Genetic Variability of *Phytophthora* Community in Natural Water Resources Assessed with Microsatellite DNA Markers

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Abstract

Polymorphism of microsatellite DNA was recorded for both species *Phytophthora plurivora* (syn *P. inflata*), and *P.* taxon salixsoil in water samples, which may suggest that investigated isolates differ and present the genetic richness leading to the potential adaptation in new environments. Results showed that locus S29-30 could be useful for analysis of *Phytophthora* species diversity. High polymorphism of microsatellite DNA was recorded for both species *P. plurivora* and *P.* taxon salixsoil. It may reflect the high potential of adaptability of *P. plurivora* to the changing environmental conditions. As they are distributed with the system of Polish rivers they may cause threat for many forest tree species.

Key words: microsatellite DNA markers, *P. plurivora, P.* taxon *salixsoil*, genetic variability

Introduction

The multitude of *Phytophthora* species in European forests have actually been found as pathologists started studying soil borne populations of *Phytophthora* species. Many species of *Phytophthora* have been identified in European forests to date, including the potentially highly destructive *P. cinnamomi* and *P. ramorum* (Brasier and Webber 2010). Distribution of *Phytophthora* species harmful to forest trees in European forests was summarized by Hansen and Delatour (1999), Oszako et al. (2005), Jung et al. (2009), Jung (2009). Several species are known to cause severe disease syndromes; other species appear to be involved in pathogen complexes and may be partly responsible for forest decline syndromes. The most affected species are broad-leaved trees like oaks, beeches and ashes (Jung et al. 1996, 2000, 2003, 2005, 2009, Oszako and Orlikowski 2005, Orlikowski et al. 2011). Many Oomycetes are found in forest and ornamental nurseries worldwide in addition to natural ecosystems and commercial forests (Mac Donald et al. 1994, Themann et al. 2002, Jung and Blaschke 2004, Orlikowski et al. 2004a, b, and 2011c, Stępniewska 2005, Schwingle et al. 2007, Brasier 2008, Moralejo et al. 2008, Jung 2009, Hulvey et al. 2010).

In Poland, *P. citricola* and *P. cambivora* have been associated with decline of beech stands and ash seedlings in nurseries (Oszako 2005, Orlikowski et al. 2004a,b and 2006). Recently, Jung and Burgess (2009) re-evaluated *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America, and finally revealed a new species, *Phytophthora plurivora.* So, in Poland, *P. plurivora* (former identified as *P. citricola*) it is the most frequent species recognized in forest nurseries (Oszako et al. 2009), as well as in ornamental ones (Orlikowski et al. 2011a).

Apart from plants for plantings, the other major source of infection are natural water courses. Nowadays is known that *Phytophthora* taxa comprises of

ca. 150 species, not all of them occur in European waterways but we can suspect that in 1 liter of water there maybe ca. 50 to 400 zoospores (Jung pers. comm.). Abundance of *Phytophthora* species in Polish forest and riparian ecosystems was revealed by Oszako and Orlikowski (2005), and Orlikowski et al. (2003, 2011a). Other studies involving water and *Phytophthora* come from USA. Reeser et al (2011) identified more than eighteen *Phytophthora* species and even one species of *Halophytophthora* in over hundred forest streams in Alaska and western and southwestern Oregon. Also, some other studies showed that *P.* taxon *salixsoil* and *P. plurivora* (previously known as *P. citricola*) are the most frequent species identified in Polish streams and rivers (Orlikowski et al. 2008). Former *P. citricola* has been the most abundant species in Polish nurseries, probably brought there with river water used for plants watering (Orlikowski et al. 2004b). Nechwatal and Mendgen (2006) have been working on *P.* taxon *salixsoil* species associated with dying plants in littoral zone of Lake Constance in Germany. The species *P.* taxon *salixsoil* belongs to the ITS Clade 6 together with many other species occurring in natural ecosystems. During surveys of dying vegetation in natural ecosystems and associated waterways in Australia many new taxa have been identified from *Phytophthora* ITS Clade 6 (Jung et al. 2011). This Clade represents probably the most numerous pathogens of forest tree species. The paper discusses the known species of *P.* taxon *salixsoil* and *P. plurivora* in Polish natural water reservoirs.

