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Isolation and genetic characterization of *Erwinia amylovora* bacteria from Kyrgyzstan

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Abstract Fire Blight, an economically relevant disease of apple, pear, and quince trees that is caused by the Gram-negative bacterium *Erwinia amylovora*, was first reported from Kyrgyzstan in 2008. One decade later, the disease has spread across the northern part of the country, affecting fruit orchards mainly in Chuy and Issyk-kul regions. Using semi-selective cultural media, bacteria have been isolated from plant material sampled in infested orchards from different locations in Kyrgyzstan, and 16S rRNA gene sequence determination together with diagnostic PCR have been used to identify *E. amylovora* bacteria among isolates. The assignment to this taxonomic species has been corroborated by phylogenetic reconstruction using multilocus sequence analysis, and a short-sequence repeat (SSR) marker has been employed to estimate genetic diversity across the isolates. CRISPR analysis has revealed both a previously unreported CRISPR-2 array pattern and a close relationship of Kyrgyz *E. amylovora* isolates to strains present in Europe and the Middle East. This study presents the first consistent molecular taxonomic characterization of *E. amylovora* bacteria from Kyrgyzstan.

Keywords Fire blight · *Erwinia amylovora* · Kyrgyzstan · Diagnostic PCR · Multilocus sequence analysis (MLSA) · Short-sequence repeat (SSR) · CRISPR arrays

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Fire Blight is a devastating disease of *Rosaceae* plants and is of economic relevance in the cultivation of apple, pear, and quince (Thompson 2000; Acimovic et al. 2015). The disease is caused by the bacterium *Erwinia amylovora (Enterobacterales; Erwiniaceae)* (Adeolu et al. 2016) that infects the host through natural openings or wounds in above-ground parts of fruit trees including blossoms, fruits, shoots, and branches (Agrios 2005 and references therein). Infected tissues typically produce a viscous bacteria-carrying exudate (termed "ooze") from that the pathogen is efficiently spread by insects, birds, wind, and rain (Slack et al. 2017). Originating from North America, Fire Blight has in the 1950s been introduced to Great Britain and in the 1970s to Northern and Central Europe (Van der Zwet and Beer 1995). The disease has subsequently continued to spread towards Southern and Eastern Europe and further to Central Asia. In 2008, Fire Blight was first reported in Kyrgyzstan and registered a

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quarantine pest in 2010 (Chakaev and Chakaeva 2010). The disease has spread within few years through the Northern part of the country, affecting apple orchards across Chuy and Issyk-kul provinces (Dootkulova et al. 2017). In 2015, *Erwinia amylovora* bacteria from infested orchards were first systematically isolated, phenotypically described and employed to evaluate antagonistic *Streptomyces* bacteria as potential Fire Blight biocontrol agents (Doolotkeldieva and Bobushova 2016). However, there is no previous consistent molecular taxonomic characterization of Fire Blight associated bacteria from Kyrgyzstan.

For the present study, plant material (twigs, branches, leaves) was collected in late summer 2018 from disease infested orchards at several locations around lake Issyk-kul, i.e. from one of the most important and most Fire Blight infested fruit production regions of Kyrgyzstan. Symptomatic samples were sterilized with sodium hypochlorite (0.5%) for 2 min, washed twice in the sterile distilled water and ground. 30 μl of a serial dilution of the macerate obtained was spread onto levan agar plates (2 g/l yeast extract, 5 g/l bactopeptone, 5 g/l NaCl, 50 g/l sucrose, agar 20 g/l, pH 7,2) (Anonymous 2013). Petri dishes were incubated at 27 ± 1 °C for 48 h, and single colonies showing the "typical" white, circular, mucoid, and curved morphology of *E. amylovora* on levan medium were further sub-cultivated on King's B (Anonymous 2013) and Miller-Schroth semi-selective agar (8 g/l nutrient broth, 50 g/l saccharose, 9 ml 0,5% bromothymol blue, 2,5 ml 0,5% neutral red, agar 20 g/l, pH 7,4) (Miller and Schroth 1972). For sub-cultivations displaying the expected appearance on both media, a single colony of appropriate morphology and color was isolated from Miller-Schroth plates. Three rounds of serial sub-cultivation on LB medium (10 g/l peptone, 5 g/l yeast extract, 10 g/l NaCl, pH 7.5) were used to generate clonally pure cultures; the isolates thus obtained were once more grown on levan and Miller-Schroth plates to assess morphologies. In total, 26 bacterial isolates were purified and provisionally termed KTMU-1 through KTMU-26 (Table 1). On levan and Miller-Schroth identification media, eleven out of these, namely isolates number 11, 14–17, 19–21 and 24–26 displayed growth characteristics consistent with amylovoran producing *Erwinia* species (Suppl. Fig. S1) and were retained for 16S rRNA gene sequencing and diagnostic PCR assays.

