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ORIGINAL ARTICLE

Characterization and phylogeny of the novel taxon of *Pseudomonas* spp., closely related to *Pseudomonas avellanae* as causal agent of a bacterial leaf blight of cornelian cherry (*Cornus mas* L.) and *Pseudomonas syringae* pv. *syringae* as a new bacterial pathogen of red dogwood (*Cornus sanguinea* L.)

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Abstract

In 2013–2018 on the leaves of *Cornus mas* L. small brownish spots with regular shape merging also to large necrotic lesions or present as extensive necrotic area from leaf petiole were observed. In 2016 on leaves of *Cornus sanguinea* L. dark red brownish spots of 4 mm in diameter with necrotic center, were observed. From samples with diseased tissue, fluorescent bacteria were isolated, showing a morphology similar to that of the genus *Pseudomonas*. The phenotypic characters using LOPAT tests [levan production from sucrose (L), presence of oxidase (O), pectolytic activity on potato (P), the presence of arginine dihydrolase (A), hypersensitivity reaction on tobacco (T)], allowed to classify the isolates to *Pseudomonas syringae* (LOPAT group Ia). The pathogenicity of isolates was proved on young leaves of *Cornus mas* and *Cornus sanguinea,* confirming the ability to infect the species. Detection of genes coding for the phytotoxins coronatine (*cfl*), syringomycin (*syrB* and *syrD*), and yersiniabactin (*irp1*) showed that none of strains from cornelian cherry nor red dogwood possessed *cfl* gene, but 5 out of 6 cornelian cherry strains contained the *irpl* gene. Only the strain 1439, isolated from red dogwood possessed the *syrD* and *syrB* genes. Sequence analysis of the 16S rRNA, and housekeeping genes *gyrB* and *rpoB* showed that strains from cornelian cherry are most closely related to the hazelnut pathogen *Pseudomonas avellane*, and that the strain from red dogwood could be identified as *Pseudomonas syringae* pv. *syringae.* Further research will determine the exact taxonomic position of the cornelian cherry strains.

Keywords *Pseudomonas* spp. · *gyrB* · *rpoB* · 16S rRNA · Phytotoxins · Taxonomy

Introduction

In recent years, the growing of cornelian cherry dogwood (*Cornus mas* L., family Cornaceae) is becoming more and more popular. The plant is native to southern Europe and SW-Asia (Lannert 1981; Lawrence 1985, Mamedov and Craker 2004), but nowadays is also commonly grown in north-eastern Europe and Great Britain, Central Asia, and South America (rev in Mamedov and Craker 2004). Its popularity in Europe is undoubtedly linked to the value and the 'advantages' of the fruits both as fresh or used for preparation of juices, syrups, wine, and traditional products (Brindza et al. 2007; Tesevic et al. 2009; Rop et al. 2010). Moreover, cornelian cherry is a prospective source for phytomedicine, possessing interesting antimicrobial properties against fungal and bacterial pathogens, e.g. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Also, it is very popular in folk medicines, especially in China (Krzyściak et al. 2011; Kyriakopoulos and Dinda 2015; Dinda et al. 2016). Cornelian cherry till now has demonstrated longevity and adaptability, and is considered as a plant that is easy to grow due to its high resistance to drought, air pollution and low susceptibility to pests and diseases.

The few most common diseases attacking cornelian cherry are caused by a number of fungal pathogens: *Discula*

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destructive (dogwood anthracnose), *Elsinoe corni* (dogwood spot anthracnose), *Septoria cornicola* (septoria leaf spot), and *Phyllactinia corni* (powdery mildew) (Redlin 1991; https://www.cabi.org/isc/datasheet/20079; http://www4.ncsu.edu/~qyxiang/cornuspathology.html; Gauthier and Stolz 2017; Bacigálová et al. 2005; Li et al. 2009, Mmbaga et al. 2016). However, very little is known about bacterial diseases occurring on cornelian cherry. Hitherto, only one report was published about occurrence of a leaf blight caused by *Pseudomonas syringae* in Tennessee, USA (Mmbaga and Sheng 2000; Mmbaga and Nodu 2006). However, the identity of isolates obtained from diseased leaves classified as *P. syringae* was confirmed only by some phenotypic characters and fatty acid analysis.

In 2013, 2014 and 2018 on leaves of *Cornus mas* plants, delivered to our laboratory from nurseries, small, regular, brownish spots ca. 3 mm in diameter sometimes merging, to form large necrotic lesions, were observed. From the symptomatic tissue fluorescent bacteria were repeatedly isolated.

Cornus sanguinea L. known as common or red dogwood belongs to the same family as cornelian cherry. It is widely distributed in almost the entire European continent and west Asia. It can grow from lowlands up to 1500 m above sea level in the Alps e.g. Switzerland and Caucasus Mountains. As an ornamental plant it is used for fences, but also grows along riversides, and is often planted as an ornamental in cities and along roads (Liesebach and Götz 2008; Wadl et al. 2013). Red dogwood is affected by only a few fungal pathogens: *Discula destructive* (dogwood anthracnose), and sometimes *Nectria* spp., *Phomopsis* spp., *Botryosphaeria* spp., *Tubercularia* spp., *Phytophthora* spp. (dieback/canker) (https://www.eppo.int/QUARANTINE/Alert_List/fungi/DISCDE.htm http://extension.illinois.edu/hortanswers/plantdetail.cfm?PlantID=372&PlantTypeID=8). Hitherto, there was no report on the occurrence of bacterial diseases on this species. In 2016 on leaves of red dogwood (*Cornus sanguinea* L.) shrubs dark red, brownish spots with regular shape of 4–5 mm in diameter with necrotic center, were observed in Poland. Although, the symptoms were first taken for those caused by *Discula destructive* causing anthracnose, fluorescent bacteria were repeatedly isolated.

