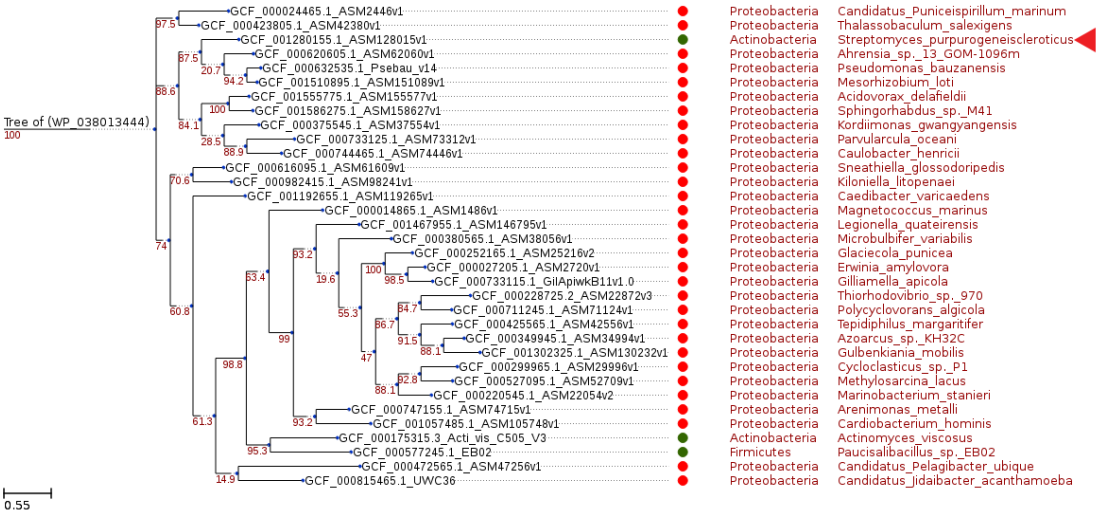
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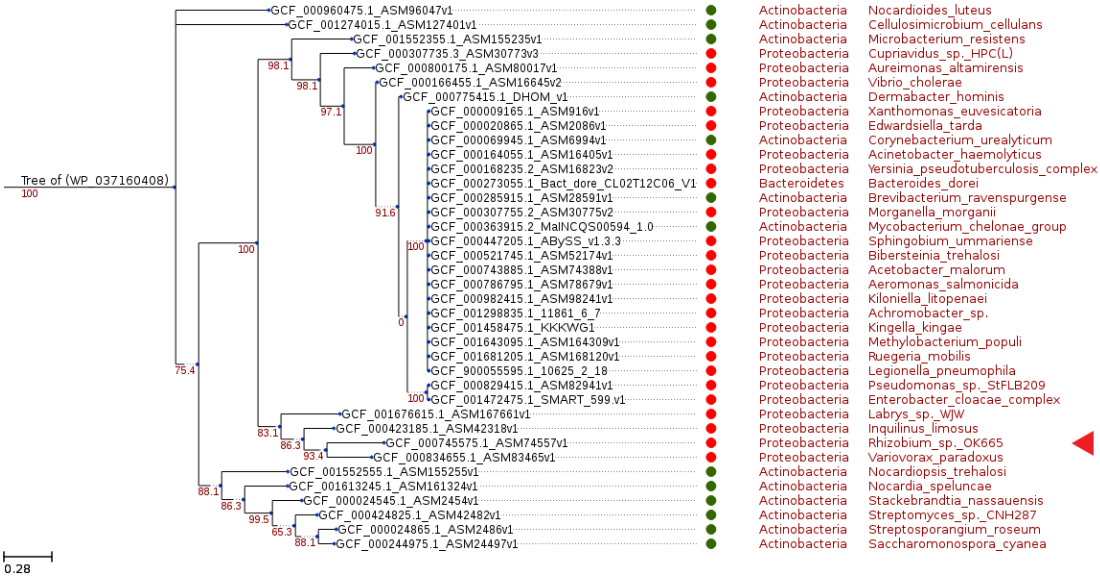
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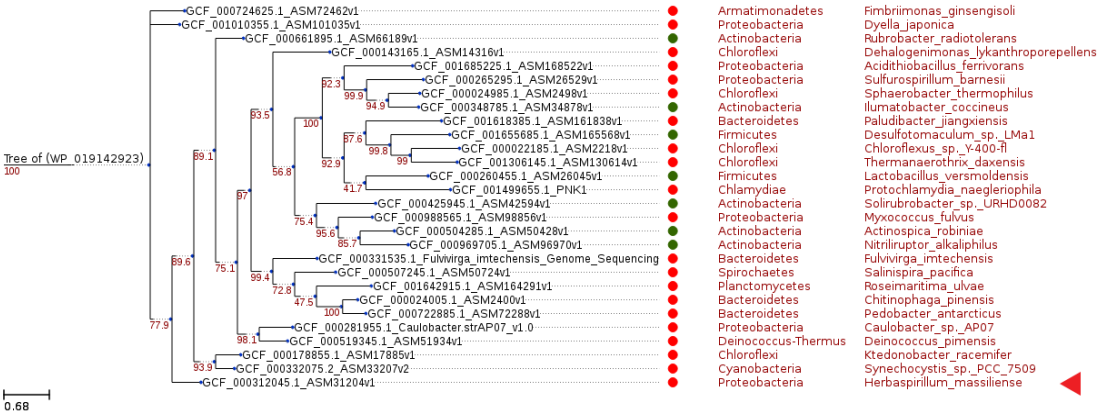
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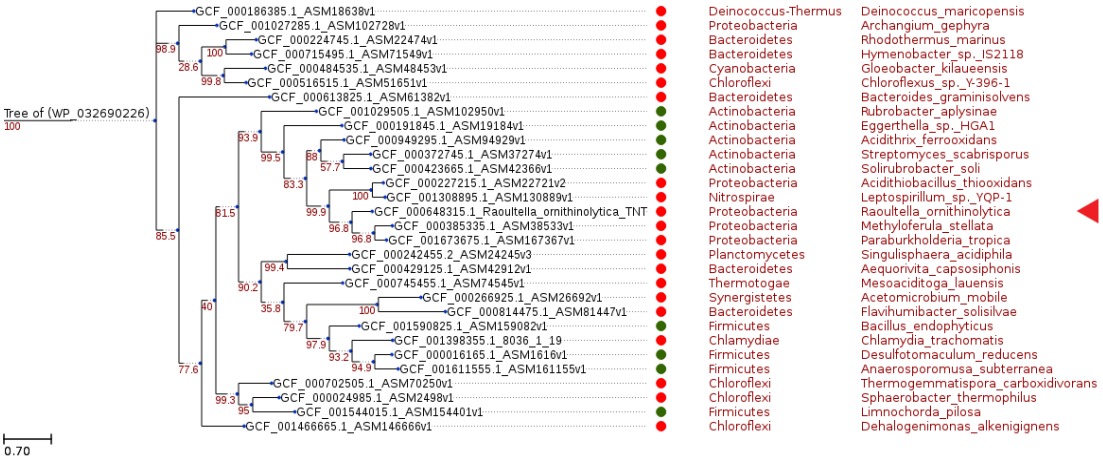
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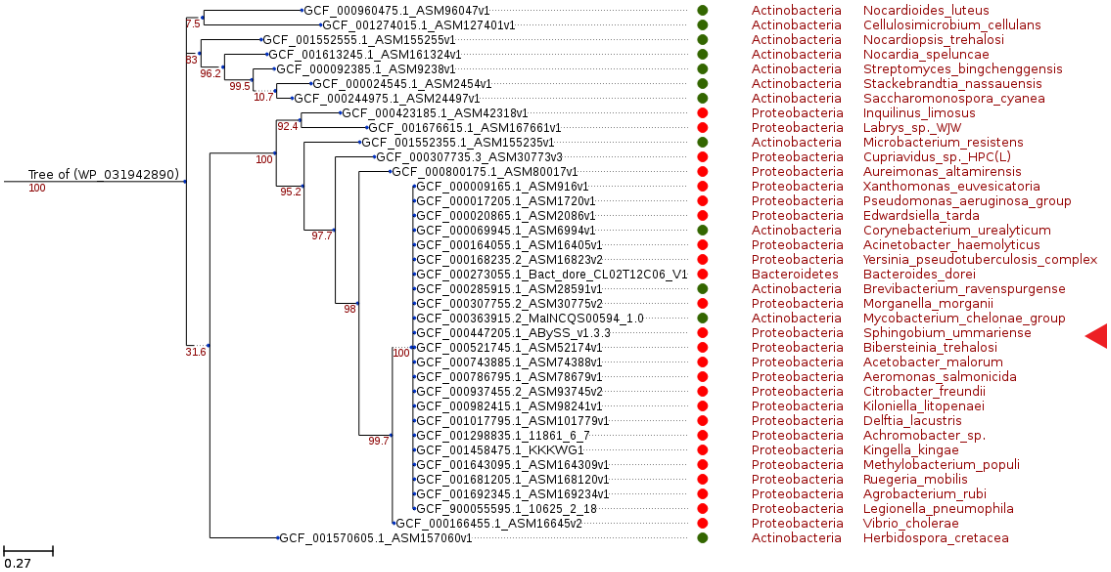
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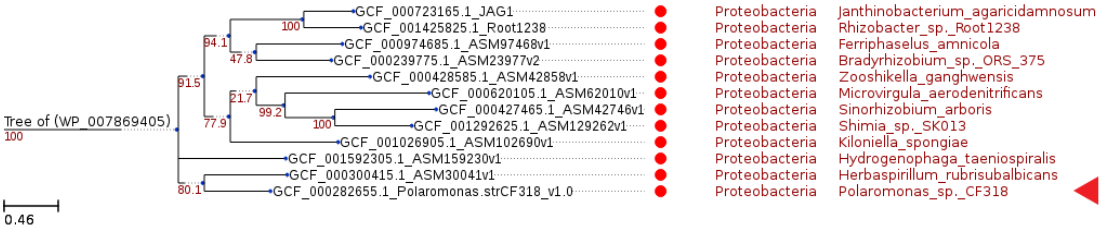
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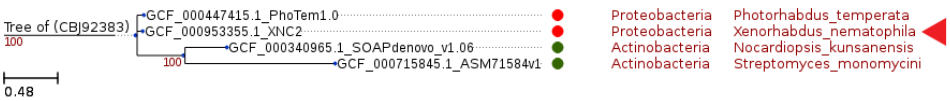
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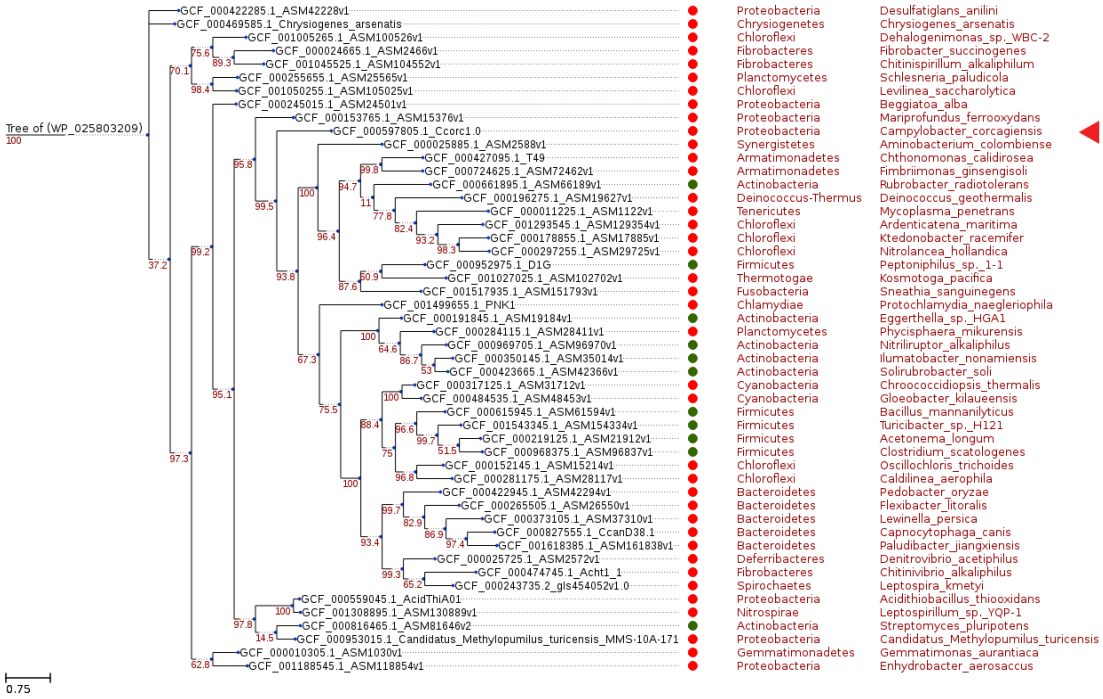
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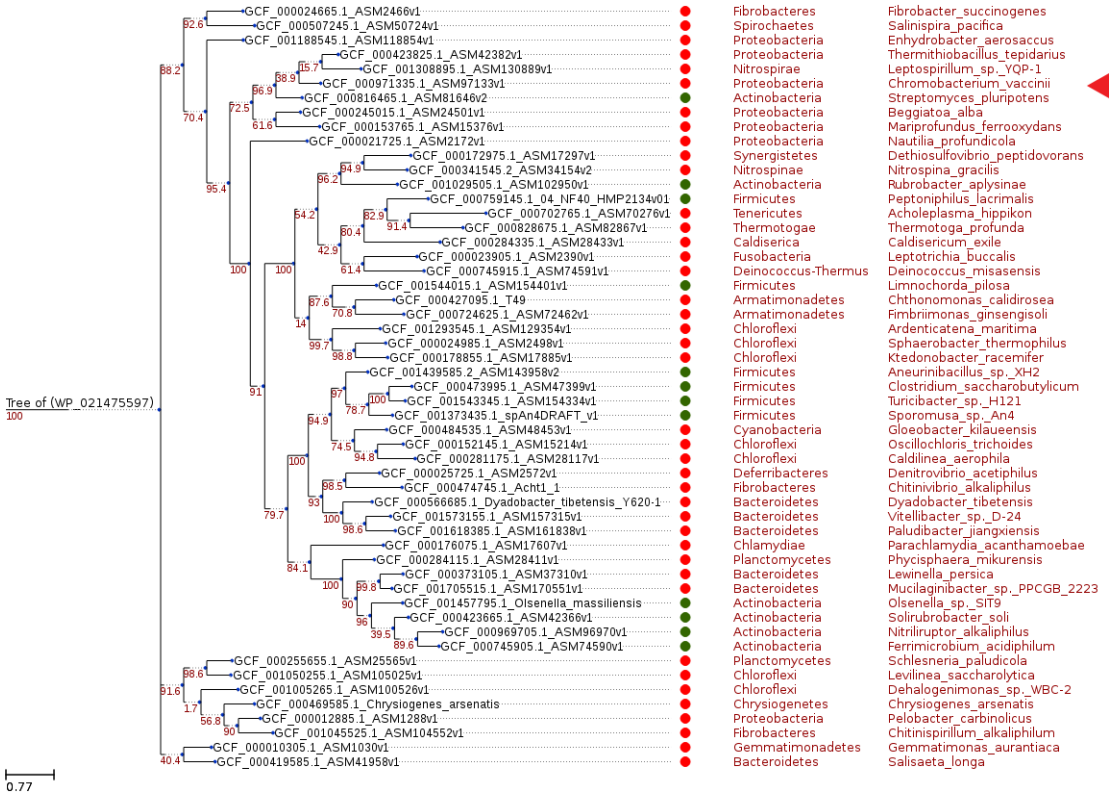
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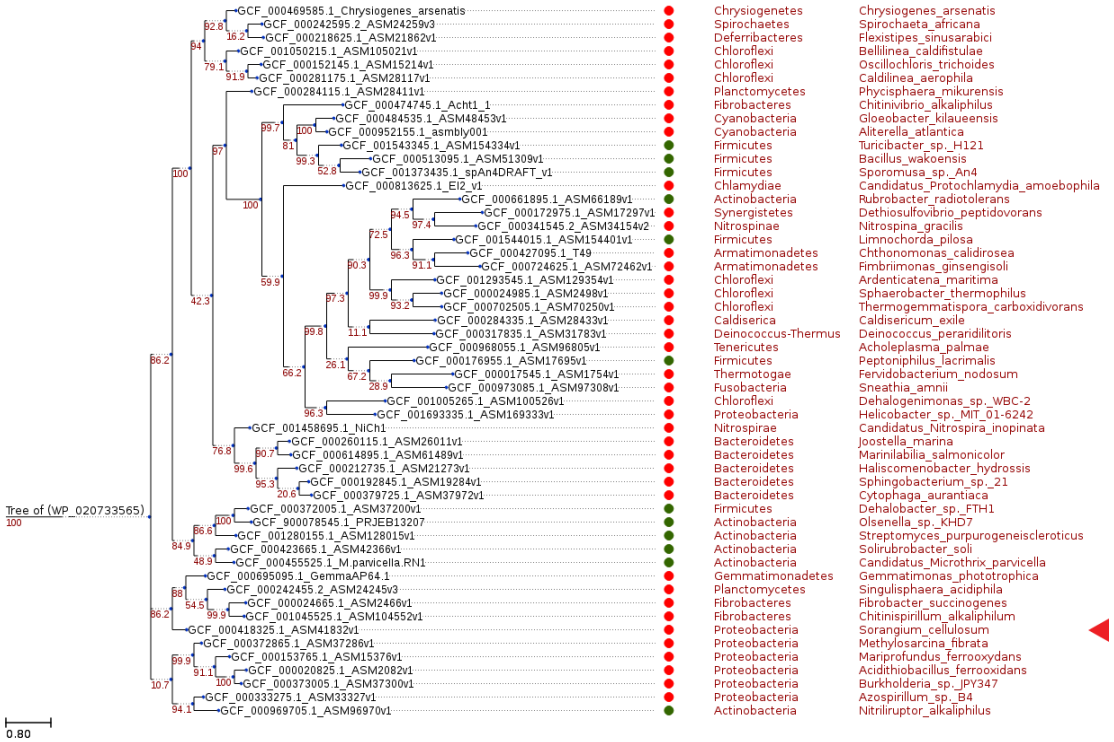
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Chloroflexi Bellilinea_caldifistulae
Chloroflexi Oscillochloris_trichoides
Chloroflexi Caldilinea_aerophila
Planctomycetes Phycisphaera_mikurensis
Fibrobacteres Chitinivibrio_alkaliphilus
Cyanobacteria Gloeobacter_kilaueensis
Cyanobacteria Aliterella_atlantica
Firmicutes Turicibacter_sp._H121
Firmicutes Bacillus_wakoensis
Firmicutes Sporomusa_sp._An4
Chlamydiae Candidatus_Protochlamydia_amoebophila
Actinobacteria Rubrobacter_radiotolerans