Materials and methods

Sampling of water from riparian ecosystems

Investigated isolates derived from streams located in Regional Forest Districts Poznań and Katowice (Western regions of Poland and Southern Silesia, respectively), as well as from several big rivers like Narew or Bug or small ones like Rządza or Kanał Królewski (Tables 1 and 2). Pathogens were isolated from water using two detection methods: baiting and filtering. In baiting method water was purred into containers and five clean rhododendron leaves (var. Nova Zembla) were floated and incubated for a few days. Leaves which were infected by *Phytophthora* species were cultured on PARP (Kannwischer et al. 1978) and potato dextrose agar (PDA) medium for 7 days at 22°C, according to Ghimire et al. (2009).

In filtering method, samples of water were filtrated through nylon filters of pore size 11, 8 and finally 5 µm (Millipore). Each filter was incubated on selective medium (PARP) for a 7 days at 22°C and next transferred on PDA medium.

DNA extraction

Total DNA was extracted from pure, seven-days cultured isolates grown on PDA. The DNA was obtained using the DNeasy Plant Mini Kit (Qiagen) according to manufacturer's procedure. The concentration and the quality of the total DNA was checked using a spectrophotometer (NanoDrop® ND-1000) and based on the electrophoretic separation in 1% agarose gel.

DNA amplification

In order to determine the level of polymorphism in soil samples, a nuclear microsatellite locus S29-30 was analyzed according to modified procedure of Schena et al. (2008). Each PCR reaction was performed in a total volume of 15 µl and consisted of 1.3 x Q buffer; 1 x Qiagen buffer, 0.13 mM of each primer, 0.11 mM dNTPs; 0.75 units of Taq polymerase (Qiagen) and 10 ng of genomic DNA. To optimize the results of amplification the gradient of annealing temperature (from 58.2ºC to 64.8ºC), the gradient of Q buffer, magnesium and Taq polymerase concentration, as well as the number of PCR cycles in the reaction were tested. Amplifications were run on PTC-200™ Programmable Thermal Controller (MJ Research, Inc.). Amplification protocol was as follows: an initial denaturation at 95ºC for 2 min, 40 cycles consisting of denaturation at 94ºC for 30 s, annealing at 58ºC for 30 s , and extension at 72ºC for 60 s. A final extension was at 72ºC for 5 min. Successful amplification was confirmed by 1.5% agarose gel electrophoresis. Finally, two-step PCR reaction was applied: after 10 cycles of amplification (according to the above-mentioned procedures), new PCR reaction was run (30 cycles) using 0.2 µl of previous reaction mixture as a template. The size of the PCR product was checked on Bioanalyser (Agilent®) using Agilent DNA 1000 Regents.

The heterozygosity level within each samples of *P. plurivora* or *P.* taxon salixsoil isolates, i.e. h (Nei 1978), observed (n_a) and estimated (n_e) number of alleles and Shannon index (I) were calculated using PopGene v.1.31 (Yeh et al. 1999) software. Polymorphism Information Content (PIC) of alleles present in *P. plurivora* and *P.* taxon salixsoil isolates was generated with MolKin v3.0 software (Gutiérez et al. 2005).

Results

The investigated *Phytophthora* species were previously confirmed by direct sequencing of ITS regions, leading to the identification of *P. plurivora* and *P.* taxon Salixsoil detected in samples taken from the natural water reservoirs (Table 1 and Table 2).

Table 1. The characteristics of investigated *P. plurivora* isolates taken from mountain streams

P. plurivora						
No.	Isolates	Accession no.	Amplicon size (bp)	Regional Directorate of State Forest	Forest district	Forest subdistrict
1	747	EU240044.1	448	Katowice	Prudnik	Wilanowice
2	778	EU240051.1	448	Katowice	Prudnik	Opawice
3	785	EU240052.1	447	Katowice	Prudnik	Biechów
4	786	EU240053.1	446	Katowice	Prudnik	Biechów
5	788	EU240054.1	449	Katowice	Prudnik	Dębowiec
6	777A	EU240080.1	448	Katowice	Prudnik	Opawice
7	769A	EU240187.1	339 and 466	Katowice	Prudnik	Pokrzywna
8	779A	EU240079.1	443	Katowice	Prudnik	Opawice
9	780A	EU240078.1	441	Katowice	Prudnik	Opawice
10	787A	EU240077.1	435	Katowice	Prudnik	Dębowiec
11	787B	EU240076.1	430	Katowice	Prudnik	Dębowiec
12	791A	EU240075.1	429	Katowice	Prudnik	Biernatów
13	754A	EU240085.1	443	Katowice	Prudnik	Buków

