

Supplementary information

Antonie van Leeuwenhoek

Duganella hordei sp. nov., *Duganella caerulea* sp. nov., and *Duganella rhizosphaerae* sp. nov., isolated from barley rhizosphere

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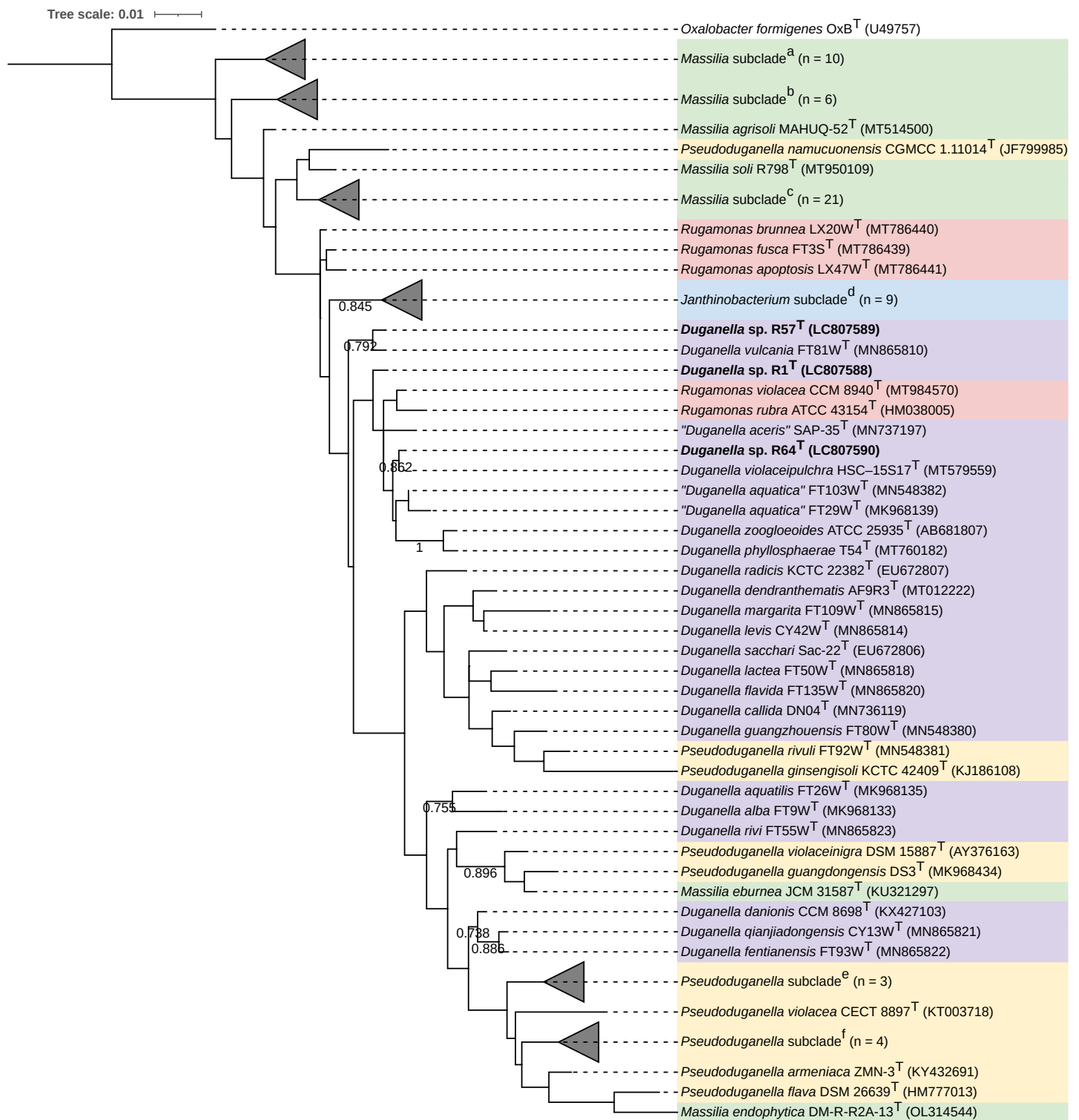


Fig. S1 Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences of R1^T, R57^T, R64^T, and their relative strains.

Oxalobacter formigenes OxB^T is used as the outgroup.

Bootstrap values (0.7-1) of 1000 replicates are shown. Bar, 0.01 substitutions per nucleotide position. *Massilia* subclade^a comprises *M. forsythiae* GN2-R2^T (MT012223), *M. rhizosphaerae* NEAU GH312^T (MW198488), *M. norwichensis* NS9^T (HG798294), *M. putida* 6NM-7^T (JQ608336), *M. pinisoli* T33^T (KU296190), *M. niastensis* 5516S-1^T (EU808005), *M. horti* ONC3^T (MT378212), *M. terrae* J11^T (KU870757), *M. solisilvae* J18^T (KU870758), and *M. agilis* J9^T (KU870756). *Massilia* subclade^b comprises *M. consociata* CCM 7792^T (MT760179), *M. yuzhufengensis* Y1243-1^T (JQ409016), *M. phyllostachyos* G4R7^T (MZ573411), *M. agri* K-3-1^T (KX672812), *M. varians* CCUG 35299^T (AM774587), and *M. alkalitolerans* YIM 31775^T (AY679161).

