Characterising the Genetic Diversity of *Pseudomonas syringae* pv. *syringae* Isolated from Rice and Wheat in Iran

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Abstract

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Sheath rot of rice and leaf blight of wheat caused by *Pseudomonas syringae* pv. *syringae* are the important bacterial pathogens of rice and wheat in Iran. The randomly amplified polymorphic DNA (RAPD) method was used to investigate the genetic diversity of 60 strains of *P. s.* pv. *syringae* obtained from rice and wheat in different growth stages. Cluster analysis by UPGMA method showed that strains were grouped into two clusters. The AMOVA analysis indicated that about 18% of the total genetic variation existed between two populations of rice and wheat, which showed the lack of host specialization in *P. s*. pv. *syringae* strains among rice and wheat. We confirmed that high genetic heterogeneity existed in the *P. s*. pv. *syringae* strains which are detectable by RAPD analysis, and that molecular and statistical analysis of RAPD fragments can be used both to distinguish between strains and to determine relatedness between them.

Key words: bacterial pathogen; population genetic structure; host preference; RAPD

Pseudomonas syringae van Hall 1902 (Palleroni 1984) is a plant-associated bacterial species which has been divided into more than 50 pathovars. *P. s.* pv. *syringae* is the causal agent of many plant disease including leaf blight of wheat (Niknejad-Kazempour *et al*. 2010) and sheath rot of rice (Khoshkdaman *et al*. 2008). *P. syringae* is a very heterogeneous group and belongs to RNA homology group I of *Pseudomonas*, which is a part of subclass g from the class of proteobacteria (Young 1991). This species is genetically diverse (Bradbury 1986) and its pathovars exhibit DNA homology at 40–100%, whereas this between 95–100% among strains of one particular pathovar (Palleroni *et al.* 1972).

Various molecular techniques that have been used to characterise *P. s.* pv. *syringae* strains showed that high genetic heterogeneity existed in the their strains, including strains isolated from the same host (Little *et al.* 1998; Scortichini *et al*. 2003; Cirvilleri *et al*. 2005, 5006). The analysis of genomic DNA using PCR-based methods has proved to be a fast, sensitive, and reliable method for determining genetic relationships among pathogenic organisms. Several methodologies are currently available for studying genetic variability among strains of the same pathovar.

The random amplified polymorphic DNA (RAPD) was a technique first described by Williams *et al*. (1990). Although there have been many debates on the reproducibility of RAPD markers, many studies have shown that RAPDs are useful molecular markers to detect genetic diversity at the population level in well-controlled experiments (Diaz *et al*. 2001; Reisch *et al*. 2003; Nybom *et al*. 2004), and also the same results as obtained by RAPD analysis were obtained by AFLP, RFLP, SSR (Powell *et al*. 1996) and isozymes (Isabel *et al.* 1995; Nadler 1995). The low reproducibility and dominant nature

are two drawbacks of RAPD markers (Williams *et al.* 1990). The low reproducibility can now be overcome by optimisation of the RAPD reaction (Rai *et al*. 2009), and the other limitation can be overcome by increasing the number of individuals assayed (by 2 to 10´ compared to isozymes and RFLPs) (Lynch & Milligan 1994) and using appropriate statistical methods e.g. analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992), which is not influenced by the dominance problem and has become an important tool for investigating the partitioning of dominant marker variation (Diaz *et al*. 2001).

The objective of this study was to use RAPD to determine the relatedness of 60 strains of *P. s*. pv. *syringae* obtained from rice and wheat in different growth stages, as well as to determine if RAPD data support the grouping of strains based upon host plants from different growth stages and explore the applicability of the RAPD technique to reveal the potential genotypic variability of *P. s*. pv. *syringae* strains.

