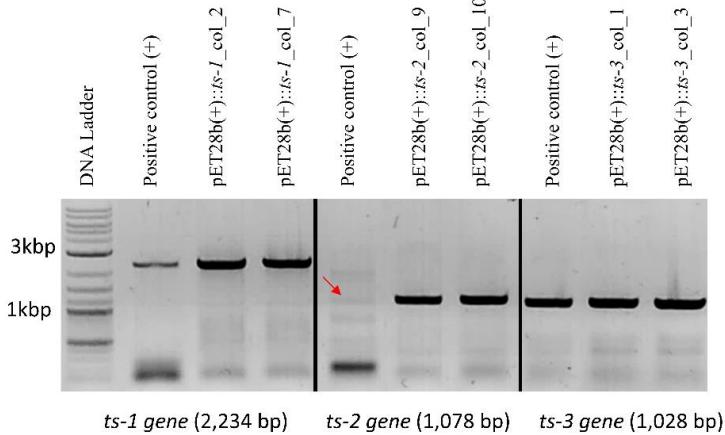


Figure S3. Confirmation of cloning of *ts-(1-3)* genes by polymerase chain reaction (PCR). The abbreviation “col” refers to colony.

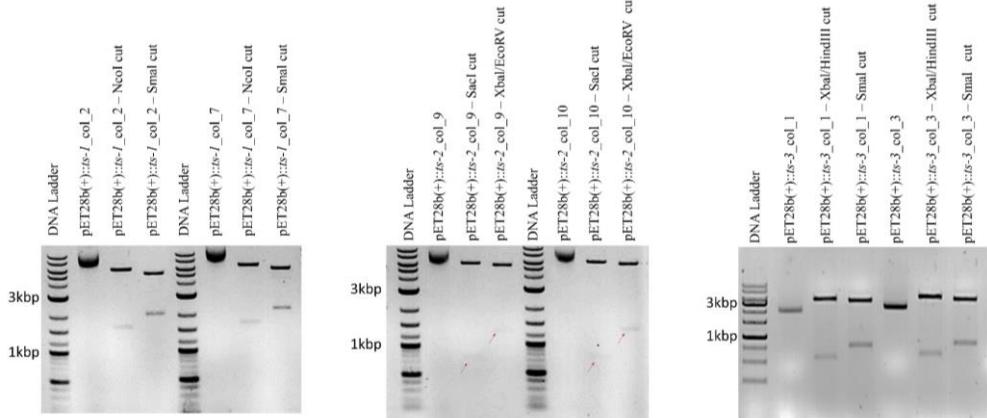


Figure S4. Confirmation of cloning of *ts-(1-3)* genes by double restriction assay. The abbreviation “col” refers to colony. *NcoI*, *SmaI*, *XbaI*, *SacI*, *EcoRV* and *HindIII* refer to restriction enzymes used in the double restriction assay.

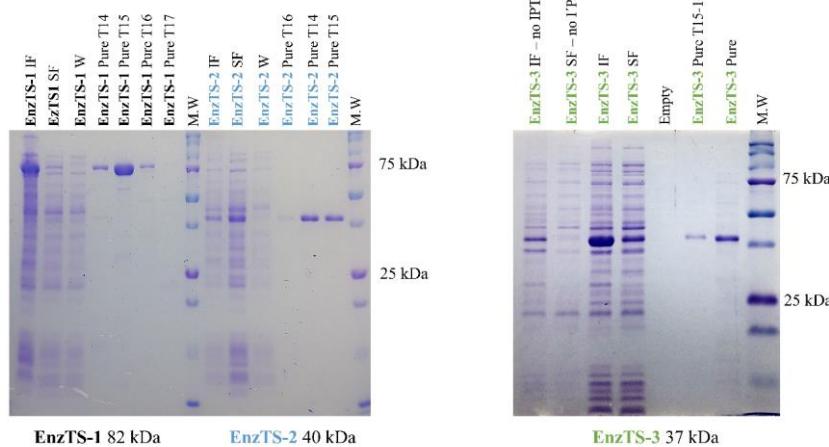


Figure S5. Production and purification of recombinant enzymes enzTS-(1-3). The abbreviations IF, SF, W and T refers to insoluble fraction, soluble fraction, waste, and tube, respectively.

