Supplementary Material belonging to

Xanthomonas hydrangeae sp. nov., a novel plant pathogen isolated from *Hydrangea arborescens*

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Table S1. Identification results of *Hydrangea* strains LMG 31884[T], LMG 31885, LMG 31886 and LMG 31887 and *X. hortorum* pv. hederae CFBP 4925[T] using a Bruker Biotyper MALDI-TOF MS instrument. Only Log(score) values above 1.7 (i.e. low and high confidence identification) were reported.

Strain	Spot no.	ID rank	Matched pattern	Log(score) value
LMG 31884[T] (CCOS 1956[T])	1	1	*Xanthomonas hortorum* DSM 50857	1.96
		2	*Xanthomonas hortorum* DSM 50859	1.93
		3	*Xanthomonas hortorum* DSM 50858	1.9
		4	*Xanthomonas cynarae* DSM 18811	1.83
		5	*Xanthomonas cynarae* DSM 16794[T]	1.73
	2	1	*Xanthomonas hortorum* DSM 50859	1.9
		2	*Xanthomonas hortorum* DSM 50858	1.84
		3	*Xanthomonas hortorum* DSM 50857	1.81
		4	*Xanthomonas cynarae* DSM 18811	1.71
LMG 31885 (CCOS 1954)	1	1	*Xanthomonas hortorum* DSM 50857	1.83
		2	*Xanthomonas hortorum* DSM 50858	1.8
		3	*Xanthomonas cynarae* DSM 18811	1.79
		4	*Xanthomonas hortorum* DSM 50859	1.78
		5	*Xanthomonas cynarae* DSM 16794[T]	1.73
	2	1	*Xanthomonas hortorum* DSM 50857	2.01
		2	*Xanthomonas cynarae* DSM 16794[T]	1.96
		3	*Xanthomonas cynarae* DSM 18811	1.96
		4	*Xanthomonas hortorum* DSM 50859	1.94
		5	*Xanthomonas hortorum* DSM 50858	1.93
		6	*Xanthomonas pisi* DSM 18956[T]	1.84
		7	*Xanthomonas campestris* DSM 50852[T]	1.7
LMG 31886 (CCOS 1957)	1	1	*Xanthomonas hortorum* DSM 50858	1.83
		2	*Xanthomonas hortorum* DSM 50857	1.82
		3	*Xanthomonas hortorum* DSM 50859	1.73
	2	1	*Xanthomonas hortorum* DSM 50857	1.96
		2	*Xanthomonas hortorum* DSM 50859	1.94
		3	*Xanthomonas hortorum* DSM 50858	1.87
		4	*Xanthomonas cynarae* DSM 18811	1.85
		5	*Xanthomonas campestris* DSM 3586[T]	1.82
		6	*Xanthomonas cynarae* DSM 16794[T]	1.76
		7	*Xanthomonas campestris* DSM 2405	1.71
LMG 31887 (CCOS 1955)	1	1	*Xanthomonas hortorum* DSM 50857	1.93
		2	*Xanthomonas hortorum* DSM 50859	1.87
		3	*Xanthomonas cynarae* DSM 18811	1.81
		4	*Xanthomonas hortorum* DSM 50858	1.8
		5	*Xanthomonas cynarae* DSM 16794[T]	1.77
	2	1	*Xanthomonas hortorum* DSM 50859	2.07
		2	*Xanthomonas hortorum* DSM 50858	2.06
		3	*Xanthomonas hortorum* DSM 50857	2.02
		4	*Xanthomonas cynarae* DSM 16794[T]	1.88
		5	*Xanthomonas cynarae* DSM 18811	1.88
		6	*Xanthomonas citri* pv. malvacearum DSM 3489	1.78
		7	*Xanthomonas arboricola* DSM 50854	1.7
X. hortorum pv. hederae CFBP 4925[T]	1	1	*Xanthomonas hortorum* DSM 50859	2.15
		2	*Xanthomonas hortorum* DSM 50857	2.11
		3	*Xanthomonas hortorum* DSM 50858	2.1
		4	*Xanthomonas cynarae* DSM 18811	2.02
		5	*Xanthomonas cynarae* DSM 16794[T]	2.01
	2	1	*Xanthomonas hortorum* DSM 50857	2.27
		2	*Xanthomonas hortorum* DSM 50858	2.26
		3	*Xanthomonas hortorum* DSM 50859	2.23
		4	*Xanthomonas cynarae* DSM 16794[T]	2.2
		5	*Xanthomonas cynarae* DSM 18811	2.05

Table S2. Fatty acid methyl ester (FAME) analysis of *Hydrangea* strains LMG 31884^T and LMG 31886, and five *Xanthomonas hortorum* pathovars (pvs. carotae, pelargonii, hederae, taraxaci and vitians). Fatty acids characteristic of the *Xanthomonas* genus are displayed in bold.

Fatty acid*	New species‡		*X. hortorum* subcluster B			*X. hortorum* subcluster A	
	LMG 31884^T	LMG 31886	CFBP 2533^{PT}	CFBP 7900	CFBP 4925^T	NCPPB 940^{PT}	LMG 938^{neoPT}
$C_{10:0}$	4.99	1.12	1.13	1.56	2.32	1.65	1.47
$C_{14:0}$	TA	2.77	1.79	2.58	3.06	2.72	2.86
$C_{16:0}$	2.68	6.53	3.9	3.79	9.41	4.48	9.96
$C_{17:0}$	TA	ND	TA	ND	TA	ND	ND
$C_{10:0}$ 3-OH	TA	TA	TA	TA	TA	TA	TA
iso-$C_{10:0}$	ND	ND	TA	ND	ND	ND	ND
$C_{11:0}$ 3-OH	TA	TA	TA	TA	TA	TA	ND
anteiso-$C_{11:0}$	TA	ND	TA	TA	TA	ND	ND
iso-$C_{11:0}$	2.88	2.44	4.01	3.74	2.24	3.76	2.99
iso-$C_{11:0}$ 3-OH	1.18	1.06	1.81	1.61	TA	2.07	1.38
$C_{12:0}$ 3-OH	1.4	2.48	2.52	3.67	2.04	2.32	2.67
iso-$C_{12:0}$ 3-OH	ND	ND	TA	ND	ND	TA	ND
$C_{13:0}$ 2-OH	TA	TA	TA	TA	TA	ND	ND
anteiso-$C_{13:0}$	ND	ND	TA	ND	ND	ND	ND
iso-$C_{13:0}$	ND	ND	TA	ND	ND	TA	ND
iso-$C_{13:0}$ 3-OH	2.02	1.89	2.12	1.97	TA	1.89	1.14
iso-$C_{14:0}$	TA	1.2	1.01	TA	1.16	2.66	TA
$C_{14:1}\omega 5c$	ND	ND	TA	ND	ND	ND	ND
anteiso-$C_{15:0}$	24.58	18.21	18.61	24.82	20.23	7.99	12.24
iso-$C_{15:0}$	18.53	21.57	23.56	18.53	14.38	29.81	20.61
$C_{15:1}\omega 6c$	2.83	2.86	1.47	1.9	5.01	1.05	TA
$C_{15:1}\omega 8c$	TA	ND	TA	ND	TA	TA	ND
iso-$C_{16:0}$	4.45	2.65	2.81	2.81	3.09	5.93	1.71
$C_{16:1}\omega 9c$	1.78	2.7	1.94	1.94	2.19	2.8	4.43
anteiso-$C_{17:0}$	1.3	ND	TA	TA	ND	ND	ND
iso-$C_{17:0}$	4.38	2.62	3.35	2	1.92	3.06	3.55
$C_{17:1}\omega 6c$	ND	ND	TA	ND	TA	ND	ND
$C_{17:1}\omega 8c$	3.16	2.49	1.68	1.45	3.83	1.33	1.29
$C_{18:1}\omega 9c$	ND	ND	ND	ND	TA	ND	ND
Summed feature 3	14.52	24.33	21.49	22.21	22.36	22.13	30.12
Summed feature 9	4.76	1.43	2.39	1.38	TA	2.07	1.78