n.a. – data not available

Optimization of PCR conditions for amplification of the SSR locus S29-30 consisted of testing three critical factors: annealing temperatures, concentration of oligonucleotide primers and quantity of Taq polymerase enzyme. Based on the obtained results, it was found that the optimum conditions for microsatellite DNA amplification took place at 58°C with the presence of 0.13 mM primers and 0.75 U of Taq polymerase, both for *P. plurivora* and *P.* taxon Salixsoil. Results of amplification of environmental samples (according to above-mentioned conditions) were confirmed by gel electrophoresis (data not shown).

Polymorphism of microsatellite DNA was showed by precise analysis of the PCR product in chip-electrophoresis (Figures 1 and 2). In the analyzed *P.*

Table 2. The characteristics of investigated *P.* taxon salixsoil isolates

P. taxon *salixsoil*						
No.	Isolates	Accession no.	Amplicon size (bp)	Regional Directorate of State Forest	Forest district	Origin of water / river name
1	WD39A	EU240100.1	481	n.a.	n.a.	Narew
2	WD39B	EU240161.1	480	n.a.	n.a.	Narew
3	WD40A	EU240125.1	481	n.a.	n.a.	Bug
4	WD40C	EU240126.1	483	n.a.	n.a.	Bug
5	WD40D	EU240127.1	483	n.a.	n.a.	Bug
6	WD40E	EU240137.1	484	n.a.	n.a.	Bug
7	WD45B	EU240101.1	483	n.a.	n.a.	Rządza
8	WD45C	EU240165.1	477	n.a.	n.a.	Rządza
9	WD47A	EU240166.1	472	Poznań	Koło	Kanał Królewski
10	WD47C	EU240144.1	470	Poznań	Koło	Kanał Królewski
11	WD37A	EU240159.1	470	n.a.	n.a.	Narew
12	WD37B	EU240099.1	468	n.a.	n.a.	Narew
13	WD37C	EU240160.1	480	n.a.	n.a.	Narew
14	WD44C	EU240164.1	478	n.a.	n.a.	Rządza
15	WD38A	EU240123.1	476	n.a.	n.a.	Narew
16	WD38B	EU240124.1	478	n.a.	n.a.	Narew
17	GD7B	EU240088.1	472 and 481	Poznań	Koło	Ner
18	GD7C	EU240089.1	473 and 481	Poznań	Koło	Ner
19	GD7G	EU240091.1	471 and 480	Poznań	Koło	Ner
20	GD7H	EU240092.1	469 and 478	Poznań	Koło	Ner
21	WD40B	n.a.	474	n.a.	n.a.	Bug
22	WD44B	EU240138.1	473	n.a.	n.a.	Rządza
23	WD43A	EU240175.1	473	n.a.	n.a.	Rządza
24	WD45A	EU240177.1	472	n.a.	n.a.	Rządza
25	920	EU240179.1	476	Poznań	Koło	lake
26	921	EU240180.1	476	Poznań	Koło	lake
27	922	EU240181.1	477	Poznań	Koło	lake
28	923	EU240197.1	470	Poznań	Koło	lake
29	B01	EU240095.1	476	Warszawa	Chojnów	Utrata
30	B02	EU240167.1	475	Warszawa	Chojnów	Utrata
31	B03	EU240036.1	473	Warszawa	Chojnów	Utrata
32	B04	EU240094.1	466	Warszawa	Chojnów	Utrata
33	B14	EU240037.1	464	Warszawa	Chojnów	Utrata
34	GD15A	EU240152.1	463	Warszawa	Chojnów	Utrata