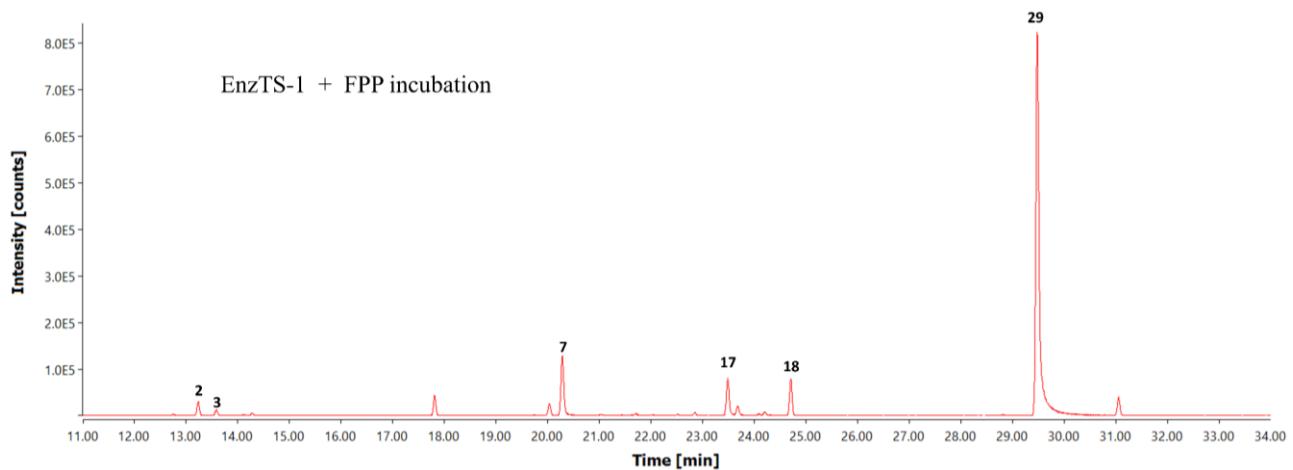


Figure S6. Chromatogram of the incubation of the recombinant enzyme EnzTS-1 with farnesyl pyrophosphate (FPP).