*Summed in feature 3: $C_{16:1}\omega 7c$/$C_{16:1}\omega 6c$; summed in feature 9: iso-$C_{17:1}\omega 9c$.

‡ TA: trace amounts (below 1%); ND: not detected.

Table S3. Phenotypic characteristics of the *Hydrangea* strains and its closest phylogenetic relative *Xanthomonas hortorum* characterized in triplicates using Biolog GEN III MicroPlates.

The substrates that can distinguish the *Hydrangea* strains from *X. hortorum* are indicated in bold. Strains: 1: LMG 31884[T]; 2: LMG 31885; 3: *X. hortorum* pv. pelargonii CFBP 2533[PT]; 4: *X. hortorum* pv. carotae CFBP 7900; 5: *X. hortorum* pv. hederae CFBP 4925[T]; 6: *X. hortorum* pv. taraxaci NCPPB 940[PT]; 7: *X. hortorum* pv. vitians LMG 938[neoPT]; 8: *X. hortorum* pv. cynarae CFBP 4188[PT]; 9: *X. hortorum* pv. gardneri ATCC 19865[PT]; 10: *X. campestris* pv. campestris ATCC 33913[T].

Characteristic	1	2	3[*]	4[*]	5[*]	6[*]	7[*]	8[*]	9[*]	10[‡]
Utilization of:										
Dextrin	w[‡]	w	-	-	w(-)	-	-	-	-	+
D-Maltose	-	-	-	-	-	-	-	-	-	+
D-Trehalose	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+
β-Gentiobiose	v	+	+	+	-(+)	+	+	-	-	+
Sucrose	+	+	+	+	+	+	+	+	+	V+
D-Turanose	-	-	-	-	-	-	-	-	-	V-
Stachyose	-	-	-	-	-	-	-	-	-	NA
D-Raffinose	-	-	-	-	+(-)	-	-	-	-	V+
α-D-Lactose	-(+)	+	-	-	+(-)	-	+(-)	-	+	-
D-Melibiose	+	+	+	+	+	+	+	+	+	+
β-Methyl-D-Glucoside	-	-	-	-	-	-	-	-	-	-
D-Salicin	-	-	-	-	-	-	-	-	-	NA
N-Acetyl-D-Glucosamine	+	+	+	+	+	+	+	+	+	-
N-Acetyl-β-D-Mannosamine	-	-	-	-	-	-	-	-	-	NA
N-Acetyl-D-Galactosamine	-	-	-	-	-	-	-	-	-	-
N-Acetyl Neuraminic Acid	-	-	-	-	-	-	-	-	-	NA
α-D-Glucose	+	+	+	+	+	+	+	+	+	-
D-Mannose	+	+	+	+	+	+	+	+	+	V+
D-Fructose	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+
3-Methyl Glucose	w(-)	-	-	-	-	-	-	-	-	NA
D-Fucose	w	w(-)	-	-	w(-)	-	-	-	-	NA
L-Fucose	+	+	+	+	+	+	+	+	+	V+
L-Rhamnose	-	-	-	-	-	-	-	-	-	-
Inosine	w	w(-)	-	-	-	-	-	-	-	V-
D-Sorbitol	-	-	-	-	-	-	-	-	-	-
D-Mannitol	-	-	-	-	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-	-	-	-	NA
myo-Inositol	-	-	-	-	-	-	-	-	-	NA
Glycerol	+	+	+	+	+	+	+	+	-	V
D-Glucose-6-PO4	-	-	-	-	-	-	-	-	-	V-
D-Fructose-6-PO4	**w**	**w**	-	-	-	-	-	-	-	**NA**
D-Aspartic Acid	-	-	-	-	-	-	-	-	-	NA
D-Serine	-	-	-	-	-	-	-	-	-	NA
Gelatin	+	+	+	+	+	+	+	+	+	NA
Glycyl-L-Proline	+	+	+	+	+	+	+	+	v	V-
L-Alanine	+	+	+	+	+	+	+	w	+	V+
L-Arginine	-	-	-	-	-	-	-	-	-	NA
L-Aspartic Acid	+	w(+)	-	+	w(-)	w(-)	v	w(-)	+	V-
L-Glutamic Acid	+	+	+	+	+	+	+	+	+	+
L-Histidine	v	+	+	-	+	+	-(+)	w(+)	+	-
L-Pyroglutamic Acid	-	-	-	-	-	-	-	-	-	-
L-Serine	+	+	+	+	+	+	+	+	+	V+
Pectin	+	+	+	+	+	+	+	-	+	NA
D-Galacturonic Acid	w	w(-)	-	-	-	-	-	-	-	-
L-Galactonic Acid Lactone	w	-	-	-	-	-	-	-	-	-

