

# Supplementary material

## S1. Average nucleotide identity (ANI) comparisons

The NCBI accession numbers of the genome assemblies used to calculate the ANI score against RON18713 chromosome in Figure 1A of the paper.

NCBI Accession	Organism Name
GCA_000005845.2	Escherichia coli str. K-12 substr. MG1655
GCA_000008865.2	Escherichia coli O157:H7 str. Sakai
GCA_003697165.2	Escherichia coli ATCC 11775
GCA_003018455.1	Escherichia coli
GCA_024300685.1	Escherichia coli
GCA_027925825.1	Escherichia coli
GCA_003018035.1	Escherichia coli
GCA_027925745.1	Escherichia coli
GCA_027925805.1	Escherichia coli
GCA_027925765.1	Escherichia coli
GCA_000091565.1	Erwinia amylovora CFBP1430
GCA_017161565.1	Erwinia amylovora
GCA_015650045.1	Erwinia amylovora
GCA_002803865.1	Erwinia amylovora
GCA_027557715.1	Erwinia amylovora
GCA_012980785.1	Erwinia amylovora
GCA_012980845.1	Erwinia amylovora
GCA_017161545.1	Erwinia amylovora
GCA_012980825.1	Erwinia amylovora
GCA_023612715.1	Erwinia amylovora
GCA_019048385.1	Pantoea agglomerans
GCA_943184895.1	Pantoea agglomerans
GCA_021397715.1	Pantoea agglomerans
GCA_001709315.1	Pantoea agglomerans
GCA_017474165.1	Pantoea agglomerans
GCA_021559955.1	Pantoea agglomerans
GCA_029016125.1	Pantoea agglomerans

NCBI Accession	Organism Name
GCA_003704305.1	<i>Pantoea agglomerans</i>
GCA_022966105.1	<i>Pantoea agglomerans</i>
GCA_020783495.1	<i>Pantoea agglomerans</i>
GCA_000233595.1	<i>Pantoea ananatis</i> PA13
GCA_004028255.1	<i>Pantoea ananatis</i>
GCA_025765855.1	<i>Pantoea ananatis</i>
GCA_013364135.1	<i>Pantoea ananatis</i>
GCA_023845475.1	<i>Pantoea ananatis</i>
GCA_002224585.2	<i>Pantoea ananatis</i>
GCA_026075575.1	<i>Pantoea ananatis</i>
GCA_023611845.1	<i>Pantoea ananatis</i>
GCA_002952035.2	<i>Pantoea ananatis</i>
GCA_001543055.1	<i>Pantoea ananatis</i>
GCA_011044475.1	<i>Pantoea stewartii</i>
GCA_025765915.1	<i>Pantoea stewartii</i>
GCA_002082215.1	<i>Pantoea stewartii</i> subsp. <i>stewartii</i> DC283
GCA_000757405.2	<i>Pantoea stewartii</i> subsp. <i>indologenes</i>
GCA_008801695.1	<i>Pantoea stewartii</i> subsp. <i>stewartii</i>
GCA_029433915.1	<i>Pantoea stewartii</i>
GCA_001310285.1	<i>Pantoea stewartii</i>
GCA_013277595.1	<i>Pantoea stewartii</i>
GCA_025599245.1	<i>Pantoea stewartii</i>
GCA_014218605.1	<i>Pantoea stewartii</i>

## S2. Electronic PCR for *P. stewartii* subspecies differentiation

To distinguish between *P. stewartii* subspecies, Pal et al. (2019) designed primer pairs targeting the intergenic region of the *cpsA* and *cpsB* genes, that are contained in the Stewartan biosynthesis operon (*wce-I*). The primer pair *cpsF* (forward) and *cpsR* (reverse) can amplify this region in *P. stewartii* regardless of its subspecies. However, a 182 bp deletion was found in the *indologenes* subspecies, resulting in an expected amplicon size of 193 bp. In contrast, for the *stewartii* subspecies, the expected amplicon size is 375 bp.

The authors designed another forward primer (*cpsAB2313F*) annealing at the divergent region to further differentiate the subspecies. When used together with *cpsR*, this primer is not expected to generate a PCR product for the *indologenes* subspecies. In contrast, a PCR product of 193 bp is expected for the *stewartii* subspecies. The primer sequences are:

<i>cpsF</i>	AAGGTGCCAGCCTCTCTCTG
<i>cpsAB2313F</i>	AGAAAACGCTGATGCCAGAC
<i>cpsR</i>	ACTATCCTGACTCAGGCACT

Pal, N., Block, C. C., & Gardner, C. A. C. (2019). A Real-Time PCR Differentiating *Pantoea stewartii* subsp. *stewartii* From *P. stewartii* subsp. *indologenes* in Corn Seed. Plant disease, 103(7), 1474–1486. <https://doi.org/10.1094/PDIS-06-18-0936-RE>

To distinguish between the subspecies in various *P. stewartii* strains, we conducted an electronic PCR (ePCR) analysis using the *cpsF*-*cpsR* and *cpsAB2313F*-*cpsR* primer pair combinations. We used NCBI genomes as templates and included type strains for both subspecies (LMG 2632=*indologenes* and CCUG 26359=*stewartii*). The table below displays the ePCR results obtained from the primersearch program of the EMBOSS package. The expected amplicon sizes described above were used to diagnose the subspecies.

