Supplementary Information

Multiple *Streptomyces* species with distinct secondary metabolomes have identical 16S rRNA gene sequences

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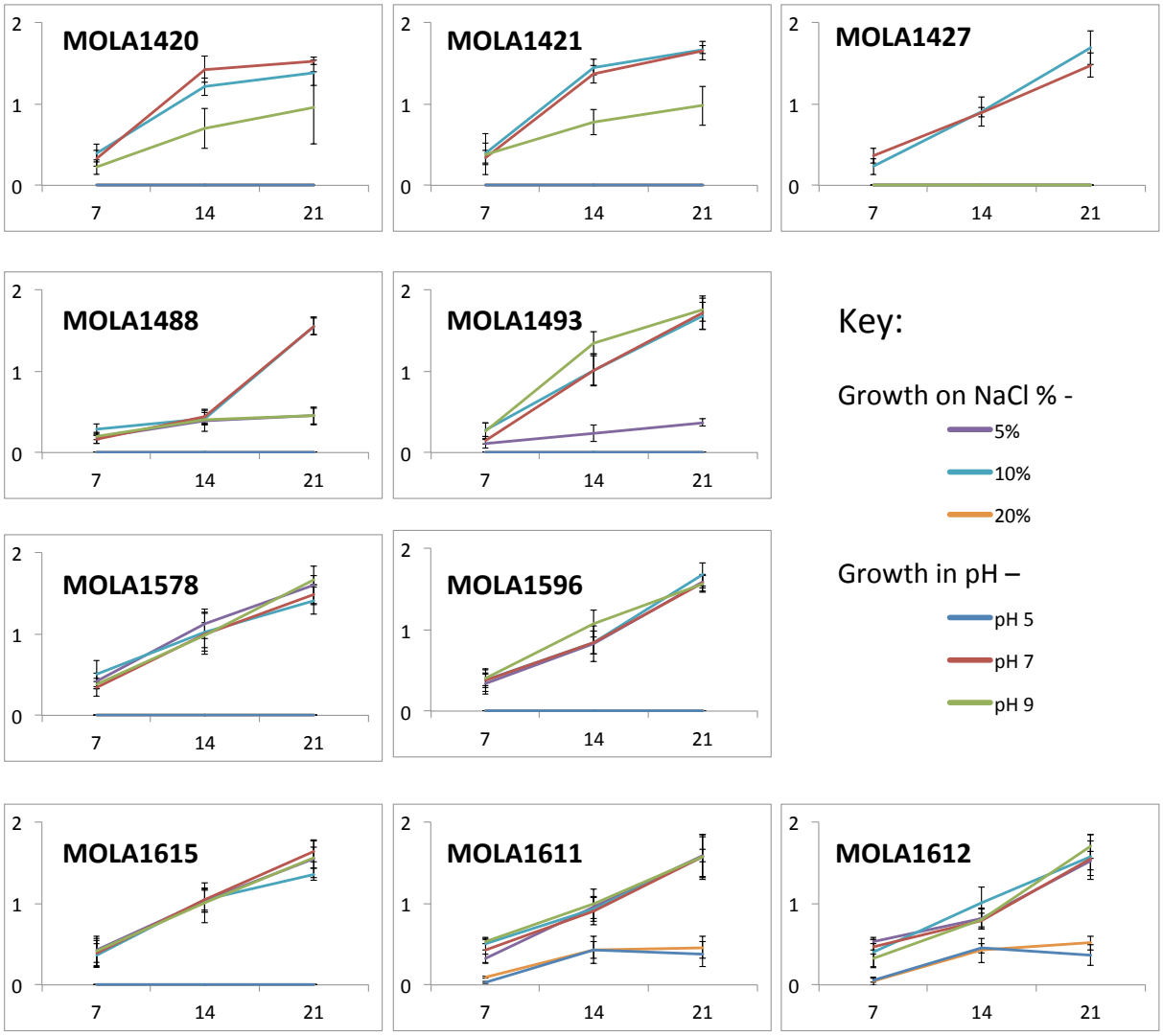
Figure S1: Growth curves of the ten tested strains in different conditions. X axis denotes the time of sampling in days (7, 14 and 21 days) and Y axis denotes dry weight biomass in grams.



