



Diversity of p_hlD alleles in the rhizosphere of wheat cropped under annual rice–wheat rotation in fields of the Indo-Gangetic plains: influence of cultivation conditions

Gwenael Imfeld, Noam Shani, David Roesti, Nathalie Fromin, Bhavdish N. Johri, Rachna Gaur, Pierre Rossi, Laurent Locatelli, Franck Poly, Michel Aragno

► To cite this version:

Gwenael Imfeld, Noam Shani, David Roesti, Nathalie Fromin, Bhavdish N. Johri, et al.. Diversity of p_hlD alleles in the rhizosphere of wheat cropped under annual rice–wheat rotation in fields of the Indo-Gangetic plains: influence of cultivation conditions. *Current Science*, 2006, 90 (11), pp.1521-1525. hal-00128260

HAL Id: hal-00128260

<https://hal.science/hal-00128260>

Submitted on 30 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

7. Weber, A., Smolander, A., Nurmiaho-Lassila, E.-L. and Sundman, V., Isolation and characterization of *Frankia* strains from *Alnus incana* and *Alnus glutinosa* in Finland. *Symbiosis*, 1988, **6**, 97–116.
8. Lumini, E. and Bosco, M., PCR-restriction fragment length polymorphism identification and host range of single-spore isolates of flexible *Frankia* sp. strain UFI132715. *Appl. Environ. Microbiol.*, 1996, **62**, 3026–3029.
9. Zhong, Z., Murry, M. A. and Torrey, J. G., Culture conditions influencing growth and nitrogen fixation in *Frankia* sp. HFPCc13 isolated from *Casuarina*. *Plant Soil*, 1986, **91**, 3–15.
10. Prin, Y., Maggia, L., Picard, B., Diem, H. G. and Goullet, P., Electrophoretic comparison of enzymes from 22 single-spore cultures obtained from *Frankia* strain ORS140102. *FEMS Microbiol. Lett.*, 1991, **77**, 223–228.
11. Wall, L. G., The actinorhizal symbiosis. *J. Plant Growth Regul.*, 2000, **19**, 167–182.
12. Clawson, M. L., Caru, M. and Benson, D. R., Diversity of *Frankia* strains in root nodules of plants from the families Elaeagnaceae and Rhamnaceae. *Appl. Environ. Microbiol.*, 1998, **64**, 3539–3543.
13. Mansour, S. R., Dewedar, A. and Torrey, J. G., Isolation, culture and behaviour of *Frankia* strain HFPCg14 from root nodules of *Casuarina glauca*. *Bot. Gaz.*, 1990, **151**, 490–496.
14. Singh, V., Nayyar, H. and Uppal, R., Germination behaviour of different biotypes of seabuckthorn (*Hippophae rhamnoides*, *Hippophae salicifolia* D. Don and *Hippophae tibetana*) growing in dry temperate Himalayas. Proceedings of the Indian Society for Tree Scientists, Solan, 2001.
15. Rongsen, Lu., In *Seabuckthorn: A Multipurpose Plant Species for Fragile Mountains* (eds ICIMOD Publications), ICIMOD Occasional Paper No. 20, Kathmandu, Nepal, 1992, pp. 2–47.
16. Sarma, H. K., Sharma, B. K. and Tiwari, S. C., A novel calcimycin antibiotic from Gram-positive actinomycete *Frankia* micro-symbiont. *Curr. Sci.*, 2003, **85**, 1401–1403.
17. Benson, D. R., Isolation of *Frankia* strains from alder actinorhizal root nodules. *Appl. Environ. Microbiol.*, 1982, **44**, 461–465.
18. Schwencke, J., Rapid exponential growth and increased biomass yield of some *Frankia* strains in buffered and stirred mineral medium (BAP) with phosphatidylcholine. *Plant Soil*, 1991, **137**, 37–41.
19. Lalonde, M. and Calvert, H. E., Production of *Frankia* hyphae and spores as an infective inoculant for *Alnus* species. In *Symbiotic Nitrogen Fixation in the Management of Temperate Forests* (eds Gordon, J. C., Wheeler, C. T. and Perry, D. A.), Forest Research Laboratory Manual, Oregon State University, Corvallis, USA, 1979, pp. 95–110.
20. Lechevalier, M. P., Cataloging *Frankia* strains. *Can. J. Bot.*, 1983, **61**, 2964–2967.
21. Haansuu, J. P., Demethyl C-11 cezomycin – a novel calcimycin antibiotic from the symbiotic nitrogen fixing actinomycete *Frankia*. Ph D Dissertationes, Biocentri Vikki Universitatis Helsingiensis, University of Helsinki, Finland, 2002.
22. Akkermans, A. D. L., Roelofsen, W., Blom, J., Huss-Danell, K. and Harkink, R., Utilization of carbon and nitrogen compounds by *Frankia* in synthetic media and in root nodules of *Alnus glutinosa*, *Hippophae rhamnoides* and *Datisca cannabina*. *Can. J. Bot.*, 1983, **61**, 2793–2800.
23. Huss-Danell, K., Roelofsen, W., Akkermans, A. D. L. and Meijer, P., Carbon metabolism of *Frankia* spp. in root nodules of *Alnus glutinosa* and *Hippophae rhamnoides*. *Physiol. Plant.*, 1982, **54**, 461–466.
24. Murry, M. A., Fontaine, M. S. and Torrey, J. G., Growth kinetics and nitrogenase induction in *Frankia* sp. HFPArl3 grown in batch culture. *Plant Soil*, 1984, **78**, 61–78.
25. Maunuksela, L., Zepp, K., Koivula, T., Zeyer, J., Haahtela, K. and Hahn, D., Analysis of *Frankia* populations in three soils devoid of actinorhizal plants. *FEMS Microbiol. Ecol.*, 1999, **28**, 11–21.
26. Sarma, G., Sen, A., Verghese, R. and Misra, A. K., A novel technique for isolation and generation of single-spore cultures. *Can. J. Microbiol.*, 1998, **44**, 490–492.
27. Racette, S. and Torrey, J. G., The isolation, culture and infectivity of a *Frankia* strain from *Gymnostoma papuanum* (Casuarinaceae). *Plant Soil*, 1989, **118**, 165–170.
28. Lundquist, O.-P. and Huss-Danell, K., Response of nitrogenase to altered carbon supply in a *Frankia*–*Alnus incana* symbiosis. *Physiol. Plant.*, 1991, **83**, 331–338.