Massilia subclade^c comprises *M. jejuensis* 5317J-18^T (FJ969486), *M. brevitalea* byr23-80^T (EF546777), *M. niabensis* 5420S-26^T (EU808006), *M. suwonensis* 5414S-25^T (FJ969487), *M. haematophila* CCM 7480^T (MT758009), *M. aurea* CCM 7363^T (MT760134), *M. timonae* UR/MT95^T (U54470), *M. oculi* CCM 7900^T (MT760187), *M. puerhi* SJY3^T (MN014073), *M. arenae* GEM 5^T (KT369857), *M. polaris* RP-1-19^T (MN685328), *M. psychrophila* B1555-1^T (KM873051), *M. eurypsychrophila* B528-3^T (KJ361504), *M. glaciei* B448-2^T (KJ755877), *M. aquatica* CCM 8693^T (MN612031), *M. mucilaginosus* CCM 8733^T (MN612043), *M. atriviolacea* SOD^T (MH551481), *M. frigida* CCM 8695^T (MN612047), *M. antarctica* CCM 8941^T (OM243916), *M. violaceinigra* B 2^T (KF267246), and *M. rubra* CCM 8692^T (MN611986). *Janthinobacterium* subclade^d comprises *J. psychrotolerans* S3-2^T (LOCQ01000055), *J. agaricidamnosum* W1r3^T (Y08845), *J. violaceinigrum* FT13W^T (MK968136), *J. fluminis* hw3^T (ON668161), *J. aquaticum* FT58W^T (MN548378), *J. kumbetense* GK^T (MZ434955), *J. rivuli* FT68W^T (MN548379), *J. tructae* SNU WT3^T (MN524134), and *J. lividum* DSM 1522^T (MN307292). *Pseudoduganella* subclade^e comprises *P. buxica* CGMCC 1.15931^T (KX944690), *P. plicata* KCTC 12344^T (MT758059), and *P. lurida* CGMCC 1.10822^T (HQ839786). *Pseudoduganella* subclade^f comprises *P. dura* DSM 17513^T (MT758057), *P. umbonata* DSM 26121^T (HM053474), *P. albidiflava* DSM 17472^T (MT758058), and *P. lutea* DSM 17473^T (AY966001).