MATERIAL AND METHODS

Bacterial strains. The strains of *P. s*. pv. *syringae* used in this study originated from various regions of the Guilan province, Iran. Thirty-eight strains were obtained from different growth stages of rice

Table 1. *Pseudomonas syringae* pv. *syringae* strains

Strain	Host	Stage of isolation	Location	Strain	Host	Stage of isolation	Location
PRN1	rice	nursery	Rasht	PRP8	rice	panicle	Lahijan
PRN2	rice	nursery	Rasht	PRP9	rice	panicle	Lahijan
PRN3	rice	nursery	Lahijan	PRP10	rice	panicle	Rasht
PRN4	rice	nursery	Roodsar	PRP11	rice	panicle	Rasht
PRN5	rice	nursery	Astaneh	PRP12	rice	panicle	Hasanrood
PRN6	rice	nursery	Astaneh	PRP13	rice	panicle	Anzali
PRN7	rice	nursery	Kalachi	PRP14	rice	panicle	Anzali
PRN8	rice	nursery	Kalachi	PRP15	rice	panicle	Fooman
PRN9	rice	nursery	Kochesfehan	PW1	wheat	field	Roodbar
PRN10	rice	nursery	Kochesfehan	PW2	wheat	field	Roodbar
PRN11	rice	nursery	Kalachi	PW3	wheat	field	Roodbar
PRN12	rice	nursery	Kalachi	PW4	wheat	field	Siahkal
PRF1	rice	field	Rasht	PW5	wheat	field	Siahkal
PRF2	rice	field	Rasht	PW6	wheat	field	Roodbar
PRF3	rice	field	Lahijan	PW7	wheat	field	Amlash
PRF4	rice	field	Roodsar	PW8	wheat	field	Amlash
PRF5	rice	field	Astaneh	PW9	wheat	field	Talesh
PRF6	rice	field	Astaneh	PW10	wheat	field	Talesh
PRF7	rice	field	Kalachi	PW11	wheat	field	Astara
PRF8	rice	field	Kalachi	PW12	wheat	field	Astara
PRF9	rice	field	Kochesfehan	PW14	wheat	field	Astara
PRF10	rice	field	Kochesfehan	PW15	wheat	field	Roodbar
PRF11	rice	field	Kalachi	PW16	wheat	field	Roodbar
PRP1	rice	panicle	Astaneh	PW17	wheat	field	Siahkal
PRP2	rice	panicle	Astaneh	PW18	wheat	field	Siahkal
PRP3	rice	panicle	Astaneh	PW19	wheat	field	Amlash
PRP4	rice	panicle	Kiashahr	PW20	wheat	field	Roodbar
PRP5	rice	panicle	Kiashahr	PW21	wheat	field	Roodbar
PRP6	rice	panicle	Kiashahr	PW22	wheat	field	Roodbar
PRP7	rice	panicle	Kiashahr				

and twenty-two strains were isolated from wheat in the field (Table 1). Strains were maintained in LP media (7 g/l yeast, 7 g/l bacto peptone and 30% glycerol) (Yessad *et al*. 1992), and subcultured on King's medium B (KB) (King *et al*. 1954) as needed.

DNA extraction. DNA was extracted from the strains that were grown overnight in nutrient broth (Merck, Darmstadt, Germany) at 26°C using the protocols described by Martins *et al.* (2005). One tube of 1.5 ml was used to centrifuge the cells at 13 000× *g* for 5 min and the pellet was suspended in 200 ml Tris 0.1 mol/l and added 200 ml of lysis solution (NaOH 0.2N and 1% SDS), mixed and deproteinased with 700 ml of phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v), homogenised and centrifuged at 13 000× *g* for 10 minutes. To precipitate DNA, 700 ml of cold isopropanol was added and spun, washed in 70% ethanol and centrifuged. Precipitated DNA was dried at room temperature and suspended in 100 ml of water. The method described by Asubel *et al*. (1996) was performed comparing 60 strains. The samples from both methods were electrophoresed on 0.8% agarose gels, stained with ethidium bromide and photographed under UV light (wavelength 312 nm).