D-Gluconic Acid	-	-	-	-	-	-	-	-	-	V-
D-Glucuronic Acid	w	w	-	-	w(-)	-	-	-	-	-
Glucuronamide	**w**	**w**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Mucic Acid	w(-)	-	-	-	-	-	-	-	-	NA
Quinic Acid	-	-	-	-	-	-	-	-	-	-
D-Saccharic Acid	-	-	-	-	-	-	-	-	-	V+
p-Hydroxy Phenylacetic Acid	-	-	-	-	-	-	-	-	-	-
Methyl Pyruvate	+	+	+	+	+	+	+	+	+	+
D-Lactic Acid Methyl Ester	-	-	-	-	-	-	-	-	-	NA
L-Lactic Acid	+	+	+	w(-)	+	+(-)	+	w	+	V+
Citric Acid	+	+	+	+	+	+	+	+	+	V+
α-Keto-Glutaric Acid	+	+	+	+	+	+	+	+	+	+
D-Malic Acid	w(-)	w	-	-	-	-	-	-	-	NA
L-Malic Acid	+	+	+	+	+	+	+	+	+	NA
Bromo-Succinic Acid	+	+	w(+)	+	+	+	+	+	+	+
Tween 40	+	+	w(+)	+	w	w(+)	+	w(-)	+	V+
γ-Amino-Butryric Acid	-	-	-	-	-	-	-	-	-	-
α-Hydroxy-Butyric Acid	+	w(+)	-	w(-)	w(-)	v	-	-	w(-)	-
β-Hydroxy-D,L-Butyric Acid	-	-	-	-	-	-	-	-	-	-
α-Keto-Butyric Acid	+	w	-	+(-)	+	-(+)	+	v	+	V+
Acetoacetic Acid	+	w(+)	w(+)	-	+	+	w(+)	w	+	NA
Propionic Acid	+	+	+	+	+	+	+	+	+	V+
Acetic Acid	+	+	+	+	+	+	+	+	+	V-
Formic Acid	+	w	+	+(-)	-(+)	+(-)	w(+)	+(-)	+	-
Growth in presence of:										
pH 6	+	+	+	+	+	+	+	+	+	NA
pH 5	w(-)	-	-	-(+)	-	-	-	-(+)	-	NA
1% NaCl	+	+	+	+	+	+	+	+	+	NA
4% NaCl	w	w	+	+	w(-)	-	+	v	+	NA
8% NaCl	-	-	-	-	-	-	-(+)	-	-	NA
1% Sodium Lactate	+	+	+	+	+	+	+	+	+	NA
Fusidic Acid	w(-)	-	-	-	-	-	-	-	-	NA
D-Serine	w(-)	-	-	-	-	-	+(-)	-	-	-
Troleandomycin	w(-)	-	-(+)	v	-	-	-	-	-	NA
Rifamycin SV	+	+	-	+	-(+)	-(+)	-(+)	+(-)	-	NA
Minocycline	w(-)	-	-	-	-	-	-	-	-	NA
Lincomycin	+	+	+	+	+	+	+	+	+	NA
Guanidine hydrochloride	w	w(-)	+(-)	+	+	+(-)	+(-)	+	+(-)	NA
Niaproof 4	w	w	+(-)	+	w(-)	v	w(-)	v	-	NA
Vancomycin	w(+)	w	v	w(-)	-	-	-	-	-	NA
Tetrazolium Violet	+	+	+	+	-(+)	+(-)	+	+(-)	+	NA
Tetrazolium Blue	+	+	+	+	+	+(-)	+	+	+	NA
Nalidixic Acid	w(-)	-	-	-	-	-	-	-	-	NA
Lithium Chloride	**-**	**-**	**+(-)**	**+**	**+(-)**	**-(+)**	**+(-)**	**+(-)**	**w(-)**	**NA**
Potassium Tellurite	-	-	+	+	w(+)	-	+	-	-	NA
Aztreonam	+	w(-)	+	+	+	+	+	+	+(-)	NA
Sodium Butyrate	w(-)	-	-	-	-	-	-	-	-	NA
Sodium Bromate	-	-	+	v	-	-	w(+)	v	w(-)	NA

[*] Phenotypic profiles of subclusters A and B of *X. hortorum* were obtained from Morinière *et al.*, 2020.

[‡] Phenotypic profiles of *X. campestris* pv. campestris ATCC 33913[T] where adapted from Vauterin *et al.*, 1995 based on the profiles of homology group 15: + or – indicate unequivocally positive or negative results, respectively while V+ or V- indicate activities in more or less than 50% of tested strains within homology group 15. Non-tested strains are noted with "NA".

[‡] Phenotypes were categorized as positive (+), weak (w) or negative (-). Triplicates were managed in the same way described by Morinière *et al.* (2020) to ensure comparability of results: + = (+/+/+) or (+/+/w), w = (w/w/w), − = (−/−/−) or (−/−/w), +(−) = (+/+/−), −(+) = (−/−/+), w(+) = (w/w/+), w(−) = (w/w/−), v = (−/w/+).

Table S4. Accession numbers of the query sequences from *Xanthomonas hortorum* pv. carotae M081 (=CFBP 7900) used to retrieve the closest orthologs of the seven housekeeping genes used in the MLSA of the 20 *Xanthomonas* genomes analyzed in this study.

Gene	Accession	Annotation
atpD	ETC89870.1	ATP synthase beta chain [*X. hortorum* pv. carotae str. M081]
dnaK	ETC88662.1	molecular chaperone DnaK [*X. hortorum* pv. carotae str. M081]
efp	ETC87912.1	elongation factor EF-P [*X. hortorum* pv. carotae str. M081]
glnA	ETC90258.1	glutamine synthetase [*X. hortorum* pv. carotae str. M081]
gyrB	ETC90400.1	DNA gyrase subunit B [*X. hortorum* pv. carotae str. M081]
lepA	ETC88931.1	GTP binding protein [*X. hortorum* pv. carotae str. M081]
rpoD	ETC85548.1	RNA polymerase sigma-70 factor [*X. hortorum* pv. carotae str. M081]

Table S5. Contig accession and position of the seven housekeeping genes used in the MLSA of the 20 *Xanthomonas* genomes analyzed in this study.