Synergistetes Dethiosulfovibrio_peptidovorans
Nitrospinae Nitrospina_gracilis
Firmicutes Limnochorda_pilosa
Armatimonadetes Chthonomonas_calidirosea
Armatimonadetes Fimbriimonas_ginsengisoli
Chloroflexi Ardenticatena_maritima
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Caldiserica Caldisericum_exile
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Tenericutes Acholeplasma_palmae
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Bacteroidetes Cytophaga_aurantiaca
Firmicutes Dehalobacter_sp._FTH1
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Planctomycetes Singulisphaera_acidiphila
Fibrobacteres Fibrobacter_succinogenes
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Proteobacteria Sorangium_cellulosum
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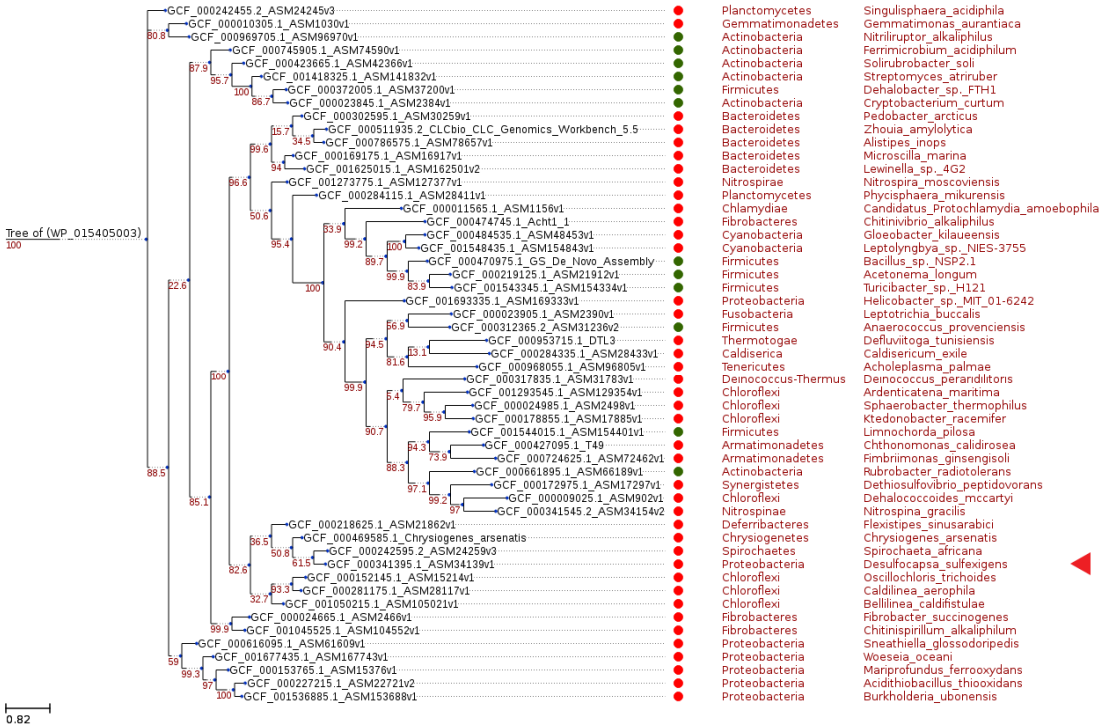
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Deferribacteres Flexistipes_sinusarabici
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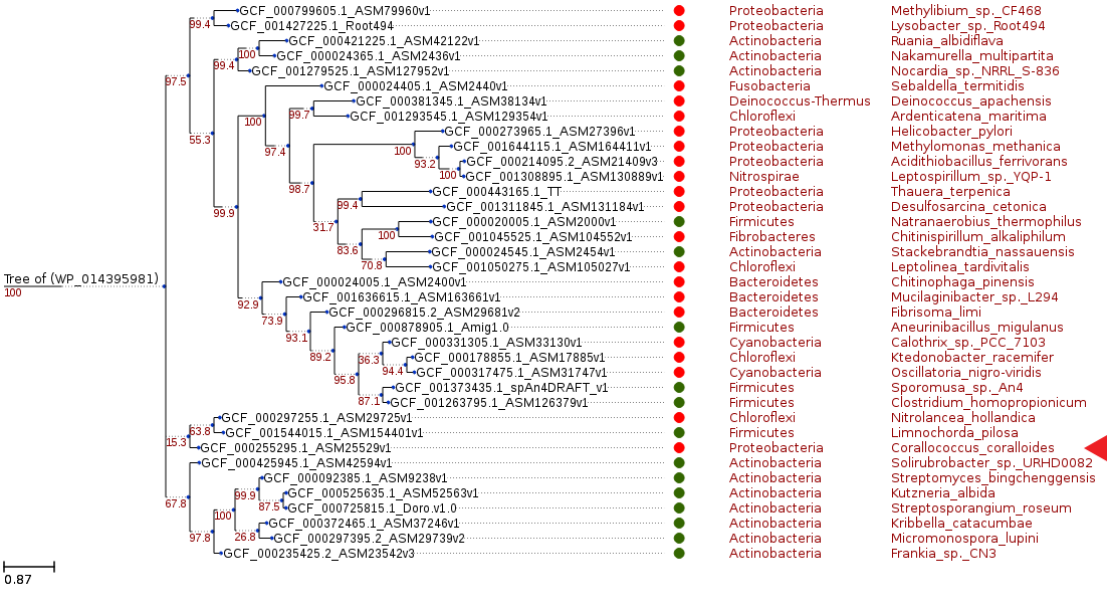
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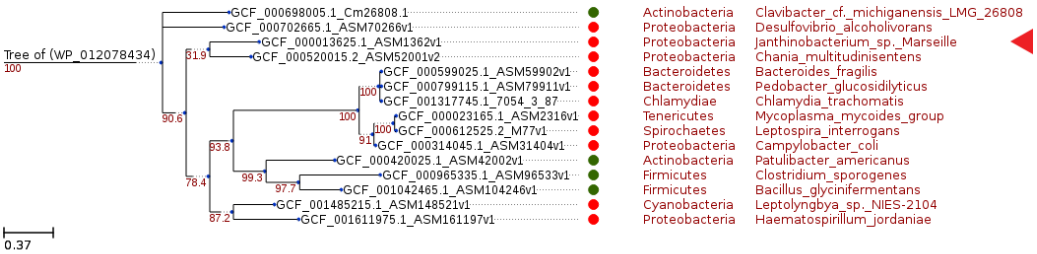
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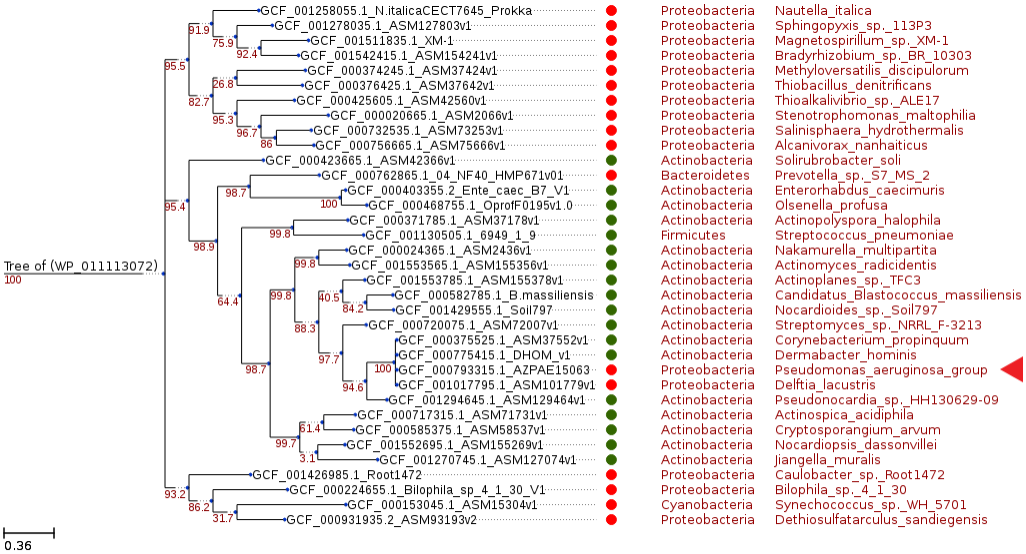
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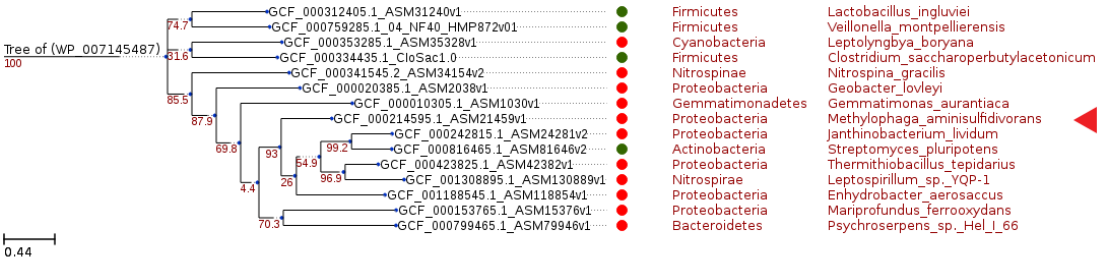
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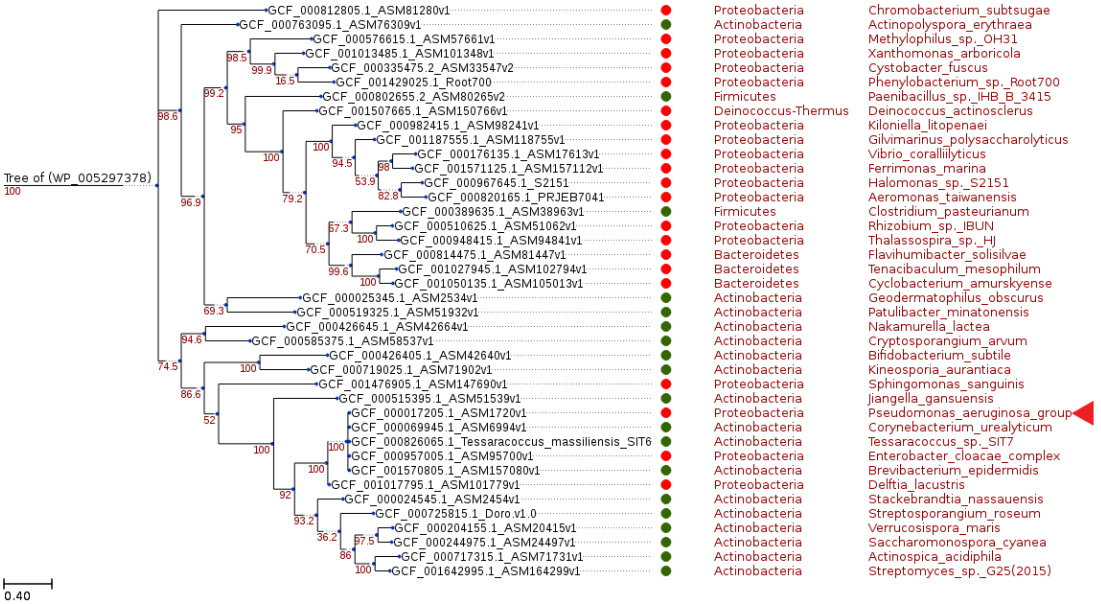