Strain	Accession	Subspecies	Amplicon		Diagnostics
			<i>cpsF</i> (bp)	<i>cpsAB2313F</i> (bp)	
RON18719	CP116285	unassigned	193	0	indologenes
A206	NZ_LIHC01000006	unassigned	193	0	indologenes
ZJ-FGZX1	NZ_CP049115	indologenes	193	0	indologenes
LMG 2632	NZ_JPKO01000031	indologenes	193	0	indologenes
DC283	NZ_CP017581	stewartii	375	256	stewartii
CCUG 26359	NZ_VZPF01000032	stewartii	375	256	stewartii

The primersearch results are shown below.

- *Pantoea stewartii* strain RON18719

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Primer name cpsF-cpsR
Amplimer 1
  Sequence: CP116285
  Pantoea stewartii strain RON18719, chromosome
  AAGGTGCCAGCCTCTCTCTG hits forward strand at 3300577 with 0
mismatches
  ACTATCCTGACTCAGGCACT hits reverse strand at [1033725] with
0 mismatches
  Amplimer length: 193 bp

Primer name cpsAB2313F-cpsR
Amplimer length: 0 bp

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- *Pantoea stewartii* strain A206

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Primer name cpsF-cpsR
Amplimer 1
  Sequence: NZ_LIHC01000006 NZ_LIHC01000006
  Pantoea stewartii strain A206 scaffold2.1, whole genome
shotgun sequence.
  AAGGTGCCAGCCTCTCTCTG hits forward strand at 486043 with 0
mismatches
  ACTATCCTGACTCAGGCACT hits reverse strand at [256853] with 0
mismatches
  Amplimer length: 193 bp

Primer name cpsAB2313F-cpsR
Amplimer length: 0 bp

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- *Pantoea stewartii* strain ZJ-FGZX1

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Primer name cpsF-cpsR
Amplimer 1
  Sequence: NZ_CP049115 NZ_CP049115
  Pantoea stewartii strain ZJ-FGZX1 chromosome, complete genome.
  ACTATCCTGACTCAGGCACT hits forward strand at 1196988 with 0
mismatches
  AAGGTGCCAGCCTCTCTCTG hits reverse strand at [3352893] with 0
mismatches
  Amplimer length: 193 bp

Primer name cpsAB2313F-cpsR
Amplimer length: 0 bp

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- *Pantoea stewartii* subsp. *indologenes* strain LMG 2632

```
Primer name cpsF-cpsR
Amplimer 1
Sequence: NZ_JPK001000031.1
Pantoea stewartii subsp. indologenes strain LMG 2632
Contig31, whole genome shotgun sequence
ACTATCCTGACTCAGGCACT hits forward strand at 139980 with 0
mismatches
AAGGTGCCAGCCTCTCTCTG hits reverse strand at [454151] with 0
mismatches
Amplimer length: 193 bp

Primer name cpsAB2313F-cpsR
Amplimer length: 0 bp
```