n.a. – data not available

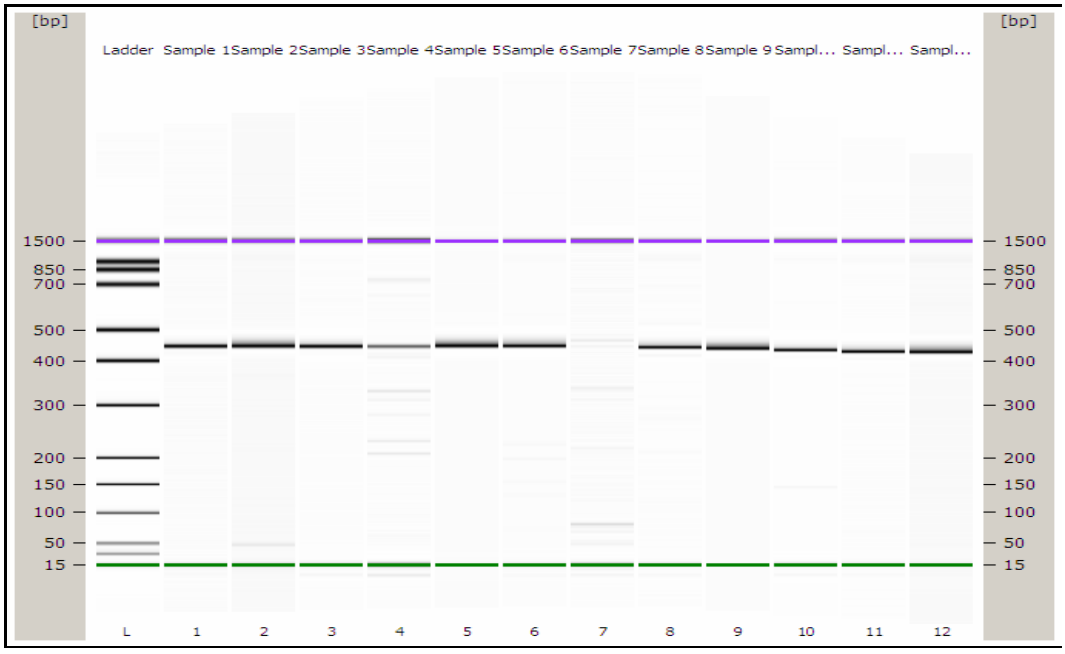

Figure 1. Size of SSR fragment of *P. plurivora* analyzed by chip electrophoresis. L - DNA marker. Numbers of samples according to positive bands from Figure 1