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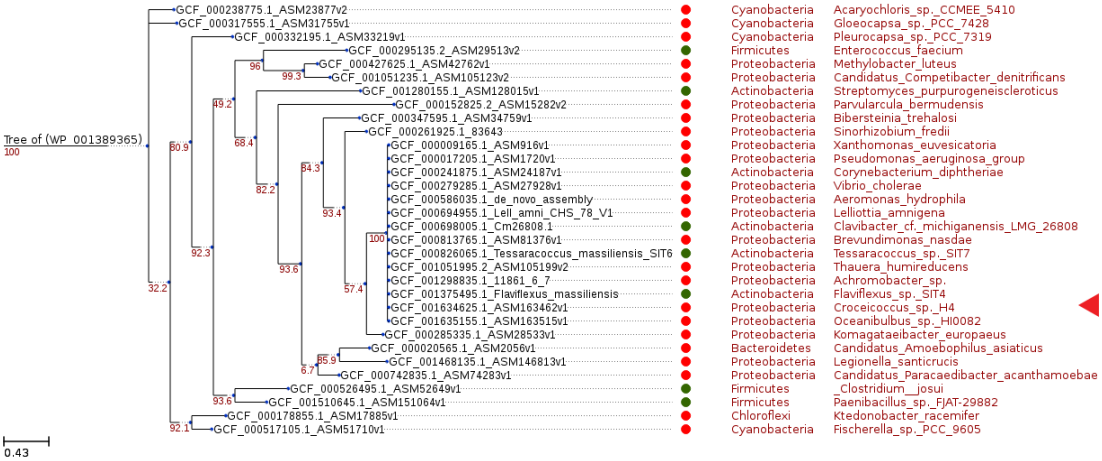
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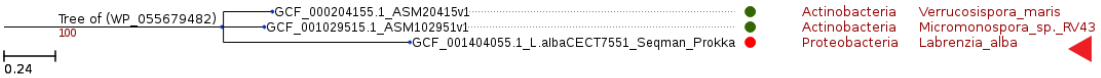
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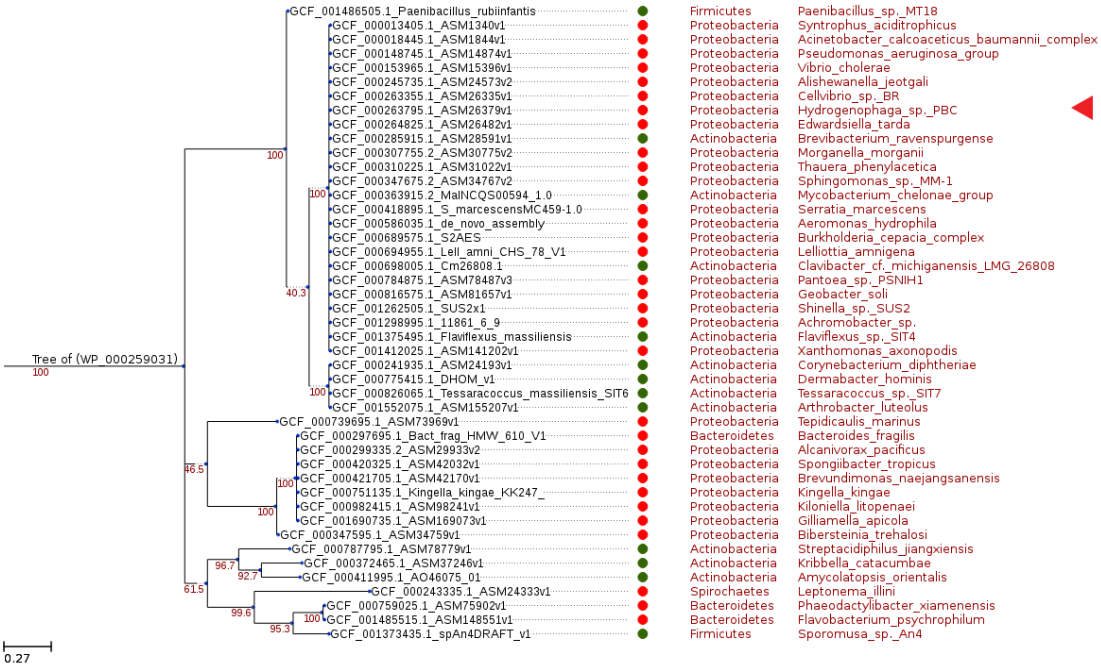
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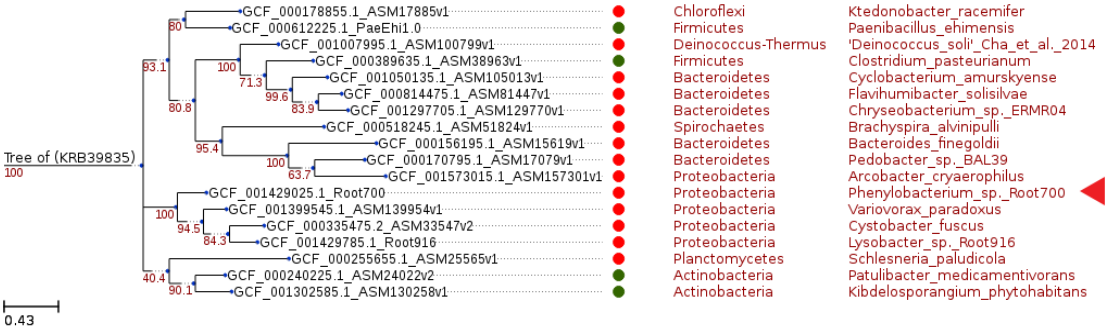
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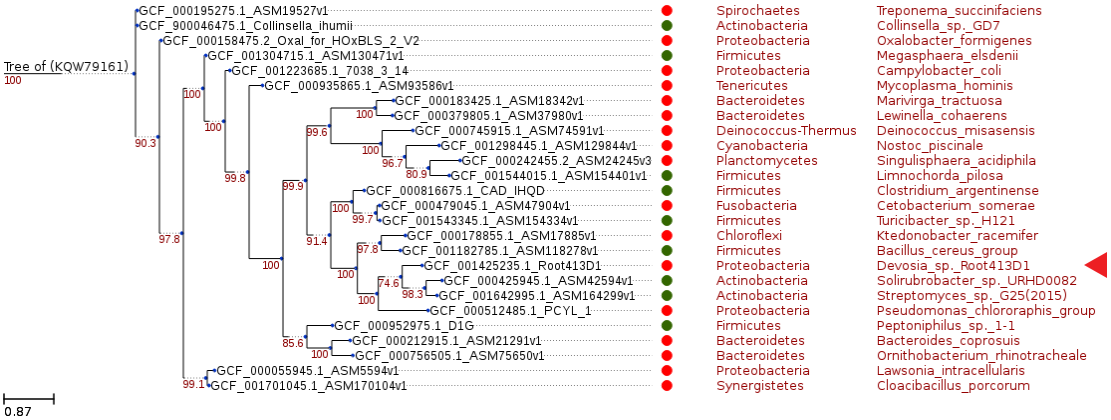
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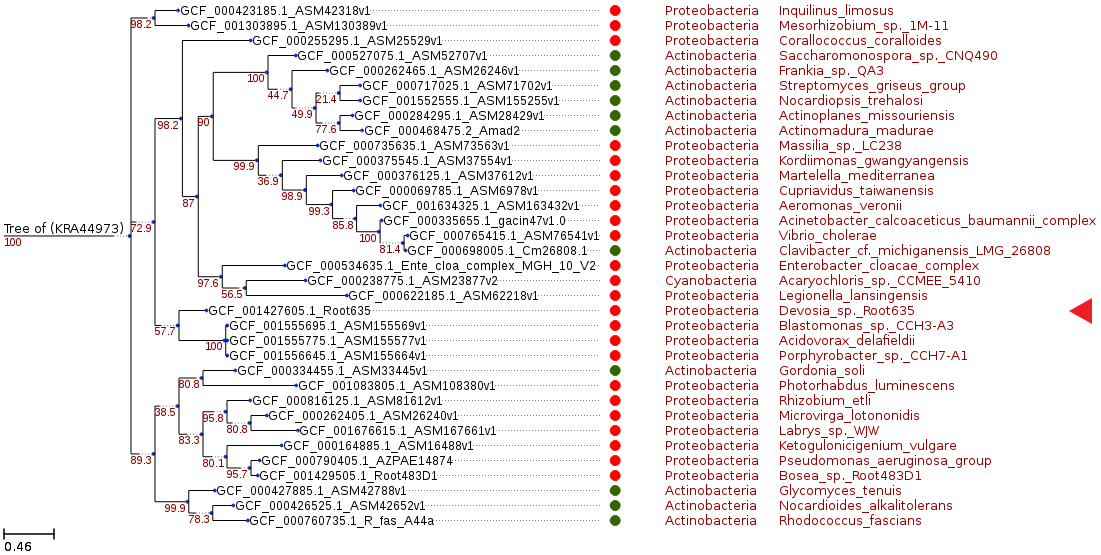
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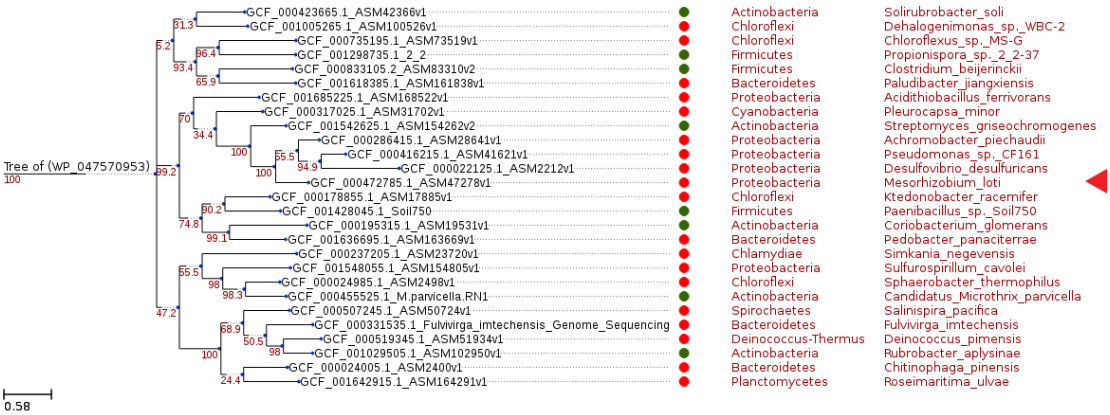
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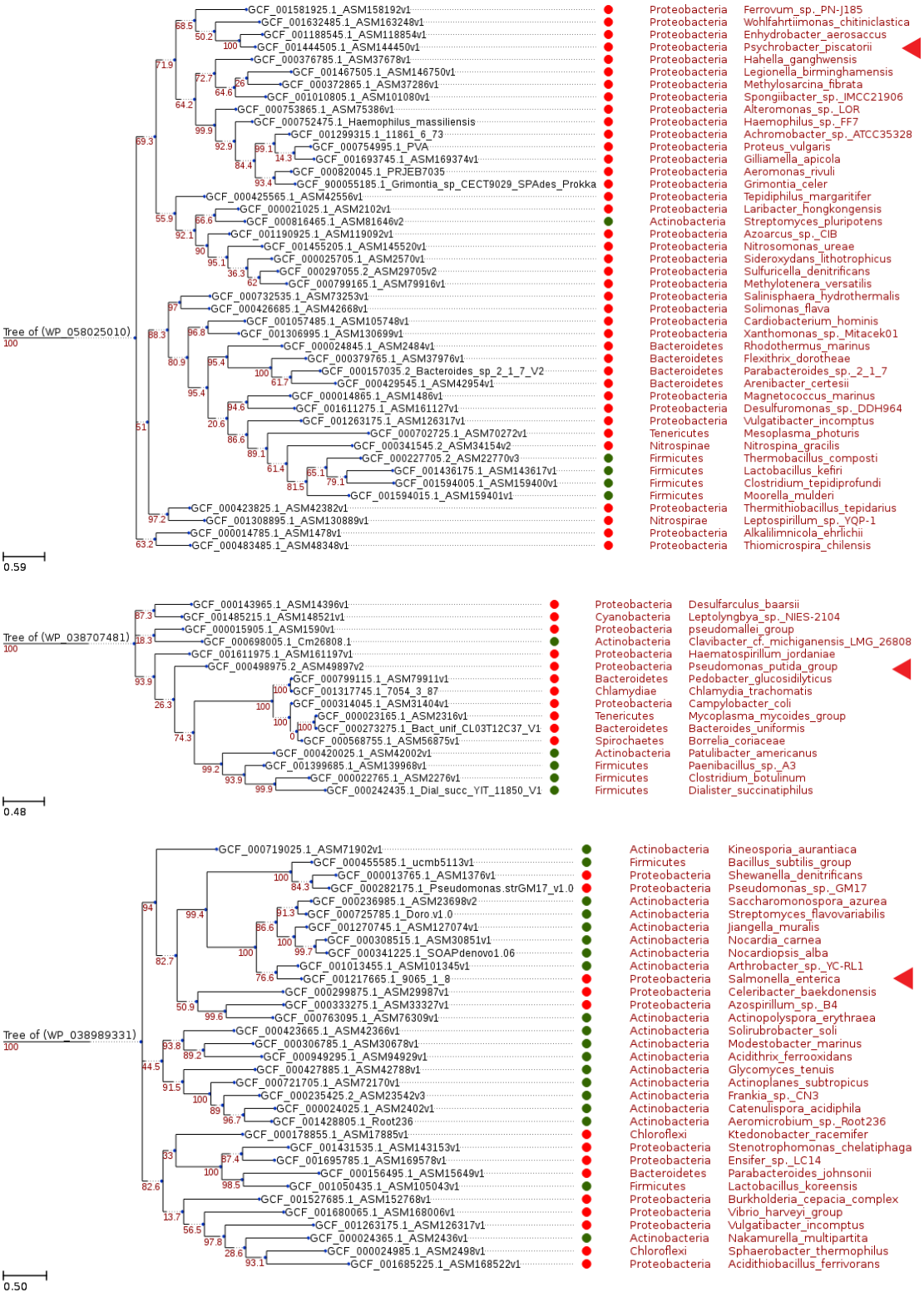
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Tree of (EFG83002)
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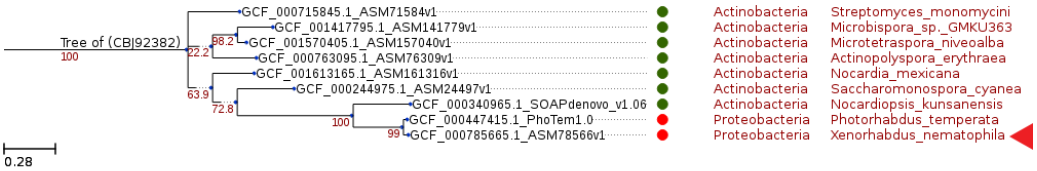
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22.2
98.2
63.9
72.8
100
99
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GCF_000340965.1_SOAPdenovo_v1.06 Actinobacteria Nocardiopsis_kunsanensis
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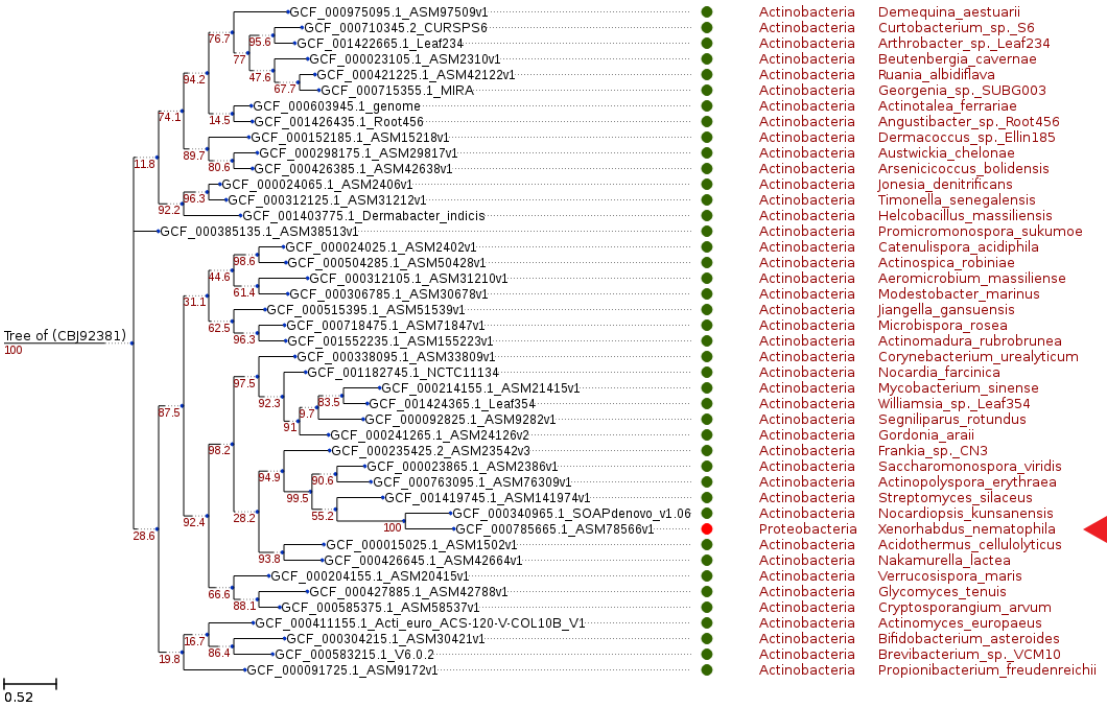
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GCF_000715355.1_MIRA Actinobacteria Georgenia_sp._SUBG003