ACKNOWLEDGEMENTS. We thank Dr Johannes Pasi Haansuu, University of Helsinki, Finland and Prof David R. Benson, University of Connecticut, USA for providing reference strains and Department of Science and Technology, New Delhi for financial assistance to H.K.S.

Received 19 August 2005; revised accepted 7 February 2006

Diversity of *phlD* alleles in the rhizosphere of wheat cropped under annual rice–wheat rotation in fields of the Indo-Gangetic plains: influence of cultivation conditions

Gwenael Imfeld¹, Noam Shani¹, David Roesti¹, Nathalie Fromin^{1,3}, Bhavdish N. Johri^{2,*}, Rachna Gaur², Pierre Rossi¹, Laurent Locatelli¹, Frank Poly^{1,4} and Michel Aragno¹

¹Microbiology Laboratory, University of Neuchâtel, Switzerland

²Department of Microbiology, GP Pant University, Pantnagar 263 145, India

³Centre d'Ecologie Fonctionnelle et Evolutive, CNRS Montpellier, France

⁴Ecologie Microbienne, UMR CNRS Université Claude Bernard, Lyon 1, France

The antibiotic 2,4-diacetylphloroglucinol is a major determinant in the biocontrol of plant growth promoting rhizobacteria associated with crops of agronomic relevance. The *phlD* gene is a useful marker of genetic and phenotypic diversity of 2,4-DAPG-producing rhizobacteria. A two-step amplification procedure was developed in order to assess directly the presence of *phlD* in environmental DNA, avoiding the tedious procedure of *phlD*-positive strain screening and isolation. We found a predominance of one or two *phlD* alleles in wheat fields cultivated in rice–wheat rotations for twenty years, suggesting that continuous rice–wheat cropping would lead to an enrichment of particular *phlD* genotypes. We also recovered new sequences with no close relative among known *phlD* sequences, indicating that part of the *phlD* allelic diversity might have been missed using standard media culture conditions.

