

Supplementary Data

Table S1. Positions of 16S rRNA genes in the genomes of four *Amycolatopsis* strains, *Saccharomonospora viridis* DSM 43017^T and *Saccharopolyspora erythraea* NRRL 2338^T.

position1	position2	position3	position4	position5	position6	position7	Genome Size
		AORI_R016 1243922-1245382(+)	AORI_R045 6528673-6530133(-)	AORI_R041 5615356-5616816(-)	AORI_R022 1947768-1949228(+)		<i>A. orientalis</i> 8,948,591
		AJAP_r33495 7182628-7184132(-)	AJAP_r10595 2149092-2150596(+)	AJAP_r14255 2961980-2963484(+)	AJAP_r30385 6468674-6470178(-)		<i>A. japonica</i> 8,961,318
		AMED_R16 1295147-1296605(+)	AMED_R25 2307316-2308774(+)	AMED_R39 6685681-6687139(-)	AMED_R49 7616847-7618305(-)		<i>A. mediterranei</i> 10,236,715
	AMETH_R14 855318-856795(+)	AMETH_R17 1112871-1114348(+)		AMETH_R33 2784936-2786413(+)			<i>A. methanolica</i> 7,237,391
	Svir_05560 564225-565751(+)	Svir_07440 767227-768753(+)		Svir_25530 2748393-2749919(-)			<i>S. viridis</i> 4,308,349
SACE_8101 1402739-1404280(+)		SACE_8105 1777032-1778573(+)		SACE_8112 5880147-5881689(-)		SACE_8116 7218077-7219618(-)	<i>S. erythraea</i> 8,212,805

+: Sense Strand; -: Antisense Strand; the sequence of AORI_R41 and AORI_45 are same; the sequence of AMED_R39 and AMED_R49 are same; the sequence of AJAP_r14255 and AJAP_r30385 are same.

Table S2. tRNA counts in the genomes of the four *Amycolatopsis* strains.

Code	number			
	<i>A. orientalis</i> HCCB10007	<i>A. mediterranei</i> U32	<i>A.methanolica</i> 239 ^T	<i>A. japonica</i> MG417-CF17 ^T
Ala tRNA	3	3	3	3
Arg tRNA	4	5	4	6
Asn tRNA	1	1	1	2
Asp tRNA	1	1	1	1
Cys tRNA	1	1	1	1
Gln tRNA	2	2	2	2
Glu tRNA	3	2	2	3
Gly tRNA	4	4	5	4
His tRNA	1	1	1	1
Ile tRNA	1	1	1	1
Leu tRNA	5	5	5	5
Lys tRNA	2	2	2	2
Met tRNA	4	4	4	4
Phe tRNA	1	1	1	1
Pro tRNA	3	3	3	3
Ser tRNA	4	4	4	5
Thr tRNA	3	3	3	3
Trp tRNA	1	1	1	1
Tyr tRNA	1	2	1	1
Val tRNA	5	5	5	5
SeC(p) tRNA	none	1	1	none
Pseudo	none	none	none	1
all	50	52	51	55

The differences are highlighted in red.

Table S3. Number of genes associated with the general COG functional categories

Code	<i>A. methanolica</i> 239 ^T		<i>A. mediterranei</i> U32		<i>A. orientalis</i> HCCB10007		<i>A. japonica</i> MG417-CF17 ^T		Description
	Value	%age	Value	%age	Value	%age	Value	%age	
Z	0	0	0	0	0	0	0	0	Cytoskeleton
Y	0	0	0	0	0	0	0	0	Nuclear structure
W	0	0	0	0	0	0	0	0	Extracellular structures
V	71	1.01	127	1.38	109	1.35	99	1.2	Defense mechanisms
U	20	0.29	32	0.35	24	0.3	19	0.23	Intracellular trafficking and secretion
T	199	2.82	402	4.36	325	4.01	202	2.44	Signal transduction mechanisms
S	368	5.21	465	5.04	361	4.45	275	3.32	Function unknown
R	615	8.7	804	8.72	557	6.86	502	6.06	General function prediction only
Q	266	3.77	254	2.76	206	2.54	213	2.57	Secondary metabolites biosynthesis, transport and catabolism
P	176	2.49	200	2.17	189	2.33	160	1.93	Inorganic ion transport and metabolism
O	133	1.89	172	1.87	134	1.66	123	1.49	Posttranslational modification, protein turnover, chaperones
N	5	0.08	11	0.12	5	0.07	2	0.03	Cell motility and secretion
M	180	2.55	279	3.03	194	2.39	152	1.84	Cell envelope biogenesis, outer membrane
L	156	2.21	195	2.12	141	1.74	129	1.56	DNA replication, recombination and repair
K	595	8.42	832	9.02	687	8.46	370	4.46	Transcription
J	186	2.63	193	2.1	192	2.37	172	2.08	Translation, ribosomal structure and biogenesis
I	300	4.25	429	4.65	308	3.8	216	2.61	Lipid metabolism
H	201	2.85	285	3.09	187	2.31	183	2.21	Coenzyme metabolism
G	332	4.7	560	6.07	324	3.99	258	3.11	Carbohydrate transport and metabolism
F	108	1.53	119	1.29	94	1.16	95	1.15	Nucleotide transport and metabolism
E	447	6.32	500	5.42	418	5.15	421	5.08	Amino acid transport and metabolism
D	36	0.51	34	0.37	27	0.34	21	0.26	Cell division and chromosome partitioning
C	434	6.14	437	4.74	307	3.79	289	3.49	Energy production and conversion
B	1	0.02	1	0.02	1	0.02	1	0.02	Chromatin structure and dynamics
A	1	0.02	1	0.02	4	0.05	1	0.02	RNA processing and modification
-	2244	31.72	2896	31.39	3327	40.97	4395	52.97	Not in COGs

The Cog annotation was built by BLAST against the Clusters of Orthologous Groups (COGs) Database:

<http://www.ncbi.nlm.nih.gov/COG/>.

Table S4. The distribution of the conserved gene AMETH_3452 in representatives of the class *Actinobacteria* with circular genomes.

Ratio	length	strain	strains_Genus	subclass	order	suborder	family
0.167	4830181	<i>Ilumatobacter coccineus</i> YM16-304 ^T	<i>Ilumatobacter</i>	Acidimicrobidae	Acidimicrobiales	Acidimicrobineae	Acidimicrobiaceae
0.56	1986154	<i>Arcanobacterium haemolyticum</i> DSM 20595 ^T	<i>Arcanobacterium</i>	Actinobacteridae	Actinomycetales	Actinomycineae	Actinomycetaceae
0.429	4669183	<i>Beutenbergia cavernae</i> DSM 12333 ^T	<i>Beutenbergia</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Beutenbergiaceae
0.784	10467782	<i>Catenulispora acidiphila</i> DSM 44928 ^T	<i>Catenulispora</i>	Actinobacteridae	Actinomycetales	Catenulisporineae	Catenulisporaceae
0.599	4123179	<i>Cellulomonas flavigena</i> DSM 20109 ^T	<i>Cellulomonas</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Cellulomonadaceae
0.612	4266344	<i>Cellulomonas fimi</i> ATCC 484 ^T	<i>Cellulomonas</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Cellulomonadaceae
0.455	2446804	<i>Corynebacterium kroppenstedtii</i> DSM 44385 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.464	2462499	<i>Corynebacterium jeikeium</i> K411	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.478	2751233	<i>Corynebacterium terpenotabidum</i> Y-11 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.483	3433007	<i>Corynebacterium variabile</i> DSM 44702	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.486	2606374	<i>Corynebacterium ulcerans</i> BR-AD22	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.49	3309401	<i>Corynebacterium glutamicum</i> ATCC 13032 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.49	3282708	<i>Corynebacterium glutamicum</i> ATCC 13032 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.493	2601311	<i>Corynebacterium resistens</i> DSM 45100 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.494	2790189	<i>Corynebacterium aurimucosum</i> ATCC 700975	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.495	3309400	<i>Corynebacterium glutamicum</i> ATCC 13032 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.498	2314404	<i>Corynebacterium pseudotuberculosis</i> 258	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.498	2320595	<i>Corynebacterium pseudotuberculosis</i> CIP 52.97	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.499	2335113	<i>Corynebacterium pseudotuberculosis</i> 1002	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.499	2310415	<i>Corynebacterium pseudotuberculosis</i> 316	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.499	2293464	<i>Corynebacterium pseudotuberculosis</i> Cp162	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.499	2335323	<i>Corynebacterium pseudotuberculosis</i> PAT10	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.5	2279118	<i>Corynebacterium pseudotuberculosis</i> 1/06-A	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.5	2337628	<i>Corynebacterium pseudotuberculosis</i> 267	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.5	2337938	<i>Corynebacterium pseudotuberculosis</i> 3/99-5	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.5	2337606	<i>Corynebacterium pseudotuberculosis</i> 42/02-A	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.5	2337913	<i>Corynebacterium pseudotuberculosis</i> FRC41	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.5	2337730	<i>Corynebacterium pseudotuberculosis</i> I19	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.5	2337657	<i>Corynebacterium pseudotuberculosis</i> P54B96	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae

0.502	2328208	<i>Corynebacterium pseudotuberculosis</i> C231	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.503	2297010	<i>Corynebacterium pseudotuberculosis</i> 31	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.505	2478364	<i>Corynebacterium diphtheriae</i> HC03	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.505	2502095	<i>Corynebacterium ulcerans</i> 809	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.507	2484332	<i>Corynebacterium diphtheriae</i> HC04	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.511	2433326	<i>Corynebacterium diphtheriae</i> CDCE 8392	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.511	2468612	<i>Corynebacterium diphtheriae</i> HC02	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.513	2395441	<i>Corynebacterium diphtheriae</i> VA01	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.513	2369219	<i>Corynebacterium urealyticum</i> DSM 7109 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.516	2530683	<i>Corynebacterium diphtheriae</i> PW8	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.517	2488635	<i>Corynebacterium diphtheriae</i> NCTC 13129	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.517	2316065	<i>Corynebacterium urealyticum</i> DSM 7111	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.519	2535346	<i>Corynebacterium diphtheriae</i> 31A	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.519	2485519	<i>Corynebacterium diphtheriae</i> BH8	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.519	3135752	<i>Corynebacterium halotolerans</i> YIM 70093 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.52	2426551	<i>Corynebacterium diphtheriae</i> 241	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.52	2427149	<i>Corynebacterium diphtheriae</i> HC01	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.522	2579188	<i>Corynebacterium ulcerans</i> 0102	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.523	2499189	<i>Corynebacterium diphtheriae</i> C7 beta	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.524	3079253	<i>Corynebacterium glutamicum</i> MB001	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.525	2449071	<i>Corynebacterium diphtheriae</i> INCA 402	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.532	2839551	<i>Corynebacterium callunae</i> DSM 20147 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.533	3314179	<i>Corynebacterium glutamicum</i> R	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.537	2787574	<i>Corynebacterium maris</i> DSM 45190 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.542	3350620	<i>Corynebacterium glutamicum</i> SCgG1	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.542	3350619	<i>Corynebacterium glutamicum</i> SCgG2	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.555	3147090	<i>Corynebacterium efficiens</i> YS-314 ^T	<i>Corynebacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Corynebacteriaceae
0.536	3614992	<i>Brachybacterium faecium</i> DSM 4810 ^T	<i>Brachybacterium</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Dermabacteraceae
0.43	2785024	<i>Kytococcus sedentarius</i> DSM 20547 ^T	<i>Kytococcus</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Dermacoccaceae
0.3	5433628	<i>Frankia</i> sp. CcI3	<i>Frankia</i>	Actinobacteridae	Actinomycetales	Frankineae	Frankiaceae
0.31	7497934	<i>Frankia alni</i> ACN14a	<i>Frankia</i>	Actinobacteridae	Actinomycetales	Frankineae	Frankiaceae
0.323	8815781	<i>Frankia</i> sp. EuI1c	<i>Frankia</i>	Actinobacteridae	Actinomycetales	Frankineae	Frankiaceae
0.689	8982042	<i>Frankia</i> sp. EAN1pec	<i>Frankia</i>	Actinobacteridae	Actinomycetales	Frankineae	Frankiaceae