GCF_000603945.1_genome Actinobacteria Actinotalea_ferrariae
GCF_001426435.1_Root456 Actinobacteria Angustibacter_sp._Root456
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GCF_000426385.1_ASM42638v1 Actinobacteria Arsenicicoccus_bolidensis
GCF_000024065.1_ASM2406v1 Actinobacteria Jonesia_denitrificans
GCF_000312125.1_ASM31212v1 Actinobacteria Timonella_senegalensis
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GCF_000385135.1_ASM38513v1 Actinobacteria Promicromonospora_sukumoe
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GCF_000504285.1_ASM50428v1 Actinobacteria Actinospica_robiniae
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GCF_000306785.1_ASM30678v1 Actinobacteria Modestobacter_marinus
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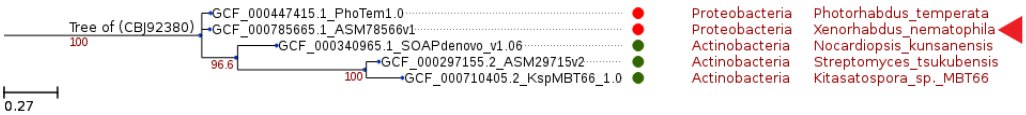
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96.6
100
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GCF_000297155.2_ASM29715v2 Actinobacteria Streptomyces_tsukubensis
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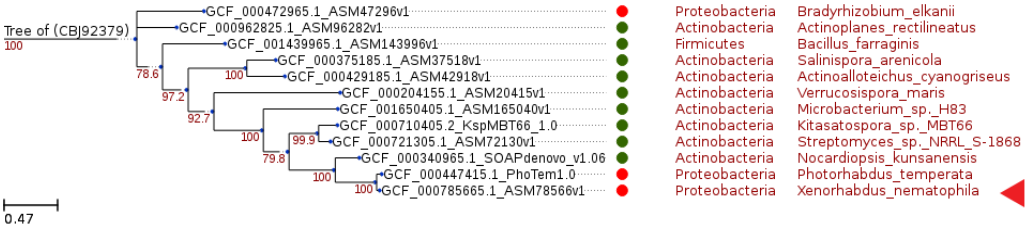
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78.6
100
97.2
92.7
100
99.9
79.8
100
100
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GCF_000375185.1_ASM37518v1 Actinobacteria Salinispora_arenicola
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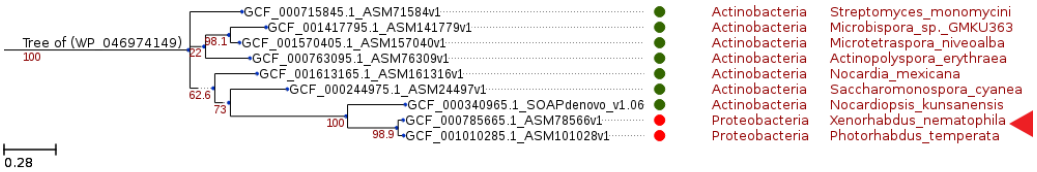
