Genetic and phenotypic microdiversity of *Ochrobactrum* spp.

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Abstract

The diversity of *Ochrobactrum anthropi, Ochrobactrum intermedium, Ochrobactrum tritici* and *Ochrobactrum grignonense* in agricultural soil and on the wheat rhizoplane was investigated. *O. anthropi* was isolated both from soil and from the rhizoplane, *O. intermedium* and *grignonense* only from bulk soil, and *O. tritici* only from the wheat rhizoplane. On the genetic level, the immunotrapped isolates and a number of strains from culture collection mainly of clinical origin were compared with rep-PCR profiling using BOX primers, and a subset of these isolates and strains using REP primers. The isolates clustered according to their species affiliation. There was no correlation between rep clusters of *O. anthropi* isolates and habitat (place of isolation). The genetic diversity of *Ochrobactrum* at the species level as well as microdiversity of *O. anthropi* (number of BOX groups) was higher in soil than on the rhizoplane. Similarity values from genetic rep-PCR profiles correlated positively with DNA–DNA reassociation percentages. Isolates with >80.7% similarity in BOX profile and >86.4% in rep profile clustered within the same species. Similarity analysis of rep-PCR profiles is hence an alternative to DNA–DNA hybridization as a genomic criterion for species delineation within the genus *Ochrobactrum*. We used the substrate utilization system BIOLOG-GN to compare the immunotrapped isolates on the phenetic level. For the isolates from bulk soil, substrate utilization versatility (number of utilized substrates) and substrate utilization capacity (mean conversion rate of substrates) were slightly but significantly higher than for the isolates from the rhizoplane. This trend was also seen using API 20E and 20NE systems. Plate counts of total bacteria and the number of immunotrapped *Ochrobactrum* isolates per gram dry weight were higher for the rhizoplane than for the soil samples. The results of genetic and phenotypic analyses indicated a 'rhizosphere effect'; the diversity and metabolic capacity of *Ochrobactrum* isolates were higher in bulk soil, and the population density was higher on the wheat rhizoplane.

Introduction

To date, the alpha proteobacterial genus *Ochrobactrum* consists of five species: *Ochrobactrum anthropi, Ochrobactrum intermedium, Ochrobactrum tritici, Ochrobactrum grignonense* and *Ochrobactrum gallinifaecis*. The closest relative to *Ochrobactrum* is the genus *Brucella*, which contains highly pathogenic strains. It has been reported that *O. intermedium* is more closely related to *Brucella* than to the other *Ochrobactrum* species (Velasco *et al*., 1998; Lebuhn *et al*., 2000; Lebuhn *et al*., 2006).

Ochrobactrum strains occur in diverse habitats including soil, plants and their rhizosphere, (waste)water, animals and humans. *Ochrobacterium anthropi* and *O. intermedium* appear to be particularly ubiquitous colonizers, and both species are of medical relevance (Jelveh & Cunha, 1999; Moller *et al*., 1999). *Ochrobactrum tritici* was originally isolated from the wheat rhizoplane (Lebuhn *et al*., 2000) and recently was also recovered from wastewater (Goris *et al*., 2003). Only a few isolates of *O. grignonense* (isolated from soil, Lebuhn *et al*., 2000) and *O. gallinifaecis* (isolated from chicken faeces, Kaempfer *et al*., 2003) have been described.

We reported previously (Lebuhn *et al*., 2000) that *Ochrobactrum* species can constitute a substantial part of soil and rhizosphere microbial communities. Their importance may have been neglected due to the inconspicuous appearance of

the colonies and misidentification of strains (Lebuhn *et al*., 2000; Lebuhn *et al*., 2006).