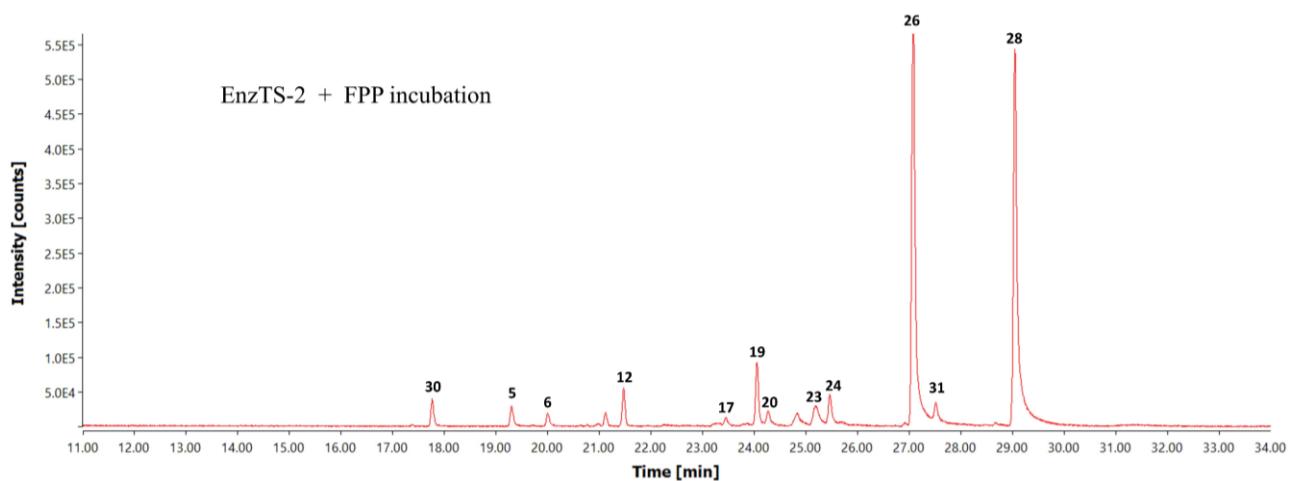


Figure S7. Chromatogram of the incubation of the recombinant enzyme EnzTS-2 with farnesyl pyrophosphate (FPP).

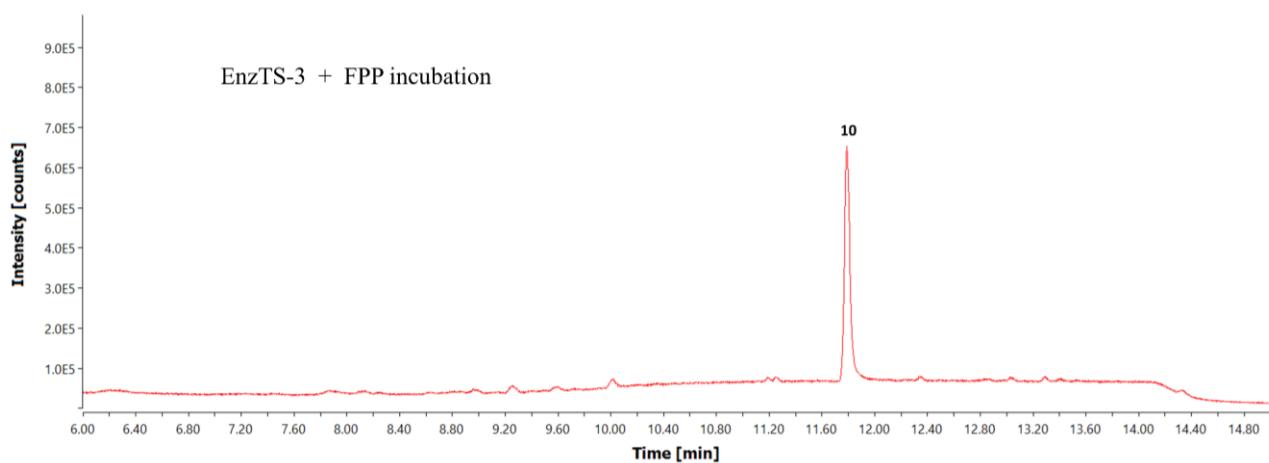


Figure S8. Chromatogram of the incubation of the recombinant enzyme EnzTS-3 with farnesyl pyrophosphate (FPP).