0.47


Tree of (WP_046974149)
100
98.1
62
62.6
73
100
98.9
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GCF_000763095.1_ASM76309v1 Actinobacteria Actinopolyspora_erythraea
GCF_001613165.1_ASM161316v1 Actinobacteria Nocardia_mexicana
GCF_000244975.1_ASM24497v1 Actinobacteria Saccharomonospora_cyanea
GCF_000340965.1_SOAPdenovo_v1.06 Actinobacteria Nocardiopsis_kunsanensis
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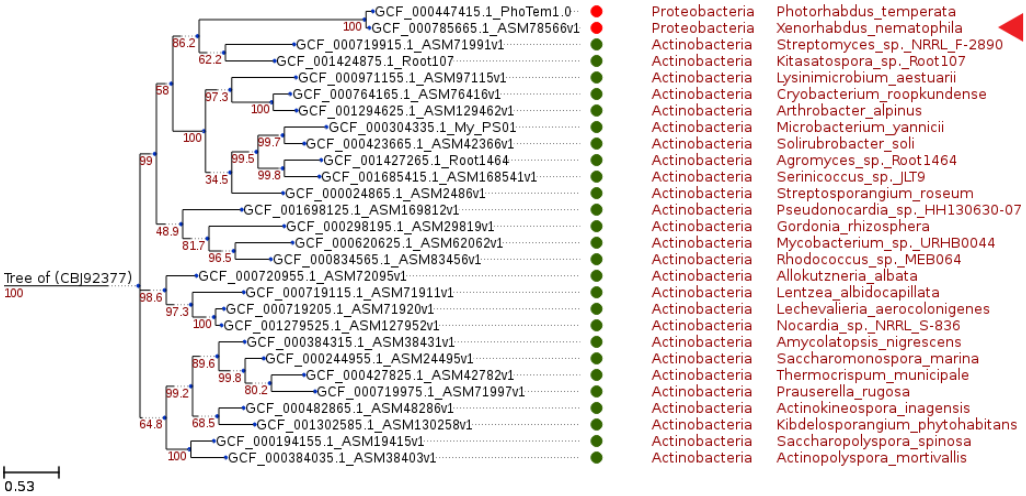
0.28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GCF_001424875.1_Root107 Actinobacteria Kitasatospora_sp._Root107
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GCF_000764165.1_ASM76416v1 Actinobacteria Cryobacterium_roopkundense
GCF_001294625.1_ASM129462v1 Actinobacteria Arthrobacter_alpinus
GCF_000304335.1_My_PS01 Actinobacteria Microbacterium_yannicii
GCF_000423665.1_ASM42366v1 Actinobacteria Solirubrobacter_soli
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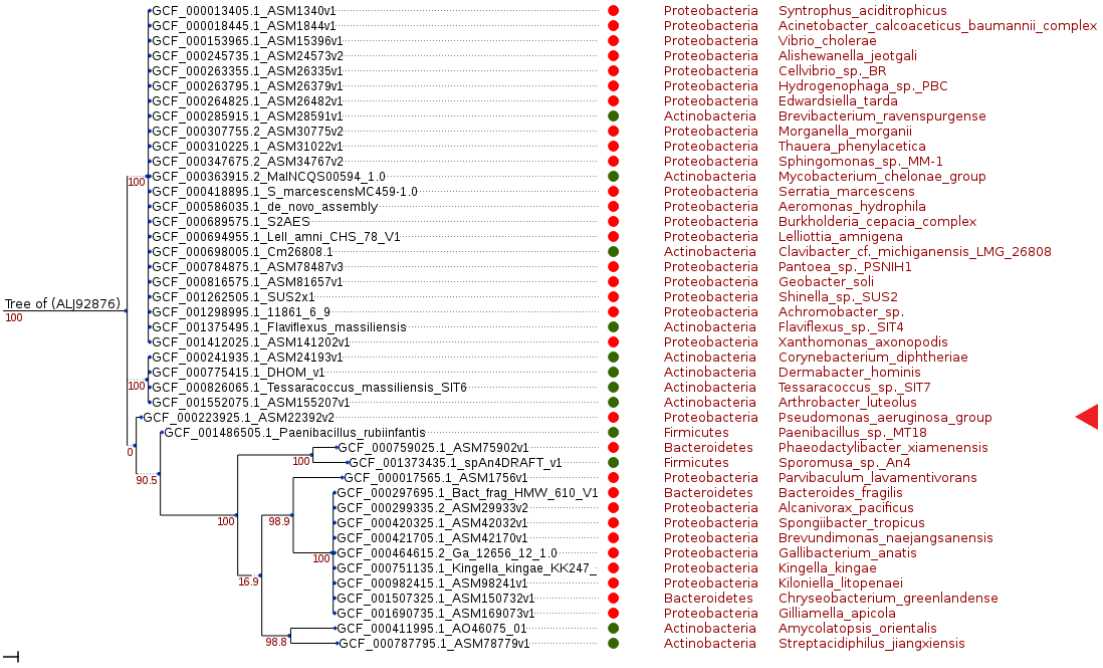
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GCF_000245735.1_ASM24573v2 Proteobacteria Alishewanella_jeotgali
GCF_000263355.1_ASM26335v1 Proteobacteria Cellvibrio_sp._BR
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GCF_000264825.1_ASM26482v1 Proteobacteria Edwardsiella_tarda