On the 16S rRNA gene level, a differentiation of *Ochrobactrum* species and their separation from *Brucella* is problematic because of their high similarity. The genera *Ochrobactrum* and *Brucella* share an overall 16S rRNA gene sequence identity of more than 96%, up to 99.28% (Lebuhn *et al*., 2000). A better discrimination between species can be achieved using PCR-based genetic fingerprinting techniques such as BOX- or REP-PCR (Schloter *et al*., 2000) because they provide resolution below the species level and are ideally suited to distinguish between genotypically related strains. Several studies have evaluated their applicability for the genetic definition of species borderlines (e.g. Rademaker *et al*., 2000; Mergaert *et al*., 2003; Lanoot *et al*., 2004) as a substitute for DNA–DNA hybridization (Rademaker *et al*., 2000; Stackebrandt *et al*., 2002).

In this paper, we address the following questions:

1. Do BOX- and REP-PCR fingerprint similarities correlate with results from (previously performed, Lebuhn *et al*., 2000) DNA–DNA hybridization experiments and can species borderlines be assigned by similarity value of rep profiles?
2. Is there a correlation between clusters of isolates derived from genetic fingerprint analyses and the habitat from which the isolates were recovered, and does the habitat influence the genetic and physiological diversity of *Ochrobactrum* spp.?

Materials and methods

Soil and root samples, bacterial isolates and cultivation

Table 1 gives a list of isolates and strains used in this study. Most soil isolates were from a typical eutrochrept (soil taxonomy) or calcic cambisol (FAO-UNESCO classification) at the Grignon experimental field of the Institut National de la Recherche Agronomique (Ile-de-France, France). The last crop before sampling was wheat. Top soil contained clay (17.5%), silt (53.0%) and sand (29.5%), organic carbon (1.35%) and nitrogen (0.12%). Water pH was 7.85 ± 0.05. In brief, pooled soil samples (1 g fresh wt.) were suspended in sodium cholate and ground in a Retsch mill (Haan, Germany). Bacteria were further removed from soil particles by stirring with Chelex 100, PEG 6000 and Na^+-Amberlite resin IR-120. After centrifugation of filtrates (5 μm), pellets were resuspended in phosphate buffered saline (PBS) solution and subjected to immunotrapping. Rhizoplane isolates were from pooled root samples of aseptically germinated wheat that was grown in Grignon soil under controlled growth chamber conditions (Lebuhn *et al*., 2000). In brief, roots were liberated from adherent soil particles by washing gently in sterile tapwater, ground four times in a mortar, suspended in 0.85% KCl solution, and subjected to immunotrapping.

Immunotrapping using a monoclonal antibody (mAb 2.11) with highest affinity to *O. anthropi* has been described previously (Lebuhn *et al*., 2000). In brief, mAb 2.11 was bound to protein A-coated microtiter plates. After washing, aliquot parallels of the soil or root suspensions were incubated in the wells, washed and treated with glycine/HCl for antibody disruption. Parallels were pooled and serial dilutions of the repetitions plated on nutrient agar. About 700 isolated colonies were purified on nutrient agar. The isolates were classified taxonomically by a polyphasic approach with priority to DNA–DNA hybridization (Lebuhn *et al*., 2000). In the current analyses, we included *Ochrobactrum* strains from different environments as available in culture collection LMG (Belgian Coordinated Collections of Microorganisms), and analyzed genetic and phenotypic diversity (see below) in relation to the different habitats.

Total cell numbers were determined by plating serial dilutions of soil and root samples on tenfold diluted tryptic soy agar.

Genetic fingerprinting

BOX-PCR was carried out using primer BoxA1R as described in Rademaker *et al*. (1998). Briefly, one reaction mix had a total volume of 25 μL and contained PCR buffer, 10% DMSO, 0.18% non-acetylated BSA, 1.25 mM of each dNTP, 6.35 mM $MgCl_2$, 6 mM of the primer, 1.5 U Taq DNA Polymerase (Eurogentec GoldStarTaq, Angers, France), and 2 μL of bacterial cell suspension in H_2O as template DNA. The thermal program used for the reaction consisted of an initial denaturation step at 94 °C for 5 min, followed by 25 cycles of 94 °C for 45 s, 50 °C for 1.5 min, and 65 °C for 8 min; with a final extension step at 65 °C for 16 min. Products were separated by electrophoresis in 2% agarose in 1× TAE buffer for 2.5 h at 100 V and visualized by staining with ethidium bromide (0.5 μg mL^{-1}).