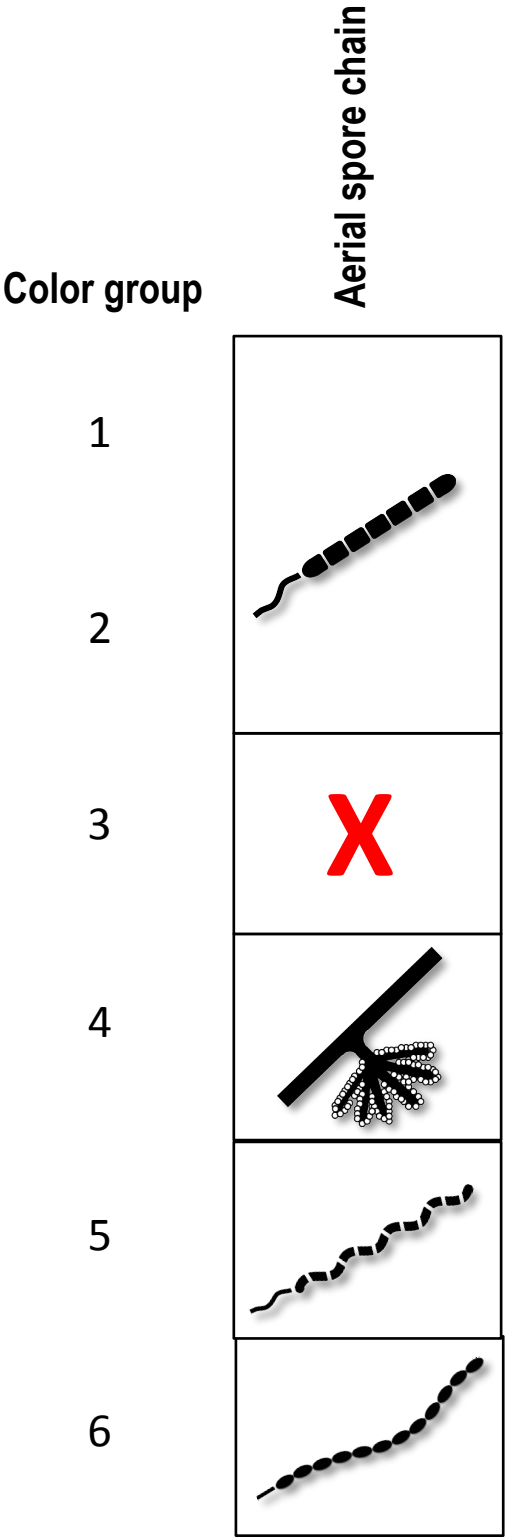


Figure S2: Micromophology of spore chain arrangement has been correlated with the color groups.