Strain	Locus	Contig accession	Position on contig
Strain LMG 31884[T]	*atpD*	LR990730.1	4491320-4492726
	dnaK		3443005-3444930
	efp		2400320-2400886
	glnA		274050-275453
	gyrB		4653-7094
	lepA		3654850-3656640
	rpoD		4689256-4691133
Strain LMG 31885	*atpD*	LR990741.1	2734342-2735748
	dnaK		3770880-3772805
	efp		4832158-4832724
	glnA		1603997-1605400
	gyrB		1868791-1871232
	lepA		3557349-3559139
	rpoD		2551426-2553303
Strain LMG 31886	*atpD*	LR990739.1	4391706-4393112
	dnaK		3354785-3356710
	efp		4832158-4832724
	glnA		270480-271883
	gyrB		4653-7094
	lepA		3568453-3570243
	rpoD		4574150-4576027
Strain LMG 31887	*atpD*	LR990736.1	4484781-4486187
	dnaK		3436316-3438241
	efp		2400535-2401101
	glnA		274069-275472
	gyrB		4653-7094
	lepA		3648159-3649949
	rpoD		4682724-4684601
Xanthomonas hortorum pv. pelargonii CFBP 2533[PT]	*atpD*	LR828261.1	4386660-4388066
	dnaK		3056803-3058731
	efp		2119935-2120501
	glnA		299855-301258
	gyrB		4652-7093
	lepA		3578157-3579947
	rpoD		4528768-4530645
Xanthomonas hortorum pv. carotae CFBP 7900	*atpD*	CAJDKC010000005.1	274062-275468
	dnaK	CAJDKC010000004.1	471200-473125
	efp	CAJDKC010000004.1	2218305-2218871

	glnA	CAJDKC010000003.1	151620-153023
	gyrB	CAJDKC010000001.1	72003-74444
	lepA	CAJDKC010000004.1	755850-757640
	rpoD	CAJDKC010000005.1	397632-399509
Xanthomonas hortorum pv. hederae CFBP 4925[T]	*atpD*	MDEF01000028.1	22052-23458
	dnaK	MDEF01000026.1	41412-43337
	efp	MDEF01000004.1	4635-5201
	glnA	MDEF01000005.1	69767-71170
	gyrB	MDEF01000069.1	23018-25459
	lepA	MDEF01000011.1	24270-26060
	rpoD	MDEF01000013.1	72447-74324
Xanthomonas hortorum pv. taraxaci NCPPB 940[PT]	*atpD*	LR828264.1	771419-772825
	dnaK		3148958-3150883
	efp		2257451-2258017
	glnA		276138-277541
	gyrB		4655-7099
	lepA		3376876-3378666
	rpoD		621099-622976
Xanthomonas hortorum pv. cynarae CFBP 2044	*atpD*	LR828251.1	4282071-4283477
	dnaK		1662490-1664415
	efp		2645167-2645733
	glnA		282580-283983
	gyrB		4654-7095
	lepA		3489241-3491031
	rpoD		4423903-4425780
Xanthomonas hortorum pv. cynarae CFBP 4188[PT]	*atpD*	MDFM01000006.1	142597-144003
	dnaK	MDFM01000026.1	25820-27745
	efp	MDFM01000003.1	118349-118915
	glnA	MDFM01000008.1	73430-74833
	gyrB	MDFM01000011.1	58502-60943
	lepA	MDFM01000014.1	114773-116563
	rpoD	MDFM01000005.1	108307-110184
Xanthomonas hortorum pv. gardneri ATCC 19865[PT]	*atpD*	AEQX01000203.1	24651-26057
	dnaK	AEQX01000108.1	12928-14853
	efp	AEQX01000194.1	4980-5546
	glnA	AEQX01000078.1	14039-15442
	gyrB	AEQX01000329.1	13807-16248
	lepA	AEQX01000153.1	33014-34804
	rpoD	AEQX01000519.1	43590-45467
Xanthomonas hortorum pv. gardneri CFBP 8129	*atpD*	LR828253.1	4338765-4340171
	dnaK		3261933-3263858
	efp		2845007-2845573

	glnA		279082-280485
	gyrB		4654-7095
	lepA		3536777-3538567
	rpoD		4496488-4498365
Xanthomonas hortorum pv. vitians ICMP 7383	*atpD*	CP018731.1	856358-857764
	dnaK		3375385-3377310
	efp		2431297-2431863
	glnA		5212489-5213892
	gyrB		71064-73508
	lepA		1692751-1694541
	rpoD		810539-812416
Xanthomonas hortorum pv. gardneri JS749-3	*atpD*	CP018728.1	1386657-1388063
	dnaK	CP018728.1	2463117-2465042
	efp	CP018728.1	2880660-2881226
	glnA	CP018728.1	288768-290171
	gyrB	CP018728.1	562165-564606
	lepA	CP018728.1	2188408-2190198
	rpoD	CP018728.1	1228320-1230197
Xanthomonas hortorum pv. vitians LMG 938[neoPT]	*atpD*	SMED01000029.1	4609-6015
	dnaK	SMED01000027.1	11560-13485
	efp	SMED01000004.1	102416-102982
	glnA	SMED01000007.1	125648-127051
	gyrB	SMED01000006.1	177554-179998
	lepA	SMED01000002.1	50908-52698
	rpoD	SMED01000023.1	33062-34939
Xanthomonas hortorum pv. vitians CFBP 498	*atpD*	LR828257.1	1615618-1617024
	dnaK		4180215-4182140
	efp		3258510-3259076
	glnA		568705-570108
	gyrB		834318-836762
	lepA		2523395-2525185
	rpoD		1478989-1480866
[*Xanthomonas campestris*] pv. nigromaculans NCPPB 1935	*atpD*	Contig 1[*]	847858-849264
	dnaK		1964947-1966872
	efp		2388552-2389118
	glnA		285486-286889
	gyrB		4654-7095
	lepA		1685571-1687361
	rpoD		4480432-4482309
Xanthomonas populi CFBP 1817[T]	*atpD*	NZ_MDEJ01000111.1	4811-6217
	dnaK	NZ_MDEJ01000011.1	10311-12236
	efp	NZ_MDEJ01000063.1	15471-16037

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	glnA	NZ_MDEJ01000004.1	45012-46415
	gyrB	NZ_MDEJ01000165.1	1800-4244
	lepA	NZ_MDEJ01000021.1	27645-29435
	rpoD	NZ_MDEJ01000012.1	1161-3038
Xanthomonas arboricola pv. juglandis CFBP 2528[T]	*atpD*	JZEF01000004.1	314563-315969
	dnaK	JZEF01000005.1	468014-469939
	efp	JZEF01000001.1	992726-993292
	glnA	JZEF01000002.1	369183-370586
	gyrB	JZEF01000002.1	140320-142764
	lepA	JZEF01000001.1	237181-238971
	rpoD	JZEF01000004.1	110228-112102
Xanthomonas campestris pv. campestris ATCC 33913[T]	*atpD*	AE008922.1	669581-670987
	dnaK		1720789-1722717
	efp		2672588-2673154
	glnA		241540-242943
	gyrB		4853-7297
	lepA		1487341-1489131
	rpoD		4442015-4443889

[*]Since the genome of this strain has not yet been made publicly available, the nucleotide sequences of these seven loci are provided as supplemental data at the end of this document.