Keywords: 2,4-DAPG, wheat rhizosphere, rice–wheat, *phlD*.

THE limited incidence of soil-borne pathogens in the rice–wheat systems, is probably due to the repeated transitions

*For correspondence. (e-mail: bhavdishnjohri@rediffmail.com)

from anaerobic (rice season) to aerobic conditions (wheat season). However, survival of pathogens during the rice phase as well as their proliferation on crop residues¹ may reduce the yield up to 20%, if their propagation occurs before leaf emergence². In the rice–wheat cropping fields of the Indo-Gangetic plains region, adoption of the raised beds system allows the upper part of the bed to remain in oxic conditions even during the rice season. The use of this technique optimizes water use efficiency, improves weed management and opens up investment opportunities by diminishing the production costs^{1,3}. Nonetheless, raised bed system, by combining reduced-tillage and a permanent oxic zone, could favour re-emergence of pests and diseases kept at low levels during the rice season. The biological approaches that are currently being developed to control a variety of phytopathogenic agents include the use of beneficial free-living bacteria, usually referred to as plant growth promoting rhizobacteria (PGPR)⁴. PGPR are involved in disease management by different mechanisms that include niche exclusion through microbial competition, stimulation of plant defence or production of antibiotics^{4,5}.

The bacterial antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) has antifungal, antibacterial, anthelmintic and phytotoxic properties⁶. Moreover, the 2,4-DAPG producing fluorescent pseudomonads from various crops have been shown to share the same biosynthetic locus⁷. The *phlD* gene is located in the *phlACBD* operon coding for the production of 2,4-DAPG⁸. In addition, the *phlD* gene was shown to be a useful marker of genetic and phenotypic diversity of 2,4-DAPG-producing rhizobacteria^{9,10}. This diversity has been studied in relation to biological control, root colonization and soil suppressiveness¹¹. To date, diversity studies of 2,4-DAPG producers are mostly based on screening approaches targeting pseudomonad isolates. Several diversity studies of functional bacterial groups showed that only a small fraction of the diversity is revealed by cultivable strains. For example, Hamelin *et al.*¹² demonstrated that the most dominant *nifH* cluster included only environmental clones and there were no sequences related to cultivable diazotrophs in the rhizosphere of *Molinia coerulea*. We can then expect that some key 2,4-DAPG-producing organisms may be ignored by culture-based experiments. Therefore, in order to avoid a cultivation bias, the investigation was aimed at assessing the allelic diversity of the *phlD* gene from DNA extracted directly from the root environment in fields under annual rice–wheat rotation. The diversity of the *phlD* sequence pools was compared in root and root-adhering soil with respect to (i) raised beds versus traditional plain fields, (ii) plain fields with the same practice but with different grain yields.

Trials were carried out in two experimental sites located in Uttar Pradesh, India. The first one was located in Bhavanipur village (Badaun district lat. 28°02'N, long. 79°10'E) and the second near Ghaziabad (Ghaziabad district, lat. 28°40'N, long. 77°28'E). Both sites have a his-

tory of twenty years of rice–wheat rotation and the same wheat cultivar UP 2338 (provided by the GB Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India) was grown for five years. Two conventionally tilled fields of 4000 m² were selected on the Bhavanipur site. They were fertilized with 50 kg ha⁻¹ urea at their preparation stage and differed mainly by their wheat grain yields^{13,14}. They were named low input low yield (LL) and low input high yield (LH). In Ghaziabad, two fields that differed only in agricultural practices were selected: one being a conventionally tilled plain field (PF) and the other a three-year-old practiced raised bed field (RB). The two Ghaziabad fields were fertilized with 120 kg ha⁻¹ urea, 40 kg ha⁻¹ diammonium phosphate, 40 kg ha⁻¹ potash mureate and 25 kg ha⁻¹ zinc.