REP-PCR was carried out as described in (Lebuhn *et al*., 2000) with a representative but minor subset of *Ochrobactrum* soil and rhizoplane isolates (also examined partially by BOX-PCR, Table 1) to compare the performance of both techniques.

Cluster analyses of the fingerprint patterns were carried out using GelCompar II (Applied Maths, Ghent, Belgium). Pairwise profile similarities were calculated using the Pearson correlation coefficient, and dendrograms were obtained applying the UPGMA clustering method.

Phenotypic analyses

To analyze different influences of soil and rhizoplane conditions on the phenotype, a representative subset of

Table 1. *Ochrobactrum* spp. isolates and strains used in this study

Isolate/strain*	BOX†	REP†	DNA†	BIO†	API†	Origin	Reference
O. anthropi							
ALM4	+					Soil	Lebuhn *et al*. (2000)
ALM13	+			+		Soil	Lebuhn *et al*. (2000)
ALM14	+			+		Soil	Lebuhn *et al*. (2000)
ALM15		+	+			Soil	Lebuhn *et al*. (2000)
ALM19		+	+	+		Soil	Lebuhn *et al*. (2000)
CLM5	+					Soil	Lebuhn *et al*. 2000)
CLM6	+					Soil	Lebuhn *et al*. (2000)
CLM7	+		+	+		Soil	Lebuhn *et al*. (2000)
CLM12	+		+			Soil	Lebuhn *et al*. (2000)
CLM14		+	+			Soil	Lebuhn *et al*. (2000)
CLM18		+		+		Soil	Lebuhn *et al*. (2000)
CLM26	+	+				Soil	Lebuhn *et al*. (2000)
DSM 14396	+	+	+	+	+	Soil, Germany	Lebuhn *et al*. (2000)
LAI12	+					Wheat rhizoplane	Lebuhn *et al*. (2000)
LAII7		+	+			Wheat rhizoplane	Lebuhn *et al*. (2000)
LAII114	+			+		Wheat rhizoplane	Lebuhn *et al*. (2000)
LMA1	+			+		Soil	Lebuhn *et al*. (2000)
LMG 34	+					Tuberculosis patient	LMG
LMG 35	+					Asthma bronchiale patient	LMG
LMG 2136	+					Sewage plant	LMG
LMG 3300	+					Cervix	LMG
LMG 3305	+					Blood culture	LMG
LMG 3329	+					Abscess	LMG
LMG 3330	+					Keratitis sample	LMG
LMG 3331^T	+	+	+	+	+	unknown	LMG
LMG 3333	+					Blood culture	LMG
LMG 5140	+	+	+	+	+	Arsenite cattle-dip tray	LMG
LMG 5440	+					Unknown	LMG
LMG 5444	+					Incision	LMG
OaA20-1		+				Soil	Lebuhn *et al*. (2000)
OaA20-2		+				Soil	Lebuhn *et al*. (2000)
OaA20-3		+				Soil	Lebuhn *et al*. (2000)
OaA20-4		+				Soil	Lebuhn *et al*. (2000)
OaA20-5		+				Soil	Lebuhn *et al*. (2000)
OaA20-6		+				Soil	Lebuhn *et al*. (2000)
OaC13a	+			+		Soil	Lebuhn *et al*. 2000)
SAI2	+					Wheat rhizoplane	Lebuhn *et al*. (2000)
SAI8	+		+	+		Wheat rhizoplane	Lebuhn *et al*. 2000)
SAII101	+	+		+	+	Wheat rhizoplane	Lebuhn *et al*. (2000)
SAIII101	+	+		+		Wheat rhizoplane	Lebuhn *et al*. (2000)
SAIII104	+	+		+		Wheat rhizoplane	Lebuhn *et al*. (2000)
SAIII108	+					Wheat rhizoplane	Lebuhn *et al*. (2000)
SCII10	+					Wheat rhizoplane	Lebuhn *et al*. (2000)
SCII22		+	+			Wheat rhizoplane	Lebuhn *et al*. (2000)
O. intermedium							
DWR (OspIndien)	+					Soil, India	AK Tripathi
LMG 379	+					Ear	LMG
LMG 3301^T	+	+	+	+	+	Blood culture	LMG
LMG 3306	+					Soil	LMG
LMG 5425	+					Urine	LMG
LMG 5426	+					Urine	LMG
OiC8-1		+		+		Soil	Lebuhn *et al*. (2000)
OiC8-2		+	+	+		Soil	Lebuhn *et al*. (2000)
OiC8-3		+		+		Soil	Lebuhn *et al*. (2000)
OiC8-4		+		+		Soil	Lebuhn *et al*. (2000)
OiC8-5		+		+		Soil	Lebuhn *et al*. (2000)