Table S6. Lengths of the housekeeping genes (in bp) and trimming settings for extended (top) and condensed (bottom) MLSA.

Locus	Length (bp)	Extended MLSA - trimming settings	
		Start	Stop
atpD	1,407	ATGAGTCAG	AAGGCCTGA
dnaK	1,926	ATGGGCAAG	AAGAAGTAA
efp	567	ATGGCCACT	TCAAGTAA
glnA	1,404	GTGGAAAAT	GCAACTGA
gyrB	2,442	ATGACCGA	CCTGGATATC
lepA	1,791	ATGCGGAA	ATAAGTAG
rpoD	1,878	ATGGCCAA	ATCGACTGA

Locus	Length (bp)	Condensed MLSA - trimming settings	
		Start	Stop
atpD	747	CATGAAGTGC	GCCGTCGGCA
dnaK	891	GATGCCGG	GGCGTCCAAG
efp	387	GGCAAGGGCC	GTGCGGTG
glnA	885	GAAGAAGG	GGCCCGCAAC
gyrB	528	ATCACCGGCG	AGCAGCTG
lepA	390	GTGGCGGTCA	GCCCGGTTTC
rpoD	874	TACGCCGAAG	CCGCTCCTGG

Table S7. fastANI, ANIm and ANIb values between *Xanthomonas hydrangeae* and *Xanthomonas hortorum* sucluster B pathovars (*X. hortorum* pv. pelargonii CFBP 2533[PT], pv. carotae CFBP 7900 and pv. hederae CFBP 4925[T]). The percentage of sequences aligned is reported for ANIm and ANIb between in brackets.

	CFBP 2533[PT]	CFBP 7900	CFBP 4925[T]	
Strain LMG 31884[T]	94.85	95.11	94.99	
Strain LMG 31885	94.99	95.16	95.19	**fastANI**
Strain LMG 31886	94.96	95.17	95.14	
Strain LMG 31887	94.84	95.07	95.01	

	CFBP 2533[PT]	CFBP 7900	CFBP 4925[T]	
Strain LMG 31884[T]	94.87 [80.8]	95.06 [80.46]	95.04 [78.75]	
Strain LMG 31885	94.97 [84.04]	95.15 [83]	95.12 [81.07]	**ANIm**
Strain LMG 31886	94.96 [84.95]	95.15 [83.92]	95.11 [81.97]	
Strain LMG 31887	94.87 [82.32]	95.06 [82]	95.04 [80.3]	

	CFBP 2533[PT]	CFBP 7900	CFBP 4925[T]	
Strain LMG 31884[T]	94.31 [78.39]	94.43 [78.47]	94.16 [75.92]	
Strain LMG 31885	94.46 [81.62]	94.5 [81.22]	94.39 [77.45]	**ANIb**
Strain LMG 31886	94.47 [82.61]	94.5 [82.06]	94.4 [78.5]	
Strain LMG 31887	94.31 [79.86]	94.43 [80.17]	94.16 [77.61]	

Table S8. Locus tags of the four specific CDS and their Prokka annotation.

CDS	Locus tags	Prokka annotation
LAMP_CDS_1	LMG31884_04510	hypothetical protein
LAMP_CDS_2	LMG31884_22160	hypothetical protein
LAMP_CDS_3	LMG31884_22170	hypothetical protein
LAMP_CDS_4	LMG31884_39740	PCP degradation transcriptional activation protein

Table S9. Strains used for testing the selectivity of the LAMP assay. The amplification (noted with a +) or lack thereof of the strains is reported in the last column.

Strain*	Organism	Isolation host	Cluster[†]	LAMP result
LMG 31884[T]	*Xanthomonas hydrangeae* sp. nov.	*Hydrangea arborescens* cv. Bella Anna	Target strain	+
LMG 31885	*Xanthomonas hydrangeae* sp. nov.	*Hydrangea arborescens* cv. Bella Anna	Target strain	+
LMG 31886	*Xanthomonas hydrangeae* sp. nov.	*Hydrangea arborescens* cv. Invincibelle	Target strain	+
LMG 31887	*Xanthomonas hydrangeae* sp. nov.	*Hydrangea arborescens* cv. Invincibelle	Target strain	+
LMG 31938	*Xanthomonas* sp.	*Hydrangea quercifolia*	Target strain	+
GBBC 2124	*Xanthomonas* sp.	*Hydrangea arborescens* cv. Bella Anna	Target strain	+
GBBC 2196	*Xanthomonas* sp.	*Hydrangea arborescens* cv. Bella Anna	Target strain	+
GBBC 2197	*Xanthomonas* sp.	*Hydrangea arborescens* cv. Annabelle	Target strain	+
GBBC 2198	*Xanthomonas* sp.	*Hydrangea arborescens* cv. Incrediball	Target strain	+
GBBC 2200	*Xanthomonas* sp.	*Hydrangea arborescens* cv. Annabelle	Target strain	+
CFBP 4188[PT]	*Xanthomonas hortorum* pv. cynarae	*Cynara scolymus* L.	*X. hortorum* slc	-
NCPPB 425	*Xanthomonas hortorum* pv. carotae	*Daucus carota*	*X. hortorum* slc	-
NCPPB 881[PT]	*Xanthomonas hortorum* pv. gardneri	*Lycopersicon esculentum*	*X. hortorum* slc	-
NCPPB 940[PT]	*Xanthomonas hortorum* pv. taraxaci	*Taraxacum kok-saghyz*	*X. hortorum* slc	-
GBBC 1934	*Xanthomonas* sp.	*Lavendula stoechas*	*X. hortorum* slc	-
GBBC 1947	*Xanthomonas* sp.	*Geranium macrrorrhizum*	*X. hortorum* slc	-
GBBC 1967	*Xanthomonas* sp.	*Hedera helix* cv. Eva	*X. hortorum* slc	-
GBBC 3001	*Xanthomonas* sp.	*Lactuca sativa*	*X. hortorum* slc	-
GBBC 950	*Xanthomonas* sp.	*Hedera helix*	*X. hortorum* slc	-
SB 3651	*Xanthomonas* sp.	*Phlox paniculata*	*X. hortorum* slc	-
SB 3701	*Xanthomonas* sp.	*Coreopsis grandiflora*	*X. hortorum* slc	-
SB 3727	*Xanthomonas* sp.	*Geranium macrorrhizum*	*X. hortorum* slc	-
SB 4092	*Xanthomonas* sp.	*Lactuca sativa*	*X. hortorum* slc	-
CFBP 4997	*Xanthomonas hortorum* pv. carotae	*Daucus carota* var. sativa	*X. hortorum* slc	-
CFBP 7900	*Xanthomonas hortorum* pv. carotae	*Dacus carota* (seed)	*X. hortorum* slc	-
CFBP 7807	*Xanthomonas hortorum* pv. carotae	Dandelion	*X. hortorum* slc	-
CFBP 7800	*Xanthomonas hortorum* pv. carotae	Grass/*Poaceae*	*X. hortorum* slc	-
CFBP 2533[PT]	*Xanthomonas hortorum* pv. pelargonii	*Pelargonium peltatum*	*X. hortorum* slc	-
CFBP 2508	*Xanthomonas hortorum* pv. pelargonii	*Pelargonium* sp.	*X. hortorum* slc	-
CFBP 2489	*Xanthomonas hortorum* pv. pelargonii	*Pelargonium* sp.	*X. hortorum* slc	-
CFBP 1718	*Xanthomonas hortorum* pv. pelargonii	*Pelargonium hortorum*	*X. hortorum* slc	-
CFBP 3994	*Xanthomonas hortorum* pv. vitians	*Lactuca sativa*	*X. hortorum* slc	-
CFBP 3972	*Xanthomonas hortorum* pv. vitians	*Lactuca sativa*	*X. hortorum* slc	-
CFBP 498	*Xanthomonas hortorum* pv. vitians	*Lactuca* sp.	*X. hortorum* slc	-
CFBP 499	*Xanthomonas hortorum* pv. vitians	*Lactuca scariola*	*X. hortorum* slc	-
CFBP 4196	*Xanthomonas hortorum* pv. cynarae	*Cynara scolymus* L.	*X. hortorum* slc	-
CFBP 4207	*Xanthomonas hortorum* pv. cynarae	*Matricaria* sp.	*X. hortorum* slc	-
CFBP 2044	*Xanthomonas hortorum* pv. cynarae	*Cynara scolymus* L.	*X. hortorum* slc	-
CFBP 4943	*Xanthomonas hortorum* pv. cynarae	*Senebiera* sp.	*X. hortorum* slc	-