- *Pantoea stewartii* subsp. *stewartii* DC283

```
Primer name cpsF-cpsR
Amplimer 1
Sequence: NZ_CP017581 NZ_CP017581
Pantoea stewartii subsp. stewartii DC283 chromosome,
complete genome.
ACTATCCTGACTCAGGCACT hits forward strand at 3252402 with 0
mismatches
AAGGTGCCAGCCTCTCTCTG hits reverse strand at [1275440] with
0 mismatches
Amplimer length: 375 bp

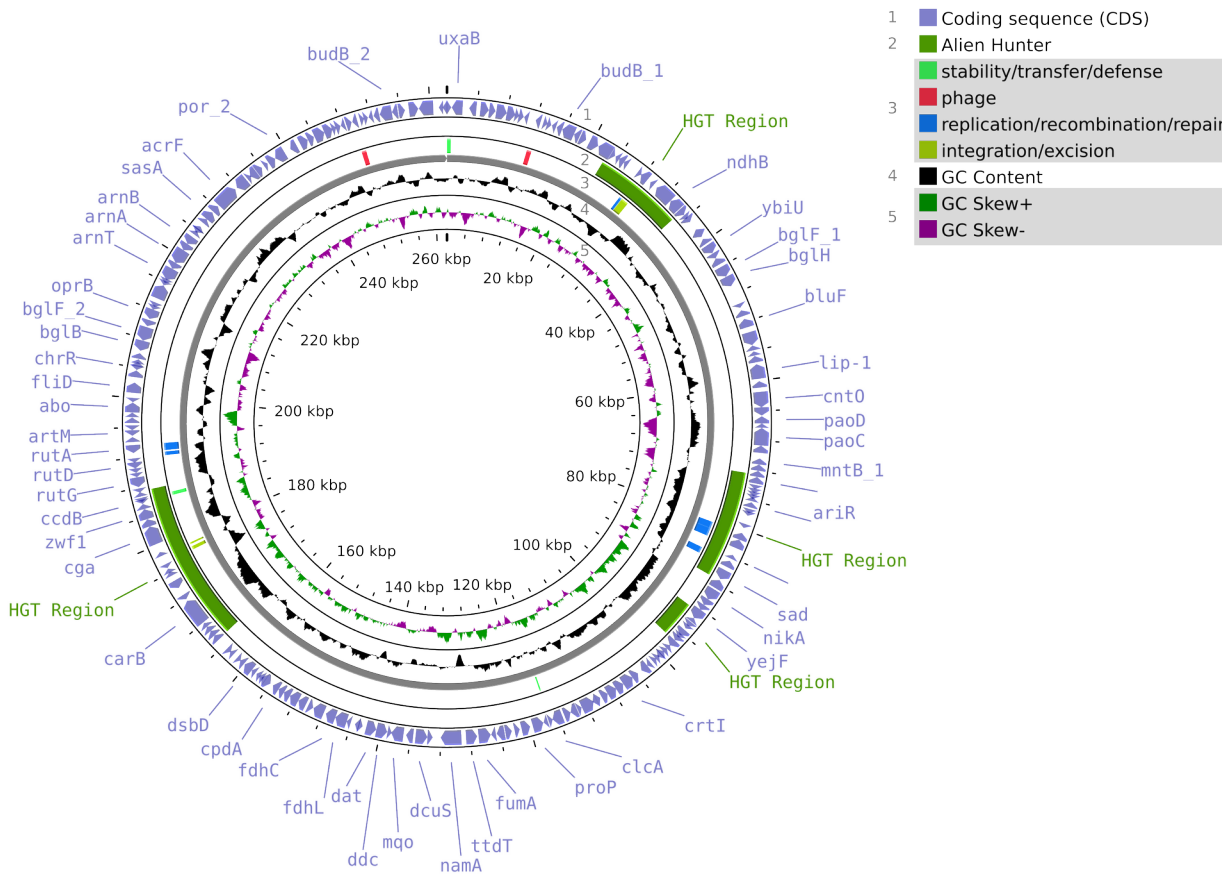
Primer name cpsAB2313F-cpsR
Amplimer 1
Sequence: NZ_CP017581 NZ_CP017581
Pantoea stewartii subsp. stewartii DC283 chromosome,
complete genome.
ACTATCCTGACTCAGGCACT hits forward strand at 3252402 with 0
mismatches
AGAAAACGCTGATGCCAGAC hits reverse strand at [1275559] with
0 mismatches
Amplimer length: 256 bp
```

- *Pantoea stewartii* subsp. *stewartii* strain CCUG 26359

```
Primer name cpsF-cpsR
Amplimer 1
Sequence: NZ_VZPF01000032.1
Pantoea stewartii subsp. stewartii strain CCUG 26359
contig_0000032, whole genome shotgun sequence
AAGGTGCCAGCCTCTCTCTG hits forward strand at 32986 with 0
mismatches
ACTATCCTGACTCAGGCACT hits reverse strand at [18045] with 0
mismatches
Amplimer length: 375 bp
```

Primer name cpsAB2313F-cpsR  
Amplimer 1  
Sequence: NZ\_VZPF01000032.1  
Pantoea stewartii subsp. stewartii strain CCUG 26359  
contig\_0000032, whole genome shotgun sequence  
AGAAAACGCTGATGCCAGAC hits forward strand at 33105 with 0  
mismatches  
ACTATCCTGACTCAGGCACT hits reverse strand at [18045] with 0  
mismatches  
Amplimer length: 256 bp

### S3. RON18713 plasmid map



*P. stewartii* RON18713 plasmid

Figure S1 - Circular representation of *P. stewartii* RON18713 assembled plasmid (GenBank: CP116286) generated by Proksee server (<https://proksee.ca>). Rings indicate the annotated coding sequences (CDS), horizontal gene transfer (HGT) events using Alien Hunter, mobile genetic elements (MGEs) using mobileOG-db (beatrix-1.6), GC content and GC skew. All annotations, except for the CDS were performed by Proksee's embedded analytical tools.