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Distribution of *Pseudomonas* populations harboring *phlD* or *hcnAB* biocontrol genes is related to depth in vineyard soils

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ABSTRACT

The abundance and population structure of pseudomonads in soils collected from long-(1006 years) and short-(54 years) term grapevine monocultures in Switzerland were examined across five soil horizons within the 1.20–1.35 m range. Soil samples were baited with grapevine, and rhizosphere pseudomonads containing the biocontrol genes *phlD* (2,4-diacetylphloroglucinol synthesis) and/or *hcnAB* (hydrogen cyanide synthesis) were analyzed by MPN-PCR. The numbers of total, *phlD*$^+$ and *hcnAB*$^+$ pseudomonads decreased with depth by 1.5–2 log (short-term monoculture) and 3–3.5 log (long-term monoculture). In addition, the percentages of *phlD*$^+$ (except in short-term monoculture) and *hcnAB*$^+$ pseudomonads were also lower in deeper horizons. RFLP-profiling of *phlD*$^+$ and *hcnAB*$^+$ pseudomonads revealed three *phlD* and twelve *hcnAB* alleles overall, but the number of alleles for both decreased in relation to depth. The only *phlD* allele found in deeper horizons was also found in topsoil, whereas one *hcnAB* allele (k) found in deeper horizons in long-term monoculture was absent in the topsoil. This suggests that certain *Pseudomonas* ecotypes are adapted to specific depths. Four *hcnAB* alleles enabled discrimination between monocultures. We conclude that soil depth is a factor selecting *phlD* and *hcnAB* genotypes, and that the allelic diversity of the two biocontrol genes decreases with depth.

1. Introduction

Most studies on soil microbes focus on surface soil layers (usually the first 30 cm), where root colonization and overall microbial density and activity are higher (Blume et al., 2002; Fierer et al., 2003; Jossi et al., 2006). This overlooks the fact that agriculturally relevant soil profiles can be more than 1 m deep and sustain significant microbial populations in subsurface horizons (Van Gestel et al., 1992; Fritze et al., 2000; Blume et al., 2002; Agnelli et al., 2004; Steenwerth et al., 2008). Microbial biomass and diversity tend to diminish with soil depth (Fierer et al., 2003; Agnelli et al., 2004; Allison et al., 2007), and there are indications that subsurface microorganisms may play an important role in soil formation, ecosystem biochemistry, contaminant degradation, and the maintenance of groundwater quality (Konopka and Turco, 1991; Hiebert and Bennett, 1992; Richter and Markewitz, 1995; Hase et al., 2001). However, little is known about the effect of soil depth on the diversity of key microbial communities, especially those relevant for plant growth and health.

Pseudomonas species constitute an important bioactive group in farm soil, because of efficient root colonization and production of antifungal compounds that are important in biological suppression of soil-borne plant diseases (Weller et al., 2002; Moënne-Loccoz and Défago, 2004; Compant et al., 2005; Haas and Défago, 2005). This is especially the case for pseudomonads that produce 2,4-diacetylphloroglucinol (Phl) and/or hydrogen cyanide (HCN), which are particularly effective in biocontrol of a wide range of soil-borne diseases on various crops (Haas and Keel, 2003; Garbeva et al., 2004; Rezzonico et al., 2007).

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Biosynthetic genes for Phl are clustered as *phlA-G* (Schnider-Keel et al., 2000; Paulsen et al., 2005), and the chalcone synthetase homolog *phlD* has become the genetic marker of choice for studying Phl-producing strains. Considerable diversity of Phl-producing pseudomonads in the rhizosphere has been revealed by sequencing or RFLP analysis of *phlD* (McSpadden Gardener et al., 2001, 2005; Ramette et al., 2001, 2006; Wang et al., 2001; Landa et al., 2006; Mazzola et al., 2004; Svercel et al., 2009). Almost all Phl-producing pseudomonads also produce HCN, whereas many HCN-producing pseudomonads are Phl-negative (Rezzonico et al., 2007). The *hcnABC* gene cluster encodes the HCN synthetase essential for HCN production (Haas and Keel, 2003; Moënne-Loccoz and Défago, 2004). RFLP analysis of *hcn* genes (especially *hcnAB*) has proved useful to document strain diversity (Ramette et al., 2003, 2006; Svercel et al., 2007), allowing for a lower detection limit for bacteria carrying the *hcnAB* genes in the soil.

In grasslands, pseudomonads are known to be an important constituent of both surface and subsurface communities in deep soils (LaMontagne et al., 2003), but little is known about the prevalence of important genotypes, especially for those with the potential to suppress pathogens, in deep agricultural soils. This is of particular relevance with deep-rooted perennial crops, where cumulative rhizosphere effects on soil bacteria may be expected even in subsurface layers, provided the crop is grown long enough. In Europe, plots with a long history (i.e., over several centuries) of the same crop can be found, notably in the case of grapevine (Schlegel, 1973).