For genetic characterization, genomic DNA was extracted from bacterial log phase liquid cultures grown over night at 27 °C in LB medium using the DNeasy Blood and Tissue kit (Qiagen) according to the standard protocol as provided by the manufacturer. *Erwinia amylovora* – specific diagnostic PCR with primer pairs PEANT-1/PEANT-2, G1-F/G2-R, and FER1-F/rgER2R (Suppl Table S1) as developed, respectively, by Llop et al. (2000), Taylor et al. (2001), and Gottsberger (2010) and validated by the European and Mediterranean Plant Protection Organization (EPPO) (Powney et al. 2011; Anonymous 2013) were performed using standard Taq polymerase (NEB) on an Eppendorf Mastercycler with identical settings consisting of 2 min initial denaturation at 95 °C, 35 amplification cycles of denaturation for 45 s at 95 °C, annealing for 45 s at 58 °C, and elongation for 1 min at 68 °C, followed by 5 min final elongation at 68 °C. Extracted DNA from the nomenclatural type strains *Erwinia amylovora* DSM 30165, *Erwinia piriflorinigrans* DSM 26166 and *Erwinia pyrifoliae* DSM 12163 was used in control reactions. When PCR product sizes were controlled by agarose gel electrophoresis, five isolates, namely KTMU-15, 16, 17, 21 and 24, together with the *E. amylovora* type strain gave rise to an expectedly sized product with each of the three primer pairs, whereas the six remaining isolates together with the *E. piriflorinigrans* and *E. pyrifoliae* type strains generated negative results for all reactions (Fig. 1).

Bacterial 16S rRNA genes were amplified with primers fD1 and rP2 (Suppl Table S1) using the above specified PCR program employing an annealing temperature of 50 °C and an elongation time of 2 min; PCR products were subcloned to a pTOPO cloning vector (Invitrogen) and purified using the Qiaprep plasmid extraction kit (Qiagen). Sanger sequencing was performed by Starseq (Mainz, Germany) using primers fD1, rP2 and the internal primer 760r (Suppl Table S1), and raw sequence data were combined into a single consensus sequence for each bacterial isolate using the MEGA program version 6 (Tamura et al. 2013), Obtained sequencing results were in line with diagnostic PCR results as 16S rRNA encoding sequences from isolates KTMU-15, 16, 17, 21 and 24 (Genbank accession numbers MK967553-MK967557) were found identical to Genbank entries representing the species *Erwinia amylovora*, whereas the further six isolates chosen for genetic analysis were found assignable to other bacterial genera. More exactly, three Kyrgyz isolates (namely KTMU-14, 19, and 20) appeared most closely related to *Pantoea* bacteria *(Enterobacterales; Erwiniaceae)* as *Pantoea agglomerans* or *Pantoea vagans*, two isolates