Sampling was performed at the tillering stage (45 d) at Bhavanipur, and at the late tillering stage (60 d) at Ghaziabad, according to Roesti *et al.*¹⁴. Briefly, three to four wheat plants with roots and soil cores were sampled under semi-sterile conditions. The bulk soil was separated from the adhering soil by shaking the plants. A fraction of the root systems with adhering soil was put into sterile 0.1 M sodium phosphate buffer (pH 7.0) and stirred to separate the rhizosphere soil (RS) from the roots. The washed roots were removed and rinsed with sterile deionized water and constituted the rhizoplane–endorrhizosphere (RE) fraction. DNA was extracted from RS and RE fractions by a bead-beater technique (Fast Prep FP120, SAVANT, BIO101, Carlsbad, USA) using a FastDNA Spin Kit (BIO101), according to the manufacturer's protocol. The extracted DNA was further purified with a GENECLEAN® II kit (BIO101) and stored at -20°C in Tris-EDTA (pH 8.0) buffer.

A two-step amplification procedure was designed to increase the *phlD* fragment yield from environmental DNA samples. The first amplification was carried out using *phl2a* and *phl2b*¹⁵ primers (Table 1). The amplification reaction mix was composed of 1X Taq DNA polymerase reaction buffer (Promega, Madison, USA), 3 mM MgCl₂ (Promega), 0.2 mM of each dNTP (Gibco, Cheshire, UK), 0.25 µM of each primer (Microsynth, Balgach, Switzerland) and 0.05 U µl⁻¹ Taq DNA polymerase in buffer B (Promega). A final concentration of 0.1–1 ng µl⁻¹ of crude extracted DNA was used as a template for a 20 µl reaction. Amplification was carried out in a PTC-200 Thermocycler (MJ Research Inc., Reno, USA), with an initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 40 s, 59°C for 30 s, and 74°C for 45 s, and completed by a final elongation step at 74°C for 10 min. The products of the first amplification were diluted tenfold in sterile, deionized water before being used for nested amplification reaction. Primers *phl310f* and *phl310chf* were mixed (50 : 50) prior to use in the PCR mix composed of 1X Taq DNA polymerase reaction buffer (Promega), 3 mM MgCl₂ (Promega), 0.2 mM of each dNTP (Gibco), 0.25 µM of the mix containing *phl310f* and *phl310chf* (Microsynth),

Table 1. List of primers used in the present study. The relative nucleotide positions were set according to the position of primer *phl2b* developed by Raaijmakers *et al.*¹⁵ on the *phlD* gene. Annealing temperatures were defined experimentally

Designation	5'-3' sequence	Size (bp)	Position	T _m (°C)	Reference
<i>phl2a</i>	GAGGACGTCGAAGACCACCA	20	728–747	59.5	15
<i>phl2b</i>	ACCGCAGCATCGTGTATGAG	21	1–20	57.5	15
<i>phl310f</i>	CTCTGCTATCAACCMCA	17	310–326	51.0	This study
<i>phl310chf</i>	CTGTGCTACCAGCCGGA	17	310–326	53.0	This study
<i>phl536r</i>	TTRATGGAGTTCATSAC	17	520–536	47.0	This study

0.25 µM of primer *phl536r* (Microsynth) and 0.05 U µl⁻¹ Taq DNA polymerase in buffer B (Promega). The nested amplification programme consisted of an initial denaturation at 95°C for 4 min, followed by 34 cycles at 94°C for 30 s, 52°C for 1 min and at 74°C for 30 s, and completed by a final elongation step at 74°C for 10 min. A DNA fragment of 227 bp in size was generated after the nested PCR. The two-step PCR assay was first validated on the 2,4-DAPG-producing strains CHA0 (ref. 16), PGNL1 (ref. 7), PGNR1 (ref. 7), Pitr2 (ref. 7), PILH1 (ref. 7), TM1'A4 (ref. 7) and TM1A3 (ref. 17). It was further tested on environmental DNA from tobacco rhizosphere planted in vineyard soils enabling to retrieve five main *phlD* clusters (F. Poly, unpublished). No PCR product was obtained from *phlD*⁻ reference strains *Escherichia coli* (Neuchâtel collection of microorganisms Neu 1006), *Pseudomonas aeruginosa* (ATCC 10145), *Alcaligenes faecalis* (Neu 1033), *Aquaspirillum autotrophicum* (ATCC 29984) and *Micrococcus luteus* (Neu 1013).