As mentioned above, *Pseudomonas syringae,* which was reported on cornelian cherry in the USA, is known as polyphagous, causing economically important diseases on many both annual and woody plants, including fruit trees, vegetables and ornamental plants (Bultreys and Kałużna 2010). From molecular studies it is known that the *P. syringae* complex comprises over 50 pathovars defined according to their pathogenic ability (Young 2010). This species is also divided into genomospecies determined by DNA:DNA hybridization (Gardan et al. 1999) and redefined into up to 13 so called phylogroups (PG) (Parkinson et al. 2011; Berge et al. 2014) based on Multi Locus Sequence Analysis (MLSA) largely corresponding to the genomospecies defined by DNA:DNA hybridization (Gardan et al. 1999).

The aim of present study was to characterize and determine the taxonomic and phylogenetic position of bacterial isolates causing necroses on leaves of cornelian cherry and red dogwood in Poland, based on phenotypic characteristics and molecular sequence analysis of 16S rRNA and the housekeeping genes *rpoB* and *gyrB*.

Materials and methods

Isolation of the bacteria and determination of their phenotypic characters Samples of cornelian cherry (*Cornus mas* L.) leaves exhibiting small brownish spots, and/or large necrotic lesions were delivered to our laboratory from nurseries in the years 2013, 2014, and 2018 in Łódzkie and Kujawsko-pomorskie voivodeships in Poland (Fig. 1; Table 1). Leaf samples of red dogwood (*Cornus sanguinea* L.) shrubs with dark red, brownish spots with regular shape of 4 mm in diameter with necrotic center were also supplied from nurseries in 2016 from Łódzkie voivodeship, Poland (Fig. 2; Table 1).

From the border between healthy and diseased tissue, that was shortly disinfected with 70% ethanol, small pieces were cut out and macerated in sterile PBS buffer (0.27% Na_2HPO_4; 0.04% NaH_2PO_4; 0.8% NaCl). The macerate was plated onto King's medium B (King et al. 1954) and incubated at 27 °C. After 48 h single, fluorescent, colonies were selected and purified on the same medium. The obtained isolates were subjected to the following tests: Gram reaction with 3% KOH (Suslow et al. 1982), and LOPAT tests [levan production from sucrose (L), presence of oxidase (O), pectolytic activity on potato (P), the presence of arginine dihydrolase (A), hypersensitivity reaction on tobacco (T)] were performed (Lelliott 1966). A culture of *Pseudomonas syringae* pv. *syringae* strain LMG 1247 was used as reference. For further analysis all isolates and the reference strain LMG 1247 were stored in a mixture of glycerol (20% *v*/v) and PBS and kept at −75 °C (Table 1).

Pathogenicity test To determine the pathogenicity of all isolates the attached young leaves of two plants of *Cornus mas* and *Cornus sanguinea* were inoculated with a water suspension of each isolate separately and the reference strain LMG 1247^T mentioned in Table 1 and at a concentration of 10^7cfu ml^{-1} was prepared from 48 h-old cultures grown on King's B. The suspension was infiltrated into leaves by a hypodermic syringe. The plants with infiltrated leaves were covered with plastic bags for 48 h to maintain high humidity conditions and then were kept in a greenhouse at 18–22 °C. Leaves were also treated by the same way with sterile distilled water as the negative control. During two weeks appearance of lesion formation and development of disease symptoms were observed

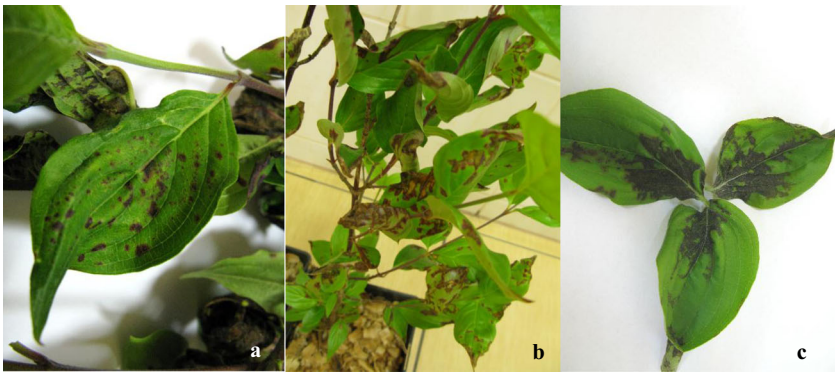

Fig. 1 Disease symptoms on *Cornus mas* L. **a** small brownish spots with regular shape of 3 mm in diameter, **b** large necrotic lesions, **c** extensive necrotic area running from leaf petiole

(Fig. 3a and b). From symptomatic tissue samples were taken for bacteria re-isolation. For confirmation of the identity of bacteria re-isolated with those used for inoculation phenotypic characters, and PCR reactions with primers Ps-for and Ps-rev were performed.

Molecular characterization and phylogenetic analysis Isolation of bacterial DNA was performed by the method described by Aljanabi and Martinez (1997) with slight modifications described in Kałużna et al. (2012). To confirm that bacteria obtained from cornelian cherry and red dogwood belong to the genus *Pseudomonas* amplification of DNA with primers Ps-for and Ps-rev (Widmer et al. 1998) according to the reaction conditions as originally described was done. DNA of reference strain LMG 1247^T was included for comparison.