RAPD analysis. Ten primers were used in this study (Table 2) which were supplied by CinnaGen Inc. (Tehran, Iran). Seventeen primers were tested for efficacy in the RAPD method, and the primers listed in Table 2 were selected because they gave reproducible results. The RAPD was performed as modified according to Martins *et al*. (2006). The amplification reactions were accomplished in a final volume of 25 ml containing 50mM Tris-HCl (pH 8.3), 3mM $MgCl_2$, 0.4mM deoxyribonucleotide triphosphates (dNTP), 0.4mM of each primer, 1 U Taq DNA polymerase (Promega, Madison, USA), and 50 ng/ml of DNA. Amplification conditions used for RAPD PCR were initial denaturation at 94°C for 1 min, followed by 40 PCR cycles at 94°C for 45 s, primer annealing at 37°C for 1 min, and primer extension at 72°C for 1 minute. Final extension at 72°C for 5 min was carried out for polishing the ends of PCR productions. PCR products were separated on a 1.5% agarose gel in 1X TBE buffer (89mM Tris base, 89mM boric acid, 2.5mM EDTA, pH 8), for 60 min at 100 V. The gels were then stained with ethidium bromide and visualised under UV light (wavelength 312 nm). Negative controls, amplification reactions without DNA, were used in all primers.

Table 2. RAPD code and sequence of primers

Primer	Sequence of nucleotide
70.8	5'CTGTACCCCC3'
70.9	5'TGCAGCACCG3'
80.7	5'GCACGCCGGA3'
80.8	5'CGCCCTCAGC3'
H3	5'AGACGTCCAC3'
H13	5'GACGCCACAC3'
G13	5'CTCTCCGCCA3'
OPA-10	5'GTGATCCCAG3'
OPA-11	5'CAATCGCCGT3'
OPA-12	5'TGGCCCTCAC3'

Analysis of molecular data. The RAPD gel images were processed using Quantity One Software Version 4.0.1 (Bio-Rad Laboratories, Hercules, USA). The bands were sized and then binary coded by 1 or 0 for their presence or absence in each strain. The scores for each primer were merged in a Microsoft excel spreadsheet. A locus was considered polymorphic if the relevant band was present in one or more, but not in all, individuals of the population. The percentage of polymorphic bands (PPB) was calculated from the matrix by the number of PBS/total number of bands × 100 (Nei *et al*. 1995). A similarity index was calculated on the basis of the formula:

$$S_{xy} = 2N_{xy}/(N_x + N_y)$$

where:

N_{xy} – number of bands of strains *x* and *y* in common

N_x – number of bands of strains *x*

N_y – number of bands of strains *y* (Nei & Li 1979) by Free Tree program (Pavlíček *et al.* 1999)

An unweighted pair group method with arithmetic average (UPGMA) algorithm was used to perform cluster analysis. The 60 strains of *P. s.* pv. *syringae* based on the host were divided into two groups: rice group (38 strains from different growth stages of rice) and wheat group (22 strains from wheat fields). The Shannon Information Index (Lewontin 1972), Nei's gene diversity (Nei 1973) and effective number of alleles (Kimura & Crow 1964) were also calculated for each population. All of these genetic diversity parameters were estimated using POPGENE program Version 1.31 (Yeh *et al.* 1998). Nei's estimate of similarity, based on the number of shared RAPD products, was used to generate similarity and distance. The

total genetic diversity (H_T), genetic diversity within each population (H_S) and Nei's coefficient of the gene differentiation (G_{ST}) were calculated using the POPGENE software, Version 1.31. The genetic variation among the populations was also estimated by the analysis of molecular variance (AMOVA) (Excoffiere *et al.* 1992), in which the total genetic variance was partitioned into among population and within population component, which were calculated by Genealex program version 6.2 (Beck *et al.* 2008; Smouse *et al.* 2008).

RESULS

RAPD-PCR using 10 primers yielded 179 reproducible bands, ranging in size from 160 bp to 5600 bp, and the number of bands for each primer was from 14 to 23, while 152 bands were polymorphic (84.92%). A representative RAPD profile is shown in Figure 1. 70.8 and 70.9 primers were appropriated respectively with 0.1247 and 0.2962 minimum and maximum amount of polymorphic information content (PIC) (Table 3). Cluster analysis was done by UPGMA using Dice coefficient. Cluster I consists of 40 strains of *P. s.* pv. *syringae* isolated from rice and wheat, and clusters II included 20 strains of *P. s.* pv. *syringae* from rice. Cluster I was further divisible into two subclusters, Ia included 22 and 7 strains isolated from wheat and panicle rice, and Ib included 11 strains from rice fields. Cluster II with 66% bootstrap consisted of two subclusters IIa and IIb that included one strain from nursery rice and 19 strains from nursery and panicle rice, respectively (Figure 2). The results of the intrapopulational diversity index of RAPD-PCR examination with 10 primers for two populations of *P. s.* pv. *syringae* strains are shown in Table 4. The largest intrapopulational diversity observed in the rice population. Genetic distance for two populations of *P. s.* pv. *syringae* was 0.0672 (Table 5). The total gene diversity (H_T = 0.2272) obtained indicates a considerable level of variation between strains isolated from wheat and rice. In addition, an H_S value of 0.1991 was obtained, a part of the total genetic diversity within each population (Table 6). G_{ST} obtained from two populations of rice and wheat was 0.1236, indicating 12% of variation among two populations of wheat and rice. The results show that most of genetic diversity is distributed within populations and that these results are consistent with the results of molecular variance. Results from AMOVA indicated that 18% of the genetic variation is attributable to