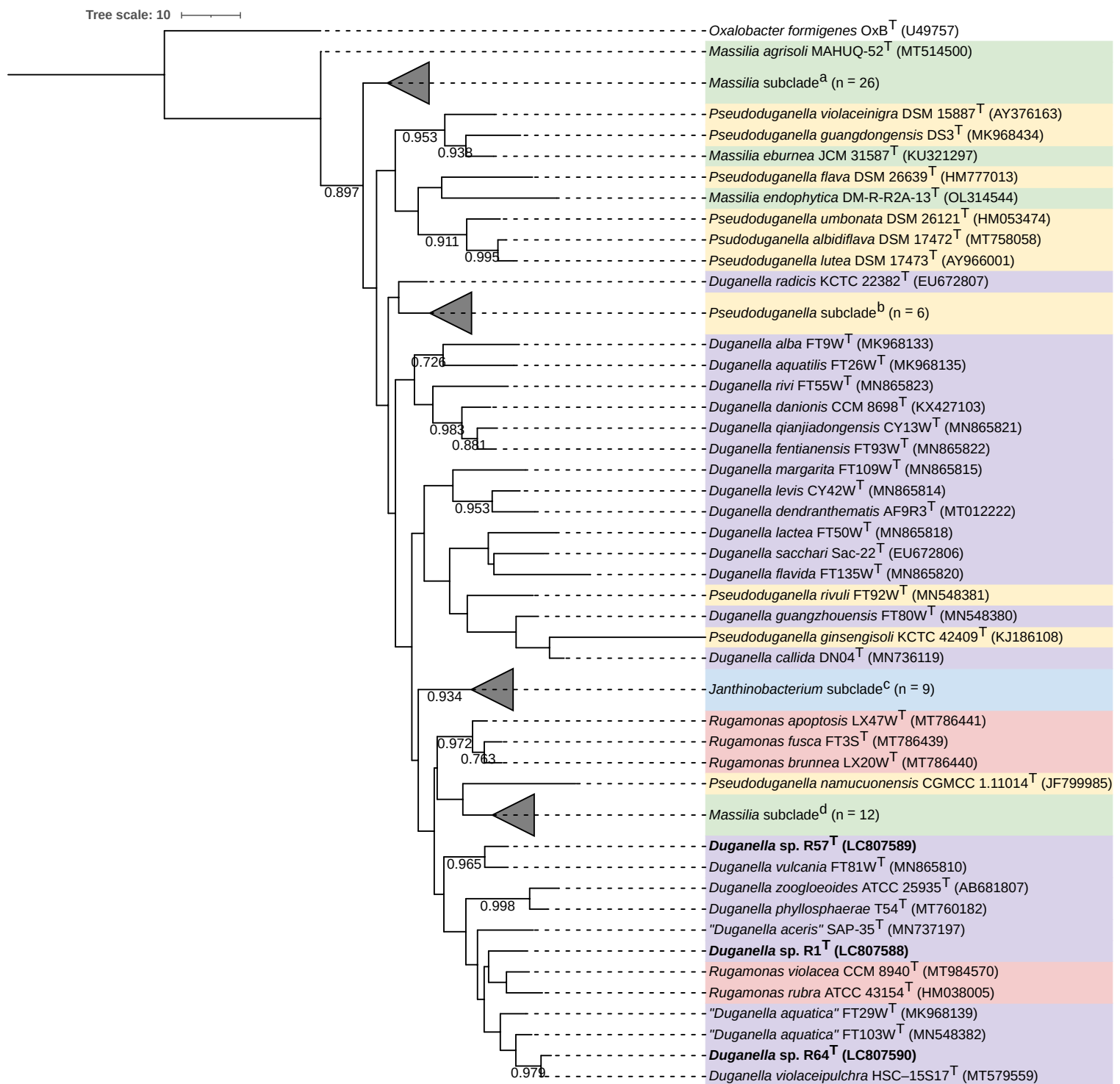


Fig. S2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of R1^T, R57^T, R64^T, and their relative strains. *Oxalobacter formigenes* OxB^T is used as the outgroup.

Bootstrap values (0.7-1) of 1000 replicates are shown. Bar, 10 substitutions per nucleotide position. *Massilia* subclade^a comprises *M. horti* ONC3^T (MT378212), *M. terrae* J11^T (KU870757), *M. solisilvae* J18^T (KU870758), *M. agilis* J9^T (KU870756), *M. consociata* CCM 7792^T (MT760179), *M. agri* K-3-1^T (KX672812), *M. yuzhufengensis* Y1243-1^T (JQ409016), *M. phyllostachyos* G4R7^T (MZ573411), *M. pinisoli* T33^T (KU296190), *M. niastensis* 5516S-1^T (EU808005), *M. forsythiae* GN2-R2^T (MT012223), *M. putida* 6NM-7^T (JQ608336), *M. rhizosphaerae* NEAU GH312^T (MW198488), *M. norwichensis* NS9^T (HG798294), *M. suwonensis* 5414S-25^T (FJ969487), *M. haematophila* CCM 7480^T (MT758009), *M. varians* CCUG 35299^T (AM774587), *M. alkalitolerans* YIM 31775^T (AY679161), *M. niabensis* 5420S-26^T (EU808006), *M. aurea* CCM 7363^T (MT760134), *M. jejuensis* 5317J-18^T (FJ969486), *M. brevitalea* byr23-80^T (EF546777), *M. oculi* CCM 7900^T (MT760187), *M. timonae* UR/MT95^T (U54470), *M. puerhi* SJY3^T (MN014073), and *M. arenae* GEM 5^T (KT369857). *Pseudoduganella* subclade^b comprises *P. violacea* CECT 8897^T (KT003718), *P. armeniaci* ZMN-3^T (KY432691), *P. lurida* CGMCC 1.10822^T (HQ839786), *P. dura* DSM 17513^T (MT758057), *P. pilcata* KCTC 12344^T (MT758059), and *P. buxica* CGMCC 1.15931^T (KX944690). *Janthinobacterium* subclade^c comprises *J. fluminis* hw3^T (ON668161), *J. psychrotolerans* S3-2^T (LOCQ01000055), *J. agaricidamnosum* W1r3^T (Y08845), *J. violaceinigrum* FT13W^T (MK968136), *J. aquaticum* FT58W^T (MN548378), *J. tructae* SNU WT3^T (MN524134), *J. kumbetense* GK^T (MZ434955), *J. rivuli* FT68W^T (MN548379), and *J. lividum* DSMZ 1522^T (MN307292). *Massilia* subclade^d comprises *M. soli* R798^T (MT950109), *M. polaris* RP-1-19^T (MN685328), *M. psychrophila* B1555-1^T (KM873051), *M. eurypsychrophila* B528-3^T (KJ361504), *M. glaciei* B448-2^T (KJ755877), *M. mucilaginis* CCM 8733^T (MN612043), *M. atriviolacea* SOD^T (MH551481), *M. aquatica* CCM 8693^T (MN612031), *M. antarctica* CCM 8941^T (OM243916), *M. frigida* CCM 8695^T (MN612047), *M. violaceinigra* B 2^T (KF267246), and *M. rubra* CCM 8692^T (MN611986).