Detection of genes coding for syringomycin, coronatine and yersiniabactin Determination of the presence of genes coding for synthesis of the bacterial phytotoxins syringomycin, coronatine and the siderophore yersiniabactin was done by PCR with primers amplifying fragments of their genes: (i) *syrB* and *syrD* encoding synthesis of syringomycin with primers syrB1/syrB2 and syrD1/syrD2 as described by Sorensen et al. (1998) and Bultreys and Gheysen (1999), respectively, (ii) *irp*1 encoding synthesis of yersiniabactin with primers PSYE2/PSYE2R from Bultreys et al. (2006) and (iii) *cfl* encoding coronatine synthesis with primers cfl1/cfl2 described by Bereswill et al. (1994). The amplifications were conducted in a Biometra T3000 thermocycler (Biometra, Germany). The conditions of all PCR reactions, with slight modifications of annealing temperatures applied are indicated in Kałużna et al. (2010). Reference strains *P. syringae* pv. *morsprunorum* race 1 – LMG 2222, *P. syringae* pv. *morsprunorum* race 2 – CFBP 3800, *P. syringae* pv. *syringae* (*Pss*) – LMG 1247^T) were included in all reactions. PCR products were separated in a 1.5% agarose gel in 0.5 x TBE buffer (0.045 M Tris – boric acid, 0.001 M EDTA, pH 8.0) (Sambrook et al. 1989) by comparison with the standard mass O'GeneRuler 100 bp DNA Ladder Plus (ThermoScientific, Lithuania) and electrophoresis run at 5–7 V/cm of gel. After staining with ethidium bromide solution (0.5 μg ml^{-1}) the obtained amplification products were visualized under UV light.

Identification of isolates based on sequence analysis of 16S rRNA, *gyrB* and *rpoB* genes All isolates obtained from cornelian cherry and the one isolate from red dogwood were identified by (partial) sequence analysis of 16S rRNA and the *gyrB* and *rpoB* housekeeping genes. For this purpose DNA was amplified with the primers fD1 and rp2 (Weisburg et al. 1991), primers gyrB-F and gyrB-R (Sawada et al. 1999) and primers LAPS and LAPS27 (Ait Tayeb et al. 2005), respectively, with PCR conditions as it was described in the above

Table 1 Strains of *Pseudomonas* spp. used in this study

Strain number	Place (voivodeship/country)	Date of isolation	Host-plant
1299a	Skierniewice, Łódzkie, Poland	2013	*Cornus mas* L.
1299b	Skierniewice, Łódzkie, Poland	2013	*Cornus mas* L.
1342	Lutobory Nowe, gm. Sadkowice, Łódzkie, Poland	2014	*Cornus mas* L.
1343	Lutobory Nowe, gm. Sadkowice, Łódzkie, Poland	2014	*Cornus mas* L.
1439	Skierniewice, Łódzkie, Poland	2016	*Cornus sanguinea* L.
1557	Dobrzyń, Kujawsko-pomorskie, Poland	2018	*Cornus mas* L.
1558	Dobrzyń, Kujawsko-pomorskie, Poland	2018	*Cornus mas* L.
LMG 1247^T	UK	1950	*Syringa vulgaris*

Fig. 2 Disease symptoms on *Cornus sanguinea* L. dark red, brownish spots with regular shape of 4–5 mm in diameter with necrotic dead center

mentioned papers. The sequences obtained were assembled using the SeqMan Lasergene package (DNASTAR, Inc., Madison, WI) and sequences compared with those deposited in NCBI GenBank (http://www.ncbi.nlm.nih.gov) and in the database of the EzTaxon server (https://www.ezbiocloud.net/; Yoon et al. 2017). Maximum Likelihood phylogenetic trees including all species of the *Pseudomonas* genus (Fig. 3) and most closely related species (Figs. 4 and 5) were constructed with the MEGAX program (Kumar et al. 2018).

A dendrogram based on the sequence analysis of the partial 16S rRNA gene for all *Pseudomonas* spp. species was constructed using the General Time Reversible evolutionary model with gamma distribution and by assuming that a certain fraction of sites are evolutionarily invariable (G + I) (found as the best substitution model) (Nei and Kumar 2000), however, with closest relatives with Hasegawa-Kishino-Yano (HKY) gamma distributed with invariant sited (G + I) (found as the best substitution model) (Hasegawa et al. 1985). A dendrogram based on sequence analysis of *gyrB* for all *Pseudomonas* spp. species was constructed using the Tamura-Nei evolutionary model with gamma distribution (+G) (Tamura and Nei 1993). For *rpoB* genes the General Time Reversible evolutionary model with gamma distribution and by assuming that a certain fraction of sites are evolutionarily invariable (G + I) was used as it was found as the best substitution model (Nei and Kumar 2000). Dendrograms based on sequence analysis for cornelian cherry, red dogwood and the closest neighbor of other species for *gyrB* and *rpoB* genes were constructed using using the Tamura 3 parameter (found as the best substitution model) (Tamura 1992). The significance of the internal branches of all dendrograms was estimated with bootstrap values expressed as percentages of

Fig. 3 **a** Pathogenicity test on *Cornus sanguinea* and *Cornus mas* plant caused by strain 1439 isolated from red dogwood and lmg 1247, 4–6 days after inoculation. A/symptoms caused by 1439 on *Cornus sanguinea,* B/symptoms caused by LMG1247 on *Cornus sanguinea,* C/symptoms caused by 1439 on *Cornus mas*, D/ symptoms caused by 1439 on *Cornus sanguinea.* **b** Pathogenicity test on *Cornus mas* L. plant caused by strains isolated from cornelian cherry, 4–6 days after inoculation. A,B, C/ symptoms caused by strains 1342, 1343, 1557, respectively, D/ symptoms caused by strain 1299b