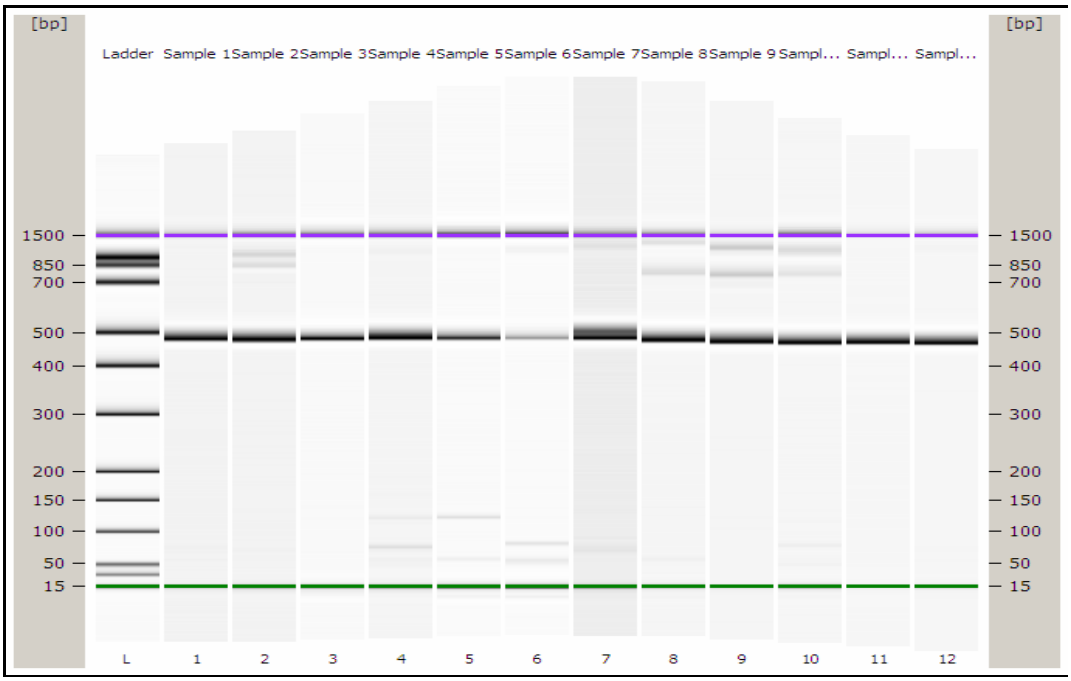

Figure 2. Size of SSR fragment of *P.* taxon salixsoil analyzed by chip electrophoresis. L - DNA marker. Numbers of samples according to Figure 2 (samples from WD39A to WD37B)

plurivora isolates 9 variants of the amplicons of S29-30 locus were found (Table 1, Figure 1), while in the isolates of *P.* taxon salixsoil 16 variants of the same locus were present (Table 2, Figure 2). The most frequent SSR alleles were 448 bp (23.1%) and 476 bp (11.7%) in isolates of *P. plurivora* and *P.* taxon salixsoil, respectively. The lowest microsatellite fragment in *P. plurivora* was 429 base-pair length and the highest – the 449 bp (Figure 3). Among the *P.* taxon salixsoil isolates the lowest and the highest SSR fragments were 463 and 484 bp, respectively (Figure 4). The double bands as a product of PCR amplification may in fact be an interesting phenomenon and could reflect hybrid isolates. The observed and effective allele number, Shannon Index, as well as heterozygosity level was lower in *P. plurivora* isolates (n_a = 11.000, n_e = 8.909, I = 2.305 and h = 0.888) comparing to n_a = 22.000, n_e = 18.050, I = 3.013 and h = 0.945 in *P.* taxon salixsoil isolates (p = 0.05). Different SSR allele sizes occurred in *P.* taxon salixsoil samples than in *P. plurivora* ones. In both kinds of isolates the polymorphism information content reached a high value of PIC = 0.75 (p = 0.5) proving high differentiation of alleles occurring in the investigated samples. Small genetic distance D_N

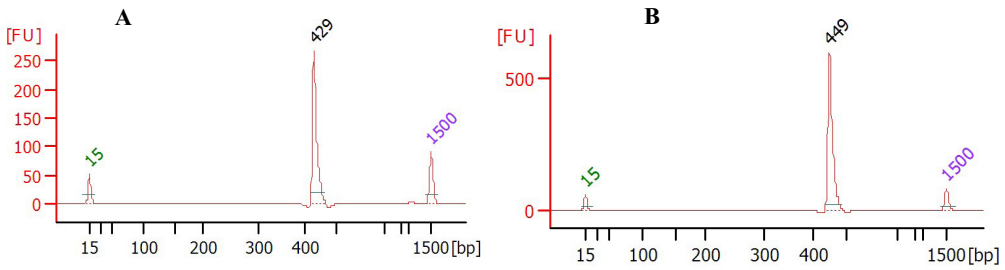

Figure 3. Electropherogram of *P. plurivora* amplicons - the lowest 429 base-pairs (A) and the highest 449 bp (B) SSR fragments generated by Agilent 2100 Bioanalyzer

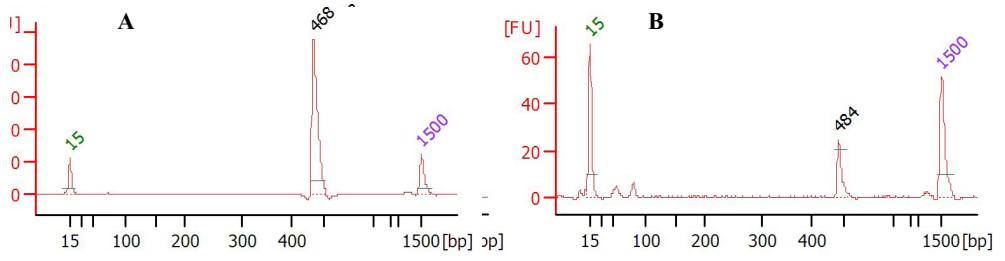

Figure 4. Agilent 2100 Bioanalyzer electropherogram of amplicons of *P.* taxon Salixsoil - the lowest (A) and the highest (B) SSR fragments

= 0.256 (p = 0.5) separated alleles frequencies from *P.* taxon salixsoil from *P. plurivora* ones.