CFBP 7992	*Xanthomonas hortorum* pv. gardneri	*Capsicum annum*	*X. hortorum* slc	-
CFBP 8000	*Xanthomonas hortorum* pv. gardneri	*Solanum lycopersicum*	*X. hortorum* slc	-
CFBP 8129	*Xanthomonas hortorum* pv. gardneri	*Solanum lycopersicum*	*X. hortorum* slc	-
CFBP 6822	*Xanthomonas hortorum* pv. gardneri	*Solanum lycopersicum*	*X. hortorum* slc	-
NCPPB 2933	*Xanthomonas hortorum* pv. gardneri	*Calendula officinalis*	*X. hortorum* slc	-
NCPPB 3959	*Xanthomonas hortorum* pv. gardneri	*Persea americana*	*X. hortorum* slc	-
NCPPB 4323	*Xanthomonas hortorum* pv. gardneri	*Lycopersicon esculentum*	*X. hortorum* slc	-
CFBP 4925[T]	*Xanthomonas hortorum* pv. hederae	*Hedera helix*	*X. hortorum* slc	-
CFBP 8163	*Xanthomonas hortorum* pv. gardneri	*Solanum lycopersicum* L.	*X. hortorum* slc	-
LMG 838[neoPT]	*Xanthomonas hortorum* pv. vitians	*Lactuca sativa*	*X. hortorum* slc	-
NCPPB 411[T]	*Xanthomonas arboricola* pv. juglandis	*Juglans regia*	Outgroup	-
CFBP 6821	*Xanthomonas euvesicatoria*	*Capsicum annuum*	Outgroup	-
CFBP 2054[T]	*Xanthomonas translucens* pv. translucens	*Hordeum vulgare*	Outgroup	-
CFBP 4645[T]	*Xanthomonas vesicatoria*	*Lycopersicon esculentum*	Outgroup	-
CFBP 2537[T]	*Xanthomonas vesicatoria*	*Lycopersicon esculentum*	Outgroup	-
CFBP 1976[T]	*Xanthomonas bromi*	*Bromus carinatus*	Outgroup	-
CFBP 4642[T]	*Xanthomonas cassavae*	*Manihot esculenta*	Outgroup	-
CFBP 4642[T]	*Xanthomonas cassavae*	*Manihot esculenta*	Outgroup	-
CFBP 4641[T]	*Xanthomonas sacchari*	*Saccharum officinarum*	Outgroup	-
CFBP 2543[T]	*Xanthomonas vasicola* pv. holcicola	*Sorghum vulgare*	Outgroup	-
CFBP 4643[T]	*Xanthomonas pisi*	*Pisum sativum*	Outgroup	-
CFBP 2542[T]	*Xanthomonas cucurbitae*	*Cucurbita maxima*	Outgroup	-
CFBP 4644[T]	*Xanthomonas melonis*	*Cucumis melo*	Outgroup	-
CFBP 1156[T]	*Xanthomonas hyacinthi*	*Hyacinthus orientalis*	Outgroup	-
CFBP 2528[T]	*Xanthomonas arboricola* pv. juglandis	*Juglans regia*	Outgroup	-
CFBP 4690[T]	*Xanthomonas codiaei*	*Codiacum variegatum*	Outgroup	-
CFBP 6165	*Xanthomonas citri* pv. phaseoli	*Phaseolus vulgaris*	Outgroup	-
CFBP 6864[T]	*Xanthomonas euvesicatoria*	*Capsicum frutescens*	Outgroup	-
CFBP 5241[T]	*Xanthomonas campestris* pv. campestris	*Brassica oleracea* var. gemmifera	Outgroup	-
NCPPB 4321[PT]	*Xanthomonas euvesicatoria* pv. perforans	*Lycopersicon esculentum*	Outgroup	-
NCPPB 4376[PT]	*Xanthomonas euvesicatoria* pv. citrumelonis	*Citrus paradisi* × *Poncirus trifoliata*	Outgroup	-
LMG 495	*X. euvesicatoria* pv. alfalfae	*Medicago sativa*	Outgroup	-
LMG 690[T]	*Xanthomonas cucurbitae*	*Cucurbita maxima*	Outgroup	-
LMG 9322[T]	*Xanthomonas citri* pv. citri	*Citrus aurantifolia*	Outgroup	-
LMG 25954	*Xanthomonas vesicatoria*	*Lycopersicon esculentum*	Outgroup	-
NCB 1082	*Xanthomonas euvesicatoria*	*Capsicum* sp.	Outgroup	-
CFBP 1156[T]	*Xanthomonas hyacinthi*	*Hyacinthus orientalis*	Outgroup	-
CFBP 4643[T]	*Xanthomonas pisi*	*Pisum sativum*	Outgroup	-
CFBP 4644[T]	*Xanthomonas melonis*	*Cucumis melo*	Outgroup	-
CFBP 7942[T]	*Xanthomonas maliensis*	*Oryza sativa* var. Kogoni	Outgroup	-
CFBP 8353[T]	*Xanthomonas prunicola*	*Prunus persica* var. *nectarina*	Outgroup	-
CFBP 8601[PT]	*Xanthomonas floridensis*	*Nasturtium officinale*	Outgroup	-