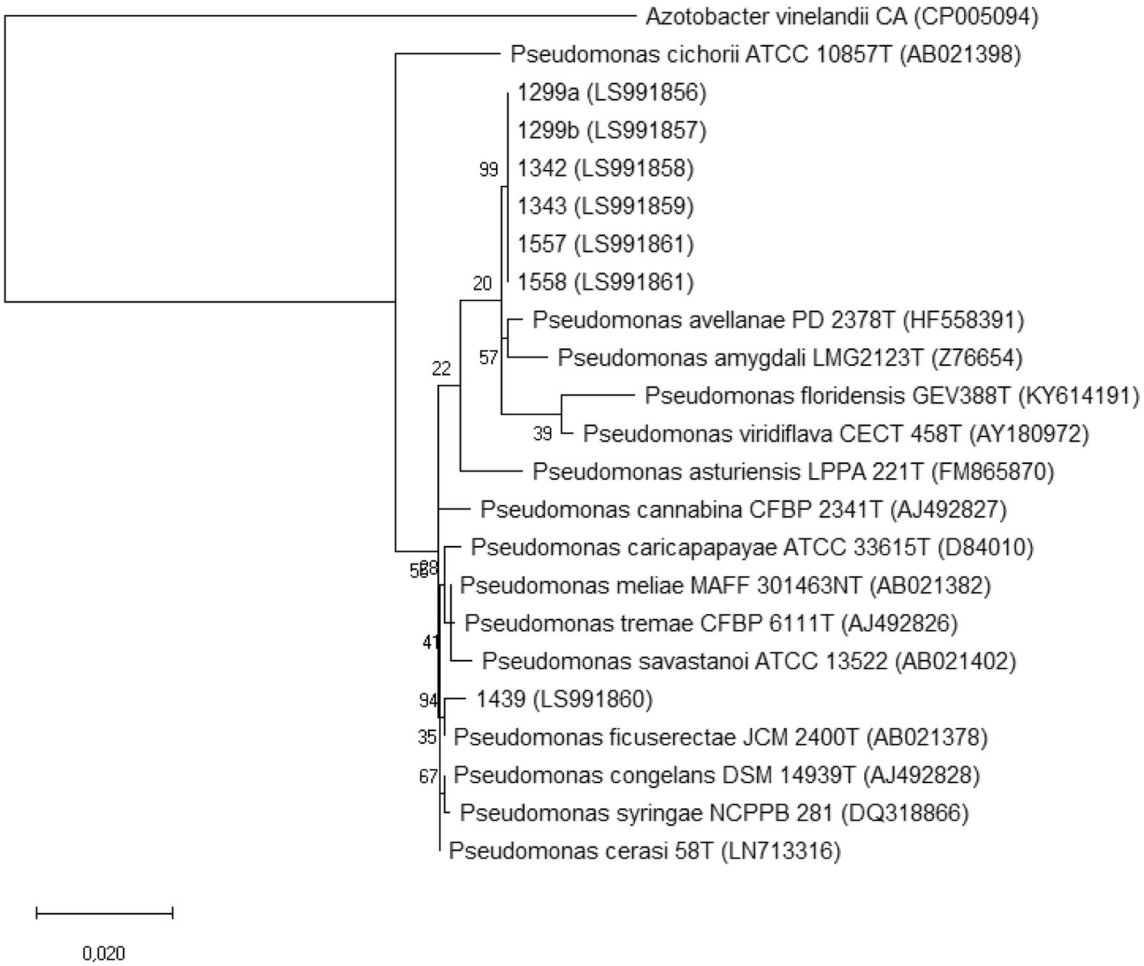

Fig. 4 Maximum likelihood tree of *Pseudomonas* isolates obtained from cornelian cherry and red dogwood and the closest neighbor of other species, based on the analysis of the 16SrRNA gene. Bar 0.020 estimated nucleotide substitutions per site. Bootstrap values (expressed as percentages of 500 replications) are indicated at each node. As an outgroup, the sequence of 16SrRNA gene of *Azotobacter vinelandii* strain CA was used

500 replications. All 21 sequences of the 3 genes analyzed for 7 strains were deposited in the NCBI database, with the following accession numbers: (ii)16S rRNA gene: LS991856-LS991862, (ii) *gyrB* gene: LS991830-LS991836 and (iii) *rpoB* gene: LS991849-LS991855.

PCR with primers specific for *P. avellanae* Based on the results obtained from the analysis of the 16S rRNA, *gyrB* and *rpoB* gene sequences (Figs. 4, 5 and 6, supplementary Fig. S1 and S2), an additional PCR using specific primers for *P. avellanae* (*Pa*): PAV 1 and PAV 22 (Scortichini and Marchesi 2001) and WA/WC (Loreti and Gallelli 2002) and *P. s.* pv. *actinidiae* (Psa) (Rees-George et al. 2010) were chosen for further identification of cornelian cherry isolates. The protocol and conditions described in original paper were used. PCR products were separated in a 1.5% agarose gel in 0.5 x TBE buffer (0.045 M Tris – boric acid, 0.001 M EDTA, pH 8.0) by comparison with the standard mass O'GeneRuler 100 bp DNA Ladder Plus (ThermoScientific, Lithuania) and electrophoresis run at 5–7 V/cm of gel. After staining with ethidium bromide solution (0.5 μg/ml) the obtained amplification products were visualized under UV light.

Results and discussion

Six fluorescent bacterial isolates were selected from those obtained from cornelian cherry and one isolate from red dogwood leaves with blight symptoms. Their colony morphology and strong blue fluorescence on King's B medium was similar to the type strain of *Pseudomonas syringae* LMG 1247^T. The affinity of isolates was confirmed by PCR with primers Ps-F and Ps-R – all gave product 967 bp characteristic for *Pseudomonas* genus.