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GCF_000307755.2_ASM30775v2 Proteobacteria Morganella_morganii
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GCF_000223925.1_ASM22392v2 Proteobacteria Pseudomonas_aeruginosa_group
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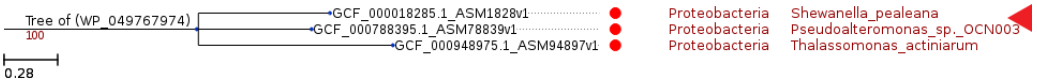
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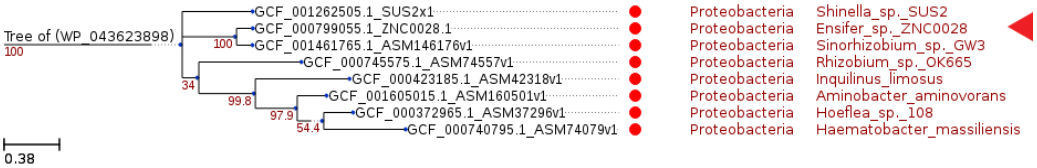
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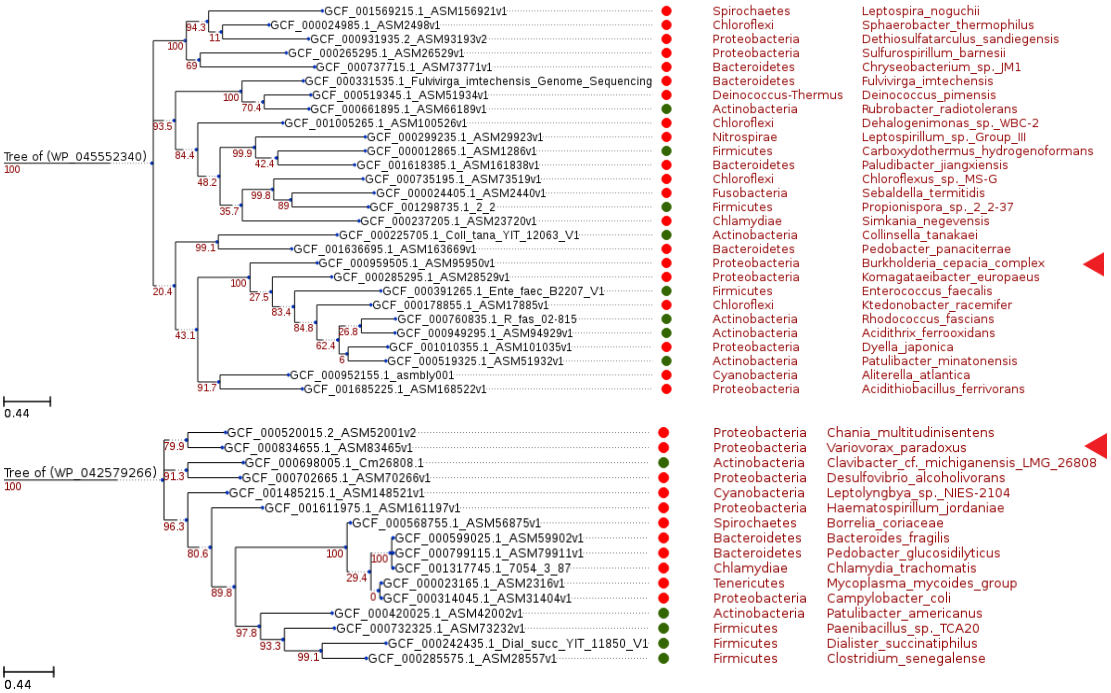
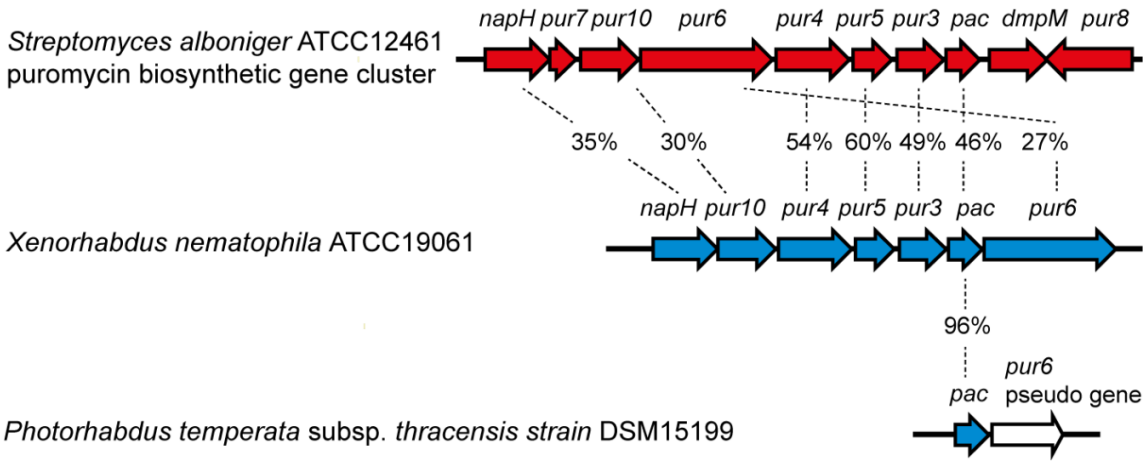
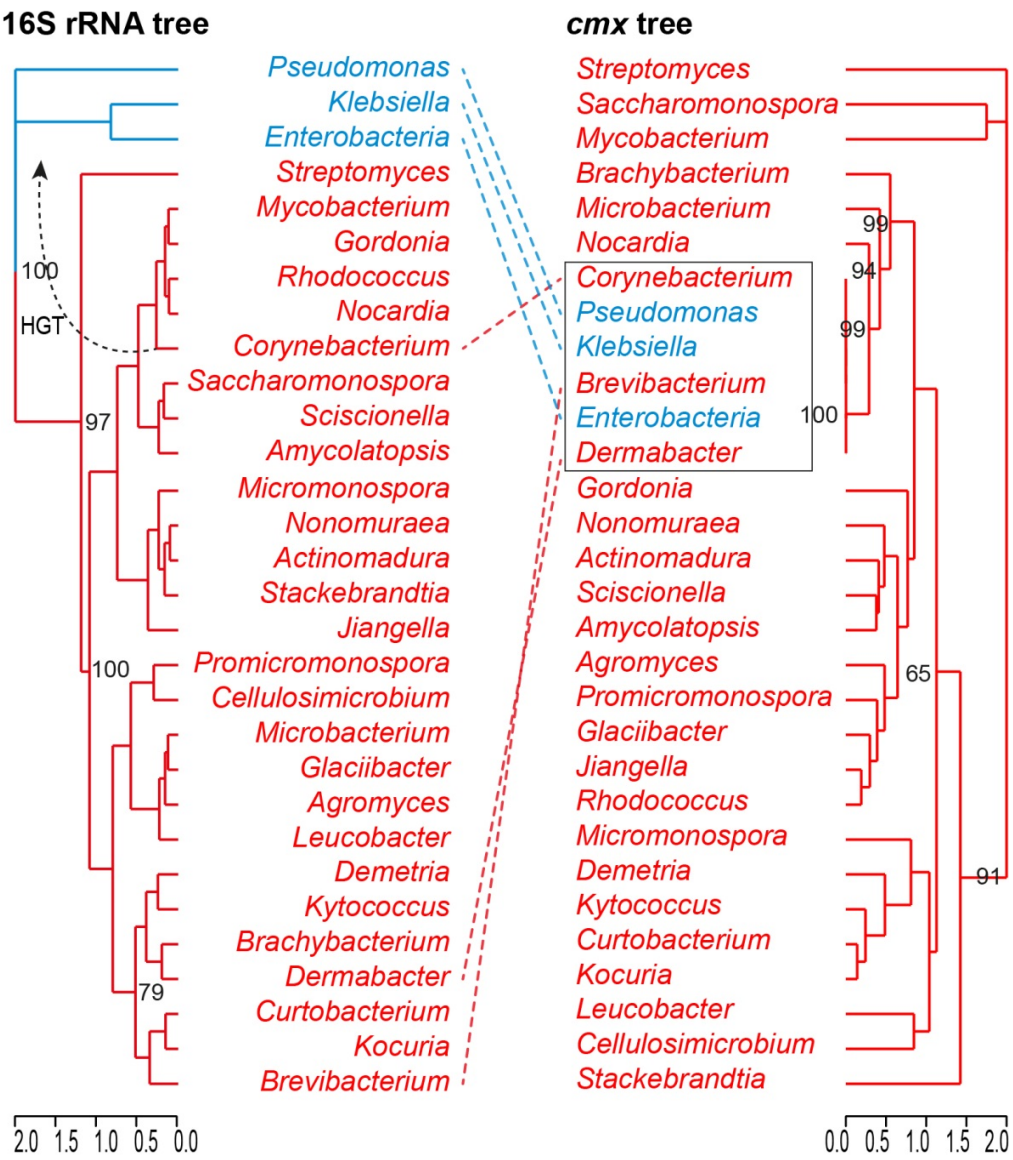


Supplementary Figure 1 | Protein phylogenetic trees. Phylogenetic trees were built for the proteobacterial proteins that show the highest sequence identity to *Streptomyces* ARG proteins, the proteins encoded in neighboring area of resistance gene *pac*, and proteins involved in *cmx* "carry back". The position of the query sequence was marked by red triangle in the trees. Gram-positive and Gram-negative bacteria are marked in red and blue respectively.

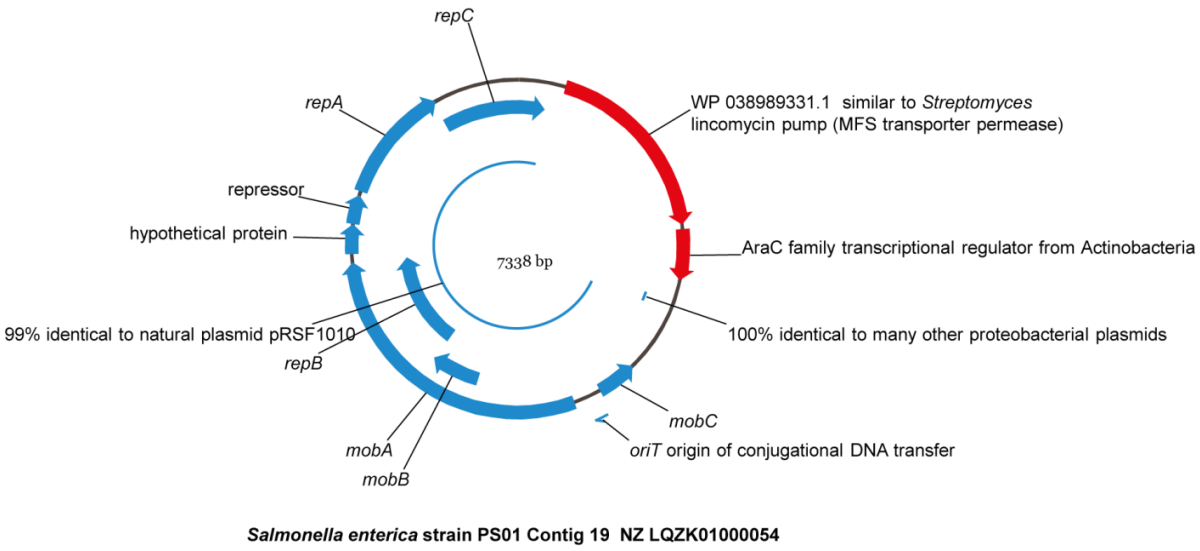


Supplementary Figure 2 | Puromycin resistance gene *pac* and neighbouring genes in actinobacteria and proteobacteria. The puromycin biosynthetic gene cluster in puromycin producer is composed of ten genes (red). Homologues (protein sequence identity value indicated) of seven of them including