PCR products were purified employing a NUCLEO TRAP-CR kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. The ligation was carried out in pGEM[®]-T Vector System (Promega), following manufacturer's protocol. Transformation was performed by electroporation using the Bio-rad Gene Pulser XCell and PC module into *E. coli* XLI-Blue. The transformed colonies were plated onto Luria-Bertani (LB) agar containing ampicillin (150 µg ml⁻¹), X-Gal (5-bromo-4-chloro-3-indolyl-*b*-D-galactopyranoside) (0.1 mM) and IPTG (isopropyl-*b*-D-thiogalactopyranoside) (0.2 mM). Plasmids were recovered from white colonies using a NucleoSpin Plasmid kit (Macherey-Nagel) according to the manufacturer's protocol. The inserts were sequenced by Synergen (Schlieren, Switzerland). *phlD* sequences were aligned using the ClustalX software¹⁸ and trees were constructed using the neighbour-joining method¹⁹ on 227 bp sequences with the NJplot software (http://pbil.univ-lyon1.fr/pub/mol_phylogeny/njplot)²⁰. The topology of the distance tree was tested by resampling data with 100 bootstraps²¹ to provide confidence estimates for tree topologies.

The amplicons obtained from environmental DNA were cloned and sequenced. All the products were related to *phlD* sequences (sequence identity > 93%). Sequencing of the 227 bp *phlD* fragment was more discriminating than RFLP analysis performed on a bigger *phlD* fragment by

Ramette *et al.*¹⁰. Figures 1 and 2 represent the phylogenetic position of the 227 bp *phlD* sequences compared to *phlD* sequences for known DAPG producers, for Ghaziabad and Bhavanipur sites, constructed according to previously defined clusters by Ramette *et al.*¹⁰. Sequences with a high level of similarity were grouped into clusters. For Ghaziabad environmental sequences, two main clusters A and B were identified (Figure 1). Cluster A included three of the seven clones of the RE fraction of wheat plant cropped under the raised beds agricultural practice and the *phlD* sequence of the *Pseudomonas* sp. biocontrol strain M196. Cluster B included sequences retrieved from RS and RE of both plain field and raised bed practices, and were not related to any reference sequences. The discrepancy between the *phlD* sequences related to plain field and raised bed practice would have been triggered by the recent introduction of the latter. This observation is consistent with several other studies, suggesting that modifications in the agronomic practices influence the bacterial community structure in the rhizosphere²². The environmental *phlD* sequences from Bhavanipur fields were grouped into three different clusters, according to the field or the origin of the root fraction (Figure 2), i.e. cluster A and B (cf. the sequences from Ghaziabad fields) and cluster C, which included CHA0 reference strain. Cluster A contained exclusively sequences of the LH field (RS and RE fractions), whereas cluster B contained sequences from the RE fraction for both LL and LH fields; cluster C contained sequences from the RS fraction of LL. In case of the LL field, a particular allele was selected with respect to the vicinity of the root. Similarly, Picard *et al.*²³ observed a spatial selection gradient for DAPG producers, which most probably resulted from the selective influence of root exudates.

Investigations carried out on pseudomonad populations suggested that strains with different *phlD* alleles may co-exist in the rhizosphere of the same dicot at a particular geographic location^{24,25}. Our field studies revealed that there was a predominance of one or two *phlD* alleles within the same field condition and rhizosphere fraction. Possibly, flooded field condition during the rice season could lower the level of *Pseudomonas* spp. population, affecting *phlD* diversity. Moreover, Mavrodi *et al.*⁹ reported that in different take-all decline fields that were subjected to wheat monoculture, up to four different *phlD* alleles