The pathogenicity test showed that these isolates caused a different kind of necrosis (Fig. 3a and b). The first symptoms were observed after 24 h from inoculation only with strain LMG 1247^T and isolate 1439 from red dogwood on both

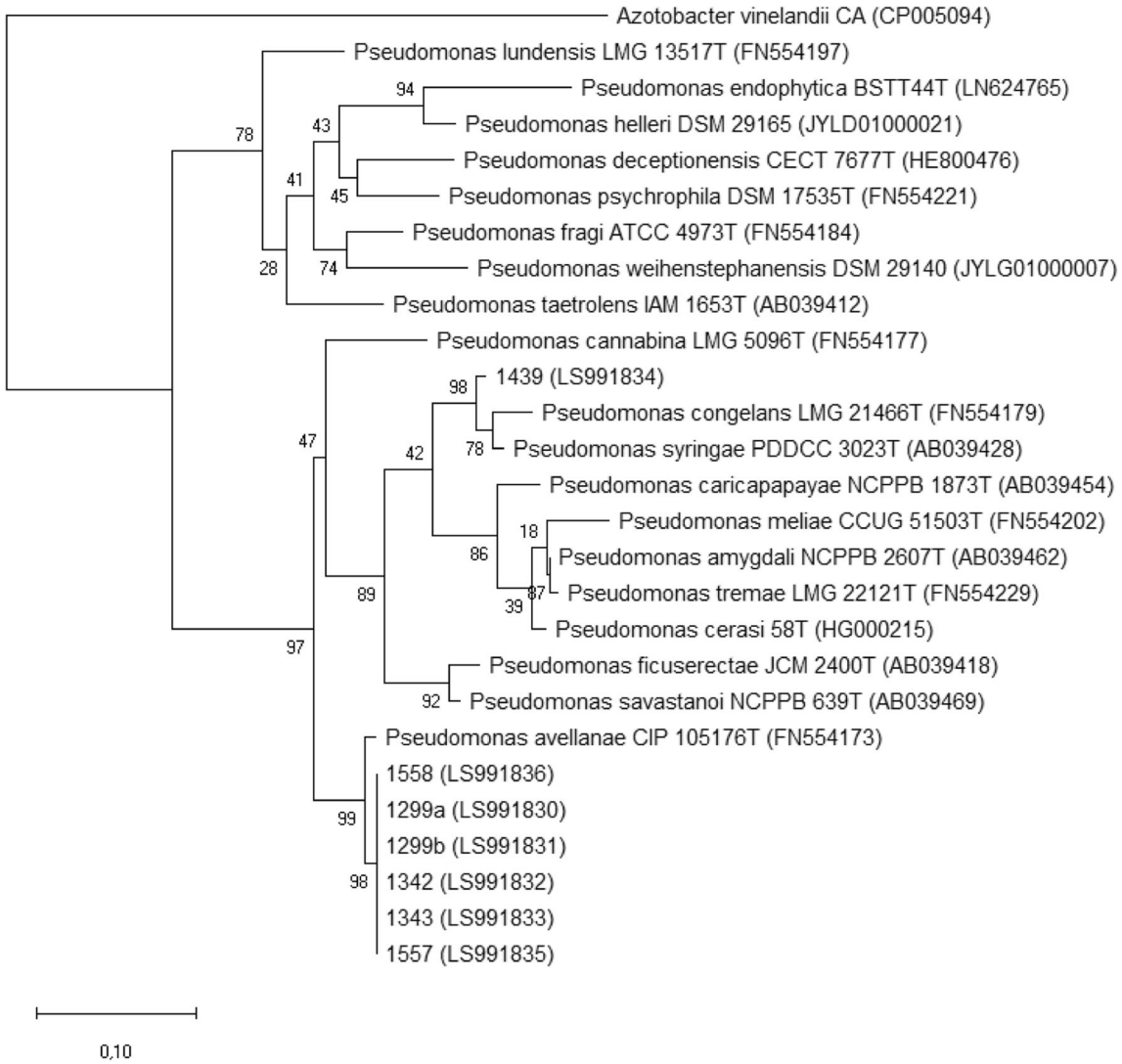

Fig. 5 Maximum likelihood tree of *Pseudomonas* isolates obtained from cornelian cherry and red dogwood and the closest neighbor of other species, based on the analysis of the *gyrB* gene. Bar 0.1 estimated nucleotide substitutions per site. Bootstrap values (expressed as percentages of 500 replications) are indicated at each node. As an outgroup, the sequence of *gyrB* gene of *Azotobacter vinelandii* strain CA was used

Cornus mas and *Cornus sanguinea* leaves. After next 48 h the symptoms caused by isolate 1439 on *Cornus sanguinea* significantly enlarged and dry necrotic areas were observed in the center of lesions (Fig. 3a), whereas the symptoms on *Cornus mas* leaves were not so expanded but also some similar dry areas could be noticed. Strain LMG 1247^{T} caused only small necrotic spots on both host but they did not enlarge since their appearance. The symptoms on both hosts did not change over a period of 5–7 days after inoculation (Fig. 3a). All isolates from cornelian cherry caused first small brownish spots 72 h after inoculation on *Cornus mas* leaves and clear brown necrotic spots, often surrounded by a chlorotic halo, similar to those observed on natural conditions appeared after next 24 h (Fig. 3b). A little different spots were observed after inoculation of leaves with isolate 1299b (Fig. 3b). To fulfill Koch's postulates, bacteria were re-isolated from leaves 8 days after the inoculations. Almost pure cultures were grown on the isolation plates. Colony morphology, other phenotypic characters i.e. LOPAT tests and PCRs done with primers for *syrB, irp1*, PAV 1/PAV 22 and WA/WC, confirmed that the re-isolates were the same as bacteria which were used for inoculation.