Table 3. The percentage of polymorphic bands (PPB), polymorphism information content (PIC) and genetic diversity based on Nei (*H*) obtained per each primer among the *P. s.* pv. *syringae* strains

Primer	PPB	PIC	*H*
70.8	18	0.1247	0.1599
70.9	16	0.2962	0.2963
80.7	19	0.2550	0.2637
80.8	22	0.2435	0.2435
H3	18	0.2642	0.2828
H13	14	0.2281	0.2618
G13	14	0.1455	0.1455
OPA-10	23	0.2330	0.2331
OPA-11	19	0.2134	0.2450
OPA-12	16	0.1541	0.2052
Average	17.9	0.2158	0.2337

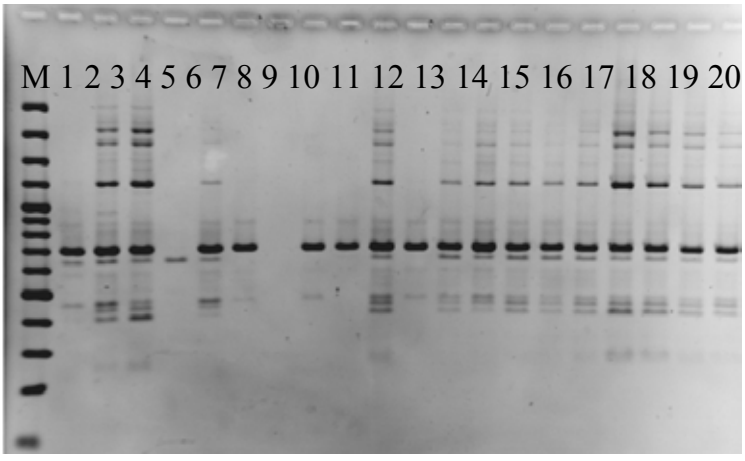

Figure 1. Gel stained with ethidium bromide showing PCR amplification products generated from the *P. s.* pv. *syringae* strains with 70.9 primer

1 – PRP8; 2 – PRP9; 3 – PRP10; 4 – PRP11; 5 – PRP12; 6 – PRP13; 7 – negative control; 8 – PRP14; 9 – PRP15; 10 – PRN1; 11 – PRN2; 12 – PRN3; 13 – PRN4; 14 – PRN5; 15 – PRN6; 16 – PRN7; 17 – PRN8; 18 – PRN9; 19 – PRN10; 20 – PRN11; M = 100 pb DNA marker