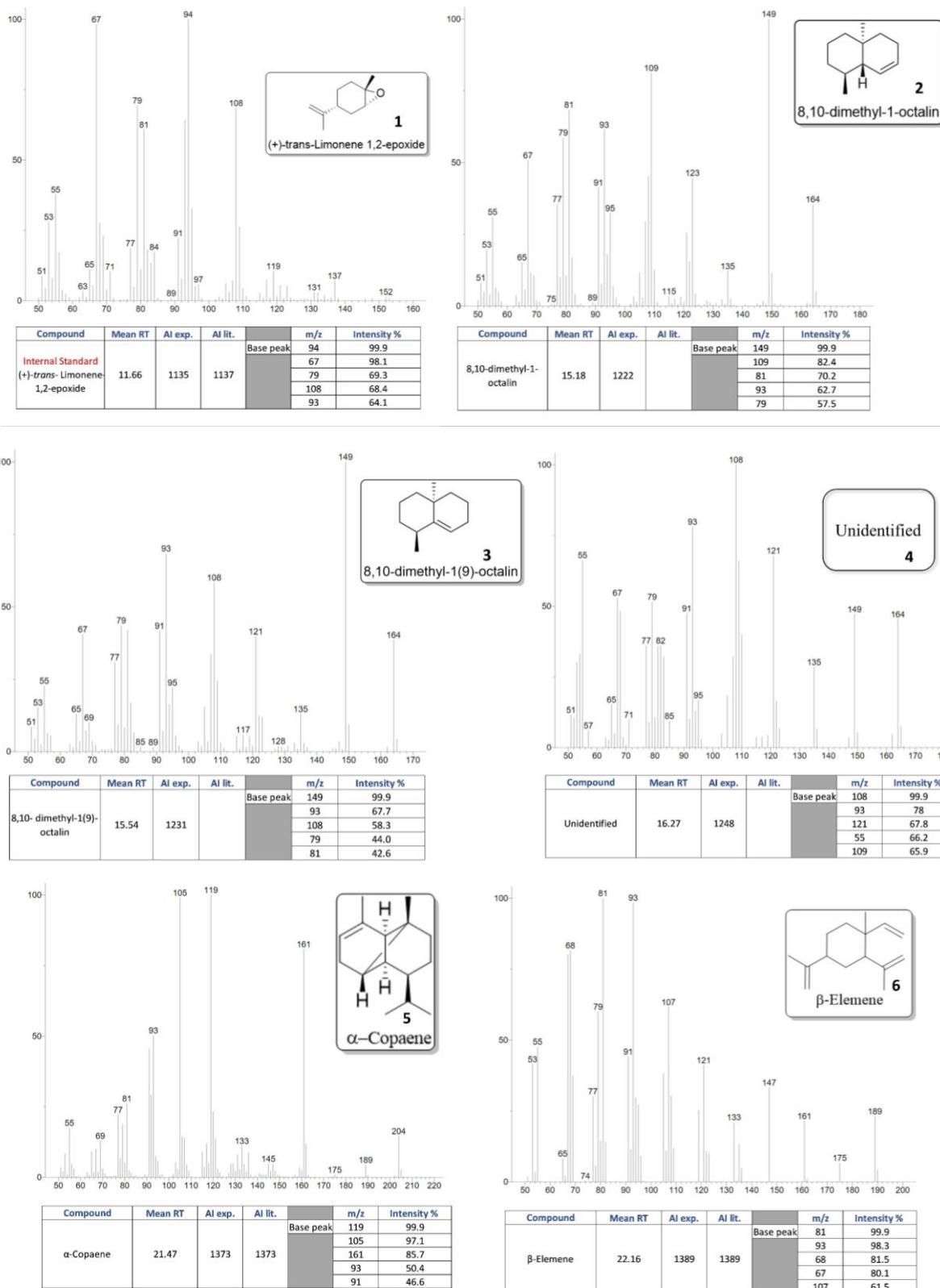


Figure S9. Mass spectrum and arithmetic index² of sesquiterpenes identified in *Streptomyces* sp. CBMAI 2042 mycelium extraction.