After a 2–4 days of incubation on King's B medium at room temperature all six isolates from cornelian cherry produced a brown pigment which changed the color of entire medium to brown after next 48 h. This feature was already observed in our previous study with a *Pseudomonas* species isolated from blueberry (*Vaccinium corymbosum*) in Poland that is also very closely related to *Pseudomonas avellanae* (Kałużna et al. 2013). No information concerning production

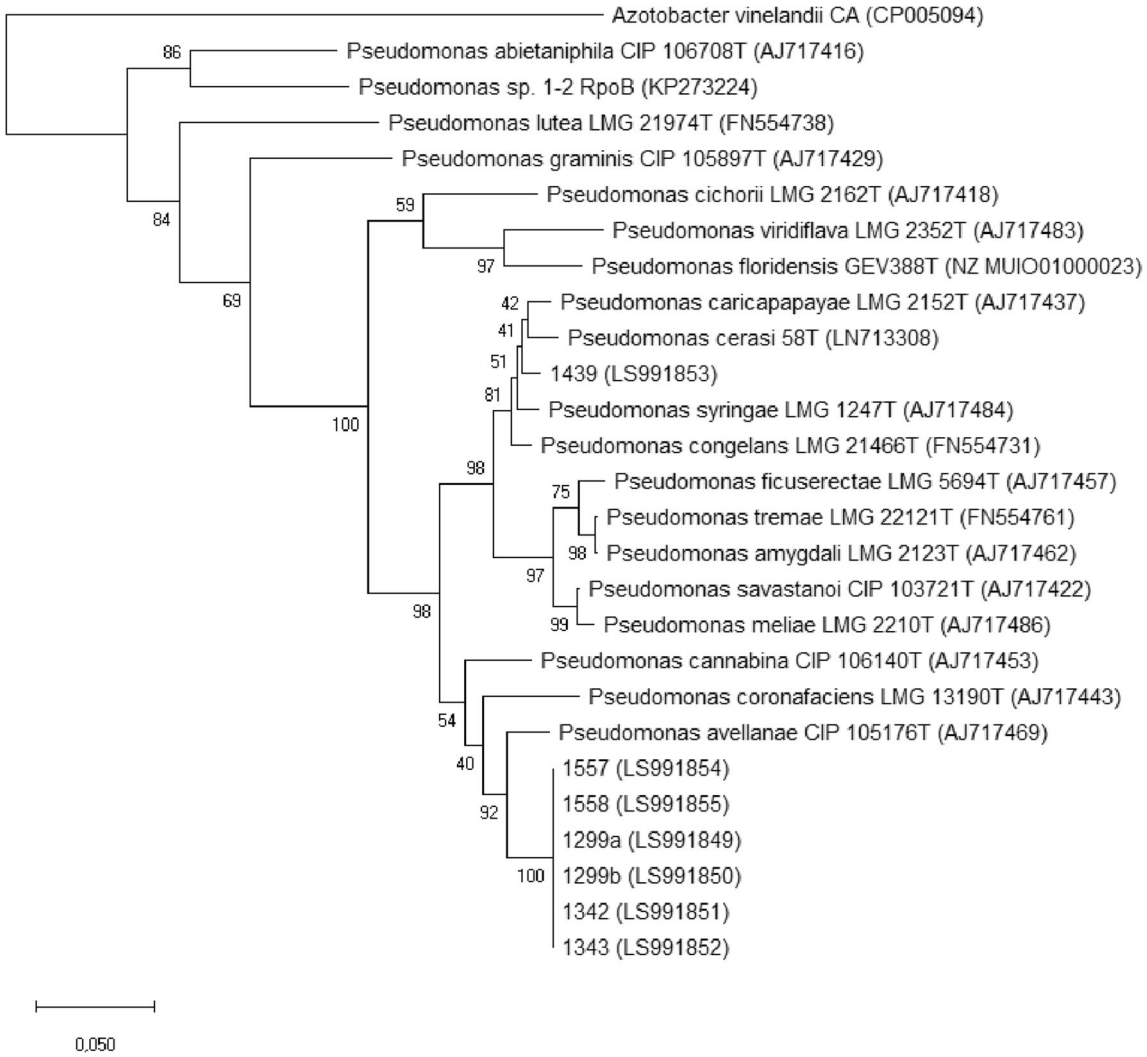

Fig. 6 Maximum likelihood tree of *Pseudomonas* isolates obtained from cornelian cherry and red dogwood and the closest neighbor of other species, based on the analysis of the *rpoB* gene. Bar 0.050 estimated nucleotide substitutions per site. Bootstrap values (expressed as percentages of 500 replications) are indicated at each node. As an outgroup, the sequence of *rpoB* gene of *Azotobacter vinelandii* strain CA was used

of a brown pigment was provided in the first report of leaf blight caused on *Cornus mas* L. from Tennessee, USA (Mmbaga and Sheng 2000). Moreover, it is not known if production of brown pigment on King's B by the US cornelian cherry isolates was not noticed by the researchers or those isolates actually do not produce it. The results from phenotypic tests presented here showed that all seven selected isolates were Gram-negative, produced levan on NSA medium [Nutrient Agar (Difco), supplemented with 5% of sucrose], induced hypersensitivity reactions on tobacco leaves cv. 'Samsun', were negative for the oxidase, arginine dihydrolase and did not caused soft rot of potato slices. These features allowed to classify them to *Pseudomonas syringae* – the LOPAT group Ia as described by Lelliott et al. (1966). As mentioned above the isolates from *Cornus mas* L. produced brown pigment similarly to isolates originating from blueberry however, the blueberry differ, at least phenotypically, because they did not produce levan (Kałużna et al. 2013). It is know from our previous study and other authors that LOPAT tests although very useful, are apart from being rather labor-intensive and time-consuming (Little et al. 1998; Vicente et al. 2004; Kałużna et al. 2013). Moreover, those tests can give sometimes questionable results, and are not sufficient for definitive identification and classification. Therefore, a broader taxonomic study was performed, including molecular characterization and phylogenetic analyses for isolates from cornelian cherry. These test results confirmed that isolates were *P. syringae* and can be considered as a strains.