0.953	4875340	<i>Blastococcus saxobsidens</i> DD2	<i>Blastococcus</i>	Actinobacteridae	Actinomycetales	Frankineae	Geodermatophilaceae
0.971	5575517	<i>Modestobacter marinus</i>	<i>Modestobacter</i>	Actinobacteridae	Actinomycetales	Frankineae	Geodermatophilaceae
0.972	5322497	<i>Geodermatophilus obscurus</i> DSM 43160 ^T	<i>Geodermatophilus</i>	Actinobacteridae	Actinomycetales	Frankineae	Geodermatophilaceae
0.681	6841557	<i>Stackebrandtia nassauensis</i> DSM 44728 ^T	<i>Stackebrandtia</i>	Actinobacteridae	Actinomycetales	Glycomycineae	Glycomycetaceae
0.596	4024382	<i>Intrasporangium calvum</i> DSM 43043 ^T	<i>Intrasporangium</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Intrasporangiaceae
0.607	2749646	<i>Jonesia denitrificans</i> DSM 20603 ^T	<i>Jonesia</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Jonesiaceae
0.321	2584158	<i>Leifsonia xyli</i> subsp. <i>xyli</i> str. CTCB07	<i>Leifsonia</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Microbacteriaceae
0.432	3258645	<i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i>	<i>Clavibacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Microbacteriaceae
0.532	2686418	<i>Leifsonia xyli</i> subsp. <i>cynodontis</i> DSM 46306 ^T	<i>Leifsonia</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Microbacteriaceae
0.643	3063596	<i>Clavibacter michiganensis nebraskensis</i> NCPPB 2581 ^T	<i>Clavibacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Microbacteriaceae
0.649	3297891	<i>Clavibacter michiganensis</i> NCPPB 382	<i>Clavibacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Microbacteriaceae
0.497	4250414	<i>Arthrobacter phenanthrenivorans</i> Sphe3 ^T	<i>Arthrobacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.498	3859257	<i>Arthrobacter arilaitensis</i> Re117 ^T	<i>Arthrobacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.507	4395537	<i>Arthrobacter chlorophenicus</i> A6 ^T	<i>Arthrobacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.534	3155250	<i>Renibacterium salmoninarum</i> ATCC 33209 ^T	<i>Renibacterium</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.535	2501097	<i>Micrococcus luteus</i> NCTC 2665 ^T	<i>Micrococcus</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.536	4597686	<i>Arthrobacter aurescens</i> TC1	<i>Arthrobacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.536	4698945	<i>Arthrobacter</i> sp. FB24	<i>Arthrobacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.542	2697540	<i>Kocuria rhizophila</i> DC2201	<i>Kocuria</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.558	4736495	<i>Arthrobacter nitroguajacolicus</i> Rue61a	<i>Arthrobacter</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Micrococcaceae
0.207	8773466	<i>Actinoplanes missouriensis</i> 431	<i>Actinoplanes</i>	Actinobacteridae	Actinomycetales	Micromonosporineae	Micromonosporaceae
0.222	9239851	<i>Actinoplanes</i> SE50/110	<i>Actinoplanes</i>	Actinobacteridae	Actinomycetales	Micromonosporineae	Micromonosporaceae
0.266	9228054	<i>Actinoplanes</i> N902-109	<i>Actinoplanes</i>	Actinobacteridae	Actinomycetales	Micromonosporineae	Micromonosporaceae
0.617	6962533	<i>Micromonospora</i> sp. L5	<i>Micromonospora</i>	Actinobacteridae	Actinomycetales	Micromonosporineae	Micromonosporaceae
0.647	5786361	<i>Salinispora arenicola</i> CNS-205	<i>Salinispora</i>	Actinobacteridae	Actinomycetales	Micromonosporineae	Micromonosporaceae
0.667	5183331	<i>Salinispora tropica</i> CNB-440 ^T	<i>Salinispora</i>	Actinobacteridae	Actinomycetales	Micromonosporineae	Micromonosporaceae
0.684	7025559	<i>Micromonospora aurantiaca</i> ATCC 27029 ^T	<i>Micromonospora</i>	Actinobacteridae	Actinomycetales	Micromonosporineae	Micromonosporaceae
0.399	4829781	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> K-10	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.41	6048425	<i>Mycobacterium</i> sp. JLS	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.421	6464916	<i>Mycobacterium smegmatis</i> JS623	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.422	4738809	<i>Amycolicococcus subflavus</i> DQS3-9A1 ^T	<i>Amycolicococcus</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.43	6491865	<i>Mycobacterium vanbaalenii</i> PYR-1 ^T	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.434	5705448	<i>Mycobacterium</i> sp. MCS	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae

0.435	5737227	Mycobacterium sp. KMS	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.437	5583723	Mycobacterium chubuense NBB4	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.443	6988208	Mycobacterium smegmatis str. MC2 155	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.443	6988209	Mycobacterium smegmatis str. MC2 155	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.445	5631606	Mycobacterium ulcerans Agy99	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.445	5521023	Mycobacterium yongonense 05-1390 ^T	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.453	5501090	Mycobacterium intracellulare MOTT-64	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.457	5589007	Mycobacterium indicus pranii MTCC 9506	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.46	5475491	Mycobacterium avium 104	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.468	5613626	Mycobacterium sp. MOTT36Y	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.473	4829424	Mycobacterium avium subsp. paratuberculosis MAP4	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.483	5402402	Mycobacterium intracellulare ATCC 13950 ^T	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.484	5409696	Mycobacterium intracellulare MOTT-02	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.486	4398250	Mycobacterium tuberculosis KZN 1435	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.486	4394985	Mycobacterium tuberculosis KZN 4207	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.486	4399120	Mycobacterium tuberculosis KZN 605	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.488	6208955	Mycobacterium liflandii 128FXT	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.511	4376711	Mycobacterium bovis BCG str. Korea 1168P	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.516	4350386	Mycobacterium bovis BCG str. Mexico	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.516	4405981	Mycobacterium tuberculosis CCDC5180	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.517	4371711	Mycobacterium bovis BCG str. Tokyo 172	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.517	4398812	Mycobacterium tuberculosis CCDC5079	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.518	4374522	Mycobacterium bovis BCG str. Pasteur 1173P2	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.518	4411128	Mycobacterium tuberculosis str.Beijing/NITR203	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.518	4392876	Mycobacterium tuberculosis CAS/NITR204	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.518	4414325	Mycobacterium tuberculosis CCDC5079	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.518	4411708	Mycobacterium tuberculosis H37Rv ^T	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.518	4411532	Mycobacterium tuberculosis H37Rv ^T	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.518	4406587	Mycobacterium tuberculosis RGTB423	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.518	4418088	Mycobacterium tuberculosis UT205	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4389314	Mycobacterium africanum GM041182	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4421197	Mycobacterium tuberculosis 7199-99	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4403837	Mycobacterium tuberculosis CDC1551	Mycobacterium	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae

0.519	4398525	<i>Mycobacterium tuberculosis</i> CTRI-2	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4391174	<i>Mycobacterium tuberculosis</i> EAI5	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4390306	<i>Mycobacterium tuberculosis</i> EAI5/NITR206	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4392353	<i>Mycobacterium tuberculosis</i> str. Erdman = ATCC 35801	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4424435	<i>Mycobacterium tuberculosis</i> F11	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4419977	<i>Mycobacterium tuberculosis</i> H37Ra	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4408224	<i>Mycobacterium tuberculosis</i> str. Haarlem	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.519	4404786	<i>Mycobacterium tuberculosis</i> str. Haarlem/NITR202	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.52	4432426	<i>Mycobacterium canettii</i> CIPT 140060008	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.52	4380119	<i>Mycobacterium tuberculosis</i> RGTB327	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.521	4482059	<i>Mycobacterium canettii</i> CIPT 140010059	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.522	4345492	<i>Mycobacterium bovis</i> AF2122/97	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.524	4524466	<i>Mycobacterium canettii</i> CIPT 140070017	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.526	4420197	<i>Mycobacterium canettii</i> CIPT 140070008	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.53	4525948	<i>Mycobacterium canettii</i> CIPT 140070010	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.548	6636827	<i>Mycobacterium marinum</i> M	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.552	4643668	<i>Mycobacterium</i> sp. JDM601	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.57	5000473	<i>Mycobacterium abscessus</i> subsp. <i>bolletii</i> 50594	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.574	5067172	<i>Mycobacterium abscessus</i>	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.583	5547747	<i>Mycobacterium gilvum</i> Spyr1	<i>Mycobacterium</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Mycobacteriaceae
0.421	6060298	<i>Nakamurella multipartita</i> DSM 44233 ^T	<i>Nakamurella</i>	Actinobacteridae	Actinomycetales	Frankineae	Nakamurellaceae
0.469	5669805	<i>Gordonia polyisoprenivorans</i> VH2	<i>Gordonia</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Nocardiaceae
0.472	5043170	<i>Rhodococcus equi</i> 103S	<i>Rhodococcus</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Nocardiaceae
0.474	6194645	<i>Nocardia cyriacigeorgica</i> GUH-2	<i>Nocardia</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Nocardiaceae
0.484	6281198	<i>Rhodococcus erythropolis</i> CCM2595	<i>Rhodococcus</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Nocardiaceae
0.495	5208602	<i>Gordonia bronchialis</i> DSM 43247 ^T	<i>Gordonia</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Nocardiaceae
0.509	6516310	<i>Rhodococcus erythropolis</i> PR4	<i>Rhodococcus</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Nocardiaceae
0.612	6021225	<i>Nocardia farcinica</i> IFM 10152	<i>Nocardia</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Nocardiaceae
0.666	9436348	<i>Nocardia brasiliensis</i> ATCC 700358	<i>Nocardia</i>	Actinobacteridae	Actinomycetales	Corynebacterineae	Nocardiaceae
0.333	7579488	<i>Kribbella flavida</i> DSM 17836 ^T	<i>Kribbella</i>	Actinobacteridae	Actinomycetales	Propionibacterineae	Nocardiodiaceae
0.408	4985871	<i>Nocardioides</i> sp. JS614	<i>Nocardioides</i>	Actinobacteridae	Actinomycetales	Propionibacterineae	Nocardiodiaceae
0.15	5767958	<i>Nocardioopsis dassonvillei</i> subsp. <i>dassonvillei</i> DSM 43111 ^T	<i>Nocardioopsis</i>	Actinobacteridae	Actinomycetales	Streptosporangineae	Nocardioipsaceae
0.632	3742776	<i>Xylanimonas cellulositytica</i> DSM 15894 ^T	<i>Xylanimonas</i>	Actinobacteridae	Actinomycetales	Micrococcineae	Promicromonosporaceae