In isolate 769A of *P. plurivora* (Table 1), as well as in GD7B, GD7C, GD7G and GD7H isolates from *P.* taxon Salixsoil (Table 2) non-specific PCR product occurred (two PCR fragments observed). Despite the modification of reaction conditions (applied gradient of annealing temperature, gradient of Q-buffer and magnesium concentration and reduction of cycles in PCR reaction) this artifact was not eliminated, suggesting that is not necessarily an artifact but was caused by diploid organism.

Discussion

It is believed that most widespread and aggressive species are considered to be exotic and invasive. *Phytophthora* species are considered to be highly invasive with wide-word distribution (Zentmyer 1988, Shearer and Tippett 1989, Erwin and Riberio 1996, Hansen et al. 2000, Rizzo et al. 2002, Jung and Blaschke 2004, Cooke et al. 2005, Evans and Oszako 2007, Hansen 2008, Jung 2009, Brasier and Webber 2010, Duran et al. 2010, Rea et al. 2010). During research on oak decline many pathogenic phytophthoras were identified, reflecting their strong involvement in this phenomenon. In Germany, 10 *Phytophthora* species were found in soil rhizosphere, 73 out of 124 oaks and in 19 out of 35 stands (Jung et al. 2000), in France - 8 *Phytophthora* species in Amance and 6 species in Illwald (Hansen and Delatour 1998). Also in Italy, 5 *Phytophthora* species were found in soil below oak trees in 19 of the 30 investigated stands (Vattraino et al. 2001), and in Turkey 7 *Phytophthora* species from soil rhizosphere in 117 out of 291 oaks and in 38 out of 51 stands (Balci and Halmschlager 2003).

The other threat of *Phytophthora* as plant destroyers come from nurseries. Testa et al. (2005) found 51 *Phytophthora* spp. in Ohio ornamental nurseries during a state-wide survey of *Phytophthora ramorum* as the causal agent of Sudden Oak Death. In this research the ITS region of all isolates was amplified using the universal primers ITS1 and ITS4 and sequenced allowing for the identification of *P. cactorum* and *P. plurivora* (former *P. inflata* and *P. citricola*) as Jung and Burgess (2009) clearly showed that *P. inflata* species was lost, there is no type isolate and all isolates designated as *P. inflata* are *P. plurivora* (Jung and Burgess 2009). However, Testa et al. (2005) also detected two occurrences of *P. plurivora* Caroselli & Tucker and one of *P. insolita* Ann & Ko. They isolated *P. inflata* (=*P. plurivora*) from two independent locations, in one case from dead twigs, and in another one from a necrotic leaf tip. Koch's postulates were satisfied by inoculating rhododendron plants with the putative pathogens. They were able to reproduce the lesions both on leaf and on twigs. The presence of the parasite was confirmed by re-isolation on sterile medium. Also, *P. plurivora* was used in a high number of pathogenicity tests performed on European oaks, beech ash and other tree species (Brasier and Jung 2003; Jung et al. 1996, 1999, 2002, 2003; Jung and Nechwatal 2008; Weiland et al. 2010; Orlikowski et al. 2011).