CFBP 8602^T	*Xanthomonas nasturtii*	*Nasturtium officinale*	Outgroup	-
PD 760	*Pseudomonas syringae* pv. syringae	*Pisum sativum* 'miranda'	Outgroup	-
DC 3000	*Pseudomonas syringae* pv. tomato	N.A.	Outgroup	-
CFBP 1323	*Pseudomonas syringae* pv. tomato	*Lycopersicon esculentum*	Outgroup	-
KD	*Pseudomonas fluorescens*	N.A.	Outgroup	-
A506	*Pseudomonas fluorescens*	N.A.	Outgroup	-
CFBP 2102^T	*Pseudomonas fluorescens* biovar 1	water reservoir	Outgroup	-

* BCCM/LMG: the Belgian Coordinated Collections of Microorganisms/ LMG Bacteria Collection in Gent, Belgium; CCOS: Culture Collection of Switzerland in Wädenswil, Switzerland; NCPPB: National Collection of Plant Pathogenic Bacteria in York, UK; CFBP: French Collection of Plant-associated Bacteria, France; GBBC: Gewasbescherming Bacteriële Collectie (English: Plant Health Unit Bacterial Collection) at ILVO, Merelbeke, Belgium; PD: Culture Collection of Plant Pathogenic Bacteria in Wageningen, The Netherlands; NCB: National Culture Bank in Udine, Italy.

† *X. hortorum* slc: *X. hortorum* species level clade, includes *X. hydrangeae* sp. nov. and *X. hortorum*.

N.A.: not available.
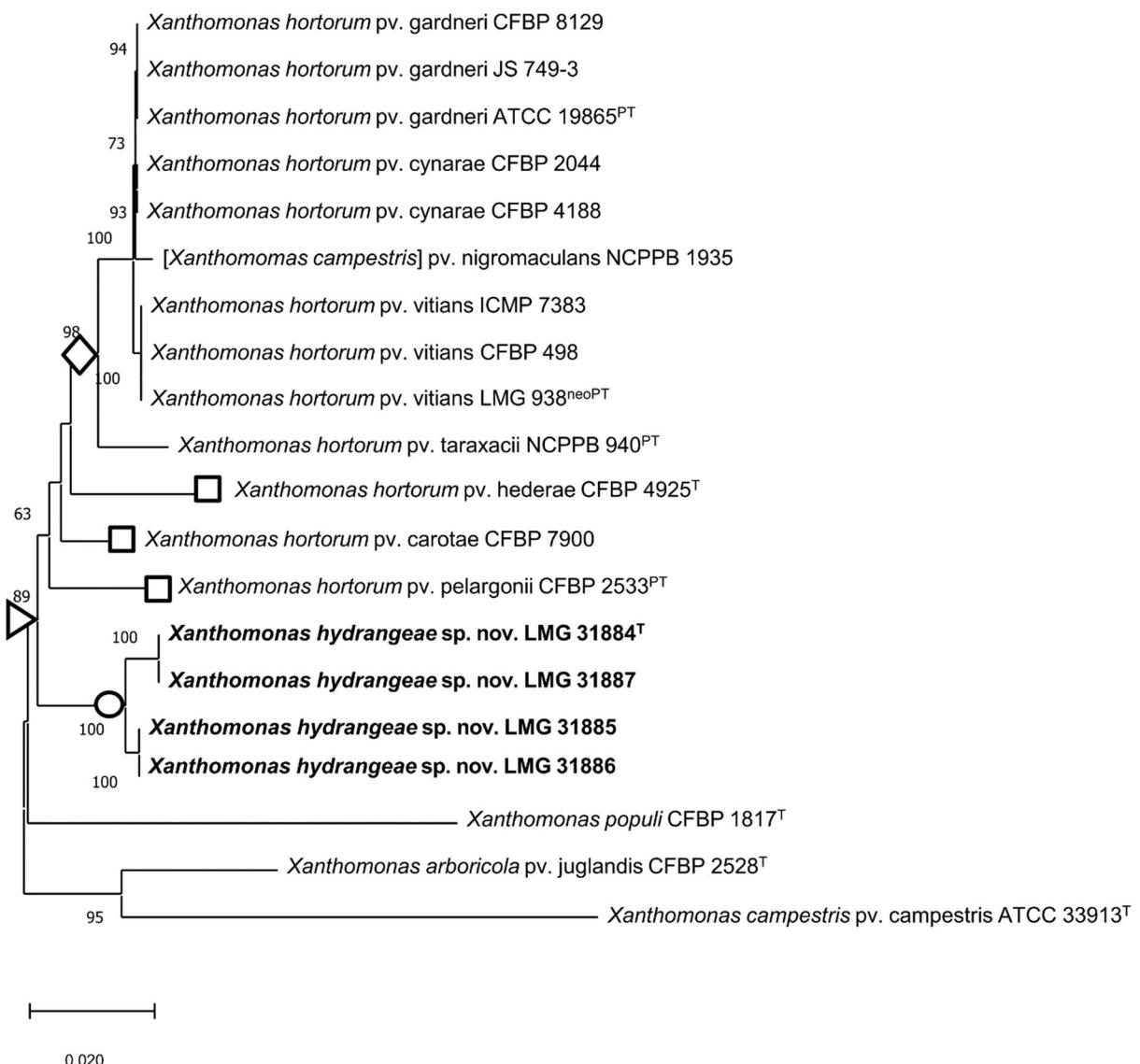
Fig. S1 Maximum-likelihood tree based on the condensed MLSA using 20 concatenated sequences of *atpD*, *dnaK*, *efp*, *glnA*, *gyrB*, *lepA* and *rpoD* (total 4,702 bp). The tree highlights the phylogenetic relationship of *Xanthomonas hydrangeae* LMG 31884[T], LMG 31887, LMG 31885 and LMG 31886 (bolded text and marked with a circle), in relation to *Xanthomonas hortorum* subclusters A and B, diamond and a square, respectively. The *X. hortorum* species level clade is marked with an arrow. Percent bootstrap support values calculated for 1,000 iterations are indicated near nodes only when over 51.