Table 1 Bacterial isolates investigated in this study

Isolate Number	Geographic Origin	Host Plant; Material of Origin
1	Ak-Suu	Currant, *Ribes nigrum*; leaves, branches
2	Ak-Suu	Dog rose, *Rosa canina*; leaves, branches, fruit
3	Ak-Suu	Dog rose, *Rosa canina*; leaves, branches, fruit
4	Ak-Suu	Rowan, *Sorbus* sp.; leaves, branches
5	Ak-Suu	Rowan, *Sorbus* sp.; leaves, branches
6	Ak-Suu	Rowan, *Sorbus* sp.; leaves, branches
7	Ak-Suu	Currant, *Ribes nigrum*; leaves, branches
8	Ak-Suu	Rowan, *Sorbus* sp.; leaves, branches
9	Ak-Suu	Hawthorn, *Crataegus altaica*; leaves, branches
10	Ak-Suu	Hawthorn, *Crataegus altaica*; leaves, branches
11	Ananyev	Apple, *Malus domestica;* leaves, branches
12	Kara–Kol	Pear, *Pyrus communis* var. Lesnaya Krasavitsa; leaves, branches
13	Kara–Kol	Pear, *Pyrus communis* var. Lesnaya Krasavitsa; leaves, branches
14	Kara–Kol	Pear, *Pyrus communis* var. Maiskaya; leaves, branches
15	Jeti Oguz	Pear, *Pyrus communis* var. Talgarskaya Krasavitsa; leaves
16	Jeti Oguz	Pear, *Pyrus communis* var. Talgarskaya Krasavitsa; leaves
17	Jeti Oguz	Pear, *Pyrus communis* var. Talgarskaya Krasavitsa; leaves, branches
18	Jeti Oguz	Apple, *Malus domestrica* var. Kripson; leaves, branches
19	Bishkek	Apricot, *Prunus* sp.; branches
20	Bishkek	Apricot, *Prunus* sp.; branches
21	Saruu	Pear, *Pyrus communis* var. Talgarskaya Krasavitsa; leaves, branches
22	Saruu	Apple, *Malus domestica*
23	Saruu	Pear, *Pyrus communis*
24	Jeti Oguz	Pear, *Pyrus communis* var. Talgarskaya Krasavitsa; leaves
25	Jeti Oguz	Pear, *Pyrus communis* var. Talgarskaya Krasavitsa; leaves
26	Jeti Oguz	Currant, *Ribes* sp.; leaves

(KTMU-25 and 26) to *Pseudomonas* bacteria (*Pseudomonadales; Pseudomonadaceae*) as *Pseudomonas orientalis* or *Pseudomonas marginalis,* and isolate KTMU-11 to bacteria of the genus *Rahnella* (*Enterobacterales; Yersiniaceae*).

To assess more accurately the systematic position of these five presumed *E. amylovora* isolates, an MLSA scheme comprising the house-keeping genes *atpD, infB,* and *rpoB* – encoding, respectively, ATP synthase subunit B, translation initiation factor IF-2, and beta subunit of RNA polymerase – was used. This MLSA marker set has previously been employed in systematic taxonomy studies of the related bacterial genera *Pantoea, Enterobacter,* and *Rahnella* (all *Enterobacterales*) (Brady et al. 2008, 2013, 2014) and has more recently been adapted to the molecular characterization of *Erwinia* bacteria (Facey et al. 2015). Internal partial sequences of the *atpD, infB*, and *rpoB* markers were PCR amplified and sequenced using PCR and sequencing primers and PCR parameters as described by Brady et al. (2008). PCR products were purified using the Qiaquick PCR purification kit (Qiagen) and sequenced. Assembly of raw sequence data gave rise to confirmed consensus sequences comprising 525 bp, 612 bp, and 489 bp in length for the *atpD, infB*, and *rpoB* markers, respectively. Pair-wise sequence similarities as calculated in a p-distance matrix (Table 2) ranged from 100% down to 99.8% and 99.4% for the *infB* and *rpoB* markers, respectively, whereas amplified *atpD* gene sequences from all isolates were found identical. Interestingly, all single nucleotide mutations detected in the *infB* and *rpoB* genes occurred in non-synonymus positions, giving rise to a single Val/Leu exchange as well as to one Met/Ile and two His/Asp