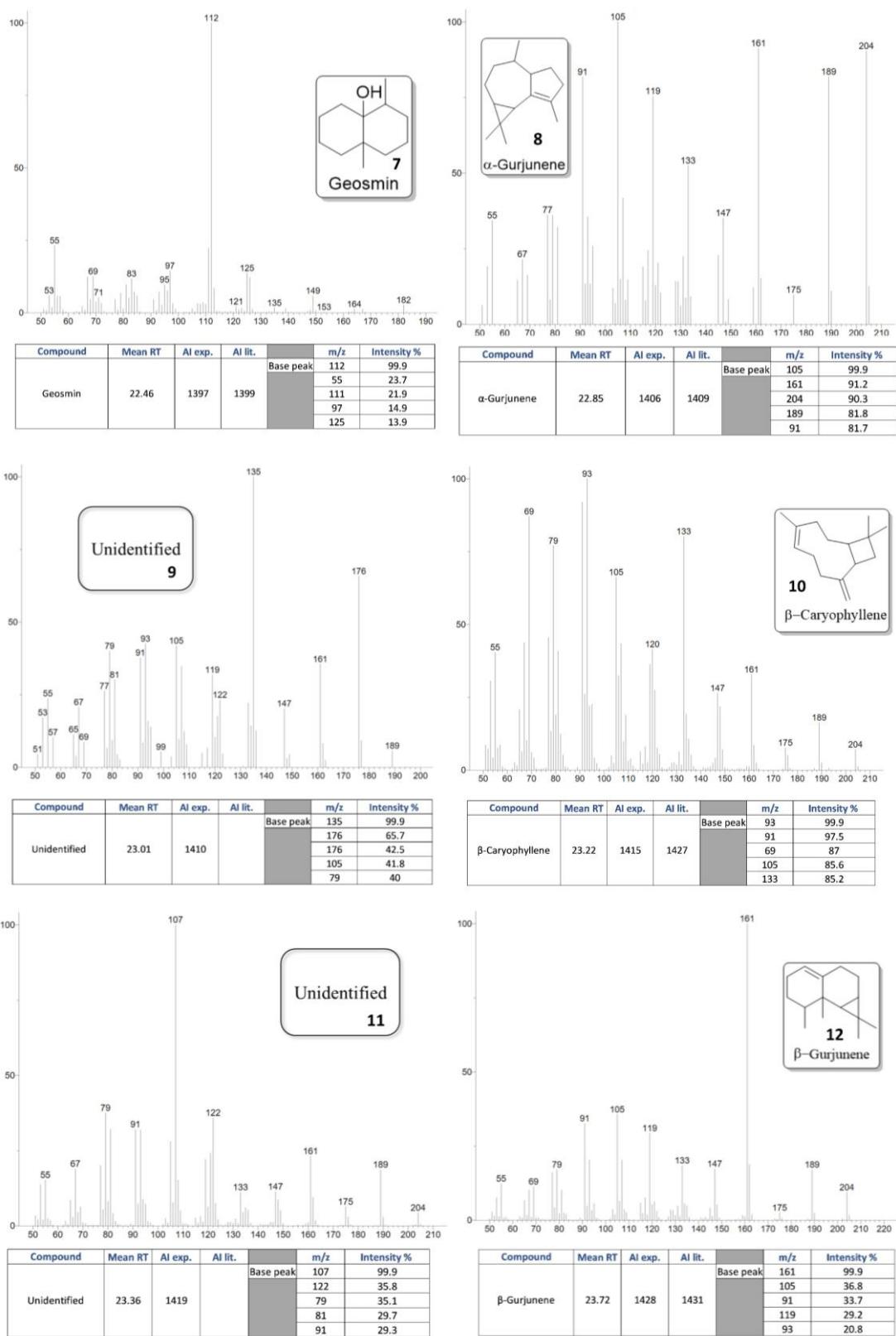


Figure S9. Mass spectrum and arithmetic index² of sesquiterpenes identified in *Streptomyces* sp. CBMAI 2042 mycelium extraction (cont.).