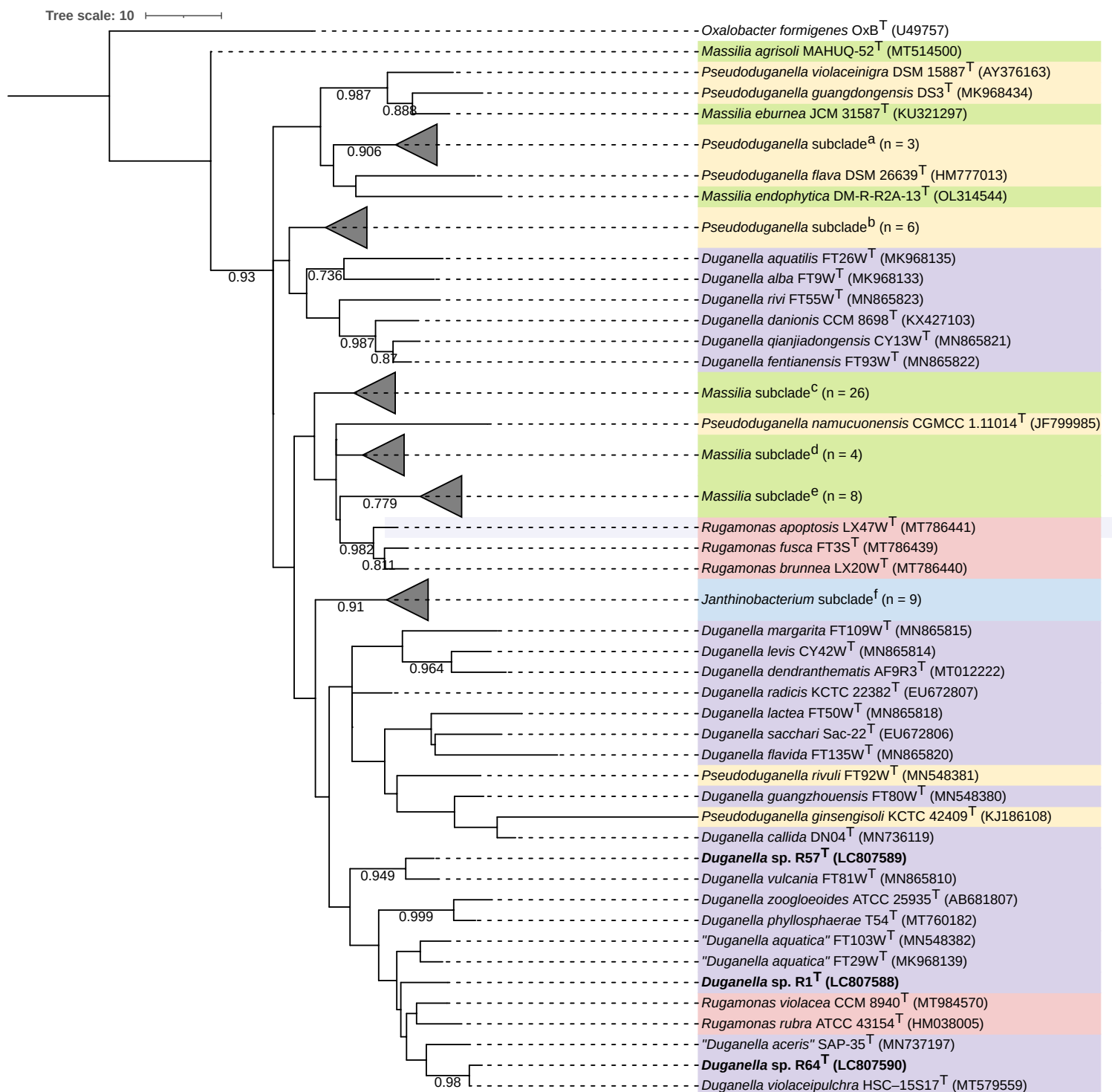


Fig. S3 Minimum-evolution phylogenetic tree based on 16S rRNA gene sequences of R1^T, R57^T, R64^T, and their relative strains. *Oxalobacter formigenes* OxB^T is used as the outgroup.

Bootstrap values (0.7-1) of 1000 replicates are shown. Bar, 10 substitutions per nucleotide position. *Pseudoduganella* subclade^a comprises *P. umbonata* DSM 26121^T (HM053474), *P. albidiflava* DSM 17472^T (MT758058), and *P. lutea* DSM 17473^T (AY966001). *Pseudoduganella* subclade^b comprises *P. violacea* CECT 8897^T (KT003718), *P. armeniacae* ZMN-3^T (KY432691), *P. lurida* CGMCC 1.10822^T (HQ839786), *P. dura* DSM 17513^T (MT758057), *P. plicata* KCTC 12344^T (MT758059), and *P. buxica* CGMCC 1.15931^T (KX944690). *Massilia* subclade^c comprises *M. niabensis* 5420S-26^T (EU808006), *M. aurea* CCM 7363^T (MT760134), *M. jejuensis* 5317J-18^T (FJ969486), *M. brevitalea* byr23-80^T (EF546777), *M. oculi* CCM 7900^T (MT760187), *M. timonae* UR/MT95^T (U54470), *M. puerhi* SJY3^T (MN014073), *M. arenae* GEM 5^T (KT369857), *M. suwonensis* 5414S-25^T (FJ969487), *M. haematophila* CCM 7480^T (MT758009), *M. varians* CCUG 35299^T (AM774587), *M. alkalitolerans* YIM 31775^T (AY679161), *M. yuzhufengensis* Y1243-1^T (JQ409016), *M. phyllostachyos* G4R7^T (MZ573411), *M. consociata* CCM 7792^T (MT760179), *M. agri* K-3-1^T (KX672812), *M. horti* ONC3^T (MT378212), *M. terrae* J11^T (KU870757), *M. solisilvae* J18^T (KU870758), *M. agilis* J9^T (KU870756), *M. pinisoli* T33^T (KU296190), *M. niastensis* 5516S-1^T (EU808005), *M. forsythiae* GN2-R2^T (MT012223), *M. putida* 6NM-7^T (JQ608336), *M. rhizosphaerae* NEAU GH312^T (MW198488), and *M. norwichensis* NS9^T (HG798294). *Massilia* subclade^d comprises *M. soli* R798^T (MT950109), *M. polaris* RP-1-19^T (MN685328), *M. psychrophila* B1555-1^T (KM873051), and *M. eurypsychrophila* B528-3^T (KJ361504). *Massilia* subclade^e comprises *M. glaciei* B448-2^T (KJ755877), *M. mucilaginis* CCM 8733^T (MN612043), *M. atriviolacea* SOD^T (MH551481), *M. aquatica* CCM 8693^T (MN612031), *M. antarctica* CCM 8941^T (OM243916), *M. frigida* CCM 8695^T (MN612047), *M. violaceinigra* B 2^T (KF267246), and *M. rubra* CCM 8692^T (MN611986). *Janthinobacterium* subclade^f comprises *J. fluminis* hw3^T (ON668161), *J. agaricidamnosum* W1r3^T (Y08845), *J. violaceinigrum* FT13W^T (MK968136), *J. aquaticum* FT58W^T (MN548378), *J. psychrotolerans* S3-2^T (LOCQ01000055), *J. tractae* SNU WT3^T (MN524134), *J. kumbetense* GK^T (MZ434955), *J. rivuli* FT68W^T (MN548379), and *J. lividum* DSMZ 1522^T (MN307292).