0.647	3307740	Isoptricola variabilis 225 Propionibacterium freudenreichii subsp.shermanii	Isoptricola	Actinobacteridae	Actinomycetales	Micrococcineae	Promicromonosporaceae
0.379	2616384	CIRM-BIA1	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.384	2522885	Propionibacterium acnes TypeIA2 P.acn17	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.386	2489623	Propionibacterium acnes TypeIA2 P.acn33	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.388	2498766	Propionibacterium acnes TypeIA2 P.acn31	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.389	2494578	Propionibacterium acnes 266	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.389	2560282	Propionibacterium acnes 6609	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.389	2560265	Propionibacterium acnes KPA171202	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.396	2519002	Propionibacterium acnes C1	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.399	2495334	Propionibacterium acnes SK137	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.417	3656170	Propionibacterium acidipropionici ATCC 4875	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.544	3449360	Propionibacterium propionicum F0230a	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.555	2494190	Propionibacterium acnes HL096PA1	Propionibacterium	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.649	5683123	Microlunatus phosphovorius NM-1 ^T	Microlunatus	Actinobacteridae	Actinomycetales	Propionibacterineae	Propionibacteriaceae
0.315	4189976	Thermobispora bispora DSM 43833 ^T	Thermobispora	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.467	8961318	Amycolatopsis japonica MG417-CF17 ^T	Amycolatopsis	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.478	8948591	Amycolatopsis orientalis HCCB10007	Amycolatopsis	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.484	8248144	Actinosynnema mirum DSM 43827 ^T	Actinosynnema	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.491	4308349	Saccharomonospora viridis DSM 43017 ^T	Saccharomonospora	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.495	7237391	Amycolatopsis methanolia 239 ^T	Amycolatopsis	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.5	9360653	Saccharothrix espanaensis DSM 44229 ^T	Saccharothrix	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.503	10236779	Amycolatopsis mediterranei S699	Amycolatopsis	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.503	10236715	Amycolatopsis mediterranei U32	Amycolatopsis	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.504	10246864	Amycolatopsis mediterranei RB	Amycolatopsis	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.504	10246920	Amycolatopsis mediterranei S699	Amycolatopsis	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.504	8212805	Saccharopolyspora erythraea NRRL 2338 ^T	Saccharopolyspora	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.506	7096571	Pseudonocardia dioxanivorans CB1190 ^T	Pseudonocardia	Actinobacteridae	Actinomycetales	Pseudonocardineae	Pseudonocardiaceae
0.355	4253413	Sanguibacter keddieii DSM 10542 ^T	Sanguibacter	Actinobacteridae	Actinomycetales	Micrococcineae	Sanguibacteraceae
0.522	3157527	Segniliparus rotundus DSM 44985 ^T	Segniliparus	Actinobacteridae	Actinomycetales	Corynebacterineae	Segniliparaceae
0.836	10657107	Streptomyces violaceusniger Tu 4113	Streptomyces	Actinobacteridae	Actinomycetales	Streptomycineae	Streptomycetaceae
0.666	5639016	Thermomonospora curvata DSM 43183 ^T	Thermomonospora	Actinobacteridae	Actinomycetales	Streptosporangineae	Thermomonosporaceae
0.497	4379918	Tsukamurella paurometabola DSM 20162 ^T	Tsukamurella	Actinobacteridae	Actinomycetales	Corynebacterineae	Tsukamurellaceae

The ratio represents the relative chromosomal positions of the genomes normalized to 0.0–1.0 with *oriC* located at the both ends. This conserved gene is located in the middle of the circular chromosomes (*i.e.*, close to the 0.5 locus) in the species belonging to mainly four families including *Pseudonocardiaceae* (13 genomes, red, except genus *Thermobispora*), *Corynebacteriaceae* (50 genomes, green), *Micrococcaceae* (9 genomes, blue) and *Mycobacteriaceae* (59 genomes, cyan).

All the genomes are available at <ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/>.

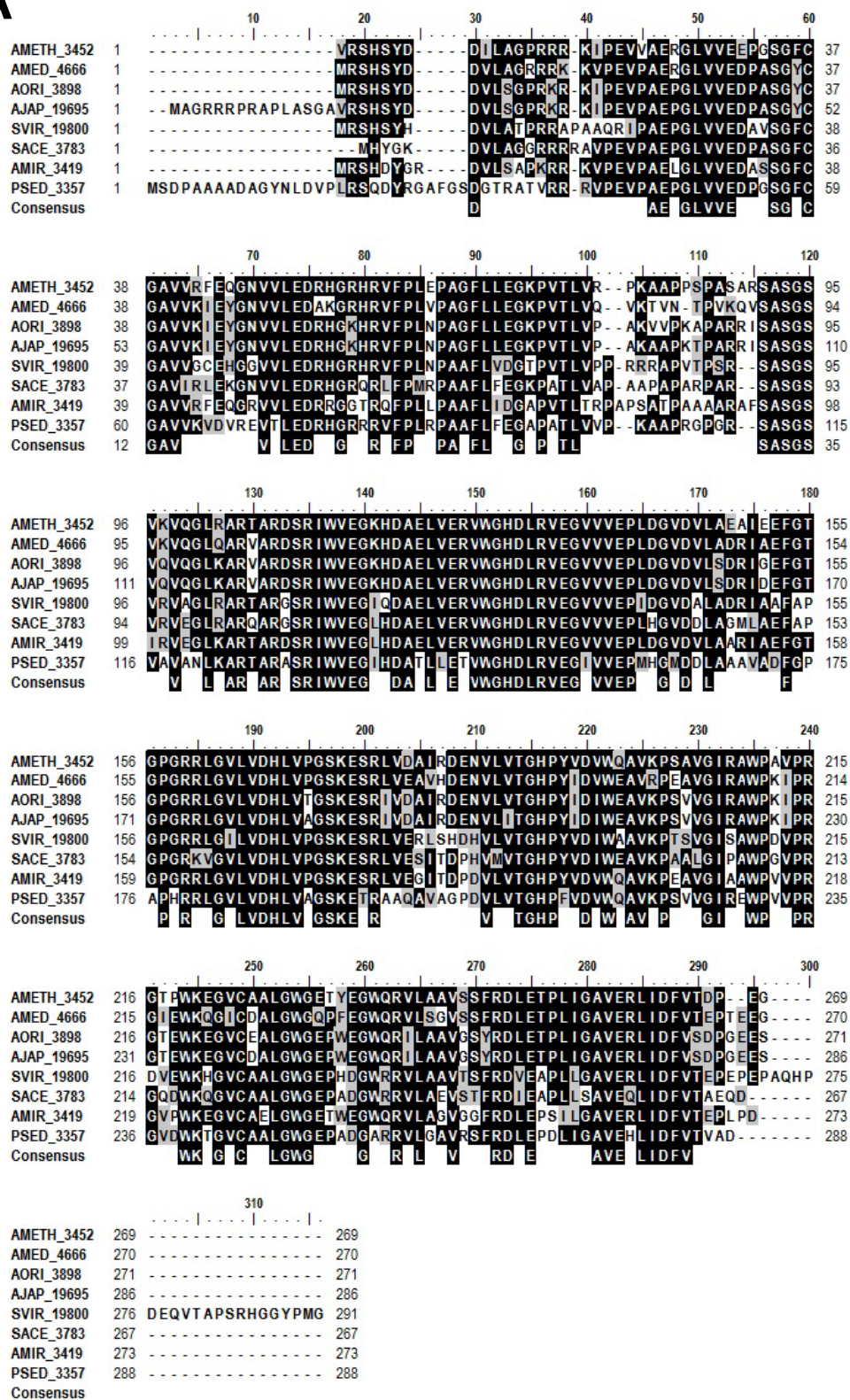
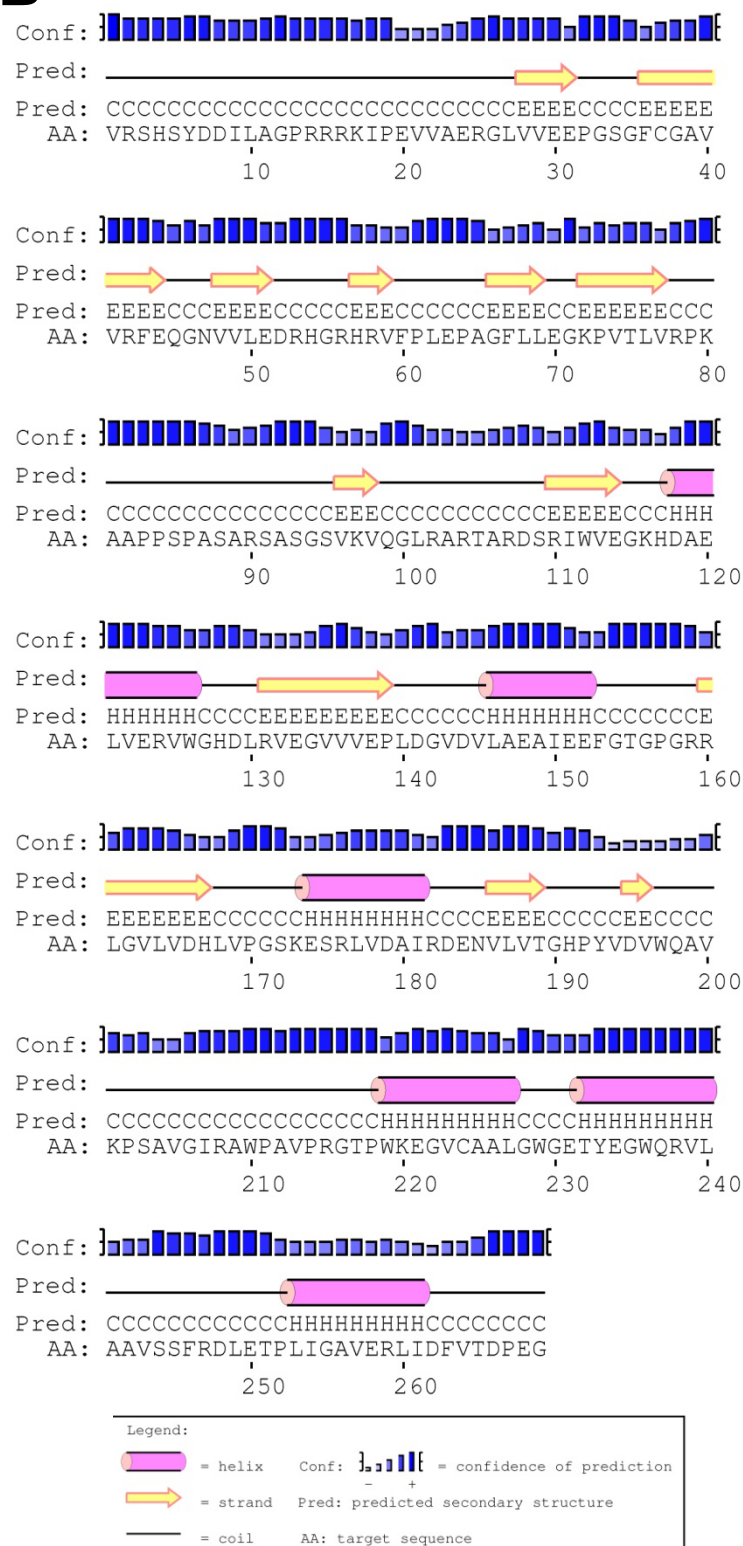
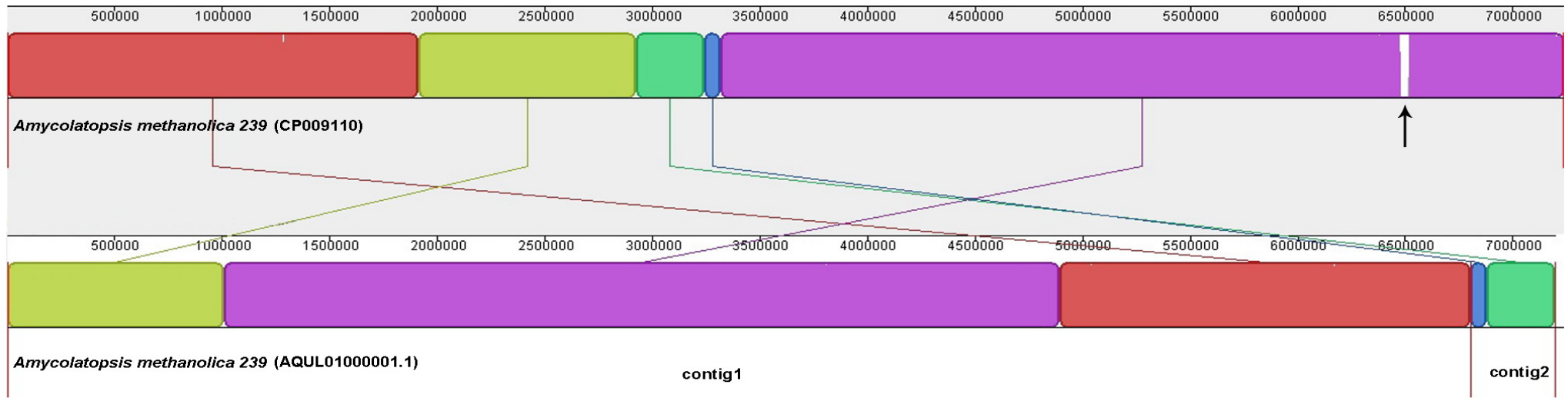
A**B**