the resistance gene *pac* (WP_046974149.1) were found in proteobacteria (blue). A standalone *pac* was also found in proteobacteria (blue). The products of all these genes were more similar to actinobacterial proteins than to proteins from any other phyla, suggesting they were co-transferred from actinobacteria to proteobacteria (Supplementary Fig. 1).

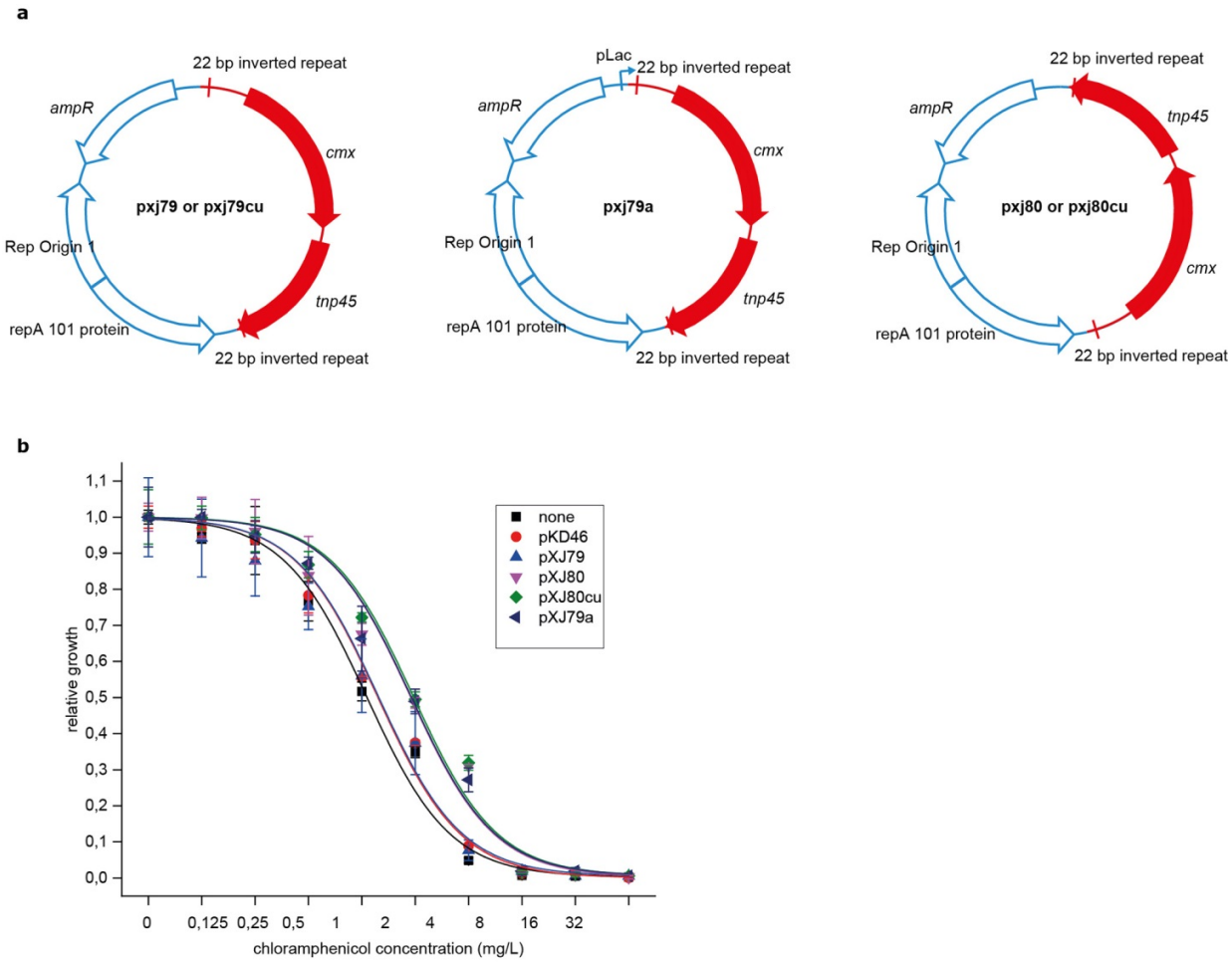
Supplementary Figure 3 | Alignment of the multi-phylum Cmx, and three other confirmed chloramphenicol resistance pumps. Alignment was performed using software Vector NTI. Non-similar residues are shown as black letters on a white background; weakly similar residues are shown as green letters on a white background; conservative residues are shown as black letters on a blue background; block of similar residues are shown as black letters on a green background; identical residues are shown as red letters on a yellow background. The multi-phylum Cmx showed 63% identity (100% coverage) to the chloramphenicol efflux pump (P31141) from the model actinobacterium *Streptomyces lividans* 66, and 52% identity (99% coverage) to the self-protecting efflux pump (WP_015032122.1) from the chloramphenicol producer *Streptomyces venezuelae*. Meanwhile, it showed only 29% identity (27% coverage) to CmlA (WP_000095725), a confirmed proteobacterial chloramphenicol efflux pump.