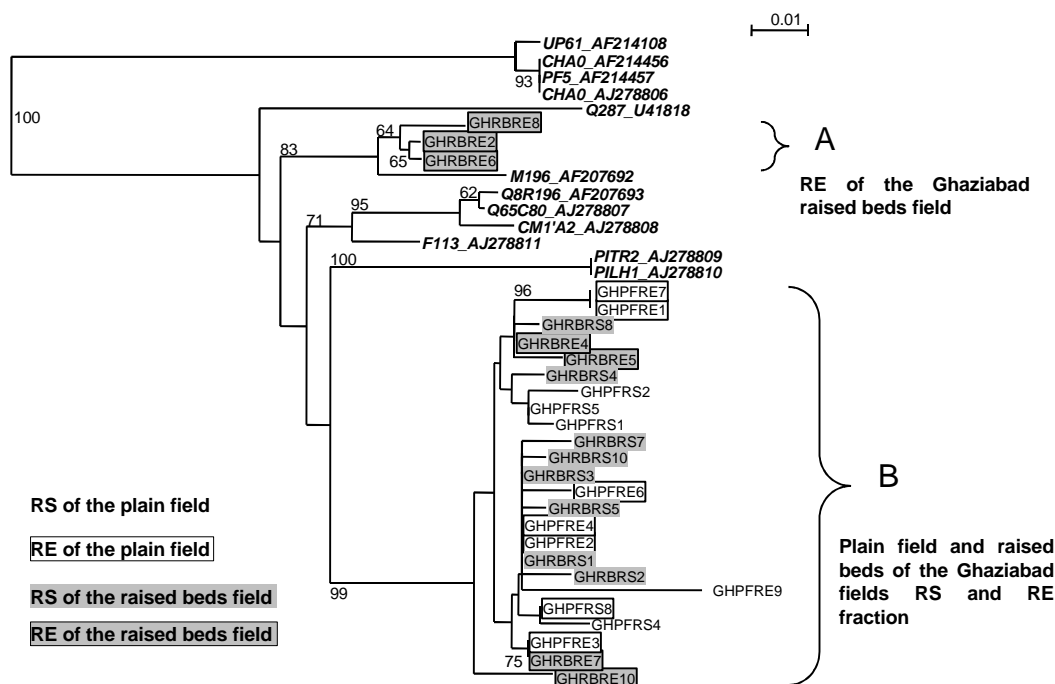


Figure 1. Phylogeny to *phlD* nucleotide sequences using partial *phlD* gene sequences from the GenBank database (bold, italics) and sequences obtained from cloning of the 227 bp amplicons from DNA extracted from the soil and root fractions of the wheat rhizosphere, on the basis of Ghaziabad raised beds and plain fields samples. The tree was constructed by neighbour-joining method¹⁹ with Njplot²⁰. Robustness of the inferred tree was assessed by 100 bootstrap replicates. Nodal support is indicated when higher than 50%. Scale bar indicates the number of substitutions per site.