Figure S1. A. The alignment of protein AMETH_3452 and its orthologs in *Pseudonocardiaceae*. B. The secondary structure prediction of the conserved CDS (AMETH_3452). The conserved protein is restricted to *Actinobacteria* after blast with NR database and all genome sequences and it belongs to pfam11296 by matching with NCBI's conserved domain database. In panel A, the hypothetical protein of 269 amino acid residues (AMETH_3452) and its orthologous protein from sequenced genomes belonging to the family *Pseudonocardiaceae* (AMED_4666, AORI_3898, AJAP_19695, SVIR_19800, SACE_3783, AMIR_3419, and PSED_3357) were aligned by Clustal X. In panel B, the secondary structure of conserved CDS in *A. methanolica* 239 was predicted by PSIPRED server (Buchan *et al.*, 2013). In addition, the protein was predicted cytoplasmic using PSLpred (Bhasin *et al.*, 2005), Psortb (Yu *et al.*, 2010) and SubLoc (Chen *et al.*, 2006), no transmembrane helices was found based on TMPred analysis (http://embnet.vital-it.ch/software/TMPRED_form.html) and no signal peptide based on SignalP-4.1 (Petersen *et al.*, 2011)

Reference:

- Bhasin, M., Garg, A., Raghava, G.P., 2005. PSLpred: prediction of subcellular localization of bacterial proteins. *Bioinformatics* 21, 2522-2524.
- Buchan, D.W., Minnici, F., Nugent, T.C., Bryson, K., Jones, D.T., 2013. Scalable web services for the PSIPRED Protein Analysis Workbench. *Nucleic Acids Res* 41, W349-357.
- Chen, H., Huang, N., Sun, Z., 2006. SubLoc: a server/client suite for protein subcellular location based on SOAP. *Bioinformatics* 22, 376-377.
- Petersen, T.N., Brunak, S., von Heijne, G., Nielsen, H., 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nature methods* 8, 785-786.
- Yu, N.Y., Wagner, J.R., Laird, M.R., Melli, G., Rey, S., Lo, R., Dao, P., Sahinalp, S.C., Ester, M., Foster, L.J., Brinkman, F.S., 2010. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics* 26, 1608-1615.

Figure S2. The differences between the sequences of the two versions (AQL00000000.1 and CP009110) of *A.methanolica* 239^T



Arrow represents a copy of the prophage which is a tandem repeat in Strain 239^T (CP009110).

Table S5. Genes related to the synthesis and transport of several compatible solutes in the AMS *A. methanolica* 239^T, *Amycolatopsis* sp. ATCC 39116 and *A. thermoflava* N1165^T versus ATS *A. taiwanensis* 0345M-7^T

AMS		ATS		gene	product
<i>A. methanolica</i>	<i>Amycolatopsis</i> sp. ATCC39116	<i>A. thermoflava</i>	<i>A. taiwanensis</i>		
ectoine/hydroxyectoine transporter					
AMETH_1367	Y	Y	Y	<i>hisJ</i>	ectoine/hydroxyectoine ABC transporter solute-binding protein
AMETH_1368	Y	Y	Y	<i>hisM</i>	ectoine/hydroxyectoine ABC transporter permease EhuC
AMETH_1369	Y	Y	Y	<i>hisM</i>	ectoine/hydroxyectoine ABC transporter, permease protein
AMETH_1370	Y	Y	Y	<i>ehuA</i>	ectoine/hydroxyectoine ABC transporter, ATP-binding protein
AMETH_5002	Y	Y	Y	<i>hisM</i>	ectoine/hydroxyectoine ABC transporter, permease EhuD
	Y	Y	-		nitrate/sulfonate/bicarbonate ABC transporter ATPase/
AMETH_2747				<i>ssuB</i>	ectoine/hydroxyectoine ABC transporter
Ectoine/hydroxyectoine synthesis					
AMETH_6462	Y	Y	Y	<i>ectD</i>	ectoine hydroxylase
AMETH_6463	Y	Y	Y	<i>ectC</i>	L-ectoine synthase
AMETH_6464	Y	Y	Y	<i>ectB</i>	diaminobutyrate/2-oxoglutarate aminotransferase
AMETH_6465	Y	Y	Y	<i>ectA</i>	L-2,4-diaminobutyric acid acetyltransferase
glycine betaine transporter					
AMETH_0110	Y	Y	Y	<i>opuBB</i>	ABC quaternary amine transporter
AMETH_0111	Y	Y	Y	<i>opuBA</i>	proline/glycine/betaine ABC transporter ATPase
AMETH_5762	Y	Y	Y	<i>opuBA</i>	D-methionine ABC transporter, ATP-binding protein
AMETH_0112	Y	Y	Y	<i>opuBB</i>	ABC-type transporter, integral membrane subunit
AMETH_0406	Y	Y	Y	<i>opuBB</i>	proline/glycine/betaine ABC transporter permease
AMETH_0407	Y	Y	Y	<i>opuBB</i>	proline/glycine/betaine ABC transporter permease
AMETH_6704	Y	Y	Y	<i>proP</i>	L-Proline/Glycine betaine transporter ProP
AMETH_0113	Y	Y	Y	<i>opuC</i>	glycine/betaine ABC transporter periplasmic protein
AMETH_0408	Y	Y	Y	<i>opuC</i>	glycine/betaine ABC transporter periplasmic protein
AMETH_5526	Y	Y	-	<i>proP</i>	proline/betaine transporter
AMETH_6721	Y	Y	-	<i>proP</i>	proline/betaine transporter
AMETH_3300	Y	Y	-	<i>betP</i>	choline/carnitine/betaine transporter
AMETH_4479	Y	Y	-	<i>betP</i>	choline/carnitine/betaine transporter
glycine betaine metabolism					
AMETH_4499	Y	Y	Y	<i>betA</i>	choline dehydrogenase
AMETH_4960	Y	Y	Y	<i>putA</i>	betaine aldehyde dehydrogenase
AMETH_4497	Y	Y	Y	<i>putA</i>	betaine aldehyde dehydrogenase
AMETH_0838	Y	Y	Y	<i>putA</i>	betaine aldehyde dehydrogenase
AMETH_5372	Y	Y	-	<i>putA</i>	betaine aldehyde dehydrogenase
AMETH_0085	Y	Y	-	<i>putA</i>	betaine aldehyde dehydrogenase
AMETH_5920	Y	Y	-	<i>putA</i>	betaine aldehyde dehydrogenase
AMETH_5579	Y	Y	-	<i>putA</i>	betaine aldehyde dehydrogenase
AMETH_2645	Y	Y	-	<i>putA</i>	betaine aldehyde dehydrogenase
trehalose synthesis					
AMETH_2758	Y	Y	-	<i>treS</i>	maltose alpha-D-glucosyltransferase
AMETH_2761	Y	Y	-	<i>treZ</i>	malto-oligosyltrehalose trehalohydrolase
AMETH_2762	Y	Y	-	<i>treY</i>	maltooligosyl trehalose synthase
AMETH_2559	Y	Y	Y	<i>terP</i>	trehalose phosphorylase
AMETH_6507	Y	Y	Y	<i>otsA</i>	alpha, alpha-trehalose-phosphate synthase
AMETH_6508	Y	Y	Y	<i>otsB</i>	trehalose-phosphatase

Figure S3. Neighbour-joining phylogenetic tree based on the fifteen 16S rDNA sequences of *A. orientalis*, *A. mediterranei*, *A. methanolica* and *A. japonica* with 16S rRNA genes from other 55 *Amycolatopsis* species.