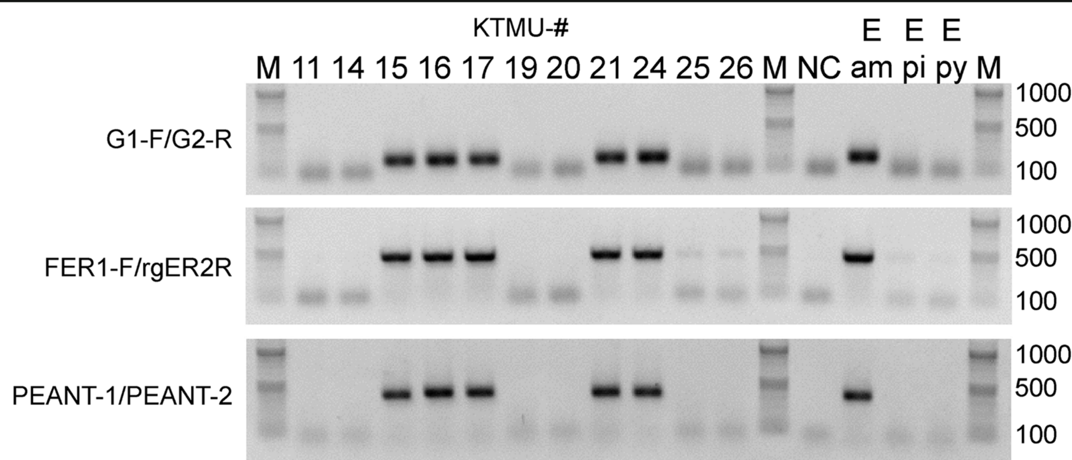

Fig. 1 Gel electrophoresis of *E. amylovora* - specific diagnostic PCR reactions with primers G1-F / G2-R (top), FER1-F / rgER2R (middle), and PEANT-1 / PEANT-2 (bottom). Bacterial isolates are identified by strain numbers corresponding to Table 1. Nomenclatural type strains of the species *E. amylovora. E. pyrifoliae, and E. piriflorinigrans* are, respectively, named "Eam", "Epy", and "Epi". "NC" denotes the negative (no DNA template) control, "M" the DNA 100 bp ladder size standard; DNA product sizes in bp are indicated at the right-hand margin

exchanges in the deduced amino acid sequences of the *infB* and *rpoB* gene products, respectively. All consensus sequences have been submitted to the Genbank database under accession numbers MK368705-MK368719.

In order to create a reference dataset for phylogenetic reconstruction for each MLSA marker, the orthologous gene sequences from the nomenclatural type strains representing *Erwinia* species spanning the full range of diversity of this genus in its current definition, namely *E. amylovora, E. piriflorinigrans, E. aphidicola, E. persicina*, and *E. oleae*, were used as queries in GenBank database searches (Altschul et al. 1997; Zhang et al. 2000). From BlastN searches for the 1000 most similar single sequence entries, all sequences marked "*Erwinia*" that covered at least 90% of the query were retained for downstream analyses. Moreover, complete and draft genome sequences from the genus *Erwinia* (taxid 551) were searched with marker sequences from the *E. amylovora* type strain DSM 30165. Sequence entries retained from these searches were combined into a single non-redundant reference data set comprising 143, 126, and 135 orthologous *Erwinia* sequences for the *atpD, infB,* and *rpoB* markers, respectively. The sequences from 117 *Erwinia* strains represented in all three single marker sets were combined into a reference set for a concatenation of the three markers. Marker sequences were codon-aligned using the CLUSTAL W function (Thompson et al. 1994) as implemented in the MEGA 6 software package. Phylogenies were reconstructed from p-distance matrices with the Neighbour Joining (NJ) method as implemented in the

Table 2 Genetic diversity across Kyrgyz *E. amylovora* isolates. Comprehensive representation of diversity found with respect to pair-wise nucleotide sequence similarity percentages of the MLSA markers *infB* (lower left triangle) and *rpoB* (upper right triangle) as calculated from a p-distance matrix, to the number of SSR motifs present in plasmid pEA29, and to the nucleotide present in the point-mutated position of spacer 68/1022 within the CRISPR-1 array

Sequence Similarity (%)	KTMU-15	KTMU-16	KTMU-17	KTMU-21	KTMU-24	*rpoB*	SSR Number	Pos. 26 of Spacer 68/1022
KTMU-15		99.8	99.6	99.8	99.8	KTMU-15	6	C
KTMU-16	100		99.4	100	100	KTMU-16	8	C
KTMU-17	100	100		99.4	99.4	KTMU-17	7	C
KTMU-21	99.8	99.8	99.8		100	KTMU-21	6	G
KTMU-24	100	100	100	99.8		KTMU-24	6	G
infB	KTMU-15	KTMU-16	KTMU-17	KTMU-21	KTMU-24			

same software tool under pair-wise deletion of alignment gaps and missing data, and tree topology confidance limits were explored in non-parametric bootstrap analyses over 1000 pseudo-replicates. In the phylogeny generated from the alignment of concatenated MLSA marker sequences, the five bacterial isolates from Kyrgyzstan were located in a single maximally bootstrap supported clade together with all reference strains representing the range of geographic origins and host plants reported to date for the taxonomic species *E. amylovora* under exclusion of all further *Erwinia* species (Fig. 2, Suppl. Fig. S2). Moreover, a corresponding *E. amylovora* clade receiving 100% bootstrap support was present in all three single marker trees (not shown).