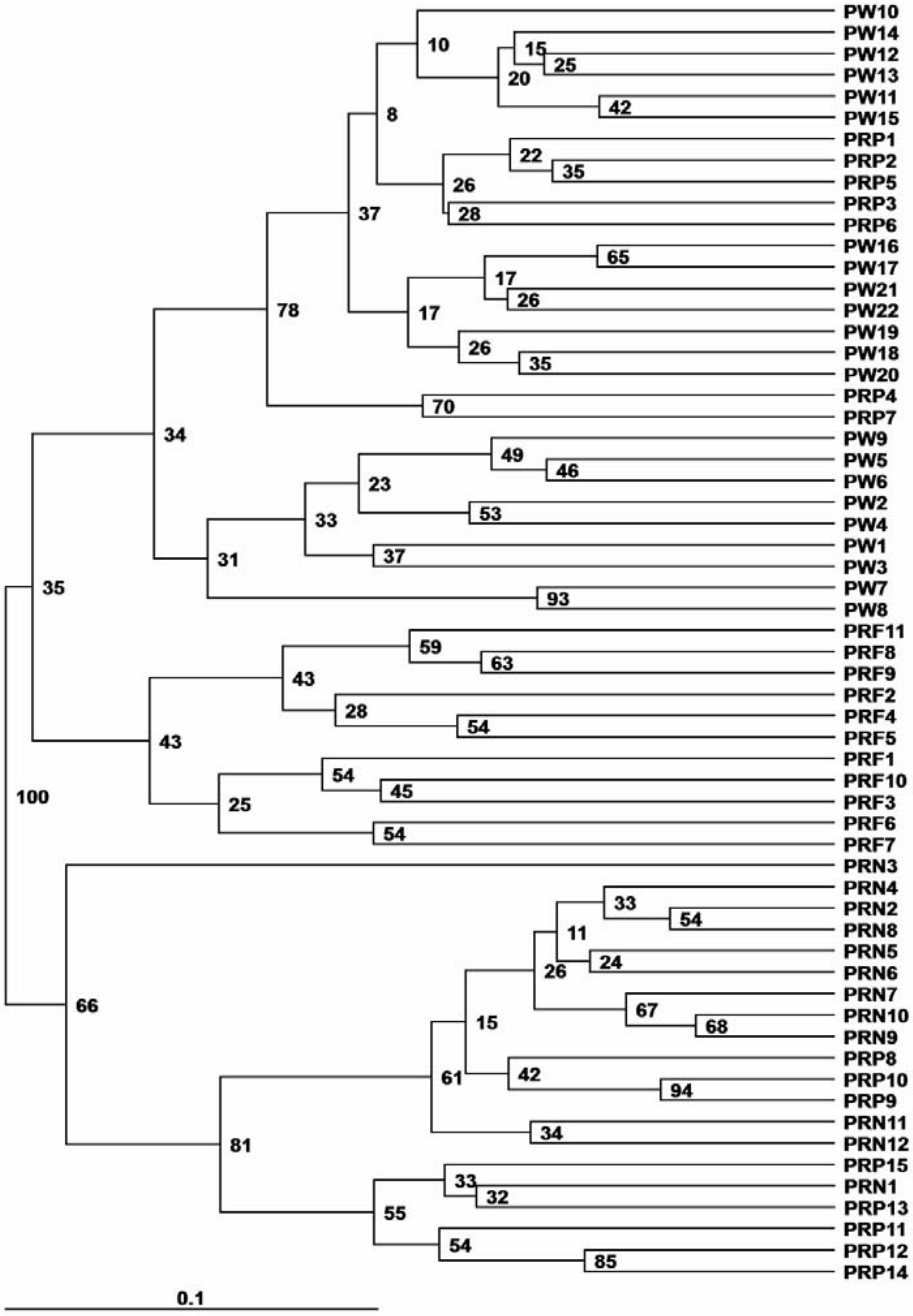

Figure 2. A dendrogram constructed using the UPGMA method shows the phylogenetic relationships of RAPD fingerprints of *P. s.* pv. *syringae* strains isolated rice and wheat; numbers at the branch points represent the bootstrap values (1000 replicates)

Table 4. Genetic variation of two populations of *P. s.* pv. *syringae* obtained from rice and wheat

Population	No. of isolates bacteria	*Na*	*Ne*	*H*	*I*	Polymorphic loci (%)
Rice	38	1.76 ± 0.42	1.39 ± 0.35	0.23 ± 0.18	0.36 ± 0.25	76.54
Wheat	22	1.51 ± 0.50	1.28 ± 0.36	0.16 ± 0.19	0.25 ± 0.28	50.84
Average	–	1.63 ± 0.46	1.33 ± 0.35	0.19 ± 0.18	0.30 ± 0.26	–

Na – observed number of alleles; *Ne* – effective number of alleles (Kimura & Crow 1964); *H* = Nei's (1973) gene diversity; *I* – Shannon Information Index (Lewontin 1972); *n* ± SD

Table 5. Ne's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) between populations of *P. s.* pv. *syringae* obtained from rice and wheat