MLSA_NCPPB1935 - Nucleotide sequences of the seven loci used in the MLSA for [*Xanthomonas campestris*] pv. nigromaculans NCPPB 1935.

```
>Xcani-NCPPB1935_1:847858-849264|atpD [Xanthomonas campestris] pv.
nigromaculans
ATGAGTCAGGGCAAGATCGTTCAGATCATCGGCGCGGTCGTCGACGTCGAATTCCAGCGCCATGAAGTGCCGAAG
GTCTATCACGCGTTGAAGGTCGAAGGCACCGAAATCACCCTGGAAGTGCAGCAGCAGCTCGGTGATGGCGTGGTG
CGCACGATTGCGCTCGGCTCCACCGACGGCCTGAAGCGCAACCTGCTGGCCACCAATACCGAGCGCGCCATTTCG
GTGCCGGTCGGCGCCGGGACGCTGGGCCGCATCATGGACGTGCTGGGTCGCCCGATCGACGAAGCCGGCGATGTG
CAGGCCTCGGACCATTGGGAAATCCATCGCGCTGCACCGTCGTACGAAGACCAGTCCTCCAGCACCGAGCTGCTG
GAAACCGGCATCAAGGTCATCGACCTGATGTGCCCGTTCGCCAAGGGCGGCAAGGTCGGCCTGTTCGGCGGCGCC
GGCGTCGGCAAGACCGTCAACATGATGGAATTGATCAACAACATCGCCAAGGCGCACAGCGGTTTGTCCGTGTTT
GCCGGCGTGGGCGAGCGTACCCGTGAGGGCAACGACTTCTACCACGAGATGAAGGACTCCAACGTGCTGGACAAG
GTCGCGATGGTGTACGGCCAGATGAACGAGCCGCCGGGCAACCGTCTGCGCGTTGCGCTGACCGGCCTGACCATG
GCCGAGTACTTCCGCGACGAGAAGGACGAGAACGGCAAGGGCAAGGACGTGCTGCTGTTCGTGGACAACATCTAC
CGCTACACGCTGGCCGGTACCGAAGTGTCCGCGCTGCTCGGCCGCATGCCGTCGGCAGTGGGCTACCAGCCGACC
CTGGCCGAGGAAATGGGCGTGCTGCAGGAACGCATCACCTCGACCAAGAGCGGTTCGATCACCTCGATCCAGGCC
GTGTACGTGCCTGCGGACGACCTGACCGACCCGTCGCCGGCGACCACCTTCGCCCACCTGGATTCCACCGTTACG
CTGAGCCGTAACATCGCCTCGCTGGGCATCTACCCGGCTGTGGATCCGCTGGATTCCACCAGCCGCCAGATGGAC
CCACTGGTGATCGGCCACGAGCATTACGACACCGCCCAGCGCGTGCAGCAGACCTTGCAGAAGTACAAGGAACTG
AAGGACATCATCGCGATCCTGGGCATGGACGAGCTGAGCGAAGAAGACAAGCAGTCGGTGTCGCGCGCGCGCAAG
ATCGAGCGCTTCTTCAGCCAGCCCTTCCACGTGGCCGAAGTGTTCACCGGCTCGCCGGGCAAGTACGTCTCGCTG
AAGGACACCATCCGCGGCTTCAAGGCGATCTGCGACGGCGAGTACGACCACCTGCCGGAGCAGGCGTTCTACATG
GTCGGCAGCATCGAAGAAGCCGTCGAGAAAGCCAACAAGATGAGCGCCAAGGCCTGA

>Xcani-NCPPB1935_1:1964947-1966872|dnaK [Xanthomonas campestris] pv.
nigromaculans
ATGGGCAAGATCATTGGTATTGACCTCGGCACCACGAACTCGTGCGTGTCGATCATGGACGGCGGCAAGGCCCGC
GTCATCGAAAATTCCGAGGGCGATCGCACCACGCCTTCGATCGTCGCCTACACCAAGGACGGCGAAGTGCTGGTC
GGTGCCTCGGCCAAGCGCCAGGCGGTCACCAACCCGAAGAACACGTTCTACGCGGTGAAGCGCCTGATCGGCCGC
AAGTTCACCGACGCTGAAGTGCAGAAGGACATCTCGCACGTGCCGTACGGCATCCTCGCCCACGACAACGGCGAT
GCCTGGGTGCAGACCAGCGATGCCAAGCGTATGGCGCCGCAGGAAATCTCTGCGCGCGTGCTGGAAAAGATGAAG
AAGACCGCCGAGGATTTCCTGGGCGAGAAGGTCACCGAGGCTGTGATCACGGTGCCGGCGTACTTCAACGACAGC
CAGCGTCAGGCCACCAAGGACGCCGGCCGCATCGCCGGTCTGGACGTCAAGCGCATCATCAACGAGCCGACAGCT
GCAGCCCTGGCCTATGGCCTGGACAAGAACGGCGGCGACCGCAAGATTGCGGTGTACGACCTGGGCGGCGGCACC
TTCGACGTGTCGATCATCGAAATCGCCGAAGTCGATGGCGAGAAGCAGTTCGAAGTGCTGGCCACCAATGGCGAC
ACCTTCCTGGGCGGCGAAGACTTCGACAACCGCGTCATCGAATATCTGGTCGACGAATTCAACAAGGATCAGGGC
ATCGATCTGCGCAAGGATCCGTTGGCGTTGCAGCGCCTGAAGGACGCCGCAGAGCGCGCCAAGATCGAGCTGTCG
ACCAGCCAGCAGACCGAAGTGAACCTGCCGTACGTCACCGCCGATGCCTCGGGCCCGAAGCACCTCAACATCAAG
TTGACCCGTGCCAAGCTCGAAGCGCTGGTGGAAGACCTGGTCAAGAAGTCGATCGAGCCGTGCCGCACCGCGTTG
AACGATGCCGGCCTGCGCGCCAGTGATGTCAACGAAGTGATCCTGGTCGGCGGTCAGACCCGCATGCCGAAGGTG
CAGCAGGCGGTTGCCGATTTCTTCGGCAAGGAACCGCGCAAGGACGTCAACCCGGACGAAGCCGTGGCCGTCGGT
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