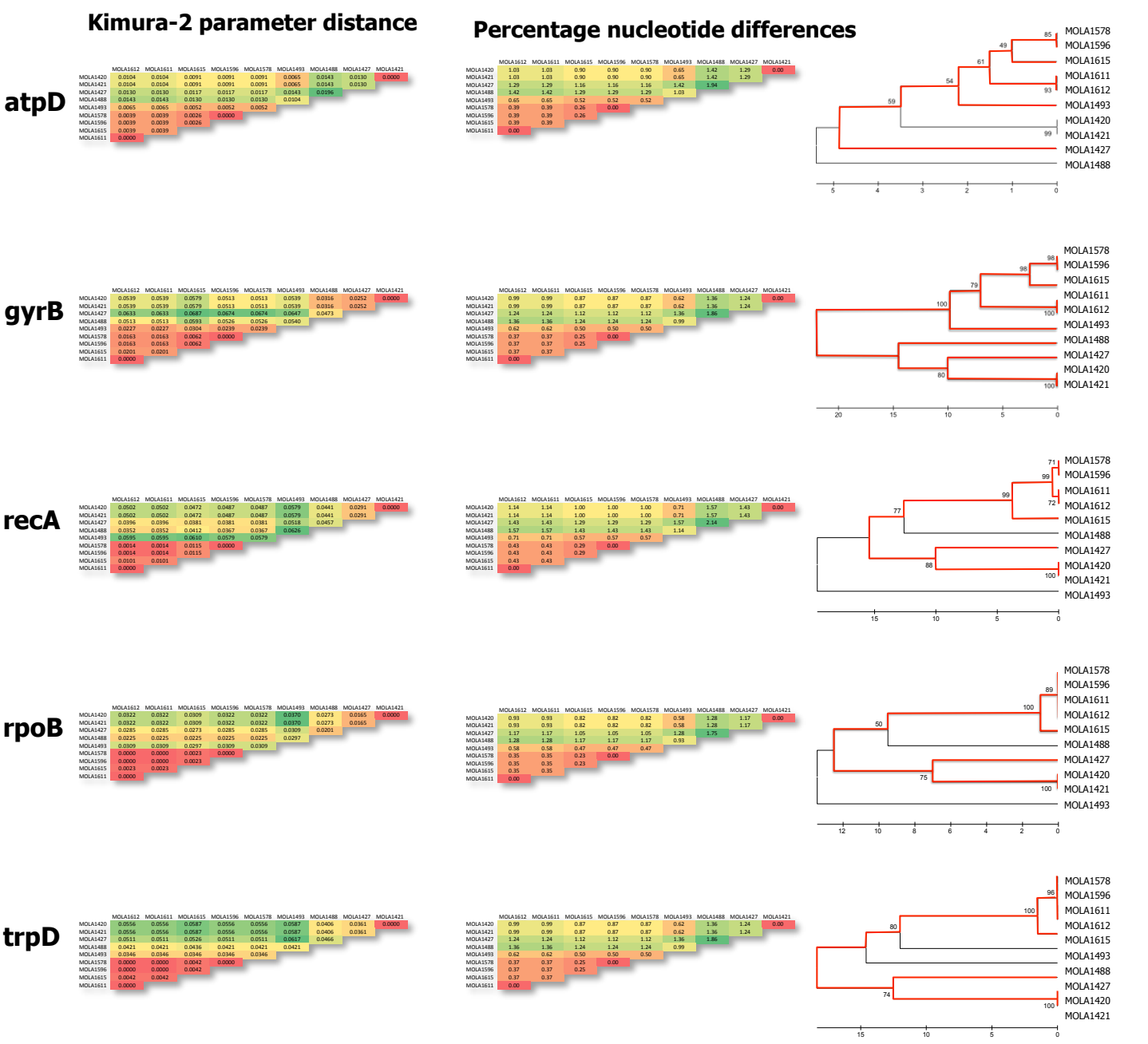
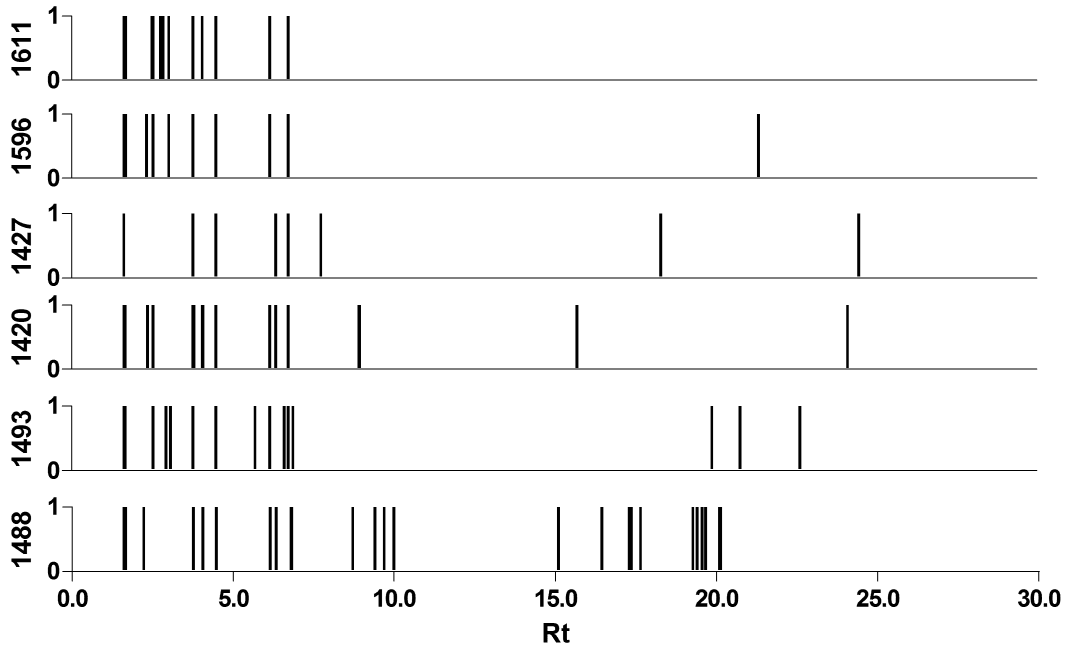


Figure S3. Properties of each of protein coding loci examined. The first two columns contain upper triangular matrices of dissimilarities based on Kimura-2 parameter and percentage of number of nucleotides. The last column shows UPGMA tree based on 1000 replicates (bootstrap). The branching patterns that were in agreement with those that were obtained by maximum parsimony, neighbor joining, maximum likelihood and minimum evolution are shown in red.

Figure S4. Aligned metabolomic profiles of actinomycete culture extracts. The x-axis indicate the retention time. Peak intensities were all set to 1. Culture medium compound peaks have been removed from the profiles so that only microbial metabolites remain.