Population	Rice	Wheat
Rice	****	0.9350
Wheat	0.0672	****

Table 6. Genetic structure of three populations of *P. s.* pv. *syringae* obtained from rice and wheat

Estimative	H_T	H_S	G_{ST}
Mean	0. 2272 ± 0. 0269**	0. 1991 ± 0. 0210	0. 1236

H_T – total genetic diversity in the pooled populations; H_S – mean diversity within each population; G_{ST} – mean coefficient of gene differentiation; **standard deviation

Table 7. Analysis of molecular variance (AMOVA) for 60 strains of *P. s.* pv. *syringae*; the total data set contains isolates from two populations

Source	df	SS	MS	Est. Var.	%
Among populations	1	139.490	139.490	4.311	18
Within populations	58	1122.304	19.350	19.350	82
Total	59	1261.794	–	23.661	100

df – degrees of freedom; SS = sum of squares; MS – mean square; Est. Var. – estimated variance; % – percentage of estimated variance; **significant at $P < 0.01$ level

differences among *P. s.* pv. *syringae* populations (rice and wheat) and 82% of the genetic variation is attributable to *P. s.* pv. *syringae* within one population (Table 7).

DISCUSSION

This study examined the ability of RAPD markers to determine genetic variability among 60 strains of *P. s.* pv. *syringae* obtained from rice and wheat in different growth stages in various regions of Guilan province, Iran. The primers were selected based on reproducibility and polymorphic pattern from this initial screening. The primers were selected based on the quantity and quality of their amplified fragments and were then used to survey all strains of *P. s.* pv. *syringae* (Clerc *et al.* 1998). All of these primers had G + C contents higher than 60% mol, while the genome G + C content of *P. s.* pv. *syringae* was 59% to 60% mol (Doudoroff & Palleroni 1974). Ten primers used were highly informative and revealed an average of 17.9 bands per primer, they also produced polymorphic banding patterns that provide information about the relatedness of *P. s.* pv. *syringae* strains (Martins *et al.* 2006).

There was a short genetic distance between two population of rice and wheat (0.0672). This short genetic distance is attributed to separating the isolates from a limited geographic area (Sisto *et al.* 2007), adapted to a specific ecological niche (Little *et al.* 1998) and isolation of strains from a narrow host range (Poaceae) (Denny *et al.* 1988; Little *et al.* 1998). Also, results from AMOVA showed that a high genetic variation occurs within *P. s.* pv. *syringae* strains of one population. This low variance between two populations of rice and wheat showed the lack of specialisation in *P. s.* pv. *syringae* strains among rice and wheat, which is advantageous for the management of breeding strategies.

Based on results from *Na*, *Ne*, *H*, *I* and polymorphic loci index, maximum intrapopulational diversity was in strains isolated from rice. One of the reasons was the isolation of rice strains from different growth stages (nursery, field-leaf and glume).

This study confirms that considerable genetic variability exists within the *P. s.* pv. *syringae* complex, and is also present when the pathogen attacks solely the host plant in different growth stages, rice in this case. This variability was in agreement with other investigations performed on the same strains using ERIC-PCR (Cirvilleri *et al.* 2005) or BOX-PCR (Scortichini *et al.* 2003; Natalini *et al.* 2006), or on other strains of *P. s.* pv. *syringae* using ERIC-PCR (Little *et al.* 1998) or AFLP analysis (Clerc *et al.* 1998; Manceau & Brin 2003; Cirvilleri *et al.* 2006).

Overall, our results suggest the high genetic similarity between two populations of rice and wheat which can be adapted to a specific ecological niche, such as *P. s.* pv. *syringae* strains in the north of Iran

(Little *et al.* 1998). These findings have practical implications in giving a better understanding of the pathogen population structure over time and space, the use of available resistant sources in disease control, understanding the impacts of host genotypes on pathogenic variability, and tracking changes in the population structure over time and space. This study shows that RAPD analysis is a useful technique to determine relatedness between strains *P. s.* pv. *syringae* from the same and different host plants in different growth stages.

Reference

Asubel F.M., Brent R., Kingston E., Moore D.D., Seidmann J.G. (1996): Current Protocols in Molecular Biology. John Wiley & Sons, New York.