Detection of genes coding for coronatine, syringomycin, and yersiniabactin showed that the product of the expected size (650 bp) obtained by amplification of *cfl* gene encoding for coronatine was obtained only for *Pss* reference strain LMG

2222. The results of PCR with primers for the gene *irp1* coding for yersiniabactin showed that this gene was present in almost all cornelian cherry strains, namely 1299a, 1342, 1343, 1557 and 1558 and in *P. syringae* pv. *morsprunorum* race 2 reference strain CFBP 3800. The syringomycin specific PCR product of 752 bp of the *syrB* gene and 1078 bp for the gene *syrD* was detected only in strain 1439 isolated from red dogwood and *Pss* reference strain LMG 1247. Based on these results strain 1439 isolated from red dogwood is identified as *Pseudomonas syringae* pv. *syringae* (*Pss*). The study of other authors indicated that *syrD* gene is most common in *Pss* strains (Abdellatif et al. 2017; Bultreys and Gheysen (1999). The presence of the genes for yersiniabactin production is widespread within the genus *Pseudomonas* and hitherto was found to be present in the pathovars *antirrhini*, *apii*, *berberidis*, *delphinii*, *lachrymans*, *morsprunorum* race 2, *passiflorae*, *persicae*, *tomato*, *viburni*, *helianthi*, *tagetis* and *theae* (Bultreys et al. 2006).

When sequences of the cornelian cherry strains were submitted to the database of the EzTaxon server (database updated in May 2018) it was found that they were most similar to *Pseudomonas avellanae* BPIC 631^T isolated from *Corylus avellana* (common hazelnut) in 1976 in Greece (over 99% of similarity), the causal agent of bacterial canker and decline of hazelnut, *Corylus avellana* L. (Janse et al. 1996) which belongs also to group LOPAT Ia and does not possess the *syrB* gene (Lelliott et al. 1966; Scortichini et al. 2002). The strain 1439 from red dogwood was most closely related (100%) to *Pseudomonas ficuserectae* JCM 2400^T isolated from *Ficus erecta* Thunb. in 1983 in Japan, (Goto 1983). Sequence similarity of cornelian cherry strains when compared to sequences available in NCBI database showed the closest similarity (99%) to *Pseudomonas syringae* pathovars *tomato*, *avii* and *maculicola*. In the NCBI database the strain 1439 from red dogwood was the most similar to *Pseudomonas ficuserectae* JCM 2402 isolated from *Ficus erecta* Thunb. in 1983 in Japan (Goto 1983) and *P. s.* pv. *syringae* Pss9097 isolated from *Prunus avium* in UK by Steve Roberts in 2010 and CFBP 2118 isolated from *Prunus avium* in France in 1979.

The dendrogram constructed on the analysis of gene sequence of the 16S rRNA including all type strains of *Pseudomonas* spp. (data not shown) showed that the all isolates from cornelian cherry revealed the highest similarity (formed separate cluster with low bootstrap value 32%) to *Pseudomonas amygdali* LMG 2123^T isolated from *Prunus dulcis* in 1975 in Crete Greece, causing leaf blight on almond (*Prunus amygdalus*) trees, and again a close relation (formed separate cluster with low bootstrap value 32%) with *Pseudomonas avellanae* PD 2378^T and *Pseudomonas asturiensis* LPPA 221^T but not to type strain of *P. s. pv. syringae* (*Pss*), was found in separate distant cluster. The strain 1439 from red dogwood was found again to be the most related to *Pseudomonas ficuserectae* JCM 2400^T. The dendrogram constructed based on the whole analyses with these closest relatives showed that the strains from cornelian cherry formed a separate cluster with high bootstrap value of 99% (Fig. 4).

The dendrogram constructed on sequence analysis of *gyrB* gene with all type strains of *Pseudomonas* spp. species (data not shown) indicated that cornelian cherry isolates were most similar to *Pseudomonas avellanae* CIP 105176^T isolated from *Corylus avellana* in Greece in 1997, but formed separate cluster with bootstrap value 98%, again a close relation also to *Pseudomonas cannabina* CIP 106140^T isolated from *Cannabis sativa* in Hongrie in 1957 (formed again separate cluster with bootstrap value 97%). The strain from red dogwood was closely related (formed separate cluster with bootstrap value 82%,) to *P. syringae* pv. *syringae* PDDCC 3023^T and *Pseudomonas congelans* LMG 21466^T isolated from *Syringa vulgaris* in UK in 1950 and from grasses, phyllosphere in Paulinenaue Brandenburg Germany, in 1994, respectively. The dendrogram constructed based on the analysis with the closest relatives showed that the strains from cornelian cherry formed a separated cluster with as closest relative *P. avellanae* CIP 105176^T with a high bootstrap value of 98% (Fig. 5).

The dendrogram constructed on sequence analysis of *rpoB* gene with all type strains of *Pseudomonas* spp. species (data not shown) indicated that cornelian cherry isolates were the again most closely related to *Pseudomonas avellanae* CIP 105176^T (but formed separate cluster with bootstrap value 100%), but also to *Pseudomonas cannabina* CIP 106140^T and *Pseudomonas coronafaciens* LMG 13190^T, so exactly the same results was obtained as for *gyrB* gene (Fig. 6). The strain from red dogwood was found in a cluster together with the type strain of *P. syringae* pv. *syringae* LMG 1247^T, *Pseudomonas congelans* LMG 21466^T, *Pseudomonas cerasi* 58^T and *Pseudomonas caricapapayae* LMG 2152^T, isolated from *Syringa vulgaris* in UK in 1950, from grasses, phyllosphere in Paulinenaue Brandenburg Germany, in 1994, *Prunus cerasus* in 2007 in Poland and from *Carica papaya* in Brasil, in 1966, respectively, so in to the *P. syringae* group (Anzai et al. 2000).

All the results from phylogeny analyses are in agreement with the study on genes coding of toxin production, i.e. cornelian cherry strains possessed *irp1* gene for yersiniabactin production, as is known for *P. avellanae* strains which also produce yersiniabactin (Marcelletti and Scortichini 2015). The strain 1439 from red dogwood is highly similar to *Pss* in possessing *syrD* genes also characteristic for this pathovar.