Stretches of clustered regularly interspaced short palindromic repeat sequences, termed CRISPR arrays, have been identified in app. 85% of archaeal and about 50% of eubacterial genomes analyzed (Shariat and Dudley 2014). The arrays consist of a variable number of direct repeat (DR) elements with neighboring DRs being separated by spacer sequences. The spacers are thought to constitute the molecular memory of a bacterial adaptive immune system targeting invasive DNAs as of bacteriophages or plasmids; the supposed directionality of spacer acquisition in CRISPR arrays has been exploited in evolutionary and subtyping studies of several bacteria (Shariat and Dudley 2014), including *Erwinia amylovora* (Rezzonico et al. 2011; McGhee and Sundin 2012). The genome of *E. amylovora* typically contains three CRISPR arrays, named CRR1, CRR2, and CRR4, with spacer elements comprising, with few exceptions, 32 bp in length (Rezzonico et al. 2011). With respect to the spacer organization within these CRR loci, several CRISPR array "patterns" (McGhee and Sundin 2012) or *E. amylovora* "genotypes "(Rezzonico et al. 2011) have been distinguished.

Complete CRR1 and CRR2 arrays from *E. amylovora* isolates KTMU-15, 16, 17, 21 and 24 were amplified and sequenced using the oligonucleotide primers indicated in Supplementary Table S1, and where found to be identical in spacer organization for these isolates. More exactly, the organization of CRISPR-1 arrays was identical to CRISPR-1 pattern 4 (McGhee and Sundin 2012) or CRR1 genotype "A" (Rezzonico et al. 2011) as previously described for *E. amylovora* isolates from Europe and the Middle East. In particular, the 2.272 bp comprising CRISPR-1 nucleotide sequences obtained from isolates KTMU-21 and 24 (MK988577) were identical to those of the *E. amylovora* strain LebA-1 isolated in Lebanon, whereas the sequences obtained from Kyrgyz isolates KTMU-15, 16, and 17 (MK988576) differed from this sequence by a single nucleotide exchange, more exactly a G to C transversion within spacer element 68 or 1022 (5´-taaatggttgtccgttcttggcgcaCacggct) according to the numbering by McGhee and Sundin (2012) and Rezzonico et al. (2011), respectively (Table 2). The mutated position is generally conserved (5´-taaatggttgtccgttcttggcgcaGacggct) in CRR1 elements across the European and Middle Eastern *E. amylovora* strains listed in Supplementary Table S2, with the exception of a quince pathogen from Lebanon, strain LebA-3, that carries a G to A transition within spacer 68/1022 (5´-taaatggttgtccgttcttggcgcaAacggct) (data not shown).

Sequencing of the CRISPR-2 arrays of Kyrgyz isolates KTMU-15, 16, 17, 21, and 24 revealed five identical consensus sequences comprising 1.950 bp in length (MK988575) with highest similarity in terms of both array organization and nucleotide sequence to repeat regions representing pattern 24 or genotypes "a" and "f" as described for European and Middle Eastern *E. amylovora* isolates including reference strain LebA-1. However, the CRR2 of Kyrgyz isolates differed from that of the reference strain in lacking one out of the latter's 35 spacer elements, namely spacer number 55/2016; apart from this deletion, sequences were 100% identical to that of strain LebA-1.