Beck N., Peakall R., Heinsohn R. (2008): Social constraint and an absence of sexbiased dispersal drive fine-scale genetic structure in white-winged choughs. Molecular Ecology, **17**: 4346–4358.

Bradbury J.F. (1986): Guide to Plant Pathogenic Bacteria. CAB International, Wallingford.

Clerc A., Manceau C., Nesme X. (1998): Comparsion of randomly amplified polymorphic DNA with amplified fragment length polymorphism to assess genetic diversity and genetic relatedness within genospicies III of *Pseudomonas syringae*. Applied and Environmental Microbiology, **64**: 1180–1187.

Cirvilleri G., Bonaccorsi A., Scuderi G., Scortichini M. (2005): Potential biological control activity and genetis diversity of *Pseudomonas syringae* pv. *syringae* strains. Phytopathology, **153**: 654–666.

Cirvilleri G., Scuderi G., Bonaccorsi A., Scortichini M. (2006): Molecular characterization of *Pseudomonas syringae* pv. *syringae* strains from different host plants using fluorescent amplified fragment length polymorphism. Plant Pathology, **88**: 329–333.

Denny T.P., Gilmour M.N., Selander R.K. (1988): Genetic diversity and relationship of two pathovars of *Pseudomonas syringae*. Genetics Microbiology, **134**: 1949–1960.

Diaz V.L., Muniz M., Ferrer E. (2001): Random amplified polymorphic DNA and amplified fragment length polymorphism assessment of genetic variation in Nicaraguan population of *Pinus occarpa*. Molecular Ecology, **10**: 2593–2603.

Doudoroff M., Palleroni N.J. (1974): Genus I. *Pseudomonas* Migula. In: Buchanan R.E., Gibbons N.E. (eds): Berg's Manual of Determinative Bacteriology. 5th Ed. Williams & Wikins, Baltimore: 217–243.

Excoffier L., Smouse P.E., Quattro J.M. (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, **131**: 479–491.

Isabel N., Beaulieu J., Bousquet J. (1995): Complete congruence between gene diversity estimates derived from genotypic data at enzyme and random amplified polymorphic DNA loci in black spruce. Proceedings National Academy of Sciences USA, **29**: 6369–6373.

Khoshkdaman M., Niknejad-Kazempour M., Ebadi A.A., Mossanejad S., Pedramfar H. (2008): Identification of causal agent of foot and sheat rot of rice in the fields of Guilan province of Iran. Agricultura Tropica et Subtropica, **41**: 17–20.

Kimura M., Crow J.F. (1964): The number of alleles that can be maintained in a finite population. Genetics, **49**: 725–738.

King E.O., Ward M.K., Raney D.E. (1954): Two simple media for the demonstration of pyocyanin and fluorescein. Journal of Laboratory and Clinical Medicine, **44**: 301–307.

Lewontin R. (1972): Apportionment of human diversity. Evolutional Biology, **6**: 381–398.

Little E.L., Bostock R.M., Kirkpatrick B.C. (1998): Genetic characterization of *Pseudomonas syringae* pv. *syringe* stains from stone fruit in California. Applied and Environmental Microbiology, **64**: 3818–3823.

Lynch M., Milligan B.G. (1994): Analysis of population genetics structure with RAPD markers. Molecular Ecology, **3**: 91–99.

Manceau C., Brin C. (2003): Pathovars of *Pseudomonas syringae* are structured in genetic populations allowing the selection of specific markers for their detection in plant samples. In: Iacobellis N.S., Collmer A., Hutcheson S.W., Mansfield J.W., Morris C.E., Murillo J., Schaad N.W., Stead D.E., Surico G., Ullrich M.S. (eds): *Pseudomonas syringae* and Related Pathogen. Kluwer Academic Publisher, Dordrecht: 503–512.

Martins M.L., de Araujo E.F., Mantovani H.C., Moraes C.A., Vanetti M.C.D. (2005): Detection of the *apr* gene in proteolytic psychorotrophic bacteria isolated from refrigerated raw milk. International Journal of Food Microbiology, **102**: 203–211.

Martins M.L., Pinto C.L.O., Rocha R.B., de Araujo E.F., Vanetti M.C.D. (2006): Genetic diversity of gram-negative, proteolytic, psychorotropic bacteria isolated from refrigerated raw milk. International Journal of Food Microbiology, **111**: 144–148.