Supplementary Figure 4 | A full tanglegram of the phylogeny of the genus representative hits of *cmx* and the host phylogeny of their corresponding 16S rRNA sequences. The red labels show the Gram-positive actinobacteria while the blue labels illustrate the Gram-negative proteobacteria. *cmx* is said to have a hit in a genome if the genome has a Cmx protein (WP_005297378.1) sequence match with e-value of 10E-50 or less, 30% BLAST identity and 80% coverage. The best hit per genus is taken as the representative hit. The hits with over 99% identity to WP_005297378.1 are highlighted with a frame.

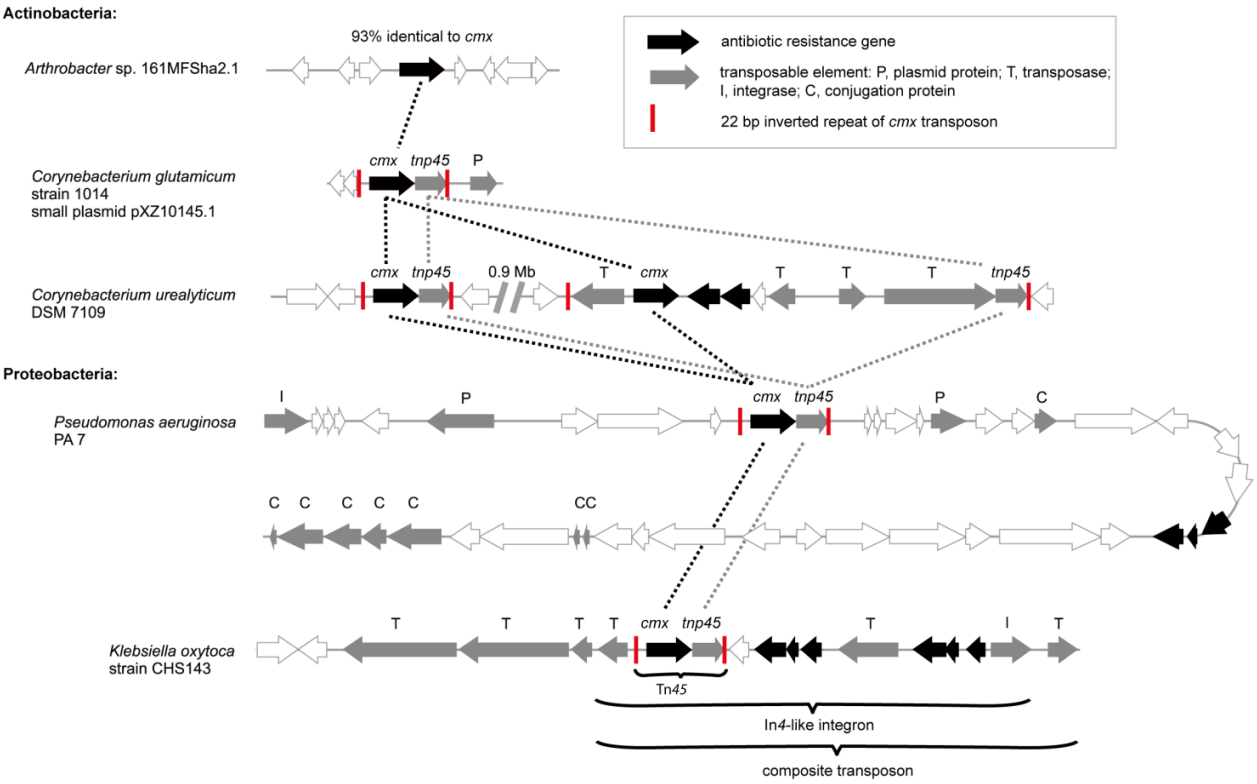


Supplementary Figure 5 |WP_038989331.1 gene from *Salmonella enterica* PS01. The predicted transporter protein WP_038989331.1 is 50% identical to the self-protecting lincomycin pump, LmrA (CAA42550), from *Streptomyces lincolnensis*. Its gene is found on a plasmid from *Salmonella enterica* PS01. Similar plasmids were also found from *Salmonella enterica* subsp. enterica serovar typhimurium str. DT104 (NZ_CTFZ01000046.1:1-9225) and *Escherichia coli* EC2_1 (NZ_JWKJ01000106.1:1-7466). The WP_038989331.1 gene and the adjacent regulator gene showed actinobacterial sequence signature (highlighted in red), suggesting that they were transferred from actinobacteria recently. The region from *mobC* to *repC* is 99% identical to the broad-host-range proteobacterial plasmid pRSF1010 (highlighted in blue). These elements are responsible for the ability of pRSF1010 to be mobilized by conjugation from *E.coli* into *Streptomyces* and *Mycobacterium* (helped by conjugative plasmid) and stably maintained there.



Supplementary Figure 6 | Cloning of *cmx* transposon and its chloramphenicol resistance activity in *E.coli*. a, Cloning of *cmx* transposon. The *cmx* transposon (highlighted in red) was PCR amplified from *P. aeruginosa* PA7 by primer xj143 (as both forward and reverse primer). Plasmid backbone (marked in blue) was amplified from plasmid pKD46 by primer xj144 and xj145. The two PCR products were assembled by Gibson reaction, transferred into *E. coli* DH5α and selected on Amp plate. Insertion direction was determined by sequencing with primer xj146. The resulted plasmids were named as pXJ79 and pXJ80. Plasmids pXJ79cu and pXJ80cu were constructed likewise and the *cmx* transposon was amplified from *C. urealyticum* DSM 7109. Plasmid pXJ79 was amplified with primer xj177and xj178, and the product was recirculated by Gibson reaction, generating pXJ79a. By this way promoter pLac (without lacO) was inserted in front of the transposon. **b**, Dose-response curves of chloramphenicol on *E.coli* DH5α with cloned *cmx* transposon. Concentrations of chloramphenicol

were included at twofold serial dilutions. Growth was conducted in quadruplicate 50 µl volumes for each antibacterial concentration in 96-well plates. After 48 h of incubation at 30°C, the OD600 was determined using a plate reader. Quadruplicate values for each drug condition were averaged and then normalized to growth levels without added drug (error bar: s.d.; n=4). Increased tolerance towards chloramphenicol was observed when a promoter was provided in front of the transposon (pXJ79a). A similar level of resistance was also observed when the transposon was inserted in the same direction as upstream native genes (pXJ80), suggesting that *cmx* can be functionally expressed in the new host by read-through of transcription from upstream native genes.



Supplementary Figure 7 | Examples of *cmx* transposon and its context in different hosts. Antibiotic resistance and mobile element genes are colored as black and grey respectively. Mobile element types are indicated. A gene 93% identical to *cmx* is found in *Arthrobacter* sp. 161MFSha2.1. No *tnp45* gene is found in the same genome. On *C. glutamicum* plasmid pXZ10145.1, a basic form of *cmx* transposon with only *cmx* and *tnp45* inside was found. On *C. urealyticum* DSM 7109 chromosome, the *cmx* transposon duplicated, and the second copy has a new transposon Tn*5393* carrying two aminoglycoside phosphotransferases inserted between *cmx* and *tnp45*. On *P. aeruginosa* PA7 chromosome, the *cmx* transposon was found within a 53 kb integrated plasmid with conjugation proteins, transposases, integrase and four other ARGs. In *K. oxytoca*, the *cmx* transposon is located in an In*4*-like integron and a composite transposon.

Supplementary Table 1 | primer sequences used in cloning and sequencing

Primer name	Primer sequence 5’-3’
Xj143	TAGGGATAACAGGGTAATCTAGCTGTGATGTCCAGGGACGTTGTT
Xj144	ATTACCCTGTTATCCCTAAAATTGGAATCAGGTTTGTGCC
Xj145	ATTACCCTGTTATCCCTACGAGTTCCGTGCCGGTTG
Xj146	AATACCGCGCCACATAGCAG
Xj171.1	CAACAGAGCCTGGCGTAATAGCGAAGAGGC
Xj172	CACCGTCACCAGCTGTTTCCTGTGTGAAATTGTTA
Xj173	GGAAACAGCTGGTGACGGTGTTCGGCATTC
Xj174.1	TATTACGCCAGGCTCTGTTGCAAAGATTGGC
Xj177	TTATGCTTCCGGCTCGTATGTTGTGTGGATTCACTTTTTCTTCACAACCGG
Xj178	ACATACGAGCCGGAAGCATAAAGTGTAAAGTCATAATAAATCGATGCAGGTGG
Xj130	GGAAACAGCTATGCCTTTTGCCCTCTACATGC
Xj131	TGCGTAAGGAGGATAGCCGTCTTCGACAATCAG
Orf5-f-1	TGCCACCCGAACCTGCG
Tnp6100-r-1	ATCGGATAGCGACAATACCAG