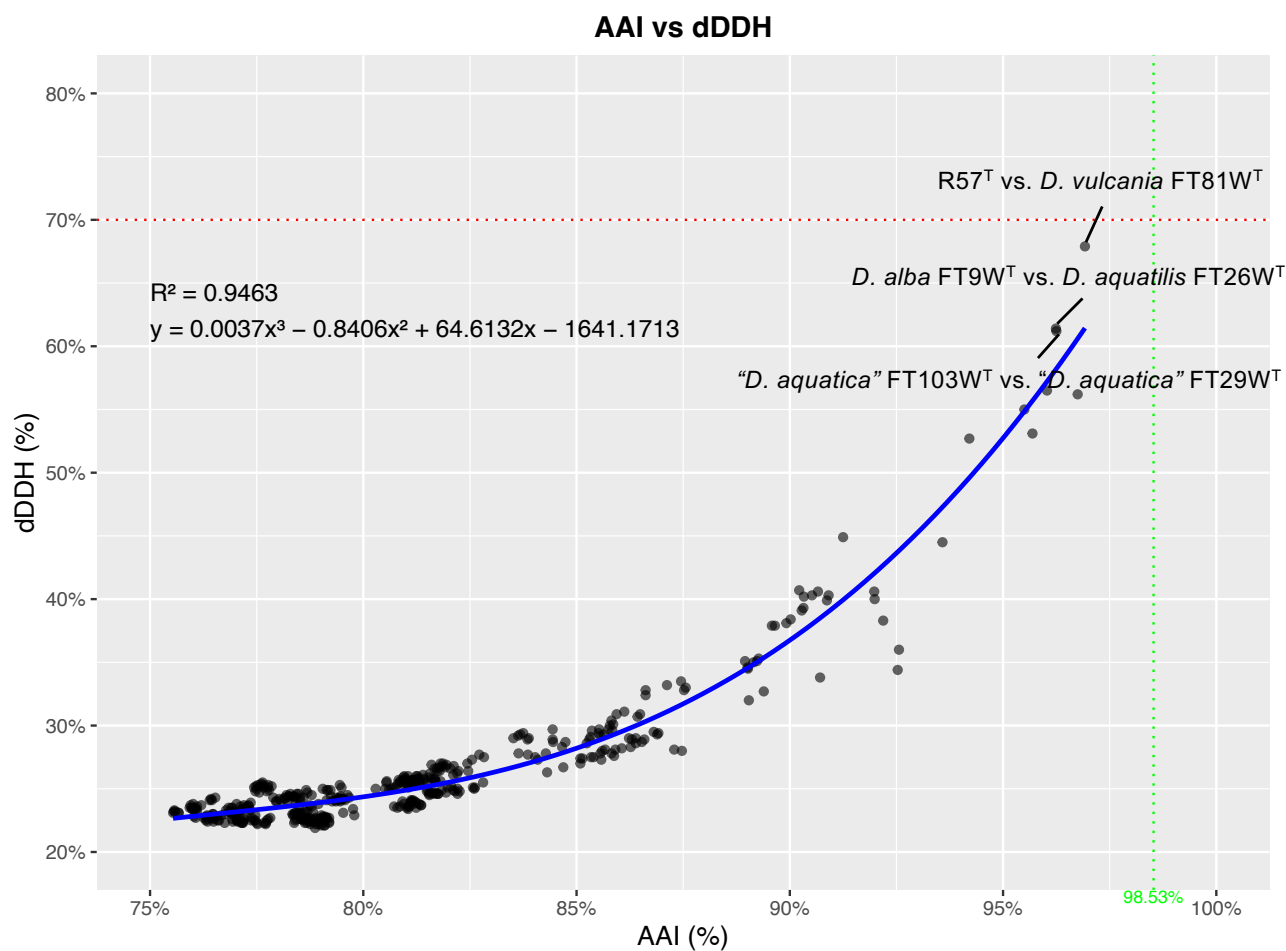
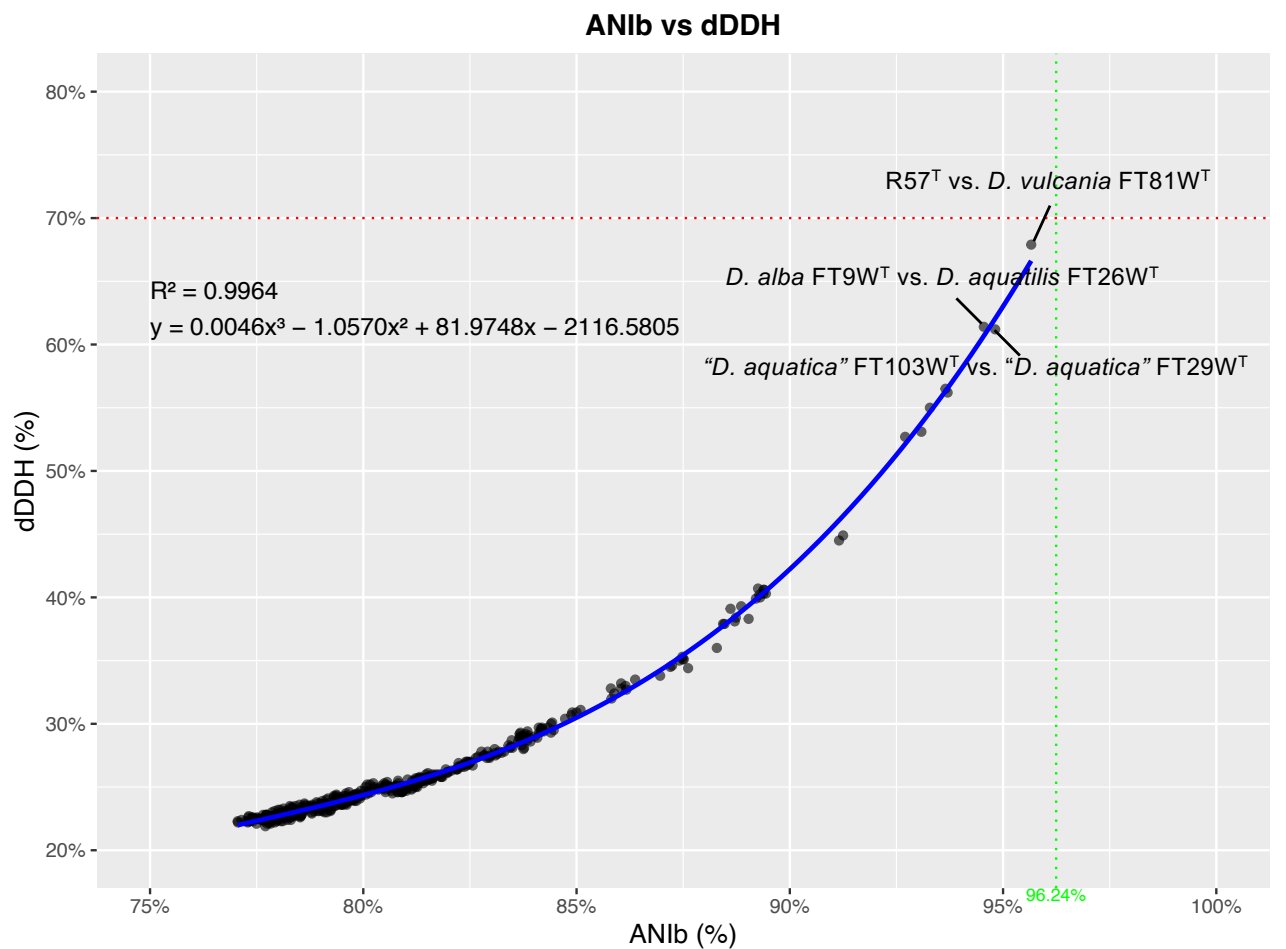