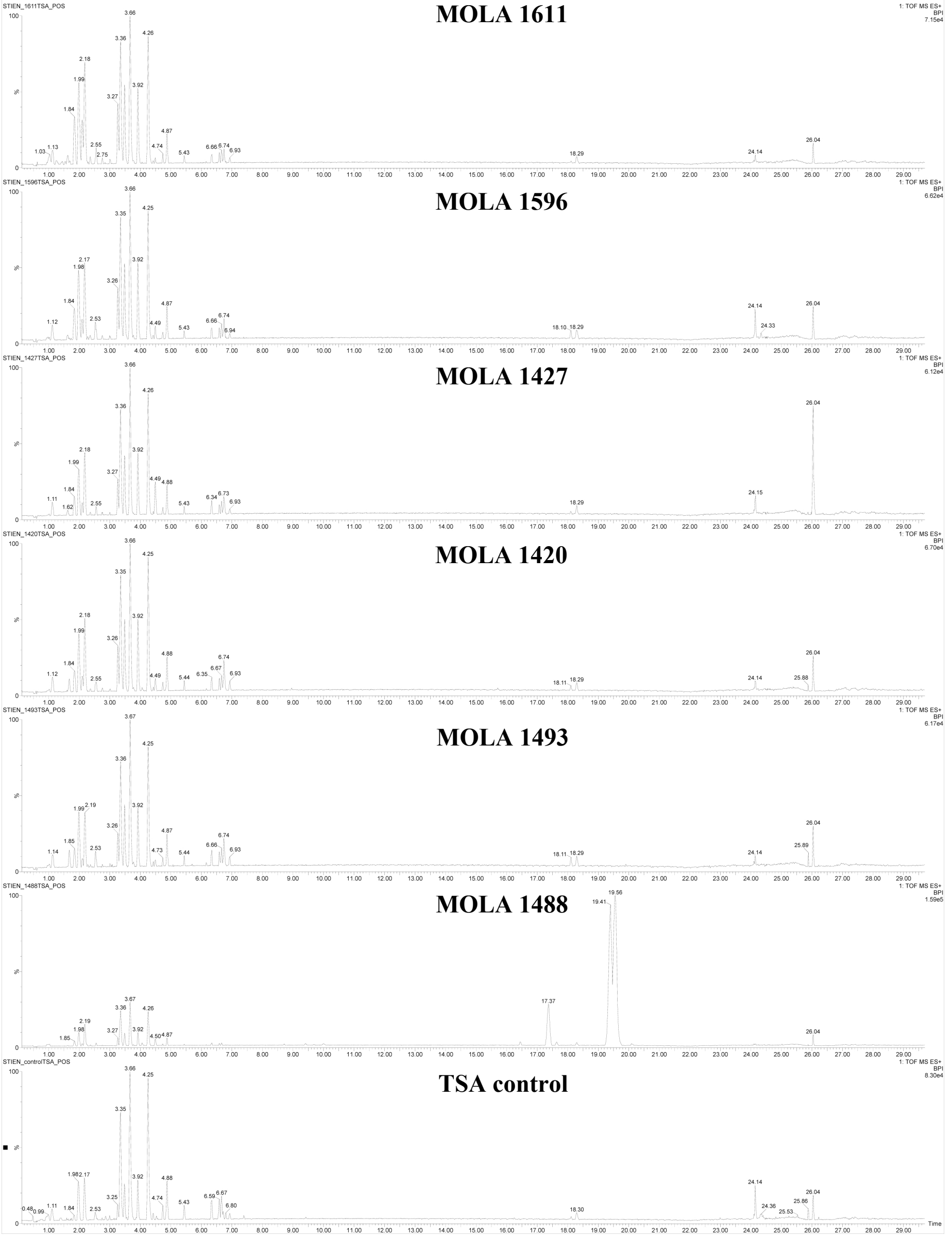


Figure S5 Aligned raw UHPLC-HRMS total ion current profiles of culture extracts of selected *S. cyaneofuscatus* strains grown in TSA. TSA medium extract profile is shown for comparison. The x-axis indicates the retention time and the y-axis the percent of peak area relative to the highest peak

Table S1: Color group characters of the 10 test strains after 21 days incubation. The numerical values denote the average red, green and blue values of colors of aerial spore mass, diffusible pigment and colony reverse on ISP 3. The colors of the average RGB values are used as fill-colors for the respective cells in the table. Melanin pigment production was concluded based on production of dark pigment on ISP 6 media.

Color group number	**Characteristics on ISP 3 (average *red:green:blue* color values)**			**Melanin pigment production on ISP 6**	**Strains**
	Aerial spore mass color	Color of diffusible pigment	Colony reverse color		
1	180:219:196	138:127:75	228:209:168	+	MOLA 1420
	195:209:205	150:134:80	215:207:165	+	MOLA 1421
2	154:184:164	112:95:46	219:184:127	+	MOLA 1427
3	201:202:165	173:129:4	241:188:46	+	MOLA 1488
4	192:209:201	88:96:89	175:158:135	+	MOLA 1493
5	165:181:155	84:101:83	178:194:169	-	MOLA 1578
	153:157:125	86:102:88	181:197:173	-	MOLA 1596
	181:192:156	85:104:91	179:196:172	-	MOLA 1615
6	111:111:77	95:112:101	159:185:173	-	MOLA 1611
	121:118:79	93:114:99	160:184:180	-	MOLA 1612

Table S2: Putative identification, sources and presence (+)/absence (-) for compounds highlighted in six *Streptomyces cyaneofuscatus* strains.