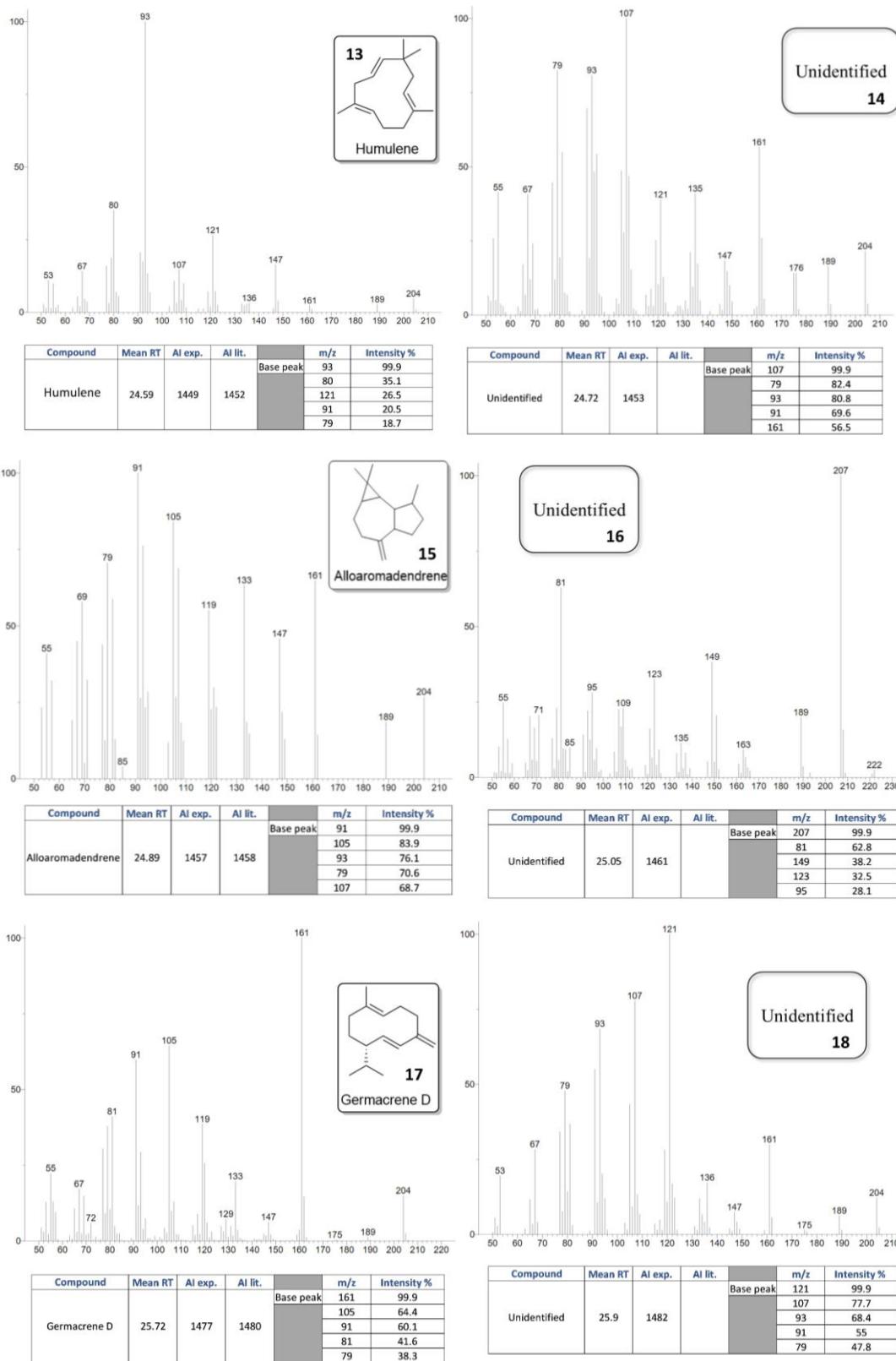


Figure S9. Mass spectrum and arithmetic index² of sesquiterpenes identified in *Streptomyces* sp. CBMAI 2042 mycelium extraction (cont.).