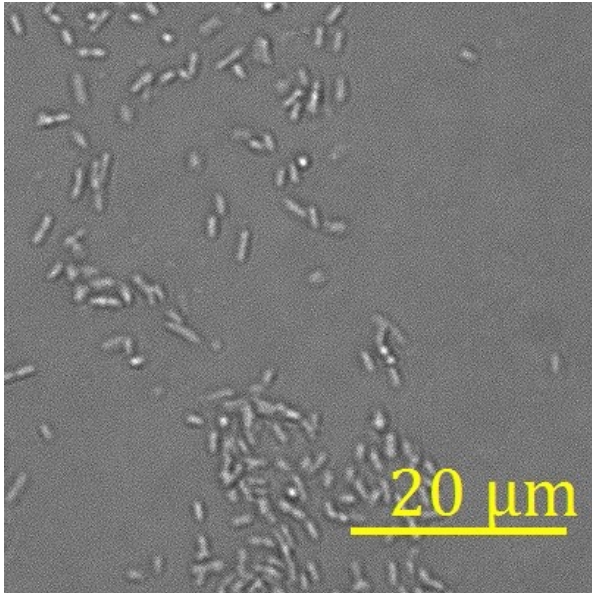
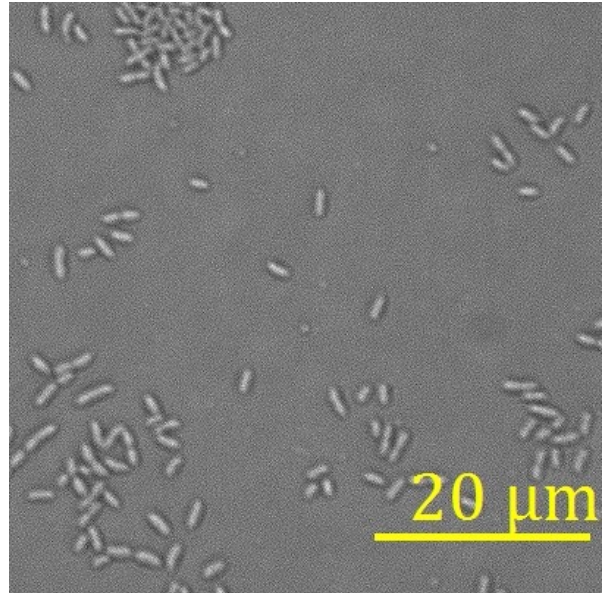