[1]Δ ppm stands for the relative m/z difference between the experimental and the calculated m/z for the $[M+H]^+$ or the $[M+Na]^+$ ion, as stated in the ion type column.
[3]The high resolution mass of this compound was incorrect due to superimposition with other signals. *N*-Methyl-dactinomycin was isolated and unambiguously identified by NMR.

Rt (min)	*m/z*	Ion type	Calcd. m/z ($[M+H]^+$ or	Putative Molecular Formula (M)	Δ ppm[1]	Putative Identification[2] and Sources	MOLA 1427	MOLA 1493	MOLA 1420	MOLA 1596	MOLA 1488	MOLA 1611	References
1.63	**146.0605**	**$[M+H]^+$**	146.0606	C_9H_7NO	0.5	*/***Indole-3-carbaldehyde (*Streptomyces zhaozhouensis*; *Aspergillus versicolor*; *Penicillium* sp.)	+	+	+	+	+	+	1-4
1.68	**212.1401**	**$[M+H]^+$**	212.1399	$C_{10}H_{17}N_3O_2$	1.0	***Bacillusamide A (*Anthocidaris crassispina* derived *Bacillus* sp.)	-	-	-	+	-	+	5
1.68	**152.0711**	**$[M+H]^+$**	152.0712	$C_8H_9NO_2$	0.4	**p*-Hydroxyphenyl-acetamide (marine *Cytophaga* sp.; marine *Vibrio parahaemolyticus*); *Anthranilic acid methyl ester (*Streptomyces avermitilis*); */***4-Hydroxyacetanilide (*Streptomyces venezuelae*); ***2-(Acetylamino)phenol (*Streptomyces* sp.; *Penicillium* sp.; *Actinomyces* sp.); ****N*-Methylanthranilic acid (*Streptomyces* sp.); ****N*-Methyl-*p*-aminobenzoic acid (*Streptomyces* sp.); ***2-(4-Hydroxyphenyl)acetamide (*Cytophaga* sp.); **/***(2-Amino-phenyl)-acetic acid (*Colletotrichum gloeosporioides*); **/***4-Hydroxyphenylacetaldehyde oxime (*Streptomyces nigellus*); */***Streptokordin (*Streptomyces* sp.)	-	+	+	-	+	-	6-18
2.24	**136.0761**	**$[M+H]^+$**	136.0762	C_8H_9NO	0.9	*/****p*-(Methylamino)-benzaldehyde (*Camarophyllus virgineus*); *2-Phenylacetamide (*Streptomyces alboniger, sponges, cyanobacterium*); *1-(2-Amino-phenyl)-ethanone (marine *Streptomyces, Saccharomyces cerevisiae*); *3-Aminophenyl 1-ethanone (*Phlebia radiata*); ***Acetanilid (*Streptosporangium* sp.); ***Benzeneacetamide (*Streptomyces* sp.; *Streptoverticillium olivoreticuli; Vibrio parahaemolyticus*); ***Agrocybyne A (*Agrocybe praecox*)	-	-	-	-	+	-	6, 19-30
2.33	**160.0767**	**$[M+H]^+$**	160.0762	$C_{10}H_9NO$	2.6	**/***4-Hydroxy-2-methylquinoline (*Pseudomonas aeruginosa*); */**/*** 4-(Hydroxymethyl)quinoline (*myxobacteria*), **/*** (*E*) or (*Z*)-3-Ethylidene-1,3-dihydroindol-2-one (*Colletotrichum fragariae* Brooks), *Quinoline-2-methanol (*Kitasatoa griseophaeus*); *8-Amino-2-naphthalenol (cyanobacterium *Aphanizomenon flos-aquae*)	-	-	-	+	-	-	20, 31-33
2.37	**185.1291**	**$[M+H]^+$**	185.1290	$C_9H_{16}N_2O_2$	0.6	*/**/***Cyclo (L-Ala-L-Leu) (various microorganisms) (e.g. *Streptomyces* sp. YIM 56130)	-	-	+	-	-	-	34
2.50	**138.0553**	**$[M+H]^+$**	138.0555	$C_7H_7NO_2$	1.5	*/**/***Pyrrole-3-yl-2-propenoic acid (*Streptomyces parvulus Tue 2480)*; */**/*** *N*-Formyl-2-aminophenol (Penicillium *notatum-Westling)*; */**/****p*-Aminobenzoic acid (Hansenula *anomala, Mycotoru*la *lipolytica, marine S* treptomyces *sp. B8459*), **/****o*-Aminobenzoic acid (various bacteria e.g. *Streptomyces* sp. IFM 11299, S. sp GW63/*1* 571 ; limnic strain GW73A, *S. staurosporeus* + tryptophan, *Paenibacillus polymyxa* +/- tryptophan); *Anthranilic acid (widespread in bacteria) ; */****p*-Hydroxybenzamide *(Streptomyces tendae);* */**/***Trigonelline and Homarine (sponges, *Polyporus sulfureus*)	-	-	-	-	-	+	19, 35-41
2.53	**229.1018**	**$[M+H]^+$**	229.1011	$C_{10}H_{16}N_2O_2S$	3.3	*/**Cyclo(L-Pro-L-Met) (sponge-associated *Pseudomonas aeruginosa*)	-	+	+	+	-	+	42
2.76	**219.1139**	**$[M+H]^+$**	219.1134	$C_{12}H_{14}N_2O_2$	2.4	*/**/***PD 125375 (*Streptomyces* sp); */***Cyclo(L-Ala-L-Phe) (various bacteria); ***(2*S*,3*R*)-threo-β-Methyl-L-tryptophan, erythro-β-Methyl-L-tryptophan*** (*Streptomyces flocculus*), β-Hydroxy-*N*-β-acetyltryptamine (*Streptomyces staurosporeus*); 5-Hydroxy-*N*-β-acetyltryptamine (*Streptomyces staurosporeus*); */*** (3a*R*,8a*S*)-1-Acetyl-1,2,3,3a,8,8a-*H*-hexahydropyrrolo[2,3-b]indol-3a-ol (*S. staurosporeus* + tryptamine hydrochloride); *(3a*R*,8a*R*)-3a-Acetoxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol (endophytic *Fusarium incarnatum* HKI00504 from mangrove) ; ***Damirone A (*Zyzzia fuliginosa*)	-	-	-	-	-	+	43-47
2.85	**128.1075**	**$[M+H]^+$**	128.1075	$C_7H_{13}NO$	0.5	no hit from microorganisms	-	-	-	-	-	+	