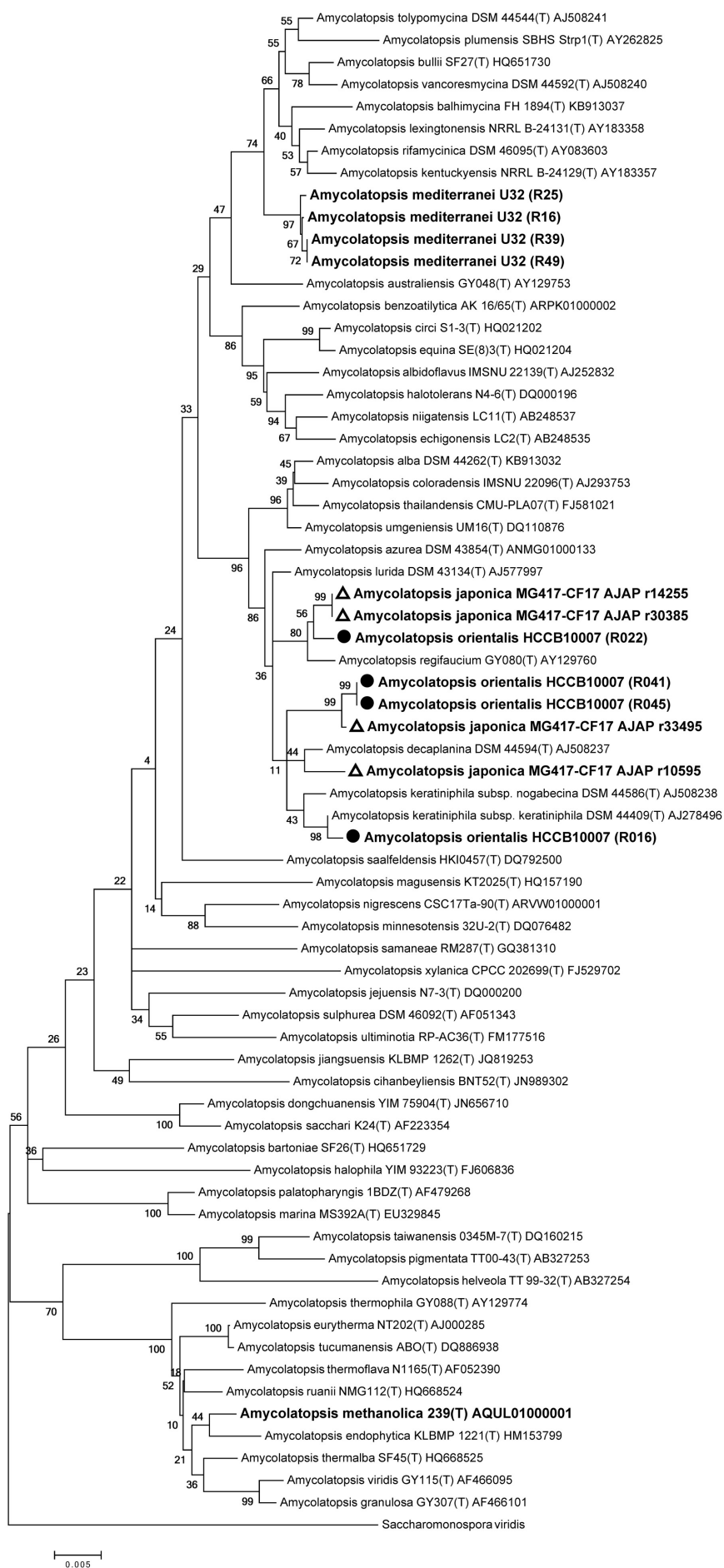
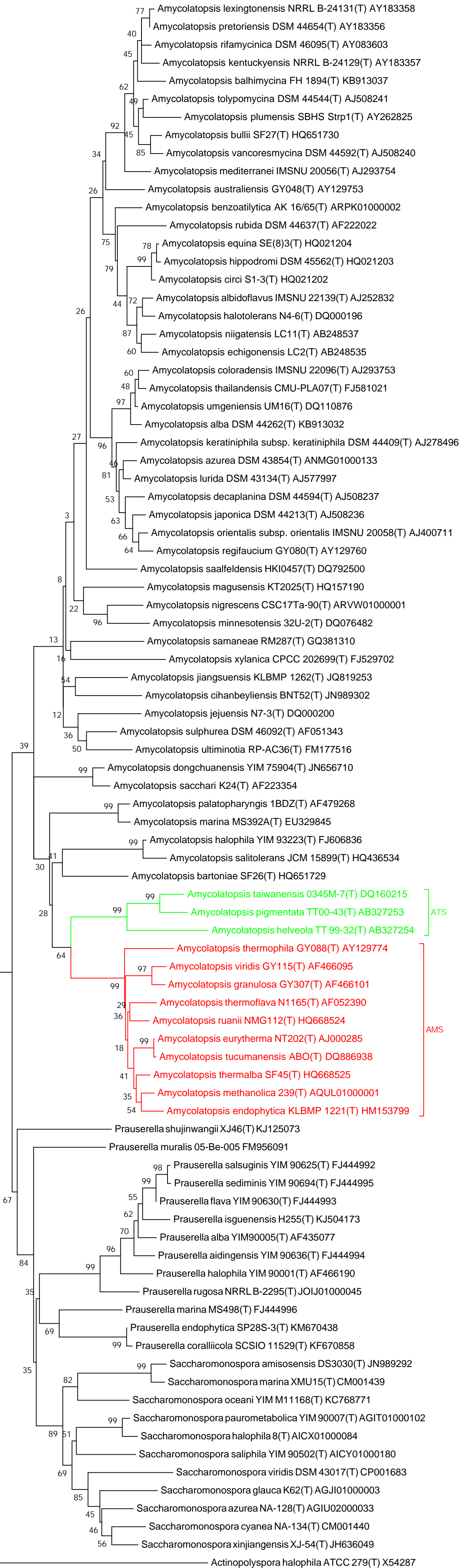


Figure S4. phylogenetic tree for 62 type strains of the genus *Amycolatopsis*, 11 type strains of the genus *Saccharomonospora* and 13 type strains of the genus *Prauserella* based on 16S rDNA sequences.



0.01

Figure S5. The phylogenetic trees for 18 type strains of the genus *Amycolatopsis* and *Saccharomonospora viridis*, *Saccharopolyspora erythraea* and *Actinosynnema mirum*. These trees were constructed using the neighbour-joining method based on the *gyrB* (A), 16S-*gyrB* (B), *recN* (C) and 16S-*recN* (D) gene sequences. The percentage bootstrap values of 1,000 replications are shown at each node. *Pseudonocardia dioxanivorans* CB1190^T was used as an outgroup. Groups A–F and J indicate the conserved clusters of strains.

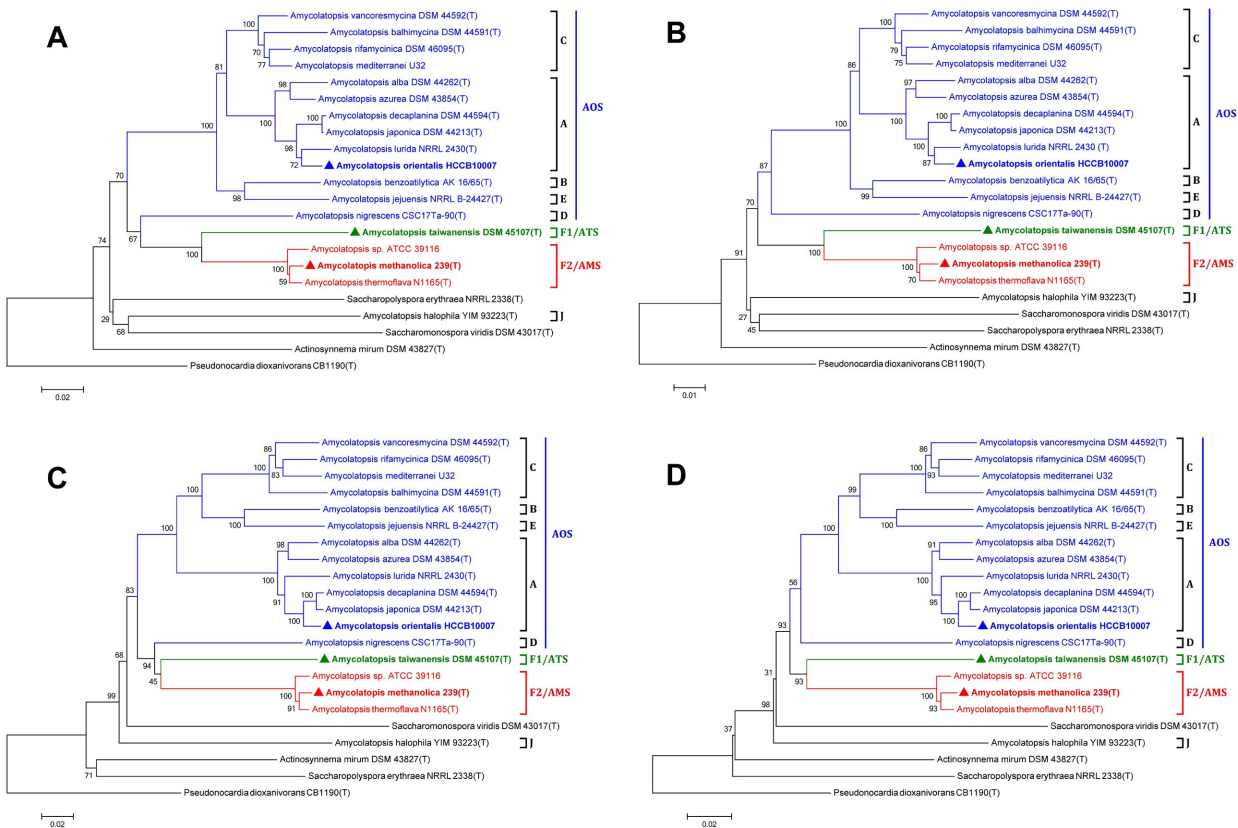


Figure S6. Phylogenetic tree of 13 AOS, 1 ATS and 3 AMS and Group J strains based on the head-tail linked 95 core protein sequences. All the sequences were obtained from the NCBI database. Subclades of the A-F and J grouping and AOS/ATS/AMS classification are shown. Red branches represent AMS and blue branches are AOS. Numbers at the nodes are the bootstrap values based on 1,000 replicates. Bootstrap values of above 60 % are shown at branch points. The tree was generated by using the maximum-likelihood method. The scale bar indicates 0.05 amino acid substitutions per amino acid position.