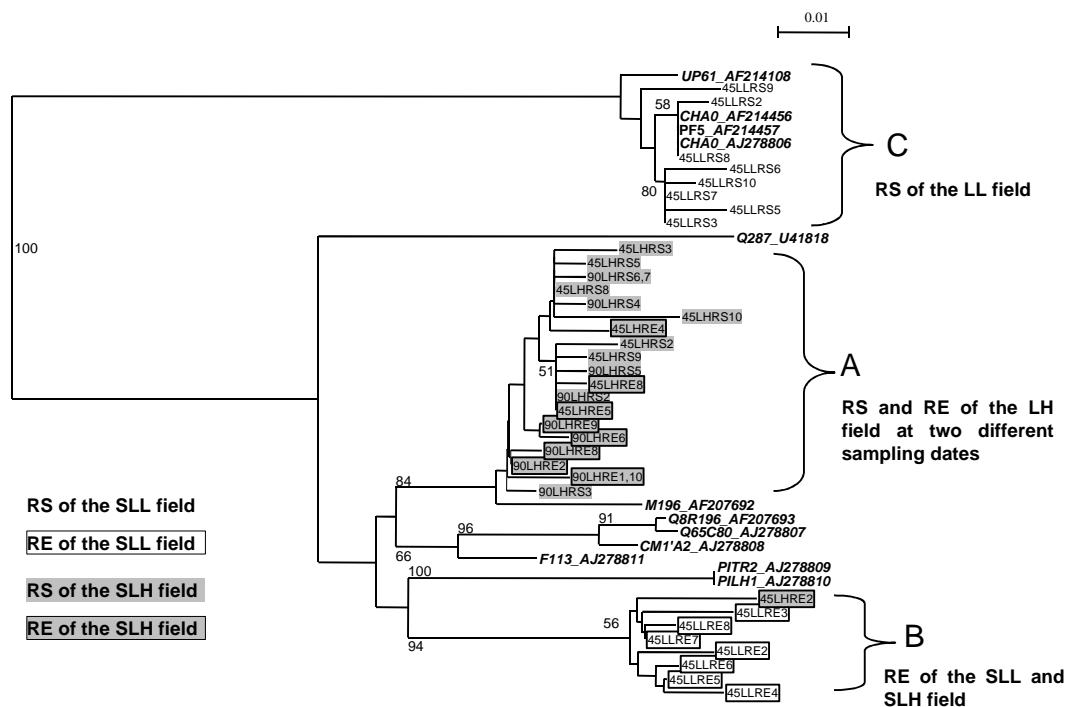


Figure 2. Phylogeny to *phlD* nucleotide sequences using partial *phlD* gene sequences from the GenBank database (bold, italics) and sequences obtained from cloning of the 227 bp amplicons from DNA extracted from the soil and root fractions of the wheat rhizosphere, on the basis of Bhavanipur samples. The tree was constructed by neighbour-joining method¹⁹ with Njplot²⁰. Robustness of the inferred tree was assessed by 100 bootstrap replicates. Nodal support is indicated when higher than 50%. Scale bar indicates the number of substitutions per site.

could be found and that a single *phlD*-based genotypic group dominated in the rhizosphere at each location studied. Their data indicated that monoculture conditions maintained for several decades might promote an enrichment of certain genotypes of DAPG-producing *Pseudomonas* spp. The continuous rice–wheat rotation for twenty years and the use of the same wheat cultivar in our studied fields might have created homogenous conditions, similar to monoculture practices.

Studies on *phlD*-harbouring bacteria have until now focused on the cultivable fluorescent pseudomonad group¹¹. However, Picard *et al.*²³ isolated *phlD*⁺ strains not only related to the fluorescent pseudomonads group, but also to the family Enterobacteriaceae. Interestingly, our molecular tools applied to environmental DNA allowed to recover new sequences with no close relative among known *phlD* sequences (cluster B). Part of the *phlD* allelic diversity might therefore have been missed using standard culture media conditions. Moreover, it is possible that non-cultivable (or not-yet cultured) bacteria could play a significant role in the biocontrol of cultivated plants.