In order to compare the CRISPR-2 sequence from Kyrgyz isolates to the full range of available CRR2 data, the Genbank and CRISPI (http://crispi.genouest.org; Rousseau et al. 2009) databases were searched for sequence entries with high similarity to the complete CRR2 element; moreover, the CRISPRs web server (https://crispr.i2bc.paris-saclay.fr; Grissa et al. 2007) was blasted for CRISPR arrays comprising the same DR element. Combination of search results gave rise to a non-redundant set of 105 sequence entries with relevant similarity to the presumably new CRR2 sequence determined (Suppl. Table S2). None of these known CRR2 arrays displayed an organization identical to that of the Kyrgyz *E. amylovora* isolates. The deletion of spacer 55/2016 from a continuous stretch of eight spacers numbered 50 through 57 (or 2014 through 2021) under conservation of the seven neighboring spacer elements appeared to be an organizational feature unique to

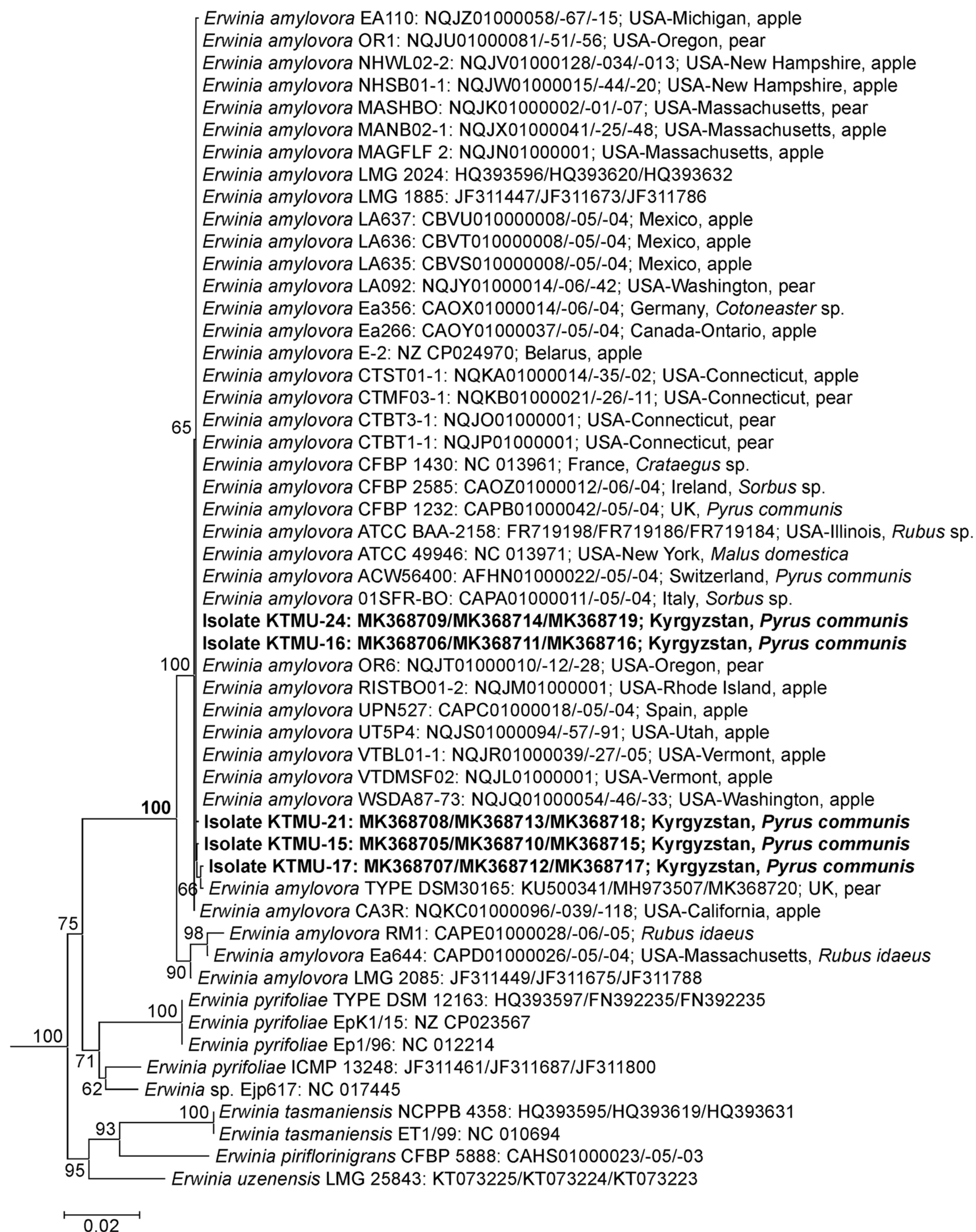

Fig. 2 The clade comprising *Erwinia amylovora* together with the phylogenetically most closely related *Erwinia* spp. as pruned from the Neighbor Joining (NJ) phylogeny of the genus *Erwinia* reconstructed from concatenated *atpD, infB,* and *rpoB* nucleotide sequences; for the full phylogenetic tree refer to Supplementary Fig. S2. Terminal branches are labelled by genus, species and strain designations followed by GenBank accession numbers for the three marker sequences in the above order as well as geographic origin and host plant indications. Data from fully annotated genome sequences are identified by the single genome accession number. The term "TYPE" denotes the nomenclatural type strain of the respective bacterial species. Bacterial isolates investigated in this study are printed in bold-type. Numbers on internal branches indicate bootstrap support percentages; the 100% bootstrapping value at the root of the *E. amylovora* clade is printed in bold type. The size bar corresponds to 2% sequence divergence

the Kyrgyz isolates. This stretch of eight spacers is conserved in its entirety in *E. amylovora* strains carrying CRR2 patterns 21, 24, 25, 26, 29, 34, and 37 and/or belonging to CRR2 genotypes “a”–“e”, “i”, and “j”, and is completely absent from CRR2 patterns 23, 27, and 28, genotypes “g” and “h” as well as several CRR2 sequences not assigned to any of these (Fig. 3). Partial deletions from this stretch of spacers give rise to CRR2 pattern 22 (deletion of spacer 50/2021), pattern 32 (spacer 53/2018) and genotype “f” (spacers 56/2015 and 57/2014); however, spacer 55/2016 that is lacking in the CRR2 from the Kyrgyz *E. amylovora* isolates is conserved in accordingly organized CRISPR-2 arrays.