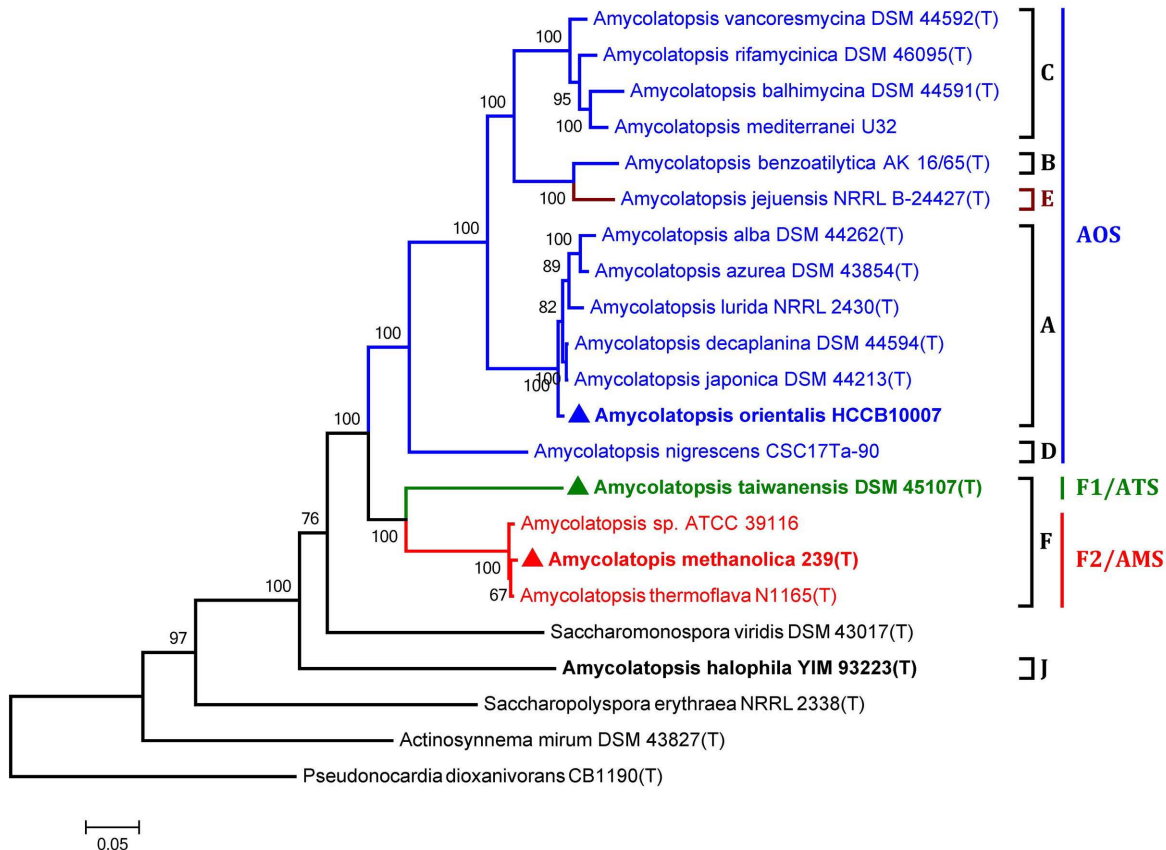


Table S6. 95 ortholog proteins in *A. methanolica* 239^T, *A. mediterranei* U32, *A. orientalis* HCCB10007 and *A. japonica* MG417-CF17^T used to generate phylogenetic trees.

<i>A.methanolica</i> 239 ^T	<i>A.mediterranei</i> U32	<i>A.orientalis</i> HCCB10007	<i>A.japonica</i> MG417-CF17 ^T	COG	category	Product
AMETH_0001	AMED_0001	AORI_0001	AJAP_00005	COG0593L	L	chromosomal replication initiator protein DnaA
AMETH_0007	AMED_0004	AORI_0004	AJAP_00025	COG1195L	L	DNA replication and repair protein RecF
AMETH_0010	AMED_0007	AORI_0007	AJAP_00040	COG0188L	L	DNA gyrase subunit A
AMETH_0624	AMED_0594	AORI_0619	AJAP_36525	COG0250K	K	transcription antiterminator NusG
AMETH_0625	AMED_0595	AORI_0620	AJAP_36520	COG0080J	J	50S ribosomal protein L11
AMETH_0626	AMED_0596	AORI_0621	AJAP_36515	COG0081J	J	50S ribosomal protein L1
AMETH_0628	AMED_0599	AORI_0624	AJAP_36500	COG0244J	J	50S ribosomal protein L10
AMETH_0629	AMED_0600	AORI_0625	AJAP_36495	COG0222J	J	50S ribosomal protein L7/L12
AMETH_0647	AMED_0672	AORI_0652	AJAP_36355	COG0048J	J	30S ribosomal protein S12
AMETH_0648	AMED_0673	AORI_0653	AJAP_36350	COG0049J	J	30S ribosomal protein S7
AMETH_0652	AMED_0677	AORI_0657	AJAP_36330	COG0087J	J	50S ribosomal protein L3
AMETH_0653	AMED_0678	AORI_0658	AJAP_36325	COG0088J	J	50S ribosomal protein L4
AMETH_0654	AMED_0679	AORI_0659	AJAP_36320	COG0089J	J	50S ribosomal protein L23
AMETH_0655	AMED_0680	AORI_0660	AJAP_36315	COG0090J	J	50S ribosomal protein L2
AMETH_0656	AMED_0681	AORI_0661	AJAP_36310	COG0185J	J	30S ribosomal protein S19
AMETH_0657	AMED_0682	AORI_0662	AJAP_36305	COG0091J	J	50S ribosomal protein L22
AMETH_0658	AMED_0683	AORI_0663	AJAP_36300	COG0092J	J	30S ribosomal protein S3
AMETH_0659	AMED_0684	AORI_0664	AJAP_36295	COG0197J	J	50S ribosomal protein L16
AMETH_0660	AMED_0685	AORI_0665	AJAP_36290	COG0255J	J	50S ribosomal protein L29
AMETH_0661	AMED_0686	AORI_0666	AJAP_36285	COG0186J	J	30S ribosomal protein S17
AMETH_0662	AMED_0687	AORI_0667	AJAP_36280	COG0093J	J	50S ribosomal protein L14
AMETH_0663	AMED_0688	AORI_0668	AJAP_36275	COG0198J	J	50S ribosomal protein L24
AMETH_0664	AMED_0689	AORI_0669	AJAP_36270	COG0094J	J	50S ribosomal protein L5
AMETH_0666	AMED_0691	AORI_0671	AJAP_36260	COG0096J	J	30S ribosomal protein S8
AMETH_0667	AMED_0692	AORI_0672	AJAP_36255	COG0097J	J	50S ribosomal protein L6
AMETH_0668	AMED_0693	AORI_0673	AJAP_36250	COG0256J	J	50S ribosomal protein L18
AMETH_0669	AMED_0694	AORI_0674	AJAP_36245	COG0098J	J	30S ribosomal protein S5
AMETH_0671	AMED_0696	AORI_0676	AJAP_36235	COG0200J	J	50S ribosomal protein L15
AMETH_0679	AMED_0704	AORI_0684	AJAP_36190	COG0099J	J	30S ribosomal protein S13
AMETH_0681	AMED_0706	AORI_0686	AJAP_36180	COG0100J	J	30S ribosomal protein S11
AMETH_0684	AMED_0709	AORI_0689	AJAP_36165	COG0203J	J	50S ribosomal protein L17
AMETH_0698	AMED_0726	AORI_0707	AJAP_36080	COG0102J	J	50S ribosomal protein L13
AMETH_0699	AMED_0727	AORI_0708	AJAP_36075	COG0103J	J	30S ribosomal protein S9
AMETH_0700	AMED_0728	AORI_0709	AJAP_36070	COG1109G	G	phosphoglucosaminemutase
AMETH_0727	AMED_0762	AORI_0751	AJAP_35915	COG0234O	O	chaperonin GroES
AMETH_0748	AMED_0782	AORI_0774	AJAP_35800	COG0519F	F	GMP synthase (glutamine-hydrolysing)
AMETH_0762	AMED_0798	AORI_0791	AJAP_35715	COG0210L	L	DNA helicase II/ATP-dependent DNA helicase
AMETH_0906	AMED_0993	AORI_0981	AJAP_34665	COG0041F	F	phosphoribosylaminoimidazole carboxylase catalytic subunit
AMETH_1139	AMED_1250	AORI_1247	AJAP_33335	COG1186J	J	peptide chain release factor RF-2
AMETH_1344	AMED_7790	AORI_6579	AJAP_06060	COG0216J	J	peptide chain release factor RF-1

AMETH_1355	AMED_7777	AORI_6562	AJAP_06145	COG0056C	C	F-type H ⁺ -transporting ATPase subunit alpha
AMETH_1357	AMED_7775	AORI_6560	AJAP_06155	COG0055C	C	F-type H ⁺ -transporting ATPase subunit beta
AMETH_1485	AMED_1669	AORI_6253	AJAP_08160	COG0272L	L	DNA ligase (NAD ⁺)
AMETH_1550	AMED_1753	AORI_6117	AJAP_08830	COG1200LK	LK	ATP-dependent DNA helicase RecG
AMETH_1565	AMED_1796	AORI_6081	AJAP_09030	COG0571K	K	ribonuclease III
AMETH_1622	AMED_1954	AORI_5924	AJAP_09830	COG0541U	U	signal recognition particle, subunit SRP54
AMETH_1626	AMED_1958	AORI_5920	AJAP_09850	COG0228J	J	30S ribosomal protein S16
AMETH_1628	AMED_1960	AORI_5918	AJAP_09860	COG0806J	J	16S rRNA processing protein RimM
AMETH_1630	AMED_1963	AORI_5916	AJAP_09870	COG0335J	J	50S ribosomal protein L19
AMETH_1640	AMED_1973	AORI_5906	AJAP_09920	COG0052J	J	30S ribosomal protein S2
AMETH_1641	AMED_1974	AORI_5905	AJAP_09925	COG0264J	J	elongation factor EF-Ts
AMETH_1643	AMED_1976	AORI_5903	AJAP_09935	COG0233J	J	ribosome recycling factor
AMETH_1732	AMED_6742	AORI_1963	AJAP_29700	COG0544O	O	trigger factor
AMETH_1778	AMED_6705	AORI_2002	AJAP_29510	COG0211J	J	50S ribosomal protein L27
AMETH_1779	AMED_6704	AORI_2003	AJAP_29505	COG0536R	R	GTP-binding protein Obg
AMETH_1792	AMED_6685	AORI_2021	AJAP_29425	COG0268J	J	30S ribosomal protein S20
AMETH_1796	AMED_6681	AORI_2025	AJAP_29405	COG0481M	M	GTP-binding protein LepA
AMETH_1928	AMED_6624	AORI_2078	AJAP_29125	COG0319R	R	metalloprotease
AMETH_1931	AMED_6621	AORI_2081	AJAP_29110	COG1159R	R	GTP-binding protein Era
AMETH_1934	AMED_6615	AORI_2088	AJAP_29075	COG1381L	L	DNA repair protein RecO
AMETH_1959	AMED_6587	AORI_2110	AJAP_28960	COG0358L	L	DNA primase
AMETH_2444	AMED_6340	AORI_2216	AJAP_27810	COG0195K	K	transcription elongation factor NusA
AMETH_2446	AMED_6338	AORI_2218	AJAP_27805	COG0532J	J	translation initiation factor IF-2
AMETH_2448	AMED_6336	AORI_2220	AJAP_27795	COG0858J	J	ribosome-binding factor A
AMETH_2459	AMED_6323	AORI_2230	AJAP_27740	COG0130J	J	tRNApseudouridine synthase B
AMETH_2463	AMED_6319	AORI_2234	AJAP_27720	COG0184J	J	30S ribosomal protein S15
AMETH_2496	AMED_6283	AORI_2265	AJAP_27535	COG0558I	I	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase
AMETH_2519	AMED_6260	AORI_2290	AJAP_27395	COG0275M	M	S-adenosyl-methyltransferase
AMETH_2525	AMED_6253	AORI_2296	AJAP_27365	COG0771M	M	UDP-N-acetylmuramoylalanine--D-glutamate ligase
AMETH_2550	AMED_2971	AORI_5593	AJAP_11515	COG0564J	J	ribosomal large subunit pseudouridine synthase D
AMETH_2615	AMED_6117	AORI_5144	AJAP_13760	COG0556L	L	excinuclease ABC subunit B
AMETH_2669	AMED_6055	AORI_5070	AJAP_14140	COG0291J	J	50S ribosomal protein L35
AMETH_2670	AMED_6054	AORI_5069	AJAP_14145	COG0292J	J	50S ribosomal protein L20
AMETH_2672	AMED_6051	AORI_5067	AJAP_14155	COG0016J	J	phenylalanyl-tRNA synthetase alpha chain
AMETH_2870	AMED_5304	AORI_4427	AJAP_16660	COG0217S	S	hypothetical protein
AMETH_2873	AMED_5300	AORI_4423	AJAP_16680	COG0632L	L	holliday junction DNA helicase RuvA
AMETH_2874	AMED_5299	AORI_4422	AJAP_16685	COG2255L	L	holliday junction DNA helicase RuvB
AMETH_2958	AMED_6138	AORI_5164	AJAP_13650	COG0749L	L	DNA polymerase I
AMETH_2970	AMED_6127	AORI_5151	AJAP_13715	COG0539J	J	30S ribosomal protein S1
AMETH_4102	AMED_2800	AORI_2787	AJAP_24855	COG0149G	G	triosephosphate isomerase
AMETH_4133	AMED_2764	AORI_2755	AJAP_25060	COG0223J	J	methionyl-tRNA formyltransferase
AMETH_4136	AMED_2759	AORI_2752	AJAP_25075	COG1198L	L	primosomal protein N'/primosome assembly protein PriA
AMETH_4139	AMED_2756	AORI_2749	AJAP_25090	COG1758K	K	DNA-directed RNA polymerase subunit omega