1. Timsina, J. and Connor, D. J., The productivity and sustainability of rice–wheat cropping systems: issues and challenges. *Field Crop Res.*, 2001, **69**, 93–132.
2. Nagarajan, S., Plant protection problems in rice–wheat rotation system: a perspective. *Oryza*, 1989, **26**, 329–333.
3. Singh, S., Yadav, A., Malik, R. K. and Singh, H., Furrow-irrigated raised bed planting system – A resource conservation technology for increasing wheat productivity in rice–wheat sequence. Paper presented at the International Workshop on Herbicide Resistance Management and Zero Tillage in Rice–Wheat Cropping System, CCS Haryana Agricultural University, Hisar, 2002.
4. Lucy, M., Reed, E. and Glick, B. R., Applications of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek*, 2004, **86**, 1–25.
5. Bloemberg, G. V. and Lugtenberg, B. J. J., Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Biotechnol.*, 2001, **4**, 343–350.
6. Thomashow, L. S., Bonsall, R. F. and Weller, D. M., Antibiotic production by soil and rhizosphere microbes *in situ*. In *Manual of Environmental Microbiology* (eds Hurst, C. J. *et al.*), ASM Press, Washington DC, 1996, pp. 493–499.
7. Keel, C., Weller, D. M., Natsch, A., Défago, G., Cook, R. J. and Thomashow, L. S., Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent *Pseudomonas* strains from diverse geographic locations. *Appl. Environ. Microbiol.*, 1996, **62**, 552–563.
8. Bangera, M. G. and Thomashow, L. S., Identification and characterization of gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. *J. Bacteriol.*, 1999, **181**, 3155–3163.
9. Mavrodi, O. V., McSpadden Gardener, B. B., Mavrodi, D. V., Bonsall, R. F., Weller, D. M. and Thomashow, L. S., Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. *Phytopathology*, 2001, **91**, 35–43.
10. Ramette, A., Moënne-Loccoz, Y. and Défago, G., Polymorphism of the polyketide synthase gene *phlD* in biocontrol fluorescent pseudomonads producing 2,4-diacetylphloroglucinol and comparison of *phlD* with plant polyketide synthases. *Mol. Plant Microb. Interact.*, 2001, **14**, 639–642.
11. Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M. and Thomashow, S., Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.*, 2002, **40**, 309–348.
12. Hamelin, J., Fromin, N., Tarnawski, S., Teyssier-Cuvelles, S. and Aragno, M., *nifH* gene diversity in the bacterial community associated with the rhizosphere of *Molinia coerulea*, an oligotrophic perennial grass. *Environ. Microbiol.*, 2002, **4**, 477–481.
13. Gaur, R., Shani, N., Kawaljeet, Johri, B. N., Rossi, P. and Aragno, M., Diacetylphloroglucinol-producing pseudomonads do not influence AM fungi in wheat rhizosphere. *Curr. Sci.*, 2004, **86**, 453–457.
14. Roesti, D., Gaur, R., Johri, B. N., Imfeld, G., Sharma, S., Kawaljeet, K. and Aragno, M., Plant growth stage, field condition and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure in rainfed wheat fields. *Soil Biol. Biochem.* (in press).
15. Raaijmakers, J. M., Weller, D. M. and Thomashow, L. S., Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. *Appl. Environ. Microbiol.*, 1997, **63**, 881–887.
16. Stutz, E. W., Défago, G. and Kern, H., Naturally-occurring fluorescent pseudomonads involved in suppression of black root-rot of tobacco. *Phytopathology*, 1986, **76**, 181–185.
17. Fuchs, J. and Défago, G., Protection of cucumber plants against black rot caused by *Phomopsis sclerotoides* with rhizobacteria. In *Plant Growth-Promoting Rhizobacteria – Progress and Prospects* (eds Keel, C. *et al.*), (IOBC/WPRS Bull XIV/8), Interlaken, Switzerland., 1991, pp. 57–62.
18. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G., The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, 1997, **24**, 4876–4882.
19. Saitou, N. and Nei, M., The neighbour-joining method – a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 1987, **4**, 406–425.
20. Perriere, G. and Gouy, M., WWW-query: An on-line retrieval system for biological sequence banks. *Biochimie*, 1996, **78**, 364–369.
21. Felsenstein, J., Confidence-limits on phylogenies – an approach using the bootstrap. *Evolution*, 1985, **39**, 783–791.
22. Alvey, S., Yang, C. H., Buerkert, A. and Crowley, D. E., Cereal/legume rotation effects on rhizosphere bacterial community structure in West African soils. *Biol. Fertil. Soils*, 2003, **37**, 73–82.
23. Picard, C., Di Cello, F., Ventura, M., Fani, R. and Guckert, A., Frequency and biodiversity of 2,4-diacetylphloroglucinol-producing bacteria isolated from the maize rhizosphere at different stages of plant growth. *Appl. Environ. Microbiol.*, 2000, **66**, 948–955.
24. Wang, C. *et al.*, Cosmopolitan distribution of dicotyledonous crop-associated biocontrol pseudomonads of worldwide origin. *FEMS Microbiol. Ecol.*, 2001, **37**, 105–116.
25. Ramette, A., Moënne-Loccoz, Y. and Défago, G., Prevalence of fluorescent pseudomonads producing antifungal phloroglucinols and/or hydrogen cyanide in soils naturally suppressive or conducive to tobacco black root rot. *FEMS Microbiol. Ecol.*, 2003, **44**, 35–43.

ACKNOWLEDGEMENTS. Financial support was provided by ISCB Project SA7 and the NCCR. Dr P. Mishra, K. Kaur and S. Sharma assisted in the design and realization of this work, including support in collecting soil samples. We also thank the farmers of Ghaziabad and Bhavanipur for their timely help during field work. Prof. G. Défago and Dr A. Ramette have kindly provided the reference strains and timely advice.

Received 27 October 2005; revised accepted 5 December 2005