Figure S4 The correlations between ANib, dDDH, from the 435 pairs of *Duganella* and *Rugamonas* species (including synonyms). The data (Table S5) are graphed. The bidirectional ANib values are averaged. The correlation was analyzed using a quadratic regression, and the regression equations and R-squared values are shown within the graphs.

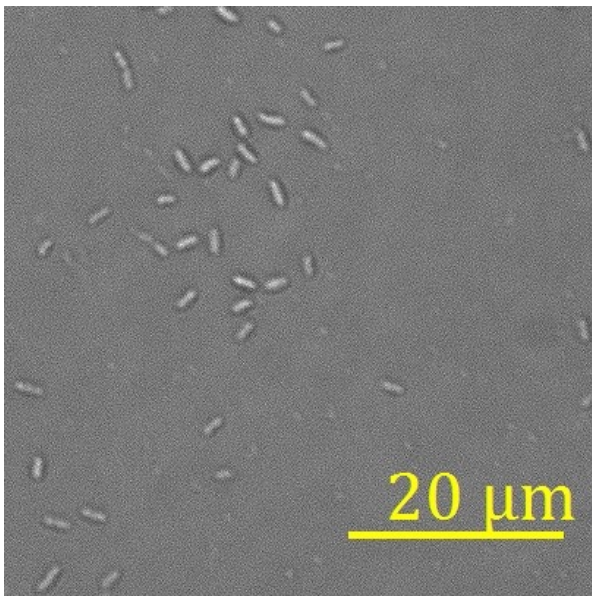
Duganella sp. R1^T



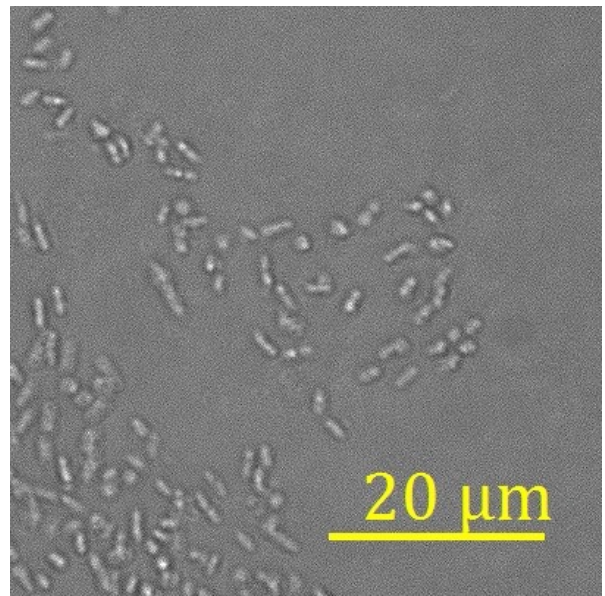
Duganella sp. R57^T



Duganella sp. R64^T



D. violaceipulchra HSC-15S17^T



D. vulcania KACC 21471^T

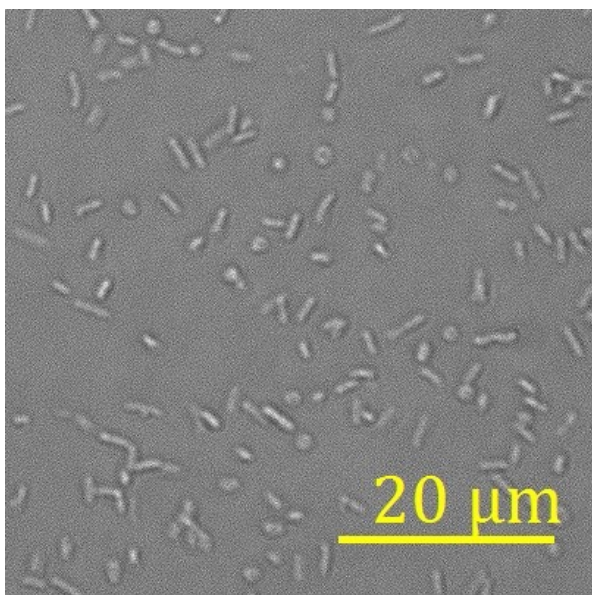


Figure S5 Microscopic images of strains R1^T, R57^T, R64^T, HSC-15S17^T, and KACC 21471^T

Cells were grown in R2A broth at 28°C for 2 days. Microscopic observations were performed using a fluorescence microscope (BZ-X700, Keyence, Japan) with a 111x immersion objective.