AMETH_4140	AMED_2755	AORI_2748	AJAP_25095	COG0194F	F	guanylate kinase
AMETH_4151	AMED_2741	AORI_2737	AJAP_25150	COG0231J	J	elongation factor EF-P
AMETH_4177	AMED_2714	AORI_2704	AJAP_25300	COG0816L	L	holliday junction resolvase
AMETH_5840	AMED_8047	AORI_6869	AJAP_04755	COG0012J	J	translation-associated GTPase
AMETH_5969	AMED_8234	AORI_7025	AJAP_04015	COG1207M	M	N-acetylglucosamine-1-phosphate uridylyltransferase
AMETH_5985	AMED_8248	AORI_7038	AJAP_03950	COG0030J	J	dimethyladenosinetransferase
AMETH_6517	AMED_8685	AORI_7442	AJAP_38915	COG0566J	J	RNA methyltransferase YjfH
AMETH_6684	AMED_8911	AORI_7684	AJAP_40065	COG0353L	L	recombination protein RecR
AMETH_6687	AMED_8918	AORI_7690	AJAP_40090	COG2812L	L	DNA polymerase III subunits gamma/tau
AMETH_7014	AMED_9296	AORI_8045	AJAP_41935	COG0360J	J	30S ribosomal protein S6
AMETH_7017	AMED_9299	AORI_8048	AJAP_41950	COG0359J	J	50S ribosomal protein L9
AMETH_7054	AMED_9346	AORI_8100	AJAP_42215	COG0617J	J	poly(A) polymerase

The Cog annotation was built by BLAST against the Clusters of Orthologous Groups (COGs) Database:

<http://www.ncbi.nlm.nih.gov/COG/>.

Figure S7. Maximum-likelihood phylogenetic tree based on the 95 core protein sequences of 123 *Actinobacteria* species

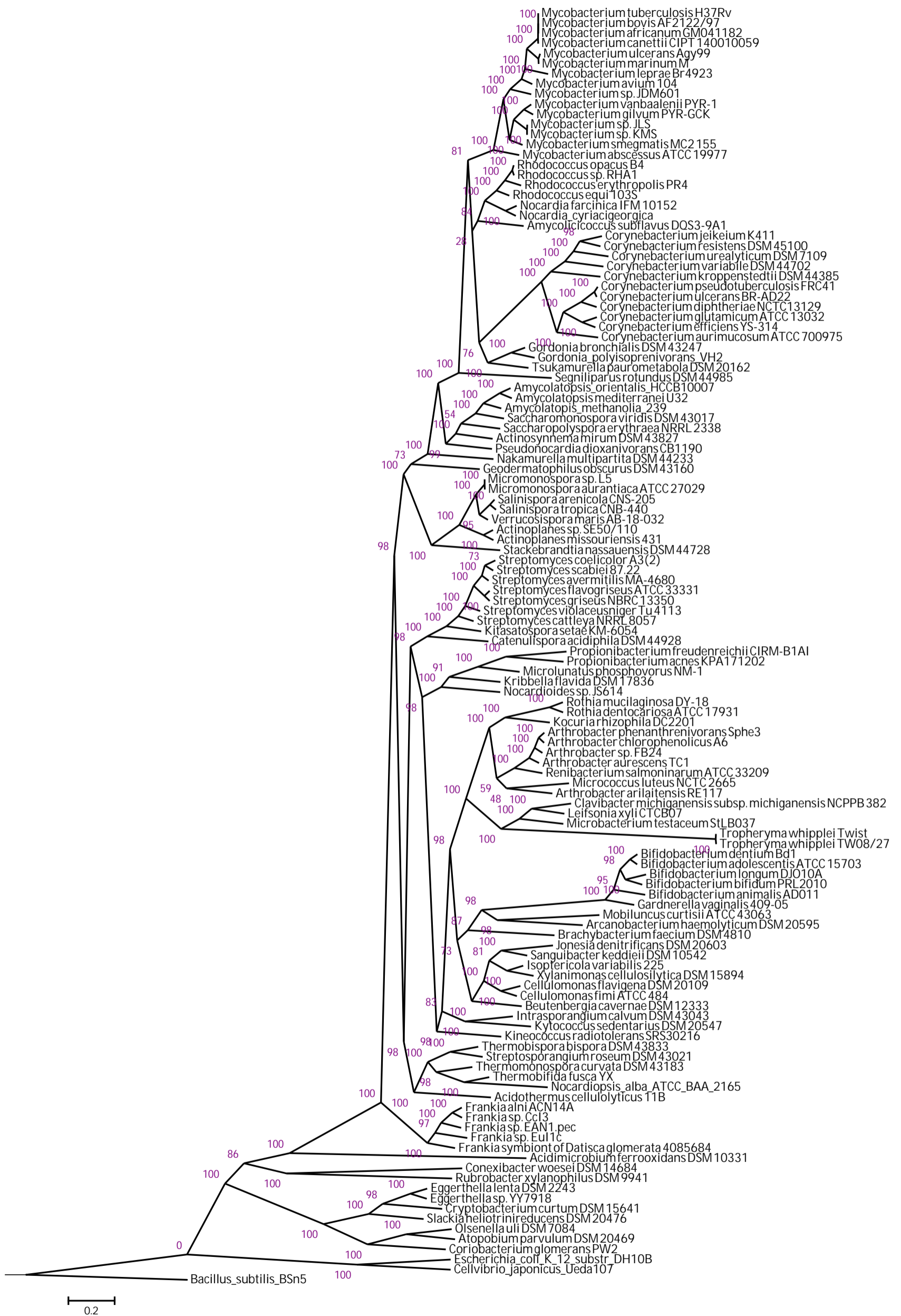
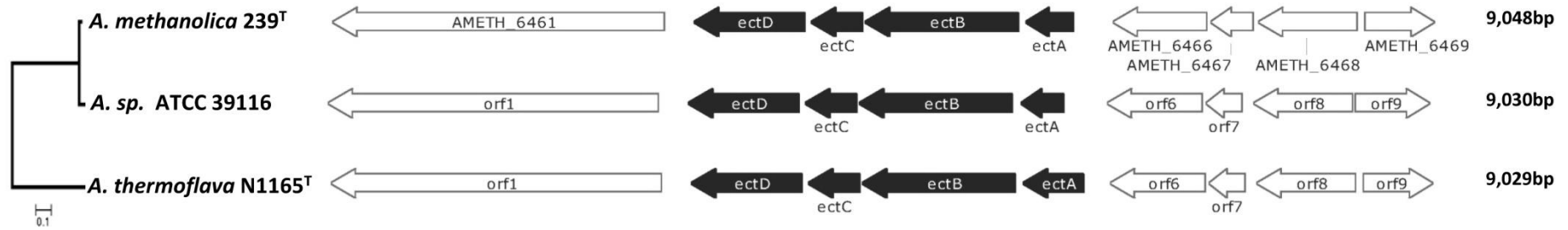


Table S7. Proposed secondary metabolite gene clusters in *Amycolatopsis* strains

Strain	Subclade	Complete/draft genome sequences	Proposed secondary metabolites		PKS-NRPS		Genome size (Mb)
			gene clusters	PKS	hybrid	NRPS	
<i>A. balhimycina</i>	AOS	Draft	29	4	7	7	10.86
<i>A. mediterranei</i>	AOS	Complete	30	5	2	8	10.24
<i>A. jejuensis</i>	AOS	Draft	25	3	1	5	10.1
<i>A. vancoresmycina</i>	AOS	Draft	55	21	3	15	9.84
<i>A. alba</i>	AOS	Draft	44	13	2	9	9.81
<i>A. azurea</i>	AOS	Draft	39	10	3	9	9.22
<i>A. rifamycina</i>	AOS	Draft	31	12	2	6	9.2
<i>A. nigrescens</i>	AOS	Draft	33	11	1	2	9.11
<i>A. lurida</i>	AOS	Draft	34	7	2	7	8.99
<i>A. japonica</i>	AOS	Complete	17	4	0	6	8.96
<i>A. orientalis</i>	AOS	Complete	32	6	4	5	8.95
<i>A. benzoatilytica</i>	AOS	Draft	23	2	1	2	8.7
<i>A. decaplanina</i>	AOS	Draft	39	10	3	6	8.53
<i>A. halophila</i>	AOS	Draft	13	2	1	2	5.55
<i>A. taiwanensis</i>	ATS	Draft	15	4	1	2	8.78
<i>A. methanolica</i> 239 ^T	AMS	Complete	12	0	0	1	7.24
<i>A. thermoflava</i>	AMS	Draft	16	3	0	1	8.69
<i>Amycolatopsis</i> sp. ATCC 39116	AMS	Draft	14	1	0	1	8.39

All of the proposed secondary metabolite gene clusters were predicted by anti-SMASH. All the genomes are available at GenBank .

Figure S8. Ectoine gene clusters in three AMS strains.



ectA, *ectB*, *ectC* and *ectD* are the key genes in ectoine gene cluster with black tags.