2.94	**118.0326**	**Fragment of $[M+H]^+$ at *m/z* 207.0636**	207.0626	$C_7H_{14}N_2OS_2$	4.9	no hit in antibase/DNP/Reaxys	-	+	-	-	-	-	
3.02	**300.1355**	**$[M+H]^+$**	300.1348	$C_{16}H_{17}N_3O_3$	2.1	*/**/***3β-Hydroxy-cyclo-L-tryptophyl-L-proline (*Aspergillus fumigatus*), **/***Cyclo(4-hydroxy-*S*-Pro-*S*-Trp) (marine-derived *Streptomyces* sp)	-	-	-	+	-	+	48, 49
3.08	**177.0671**	**n.d.**	n.d.	n.d.	n.d.	n.d.	-	+	-	-	-	-	
3.78	**217.0983**	**$[M+H]^+$**	217.0977	$C_{12}H_{12}N_2O_2$	2.5	*/**/****N*-Acetyl-β-oxotryptamine (Marine *Janibacter limosus*; *Streptosporangium* sp.; *Bacillus pumilus*); */**/***Damirone A (Sponges); */**/***Mansouramycin A (Marine *Streptomyces*); *cycloanthranilylproline (*Fuligo candida*); *Sannanine (*Streptomyces sannanensis*); */**/***Caerulomycin F (Marine-derived *Acti,oalloteichus cyanogriseus*); ***(2-aminophenyl)(2-ethyloxazol-5-yl)methanone (*Streptomyces* sp.)	+	+	+	+	+	+	23, 50-59
3.80	**231.1147**	**$[M+H]^+$**	231.1134	$C_{13}H_{14}N_2O_2$	4.5	***(3*S*,8a*S*)-3-Phenyloctahydropyrrolo[1,2-a]pyrazine-1,4-dione (*Penicillium cluniae* Quintanilla (CECT 2888), *Cyclo(Phe-Dehydroabu) (*Streptomyces* sp. Goe 40/14); Tetrahydro-1-methyl-β-carboline carboxylic acid (*Amanita muscaria*)	-	-	+	-	-	-	41, 60, 61
4.06	**130.1229**	**$[M+H]^+$**	130.1232	$C_7H_{15}NO$	2.5	*Isopentyl acetamide (Marine bacteria)	-	-	+	-	+	+	62
4.08	**171.1502**	**$[M+H]^+$**	171.1497	$C_9H_{18}N_2O$	2.6	no hit in antibase/DNP/Reaxys	-	-	+	-	+	-	
4.49	**164.1077**	**$[M+H]^+$**	164.1075	$C_{10}H_{13}NO$	1.2	*3-Acetamide derivatives; */***Streptazone D (*Streptomyces* FORM5 and *Streptomyces* A1); */**Dihydroabikoviromycin (*Streptomyces olivaceus, S. vinidochromogenes, S. reticuli, S. anulatus*); ***Methyl-*N*-(benzyl-methyl)-formamide (*Actinomadura* sp.; cultures of limnic strains GW102a and GW73a)	+	+	+	+	+	+	20, 36, 63-66
5.70	**437.2023**	**$[M+Na]^+$**	437.1999	$C_{17}H_{34}O_{11}$	3.9	no hit in antibase/DNP/Reaxys	-	+	-	-	-	-	
6.16	**255.0666**	**$[M+H]^+$**	255.0657	$C_{15}H_{10}O_4$	3.4	**1,8-Dihydroxy-4-methylanthraquinone (Cyanobacteria); */***Chrysophanol (*Penicillium* sp.); *Daidzein (*Micromonospora halophytica*, *Streptomyces* sp.)	-	+	+	+	+	+	67-69
6.34	**268.2031**	**$[M+H]^+$**	268.2025	$C_{14}H_{25}N_3O_2$	2.1	no hit in antibase/DNP/Reaxys	+	-	+	-	+	-	
6.61	**303.1711**	**$[M+H]^+$**	303.1709	$C_{17}H_{22}N_2O_3$	0.8	*/**/***Trichostatin (*Streptomyces hygroscopicus* a-300 (FERM-p 1312), *Streptomyces* sp. RK98-A74); ***Brocaeloid B (*Penicillium brocae* MA-192), **Terretrione B (mangrove-derived *Aspergillus terreus* A8-4)	-	+	-	-	-	-	70-73
6.74	**280.1240**	**$[M+H]^+$**	280.1232	$C_{12}H_{17}N_5OS$	2.8	no hit in antibase/DNP/Reaxys	+	+	+	+	-	+	
6.81	**544.2379**	**$[M+Na]^+$**	544.2370	$C_{23}H_{39}NO_{12}$	1.7	no hit in antibase/DNP/Reaxys	-	-	-	-	+	-	
6.83	**504.2800**	**$[M+Na]^+$**	504.2785	$C_{22}H_{43}NO_{10}$	3.1	no hit in antibase/DNP/Reaxys	-	-	-	-	+	-	