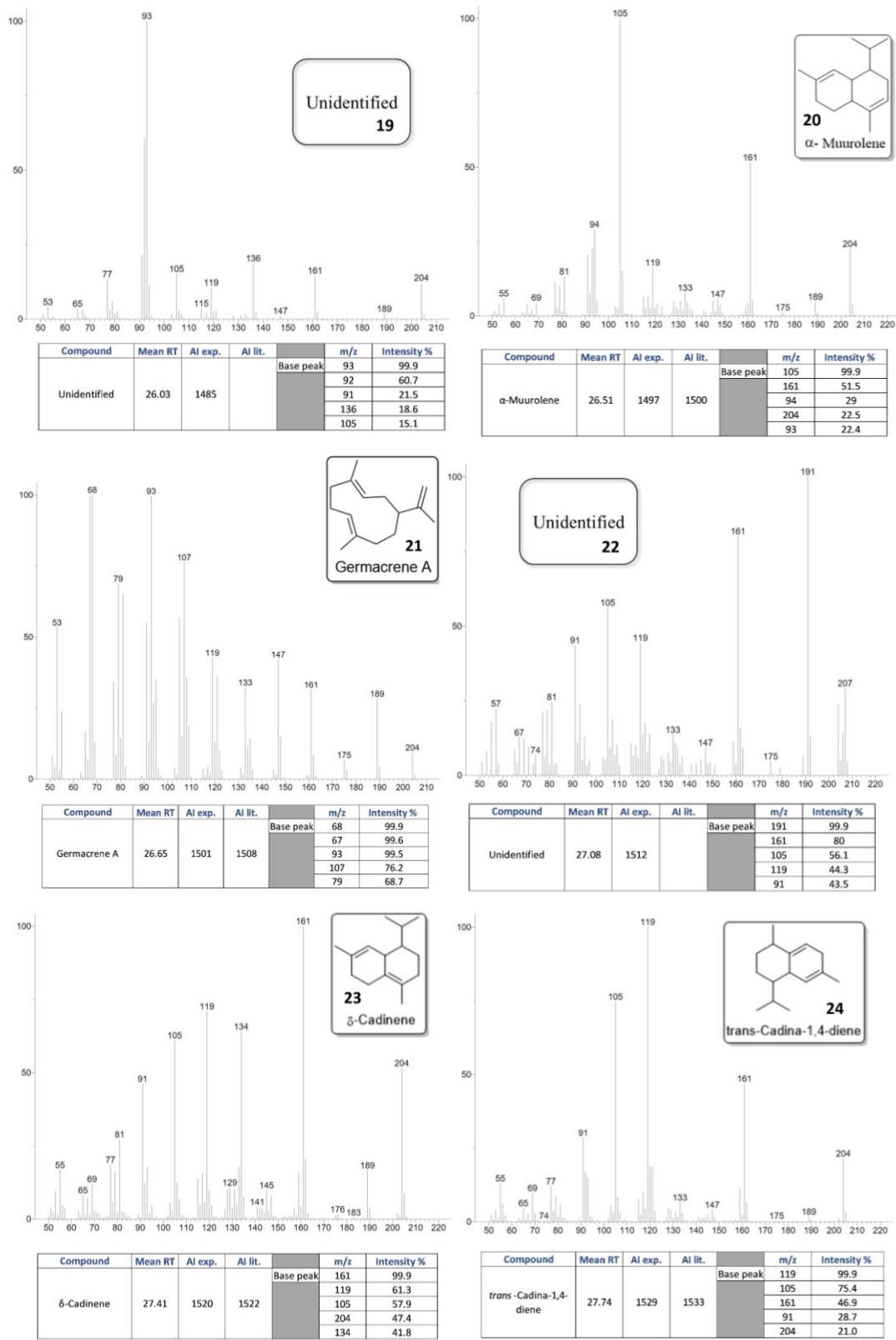


Figure S9. Mass spectrum and arithmetic index² of sesquiterpenes identified in *Streptomyces* sp. CBMAI 2042 mycelium extraction (cont.).