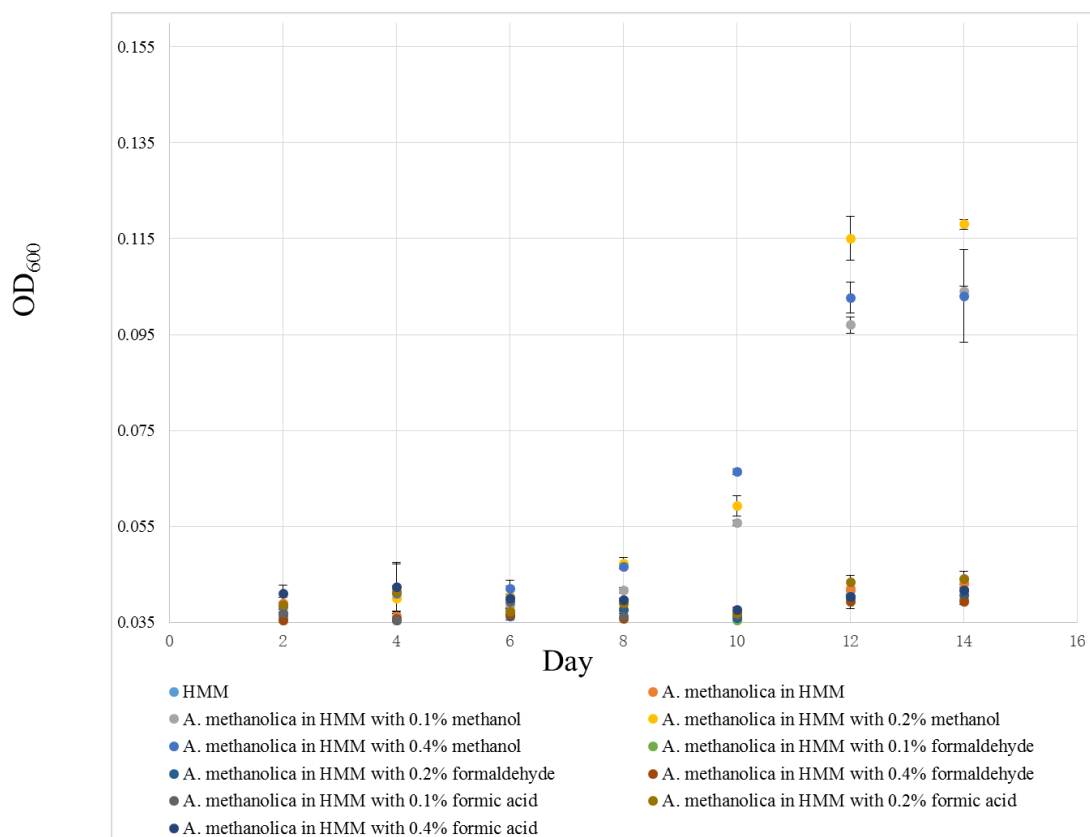


Figure S9. Survival curve of *A. methanolica* 239^T in basic medium HMM with different types and concentration of one carbon source. *A. methanolica* 239^T grew logarithmically at day 8 in HMM with methanol and achieved steady state after day 12 under the supplementation of methanol.

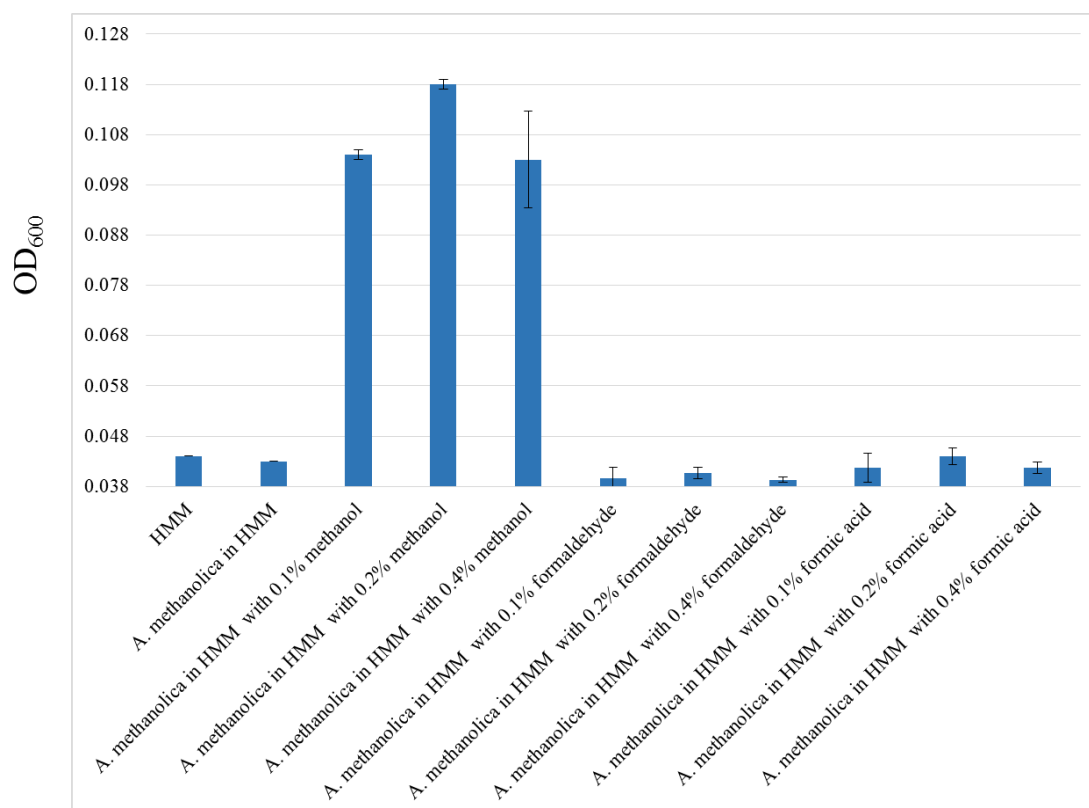


Figure S10. Growth status of *A. methanolica* 239^T in basic medium HMM supplemented with different types and concentration of one carbon sources at day 14. The growth status was evaluated by measurement of OD₆₀₀ values. *A. methanolica* 239^T grew well in HMM with methanol, while restricted in with formaldehyde or formic acid or without any carbon sources.

Table S8. Survival state of *A. methanolica* 239^T under the three one-carbon source

Liquid broth	Survival state ^a
HMM	+
HMM with 0.1% methanol	+
HMM with 0.1% formaldehyde	-
HMM with 0.1% formic acid	+

a “+”, strain from the culture can grow on GT plate; “-”, strain from the culture cannot grow on GT plate.

Method: One-carbon source utilization experiment of *A. methanolica*

A. methanolica 239 was maintained on GT plate (20 g/L starch, 0.5 g/L L-asparagine, 1 g/L KNO₃, 0.5 g/L K₂HPO₄•H₂O, 0.5 g/L NaCl, 0.5 g/L MgSO₄•7H₂O, 1 g/L CaCO₃, and 2 g/L agar, pH 7.5) at 28 °C for 3 days. Then a single colony was picked into 40 ml ISP2 liquid (0.4 % Yeast Extract, 1% Malt Extract, and 0.4 % glucose) and incubated at 28 °C for 4 days as seed. To test the survivals, we put 2 mL seed in the eppendorf tubes, washed it by HMM medium, which was prepared by mixing of (1) 100 mL phosphate salts solution (25.3 g/L K₂HPO₄ and 22.5 g/L Na₂HPO₄), (2) 100 mL sulfate salts solution (5 g/L (NH₄)₂SO₄ and 2 g/L MgSO₄•7H₂O), (3) 799 mL of deionized water, and (4) 1 mL of trace metal solution (0.177 g/L ZnSO₄•7H₂O, 1.466 g/L CaCl₂•2H₂O, 0.107 g/L MnCl₂•4H₂O, 2.496 g/L FeSO₄•7H₂O, 0.177 g/L (NH₄)₆Mo₇O₂₄•4H₂O, 0.374 g/L CuSO₄•5H₂O, 0.238 g/L CoCl₂•6H₂O and 0.1 g/L Na₂WO₄•2H₂O), for 2 times, followed by resuspending with 1mL HMM. Then, 40 µL of suspension was inoculated into 15mL HMM liquid containing 0.1%, 0.2% or 0.4% of methanol, formaldehyde and formic acid (adjusted the pH to 7.5 before use) as the sole carbon source. HMM without any additives acted as control. For the volatile methanol, a continuous feeding of methanol was performed every two days. Optical density at 600 nm (OD₆₀₀) was also measured every two days. After 14 days of incubation, 10 µL of each liquid broth were spread on the GT plate to evaluate survival state of *A. methanolica* 239. The two ones, *A. methanolica* in HMM with methanol and in HMM without any additives, were served as controls.

Table S9. Homologous proteins of AMETH_4518, AMETH_4520 and AMETH_4529 in 6 strains of *Pseudonocardiaceae*, 2 strains of *Actinobacteria* and *Bacillus subtilis* BSn5

	strain	RuMP cluster		genome		
		protein	identity	protein	identity	
AMETH_4518 <i>tkt</i>	class <i>Actinobacteria</i>	<i>A. methanolica</i> 239 ^T	AMETH_4518	100	AMETH_4092	77.63
		<i>A. benzoatilytica</i> AK 16/65 ^T	WP_020661080.1	86.17	WP_020658161.1	77.42
		<i>Saccharomonospora marina</i> XMU15 ^T	SACMADRAFT_RS11650	88.5	SACMADRAFT_RS16110	78.05
		<i>A. orientalis</i> HCCB10007			AORI_2796	74.56
		<i>A. mediterranei</i> U32			AMED_2809	74.78
		<i>A. japonica</i> MG417-CF17 ^T			AJAP_24810_4870	74.85
		<i>Arthrobacter aureescens</i> TC1			AAur_2097	66.33
		<i>Rhodococcus jostii</i> RHA1			RHA1_ro07186	75.43
		<i>Bacillus subtilis</i> BSn5			BSn5_00280	44.94
AMETH_4520 <i>rpe</i>	class <i>Actinobacteria</i>	<i>A. methanolica</i> 239 ^T	AMETH_4520	100	AMETH_4130	80.29
		<i>A. benzoatilytica</i> AK 16/65 ^T	WP_020661082.1	80.65	WP_020658131.1	78.73
		<i>Saccharomonospora marina</i> XMU15 ^T	SACMADRAFT_RS11640	78.22	SACMADRAFT_RS16305	78.37
		<i>A. orientalis</i> HCCB10007			AORI_2761	77.03
		<i>A. mediterranei</i> U32			AMED_2771	77.83
		<i>A. japonica</i> MG417-CF17 ^T			AJAP_25030_4913	77.48
		<i>Arthrobacter aureescens</i> TC1			AAur_1824	55.02
		<i>Rhodococcus jostii</i> RHA1			RHA1_ro07167	72.56
		<i>Bacillus subtilis</i> BSn5			BSn5_20010	48.34
AMETH_4529 <i>rpi</i>	class <i>Actinobacteria</i>	<i>A. methanolica</i> 239 ^T	AMETH_4529	100	AMETH_1699	66.67
		<i>A. benzoatilytica</i> AK 16/65 ^T	WP_027927817.1	79.87	WP_020661131.1	65.22
		<i>Saccharomonospora marina</i> XMU15 ^T	SACMADRAFT_RS11605	76.13	SACMADRAFT_RS07965	63.87
		<i>A. orientalis</i> HCCB10007			AORI_1928	61.94
		<i>A. mediterranei</i> U32			AMED_6811	68.38
		<i>A. japonica</i> MG417-CF17 ^T			AJAP_29895_5868	61.94
		<i>Arthrobacter aureescens</i> TC1			AAur_2387	51.63
		<i>Rhodococcus jostii</i> RHA1			RHA1_ro01378	64.97
		<i>Bacillus subtilis</i> BSn5			BSn5_09415	35.86