6.89	**341.1490**	**[M+Na]$^+$**	341.1477	$C_{17}H_{22}N_2O_4$	3.8	*/**Phomamide (*Phoma lingam*) ; */**Tomaymicin (*Nocardia* sp. C-15003, *Streptomyces achromogenes* var. *tomaymyceticus*) ; */**Terragine B (DNA isolated from soil expressed in a *Streptomyces lividans* host) ; */**Daryamide C (marine-derived *Streptomyces* strain CNQ-085) ; */**Fusaperazine C (endophytic *Colletotrichum gloeosporioides*, *Penicillium crustosum*, *Fusarium* sp.)	-	+	-	-	-	-	74-80
7.75	**513.2674**	**[M+H]$^+$**	513.2673	$C_{22}H_{36}N_6O_8$	0.1	no hit in antibase/DNP/Reaxys	+	-	-	-	-	-	
8.71	**670.3077**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
8.94	**247.1322**	**[M+Na]$^+$**	247.1310	$C_{13}H_{20}O_3$	4.7	*/**Hyalopyrone (*Ascochyta hyalospora, Sporormiella teretispora* and marine-derived *Ascochyta salicorniae*) ; */**Similin-A (*Sporormiella similis*) ; *5-(6'-Methyl-7'-oxo-octyl)-(5*H*)-furan-2-one or MKN-003A (marine *Streptomyces* sp. B 3497, B8042) ; */**11-Hydroxy-4-methyl-2,4,6-dodecatrienoic acid (*Mucor* sp. KL 94-42) ; */**Guaymasol and epiguaymasol (marine deep-sea *Bacillus*) ; */**4,5-Didehydroacaterin (*Pseudomonas* sp. A92 and *P. jessenii*) ; */**Apiosporic acid (marine endophytic *Apiospora montagnei*) ; */**2,4-Dimethoxy-6-pentylphenol (endophytic fungus *Botryosphaeria mamane* PSU-M76) ; **N*-[5-(2-Formyl-5-hydroxymethyl-pyrrol-1-yl)-pentyl]-acetamide (endophytic *Fusarium incarnatum* (HKI00504) from mangrove) ; */**Cyclopenicillone (*Penicillium decumbens*); ** Petrynol (*Petrosia* sp.); ** Mollipilin D (*Chaetomium mollipilium*); **3-Hydroxy-5-magastigmene-7,9-dione (*Prorocentrum minimum*)	-	-	+	-	-	-	81-94
8.95	**288.1585**	**[M+H]$^+$**	288.1600	$C_{17}H_{21}NO_3$	4.7	*/**/***Trichostatic acid (*Streptomyces sioyaensis*; *Streptomyces* sp.), *3-(2'-Acetoxy-3'-oxo-4'-methylhexyl)-indole (bacterial symbiont *Xenorhabdus bovienii* A2), *Solanapyrone G (marine filamentous fungus)	-	-	+	-	-	-	95-98
9.41	**624.3024**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
9.69	**668.2924**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
10.00	**654.3116**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
15.10	**1013.4707**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	*** New compound based on high resolution mass	-	-	-	-	+	-	
15.71	**205.1963**	**[M+H]$^+$**	205.1956	$C_{15}H_{24}$	3.3	Sesquiterpenes e.g. */**/*** pentalenene (*Streptomyces griseochromogenes*), *Bicyclogermacrene, δ-elemene, eepldozene, β-bourbonene (*S. griseus* CBS 109.60), *α-Gurjunene, kelsoene, bourbon-11-ene, cadina-3,5-diene, ε-cadinene, β-muurolene, cadina-1,4-diene, eudesma-5,11-diene (marine *Streptomyces* sp. GWS-BW-H5)	-	-	+	-	-	-	22, 99-101
16.44	**1279.5986**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
17.29	**1293.6152**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
17.37	**1271.6277**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
17.64	**1263.6039**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
18.32	**279.1614**	**[M+H]$^+$**	279.1596	$C_{16}H_{22}O_4$	3.1	**Coibanole A (*Pycnoporus sanguineus*); */**Oncorhyncolide (Marine bacterium); **Penicitrinol D (marine-derived *Penicillium citrinum*); **Guignardone D (endophytic fungus A1 from *Scyphiphora hydrophyllacea*); */*** (*R*)-de-*O*-Methyllasiodiplodin (endophytic fungus *Lasiodiplodia* sp.); *Isoacrostalidic acid (*Acrostalagmus* sp.); *1-(3,5-Dihydroxyphenyl)-10'-hydroxy-1-undecen-6'-one (*Gliocladium reseum*); *7-Oxo-brefeldin (*Penicillium* sp.); *Dechloromonilidiol (*Monilinia fructicola*); * Microsphaeropsisin (*Microphaeropsis* from marine sponges); *Cytosporone C (*Cytospora* sp.); *Hirsutic acid C (*Stereum hirsutum*); *Oblongolide G (*Phomopsis* sp.)	+	-	-	-	-	-	102-114
19.27	**1291.5997**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
19.41	**1269.6169**	**[M+H]$^+$**	1269.6519	$C_{63}H_{88}N_{12}O_{16}$	24.2	*N*-Methyl-dactinomycin[3]	-	-	-	-	+	-	50, 115-120