The potential source of *Phytophthora* infection could be the infestation of the soil during flooding periods, introduction of pathogens with road building material and soil adhering to vehicles and boots, and from infected seedlings coming from nurseries (Shearer and Tippett 1989, Erwin and Ribeiro 1996, Jung and Blaschke 2004, Jung 2009). Certainly species of *Phytophthora* were also introduced with infested nursery stock coming from other nurseries via trading. In the soils tested for the presence of *Phytophthora* species, it was found that *P. quercina* and *P. cambivora* are very aggressive species and are responsible for deterioration of offspring oak seedlings (*Quercus robur* L.) (Jung et al. 1996, 1999, 2000, 2003). In Poland, *P. gonapodyides, P.* taxon salixsoil and *P.* taxon oaksoil species were also found in nurseries and broad-leaved stands (data not published). The possible pathways of pathogenic phytophthoras are rivers, nurseries and forest plantations. Especially, infected water (e.g. via planting disease seedlings along river banks) create an enormous menace for riparian ecosystems themselves but also for forest and ornamental nurseries which use river water for watering plant seedlings. That hypothesis supports research done in Serbia in alluvium of Sawa river (Milenkovic et al. 2011). They point out the occurrence of *Phytophthora* species in hydrophilic forests, *Fraxino angustifoliae-Quercetum roboris* under the impact of flooded water from rivers. In our research performed in streams of Prudnik forest district, *Phytophthora* species were also identified by morphology and DNA characterization with a single strand conformational polymorphism, COX spacer sequence and ITS sequence (data deposited in the NCBI gene bank, Table 1). The species from ITS Clade 6 were most abundant overall, including *P. gonapodyides* (37% of all isolates), *P.* taxon salixsoil, *P.* taxon oaksoil and additionally *P. pseudosyringae* (Clade 3).

The purpose of this study was to recognize the variability of these pathogenic Oomycetes originated from different natural water courses in Poland. In order to achieve this goal the molecular biology techniques were applied. Microsatellite markers are a powerful tool in the study of *Phytophthora* population

biology, epidemiology, ecology, genetics and evolution. The SSR sequences present many advantages, e.g. they are equally distributed across the genome, are co-dominant, present high polymorphism in the nucleotide composition, and are suitable for genotyping in automatic sequencer. Among several SSR markers originally developed for *P. sojae*, the marker S29-30 was the most appropriate to assess the genetic variability of *P. alni* subsp. *alni*, *P. cambivora*, *P. cinnamomi* and *P. citricola* (Schena et al. 2008). In our study, this locus revealed quite high polymorphism in *P. plurivora* isolates, and in *P.* taxon salixsoil isolates (h=0.888 and h= 0.945, respectively). That proved the usefulness of the S29-30 marker in the population variability study of the above species. The variability of isolates may suggest that there were several introductions into Poland in the past and that water was a good vector to spread those pathogens. In addition the variability of isolates may also reflect recent adaptation to the multitude of new host species that *P. plurivora* invaded after its relatively recent introduction to Europe. If river passes through forest stands, all growing species can be endangered by new comers. Global warming may accelerate their development, as the optimal growth of e.g. *P. ramorum* oscillates around 30°C, which is still unusual for Poland. However, the summer temperatures in Poland have an average only increased by less than 1°C during last century, which did not influence phytophthoras spread, so far. Much more important influence could have an increase in mean winter temperatures insuring better survival of *Phytophthora* species. Moreover, the changes of precipitation patterns towards more severe heavy rain events alternating with severe droughts may favor *Phytophthora* diseases as suggested by Jung (2009) for beech and oak decline and European Environment Agency (2011). It has been supposed the southern part of Eastern Europe will experience decreases of up to 10% in annual mean precipitation, while the northern part will see increases of up to 10% (EEA report 2011). Consequently, riparian ecosystems may suffer from damage caused by *Phytophthora* species.