Because based on the phylogeny, cornelian cherry strains showed their close relationship to *P. avellanae* a PCR with a primer pair PAV1 and PAV22 specific for *P. avellane* (Scortichini and Marchesi 2001) was additionally performed. These PCR results proved to be positive for all isolates from cornelian cherry. Similar results were obtained with *Pseudomonas* spp. strains isolated in Poland from blueberry.

However, when the phylogenetic study was done on cornelian cherry and blueberry isolates based on all three genes i.e. 16S rRNA, *gyrB* and *rpoB* the cornelian cherry strains formed separate cluster from blueberry ones in 100% bootstrap value. It is known from literature that the primers specific for *P. avellanae* (Scortichini and Marchesi 2001) also amplify DNA of other taxa, namely *P. s.* pv. *actinidiae* and pv. *theae* (Pst) (Scortichini et al. 2002) and others i.e. selected strains of *Pseudomonas fluorescens*, *P. marginalis, P. syringae* pvs. *Papulans*, *syringae, actinidiae* and *tomato* (Rees-George 2010).Thus it is possible that also here such situation appear and exact identification awaits an in depth taxonomic study (Scortichini et al. 2002). Based on these results in the work on blueberry isolates, an additional dendrogram using sequences of strains belonging to pathovars of *P. syringae* was constructed which showed a closer similarity of these strains to *P. s.* pv. *actinidiae* causing bacterial canker disease of kiwifruit and pv. *theae,* the causal agent of bacterial shoot blight on tea plants, than to *P. avellanae* (Kałużna et al. 2013). Here, in this work, when the sequences of the *rpoB* gene of pathovars *Psa* and *Pst* were added to the sequence analysis of this gene from cornelian cherry, red dogwood and the closest neighbor of other species, the cornelian cherry strains were also more closely related to Psa and Pst than to *P. avellanae* (Supplementary Fig. S1). Based on this finding, PCR with primers specific to Psa with primers (Rees-George et al. 2010) showed that all the cornelian cherry strains gave a characteristic product 280 bp with primers PsaF1/R2. When additionally sequences of the *rpoB* gene of some blueberry strains were included, the cornelian cherry strains were more similar to *P. avellanae* (but separated from this species with high bootstrap value of 100%). In this final dendrogram blueberry strains grouped more with Psa (but also formed separate cluster form them with high bootstrap value of 98%) (Supplementary Fig. S2). The results of similarity of other taxa to *P. avellanae* were described already by Scortichini et al. (2013). The conducted genomic analyses on redefinition of *P. avellanae* using the average nucleotide identity (ANI) analysis and the tetranucleotide frequency correlation coefficients (TETRA) value methods demonstrated the existence of a well demarcated genomic cluster that includes strains classified as *P. avellanae*, *P. syringae* pv. *theae*, *P. s.* pv. *actinidiae* and one *P. s.* pv. *morsprunorum* strain, all belonging to *P. avellanae* sensu *lato*. Moreover, as stated by Scortichini and coworkers, some strains of *P. s.* pv. *tomato* and one *P. s.* pv. *lachrymans* strain, are also closely related to *P. avellanae* (Scortichini et al. 2013). Because the primer pair PAV1 and PAV22 proved not to be specific for Pa strains only, we have performed an additional PCR with primer set WA/WA, designed to the *hrpW* gene, which is specific for virulent Pa strains only (Loreti and Gallelli 2002). The results showed that specific product 350 bp was present in some cornelian cherry strains, namely 1299a, 1342, 1343, 1557 and 1558. Only 1299b strain was negative so the same as in case of PCR for *irp1* coding for yersiniabactin. Strain 1299b also showed different symptoms in pathogenicity test. It is known from literature that bacterial populations, that have the *hrp* genes encoding for the harpin proteins are very virulent to hazelnut germplasm (Loreti et al. 2001). Moreover, as stated by authors, specific primer set WA/WC targeted to *hrpW* gene, enables the discrimination between *P. avellanae* and other pseudomonads associated with hazelnut decline (Loreti and Gallelli 2002; Scortichini et al. 2002). Therefore, it appears that cornelian cherry strains, except 1299b, possess a gene associated coding for high virulence.

When *gyrB* gene sequences, obtained in an earlier study (Kałużna et al. 2014) of causal agents of bacterial canker of stone fruit trees in Poland were compared with sequences of the same gene fragment of all available *Pseudomonas* type strains present in GenBank, the Polish strains did not form a monophyletic group, which is the basic criterion for classification into one species. Strains of *P. syringae* pv. *morsprunorum* race 2 were grouped with *P. avellanae* CIP 105176^{T} (Kałużna et al. 2014).When sequences of all pathovars of *P. syringae* were added to the analysis the grouping of strains appeared even the more complex (data not shown). However, no Psa or Pst strains were found to be related to *P. syringae* pv. *morsprunorum* race 2.

As far as is known, this is the first report on the occurrence of a bacterial leaf blight on cornelian cherry (*Cornus mas*) in Poland. The causal agent is a *Pseudomonas* species, closely related to *P. avellanae*. Results till so far, however, do not allow definitive taxonomic placement and identification yet. Therefore, further taxonomic study using the polyphasic approach including phenotypic characterization, BIOLOG, genome sequencing, ANIb, GGDC, DNA–DNA hybridization, sequence analysis of 16S rDNA and more housekeeping genes – MLSA, determination of G + C content, FAME and others enabling definitive classification and determination of taxonomic position of these isolates and those of *Vaccinium corymbosum* (Kałużna et al. 2013) and the related pathovars (Scortichini et al. 2013; Kałużna et al. 2014) will be continued. This paper presents furthermore the first report on the occurrence of *P. s.* pv. *syringae* on red dogwood (*Cornus sanguinea* L.).

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Compliance with ethical standards

Conflict of interest Author declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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