Taking these data together, the spacer organization of the CRISPR-2 arrays sequenced was found to be unique to the Kyrgyz isolates. Moreover, CRISPR-1 and CRISPR-2 array organization and nucleotide sequences were most closely related to repeat regions reported from *E. amylovora* strains isolated from across Europe (UK, France, Spain, Germany, Serbia-Montenegro, Belarus) as well as from the Middle East (Lebanon, Israel) and New Zealand, whereas more distantly related CRISPR-2 genotypes and patterns were almost exclusively found in North American and East Asian *Erwinia* isolates (Suppl. Table S2).

A region within the *E. amylovora* low copy number plasmid pEA29 comprising variable numbers of a 8 bp short-sequence repeat (SSR) motif (ATTACAGA) has been used previously for comparative characterization of *E. amylovora* strains (Lecomte et al. 1997; Kim and Geider 1999; Llop et al. 2000; Barionovi et al. 2006; Jock et al. 2013). The SSR motif region is situated between diagnostic PCR primers PEANT-1 and PEANT-2, and tandem repeat copy number variation within a sample causes product length polymorphism in PCR reactions targeting this region (Kim and Geider 1999; Llop et al. 2000; Stöger et al. 2006). Investigation of the stability of the repeat copy number within a single strain has led to controversial results (Schnabel and Jones 1998; Jock et al. 2003; Ruppitsch et al. 2004). However, whereas the length of the SSR array tends to change under stress conditions, it appears much more stabile under normal growth conditions as, e.g., in laboratory culture. The SSR array does, therefore, not appear to be a suitable marker for strain identification; but can be used to assess the micro-diversity across a set of isolates as, e.g., those stemming from the same area or location (Jock et al. 2003). This is particularly interesting for European strains of *E. amylovora* that with respect to other discriminative methods as PFGE or *hrpN* sequence analysis show a high degree of relatedness as, e.g., compared to American isolates (Jock and Geider 2004).