Similarly, when water is taken from rivers for watering seedlings in nurseries it menaces both ornamental and forest plant production. Nechwatal and Mendgen (2006) noticed that an unnamed *Phytophthora* from ITS Clade 6 was frequently isolated from the soil rhizosphere of reed (*Phragmites australis*) growing on the littoral zone of Lake Constance (Germany). The isolates closely resembled *P. gonapodyides*, having internally proliferating, non-papillate sporangia, a rather high temperature optimum for growth (30°C), and being sexually sterile. The taxon was isolated from permanently, as well as occasionally flooded reed sites using standard baiting procedures, indicating a wide distribution in the Lake Constance littoral zone. ITS sequence analysis revealed that isolates were identical to the as yet unnamed *P.* taxon *salixsoil*, originally isolated from willow (*Salix*) roots in the UK and alder (*Alnus*) debris in Denmark. In an in vitro leaf inoculation assay *P.* taxon *salixsoil* proved to be more aggressive towards willow *Salix alba* than *P. gonapodyides*. The new taxon belonging to Clade 6 may be of significance as a root pathogen of many woody plants in moist or flooded situations occurring in alluvial forest communities. Due to its close resemblance to *P. gonapodyides* the taxon might have passed unnoticed in the past, and possibly has been much more widely distributed than previously recognized (Nachwatal and Mendgen 2006). Our studies support this idea showing the abundance of *P.* taxon *salixsoil* in Polish water coming from natural reservoirs. The pathogenicity tests including *P.* taxon *salixsoil* and *P. plurivora* were performed by Jung and Nechwatal (2008) and Orlikowski et al. (2011) on many European tree and shrub species. Also, multiple new *Phytophthora* species from the same Clade 6 were found in natural ecosystems in Australia causing ecological implications (Jung et al. 2011). The number of *Phytophthora* propagules in water depends on the period of vegetation season (Orlikowski et al. 2007, 2008). In this research rhododendron leaves were used as baits for *Phytophthora* spp. isolated from river, nursery water pond and canal. Among already identified species, *P. plurivora* was detected most often from 3 sources of water, whereas *P. cinnamomi* - in nursery pond and canal in March and May, and *P. citrophthora* only in a nursery canal in June (Orlikowski et al. 2007, 2008). Three above *Phytophthora* species colonized leaves and 1-year-old stem parts of birch and alder with the quickest development of necrosis on leaf blades of birch. It is possible that parts of birch and alder are the source of *Phytophthora* in water and the place of pathogen surviving. It is interesting that *P. plurivora* is a species affecting relatively small number of host plants while *P. cinnamomi* being much less genetically variable is more pathogenic even up to 950 host plants.

Above records prove that *Phytophthora* species were introduced to nurseries from natural water resources used for plant watering. Further, infected seedlings were important source of *Phytophthora* infection in riparian ecosystems, plantations and forest stands. Taking into consideration the pathogenicity of above *Phytophthora* species widely distributed in Polish rivers, there is a high potential risk of damage to forest ecosystems, which can be triggered by observed climatic changes.

Conclusions

1. The studies showed the abundance of *P. plurivora* and *P.* taxon *salixsoil* in Polish natural water resources.

2. Results revealed usefulness of the *locus* S29-30 for *Phythophtora* species genetic diversity analysis.

3. *P. plurivora* and *P.* taxon salixsoil reveal high polymorphism of microsatellite DNA. It probably reflects high potential of adaptability of *P. plurivora* to the changing environmental conditions.

4. Considering above findings we presume that pathogenic *Phytophthora* species distributed in Polish rivers cause significant threat to forest ecosystems, especially under observed climatic changes.

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ГЕНЕТИЧЕСКОЕ РАЗНООБРАЗИЕ РОДА *PHYTOPHTHORA* В ПРИРОДНЫХ ВОДНЫХ ИСТОЧНИКАХ, ПРОАНАЛИЗИРОВАННОЕ С ПОМОЩЬЮ МИКРОСАТЕЛЛИТНЫХ МАРКЕРОВ ДНК

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Резюме

В ходе микросателлитного анализа изолятов *Phytophthora plurivora* (син. *P. inflata*) и *P. taxon* Salixsoil, полученных из водных источников, была выявлен генетический полиморфизм. Полученные данные указывают как на генетическую гетерогенность изученных изолятов, так и свидетельствуют о высоком уровне генетического разнообразия, определяющего адаптационную способность живых организмов к изменяющимся условиям окружающей среды. В ходе исследований продемонстрирована перспективность использования микросателлитного маркера S29-30 для изучения генетического разнообразия видов рода *Phytophthora*. Наибольший уровень генетического полиморфизма был выявлен среди изолятов *P. plurivora* по сравнению с *P. taxon* Salixsoil, что, по всей видимости, связано с более высокой адаптивной способностью данного вида. Патогенные виды рода *Phytophthora* Азиатского происхождения распространяются по речной системе Польши и представляют собой угрозу для многих сельскохозяйственных и лесных видов.

Ключевые слова: микросателлитные маркеры, *P. plurivora, P.* taxon Salixsoil, генетическая изменчивость