The objective of this work was to assess the effect of soil depth on the abundance and population structure of pseudomonads harboring *phlD* or *hcnAB* biocontrol genes in vineyard soils. To this end, five soil horizons (down to 1.35 m depth) of two neighboring plots under long-(1006 years) or short-(54 years) term grapevine monocultures in Switzerland were sampled and baited with grapevine to recover rhizosphere pseudomonads, prior to assessment of $phlD^+$ and $hcnAB^+$ subpopulations.

2. Materials and methods

2.1. Soil sampling

Two neighboring vineyards at Bevaix (near Neuchâtel, Switzerland) and described by Svercel et al. (2009) were used. One was under short-term (54 years) monoculture and the other under long-term (1006 years) monoculture. The current grapevines were of the same variety (cv. Chasselas) but grafted onto different rootstocks (3309 in short-term and 5c in long-term vineyard) and of different ages (respectively 9 and 28 years old). Both vineyards had a green cover.

In July 2003, two soil profiles (i.e., SA and SB) were excavated in the short-term vineyard and two others (i.e., LA and LB) in the long-term vineyard (details of field locations are given in Fig. S1). All four profiles displayed the same five soil horizons (although not necessarily of the same thickness; Fig. 1) based on morphology analysis. Soils were classified as anthropic brunisols. Grapevine roots were present in each horizon.

At each of the four profiles, about 10 kg of soil were taken from each soil horizon. Plant roots and stones were removed, soil was homogenized, air dried and stored at 4 °C until analysis. Part of the soil was used for analysis (Geology Institute of the University of Neuchâtel, Switzerland) of chemical and physical properties (Tables 1 and S1).

2.2. Plant experiment

Soil was used to fill 300-cm^3 plastic pots with drainage holes at the bottom. Each of the 10 treatment combinations (i.e., 5 soil horizons × 2 monoculture types) was studied using 8 pots for each of the 2 profiles. One grapevine baiting-plant (*Vitis riparia* Michx × *Vitis rupestris* Scheele 3309 accession RAC 1.1, corresponding to current grapevine rootstock in the short-term vineyard), obtained as described by Svercel et al. (2009), cultivated in semi-sterile conditions and checked for lack of *Pseudomonas* spp.

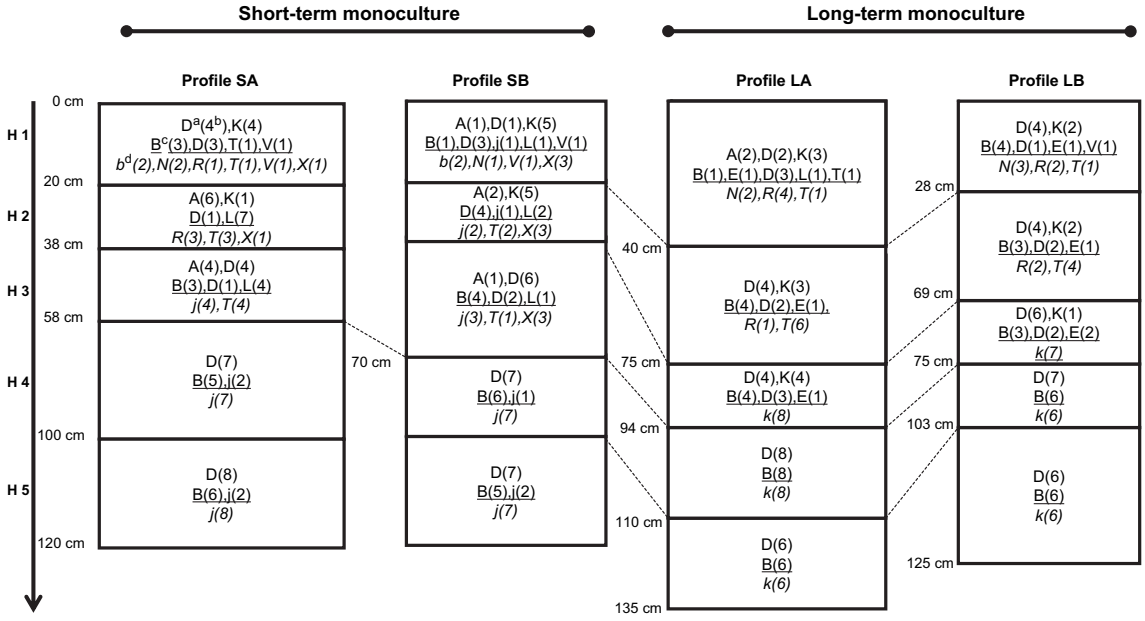

Fig. 1. Soil horizons H1 to H5 in the soil profiles studied in the short-term and the long-term vineyards (two profiles per vineyard). Dominant *phlD* alleles, *hcnAB* alleles for $phlD^+$ pseudomonads (underlined) and *hcnAB* alleles for $phlD^-$ pseudomonads (in italics) obtained from rhizosphere samples are indicated for each soil layer within each soil profile. [a]*phlD* allele; [b]number of baiting plants from which the allele was found; [c]*hcnAB* alleles (in the *phlD*-positive wells); [d]*hcnAB* alleles (in the *phlD*-negative wells).