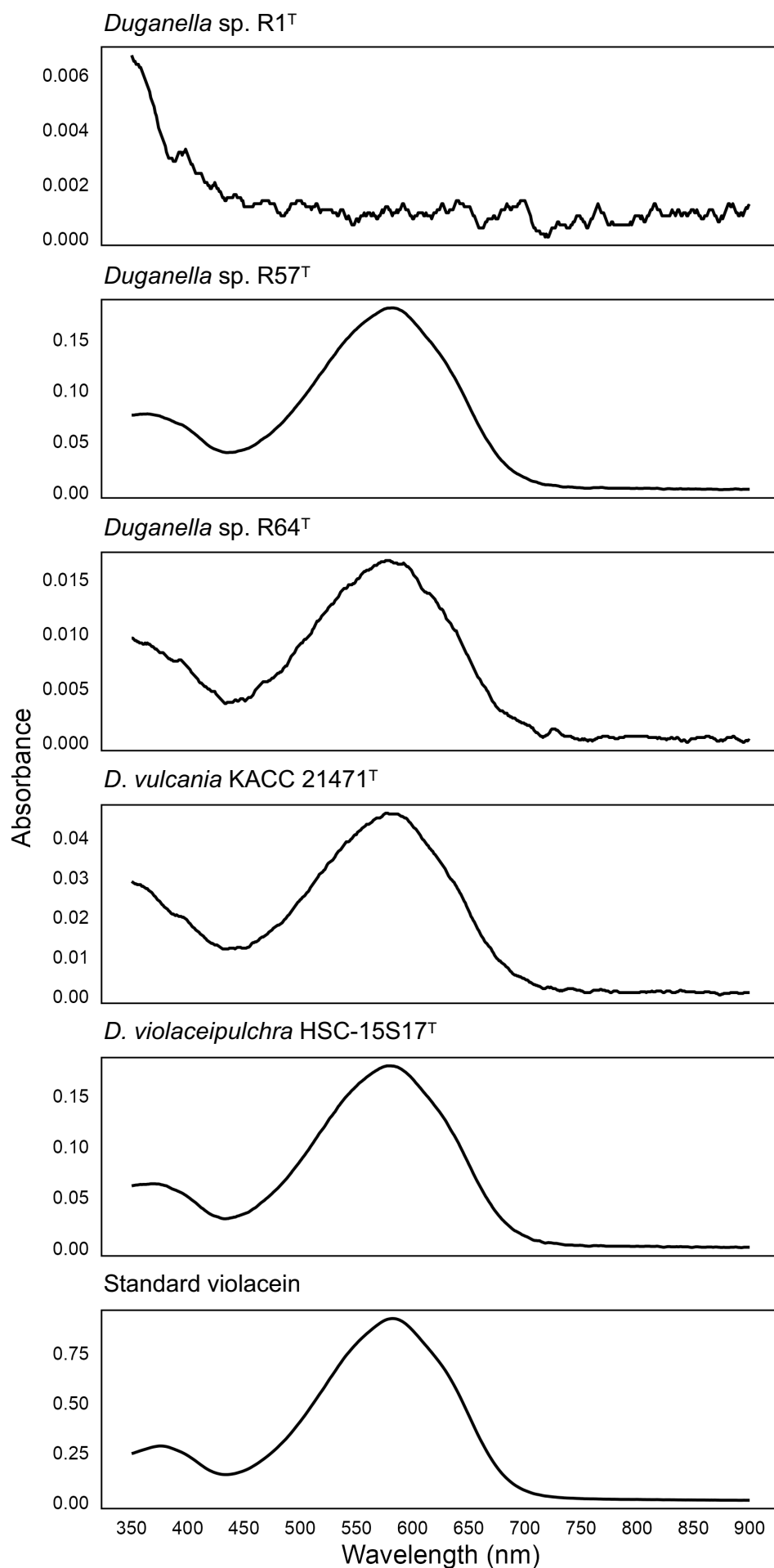


Figure S6 Absorption spectra of 1-butanol extracts of R1^T, R57^T, R64^T, KACC 21471^T, HSC-15S17^T, and standard violacein. Standard 0.1 mg/mL violacein (Cayman Chemical, USA) was dissolved in 1-butanol. The extracts were obtained by extracting strains with 1-butanol. Spectra were recorded using a microplate reader (DS Pharma Biomedical). All spectra, except that of *Duganella* sp. R1^T, exhibited absorption peaks at approximately 583 nm. *Duganella* sp. R1^T showed no detectable peak.