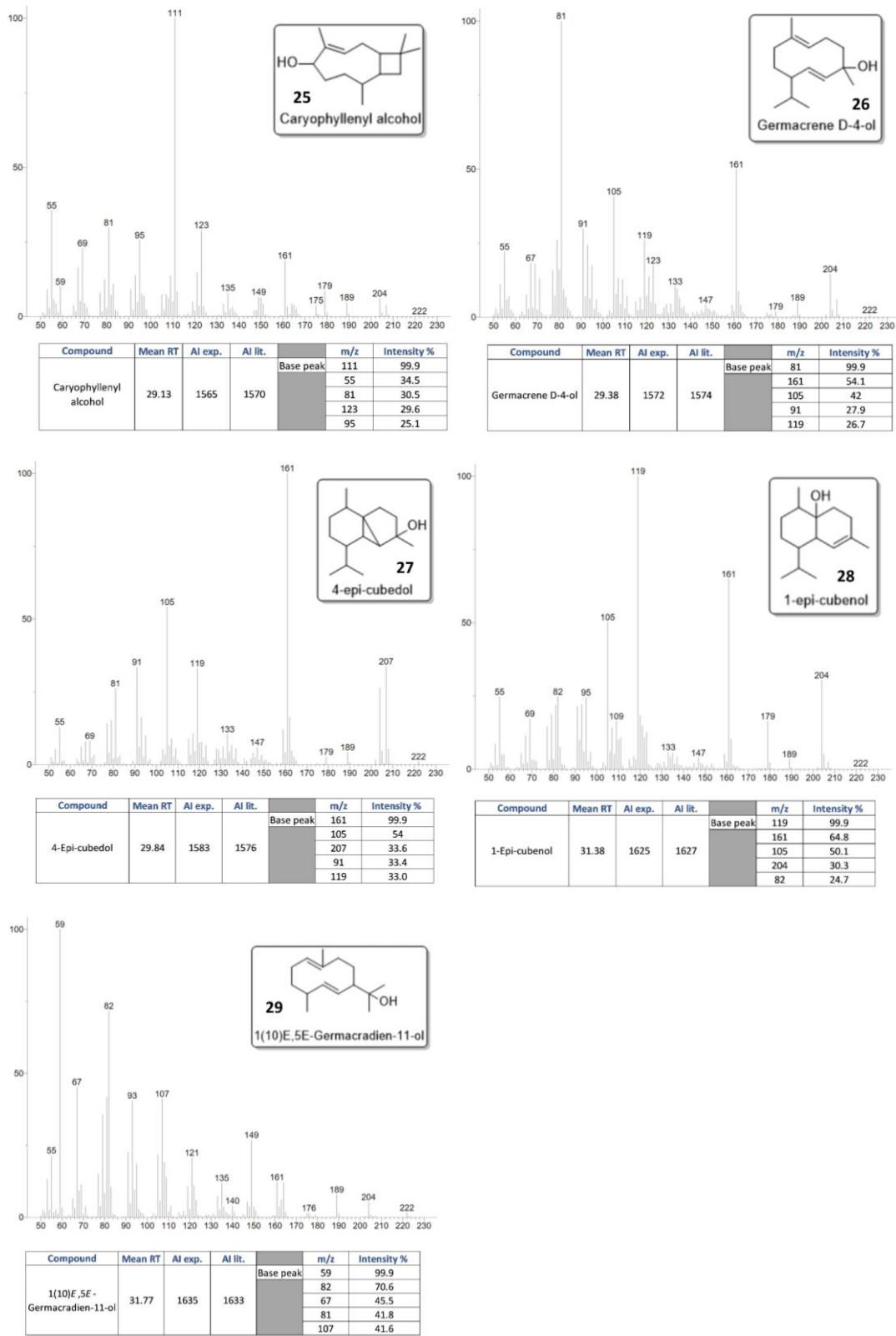


Figure S9. Mass spectrum and arithmetic index² of sesquiterpenes identified in *Streptomyces* sp. CBMAI 2042 mycelium extraction (cont.).

References

1. Blin, K.; Shaw, S.; Kloosterman, A. M.; Charlop-Powers, Z.; van Wezel, G. P.; Medema, M. H.; Weber, T.; *Nucleic Acids Res.* **2021**, *49*, W29.
2. Adams, R. P.; *Identification of Essential Oil Componentes by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing: Carol Stream, 2007.



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