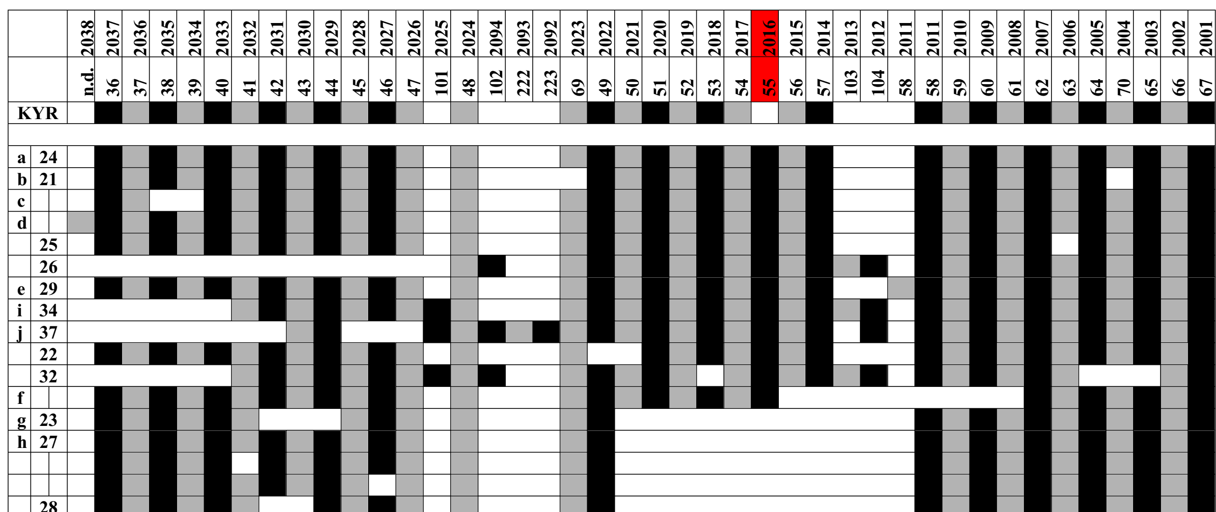

Fig. 3 Graphic representation of patterns of spacer organization for the CRISPR-2 arrays from 95 *Erwinia amylovora* strains (Suppl. Table S2). The top rows indicate the two alternative systems of spacer numbering. CRR2 genotypes “a”–“j” and spacer patterns 21–37 are indicated at the left margin; “KYR” designates the organizational pattern found in Kyrgyz isolates. Alternating black and grey fields indicate the presence, white fields the absence of the respective spacer. The position of spacer 55/2016 is highlighted in red colour

Sequencing of the SSR marker region revealed repeat copy numbers of six (KTMU-15, 21 and 24), seven (KTMU-17), and eight (KTMU-16) for the five Kyrgyz *E. amylovora* isolates (Table 2).

In conclusion, MLSA using the *atpD, infB*, and *rpoB* markers unequivocally demonstrated that five of the bacterial isolates purified on selective media from fire blight infested pear orchards in the Issyk-kul region of Kyrgyzstan and identified by diagnostic PCR belong to the taxonomic species *Erwinia amylovora*. This is the first consistent molecular taxonomic characterization of presumed *E. amylovora* bacteria from Kyrgyzstan since the first introduction of Fire Blight to Central Asia a decade ago. Sampling sites of these strains were all situated on the Southern shore of lake Issyk-kul, with isolates KTMU-15, 16, and 17 stemming from different pear trees within the same highly infested nursery with 2-year old trees, whereas KTMU-24 was isolated from an older pear tree in a private garden app. 2 km away from the nursery. Strain KTMU-21 was isolated from a pear tree in Saruu village at a distance of about 70 km from the Jeti-Oguz sampling sites.

The presence of a new unique spacer organization in the CRISPR-2 arrays of all Kyrgyz isolates together with a previously unreported single nucleotide exchange within the CRISPR-1 array of three out of the five of these and a comparatively high level of genetic variation present in amplified *infB* and *rpoB* partial sequences (Table 2) might be most parsimoniously explained by a single introduction event into the region that has been followed by an increased level of divergence as a response to environmental stress and niche establishment. In the case of both the *infB* marker and the point-mutated spacer within the CRISPR-1 array, genetic variation positively correlates with the geographic origin of the samples. Moreover, the observed variability of the SSR marker across strains stemming from different trees within the same pear nursery is fully consistent with an adaptive response to elevated levels of environmental stress.

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Compliance with ethical standards

Conflict of interest Each of the five authors, namely Tinatin Doolotkeldieva, Saikal Bobushova, Christina Schuster, Mahabat Konurbaeva, and Andreas Leclerque, declares that he/she has no conflict of interest.

Research involving humans or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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