Natalini E., Rossi M.P., Barionovi D., Scortichini M. (2006): Genetic and pathogenetic diversity of *Pseudomonas syringae* pv. *syringe* associated with bud necrosis and leaf spot of pear in a single orchard. Plant Pathology, **88**: 219–223.

Nei M. (1973): Analysis of gene diversity in subdivided populations. Proceeding National Academy Science USA, **70**: 3321–3323.

Nei M., Li W.H. (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceeding National Academy Science USA, **76**: 5269–5273.

Nei M., Takezaki N., Sitnikova T. (1995): Assessing molecular phylogenies. Science, **267**: 253–254.

Niknejad-Kazempour M., Khergoo M., Pedramfar H., Rahimian H. (2010): Isolation and identification of bacterial glum blotch and leaf blight on wheat (*Triticum aestivum* L.) in Iran. African Journal of Biotecnology, **9**: 2860–2865.

Nadler S.A., Lindquist R.L., Near T.J. (1995): Genetic structure of midwestern *Ascaris suum* populations: a comparsion of isoenzyme and RAPD markers. Journal of Parasitology, **81**: 385–394.

Nybom H. (2004): Comparsion of different nuclear DNA markers for estimating intraspecific genetic diversity in plant. Molecular Ecology, **13**: 1143–1155.

Palleroni N.J. (1984): Genus *Pseudomnas* Migula. In: Krieg N.R., Holt J.G. (eds): Bergey's Manual of Systematic Bacteriology. Vol. 1. Williams and Wilkins Co., Baltimore: 141–199.

Palleroni N.J., Ballard R.W., Rolstone E., Doudoroff M. (1972): Deoxyribonucleic acid homology among some *Pseudomonas* species. Bacteriology, **110**: 1–11.

Pavlíček A., Hrdá S., Flegr J. (1999): Free-Tree – freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus *Frenkelia*. Folia Biologica (Praha), **45**: 97–99.

Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S., Ralfski A. (1996): Comparsion of RFLP, RAPD, AFLP and SSR (micro satellite) markers for germplasm analysis. Molecular Breeding, **2**: 225–238.

Rai A.R., Meshram S.U., Donger A.B. (2009): Optimization of RAPD-PCR for discrimination of different strains of *Bacillus thuringensis*. Romanian Biotechnological Letters, **14**: 4307–4312.

Reisch C., Poschlod P., Wingender R. (2003): Genetic differentiation among populations of *Sesleria albicans* Kit. ex Schultes (Poaceae) from ecologically different habitats in central Europe. Heredity, **91**: 519–527.

Scortichini M., Marchesi U., Dettori M.T.. Rossi M.P. (2003): Genetic diversity, presence of the *syrB* gene, host preference and virulence of *Pseudomonas syringae* pv. *syringe* strains from woody and herbaceous host plant. Plant Pathology, **52**: 277–286.

Scortichini M. (2005): The population structure of some plant pathogenic bacteria: An ecological and adaptive perspective. Plant Pathology, **87**: 5–12.

Sisto A., Cipriani M.G., Tegli S., Cerboneschi M., Stea G., Santtili E. (2007): Genetic characterization by fluorescent AFLP of *Pseudomonas savastanoi* pv. *savastanoi* strains isolated from different host species. Plant Pathology, **56**: 366–372.

Smouse P.E., Peakall R., Gonzales E. (2008): A heterogeneity test for fine-scale genetic structure. Molecular Ecology, **17**: 3389–3400.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, **18**: 6531–6535.

Yeh F.C., Rong-Cai Y., Boyle T. (1998): POPGENE. Version 1.31. University of Alberta, Center for International Forestry Research, Edmonton.

Yessad S., Manceau C., Luisetti J. (1992): A detached leaf assay to evaluate virulence and pathogenicity of strains of *Pseudomonas syringae* on pear. Plant Disease, **76**: 370–373.

Young J.M. (1991): Pathogenicity and identification of the lilac pathogen, *Pseudomonas syringae* pv. *syringe* van Hall 1902. Annual of Applied Biology, **118**: 283–298.

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