19.56	**1255.6405**	**[M+H]$^+$**	1255.6363	$C_{62}H_{86}N_{12}O_{16}$	3.3	*/**/***Actinomycin D (*Streptomyces* sp.); *Aurantin II (*Streptomyces aurantiacus*)	-	-	-	-	+	-	114-119
19.67	**1277.6223**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
19.91	**283.2763**	**[M+H]$^+$**	283.2749	$C_{17}H_{34}N_2O$	4.8	no hit in antibase/DNP/Reaxys	-	+	-	-	-	-	
20.09	**1291.6429**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
20.14	**1291.6298**	**[M+Na]$^+$**	n.d.	Multiple choices	n.d.	n.d.	-	-	-	-	+	-	
20.79	**317.2700**	**[M+H]$^+$**	317.2692	$C_{18}H_{36}O_4$	2.5	**/***2,3-Dihydroxypropylpentadecanoate, 1-*O*-(13-methyltetradecanoyl)-sn-glycerol (sponge *Stelletta* sp.), */*** Aggreceride A (*Streptomyces* strain om-3209; *(Streptomyces* sp.)	-	+	-	-	-	-	120, 121
21.35	**297.2905**	**[M+H]$^+$**	297.2906	$C_{18}H_{36}N_2O$	0.3	no hit in antibase/DNP/Reaxys	-	-	-	+	-	-	
22.64	**436.2484**	**[M+H]$^+$**	436.2479	$C_{22}H_{34}N_5O_2Cl$	1.2	no hit in antibase/DNP/Reaxys	-	+	-	-	-	-	
24.12	**434.3253**	**[M+H]$^+$**	434.3270	$C_{26}H_{43}NO_4$	4.1	no hit in antibase/DNP/Reaxys	-	-	+	-	-	-	
24.47	**226.0144**	**[M+H]$^+$**	226.0132	$C_7H_4N_5O_2Cl$	5.0	no hit in antibase/DNP/Reaxys	+	-	-	-	-	-	

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