Table 1. Continued.

Isolate/strain*	BOX†	REP†	DNA†	BIO†	API†	Origin	Reference
OiC8-6	+	+	+	+	+	Soil	Lebuhn *et al*. (2000)
OiC8a		+	+	+		Soil	Lebuhn *et al*. (2000)
O. tritici							
LAIII106	+	+	+	+	+	Wheat rhizoplane	Lebuhn *et al*. (2000)
LMG 2134‡	+					Arsenite cattle-dip tray	LMG
LMG 2320‡	+					Arsenite cattle-dip tray	LMG
SAI110		+	+	+		Wheat rhizoplane	Lebuhn *et al*. (2000)
SCII24^T	+	+	+	+	+	Wheat rhizoplane	Lebuhn *et al*. (2000)
O. grignonense							
OgA9a^T	+	+	+	+	+	Soil	Lebuhn *et al*. (2000)
OgA9c	+			+	+	Soil	Lebuhn *et al*. (2000)

*The following isolates from Lebuhn *et al*. (2000) were additionally tested in BIOLOG analyses: *O. anthropi* from soil: ALM8, ALM16, ALM27, CLM13, CLM16, LMA6, LMA7, LMC2, OaA14-1, OaA19-1, OaC6, OaC17-1; *O. anthropi* from the wheat rhizoplane: LAI4, LAI108, LAII4, LAII104, RPSCII9; *O. tritici* from the wheat rhizoplane: LAIII8, LAIII104, LAIII108, LAIII109, LAIII111, LAIII113, LAIII115, LAIII116, SAI12, SAI15, SAI105, SAI107, SAI113, SAI116. The following isolates from Lebuhn *et al*. (2000) from the rhizoplane were additionally tested in API analyses: *O. anthropi*: LAI4, *O. tritici* SAI12.
†BOX: BOX-PCR, REP: REP-PCR, DNA: DNA–DNA hybridization, BIO: BIOLOG GN, API: API 20NE and API 20E.
‡Originally listed in the LMG collection as *O. anthropi*.
LMG: Belgian Co-ordinated Collections of Micro-organisms (BCCM^{TM}-LMG).

Ochrobactrum isolates (with a focus on *O. anthropi*) was analyzed with regard to substrate utilization patterns using BIOLOG-GN microtitre plates (Garland & Mills, 1991) as suggested by the manufacturer. To evaluate whether this indirect determination of substrate utilization might create bias and to increase the number of different substrates, we included respective direct data from determination of assimilation by measurement of turbidity using API 20NE and API 20E substrate assimilation/utilization systems (bioMérieux, Marcy l'Etoile, France) (Lebuhn *et al*., 2000) for a representative subset of the isolates in the analyses as suggested by the manufacturer. We determined substrate utilization capacity (SUC) and substrate utilization versatility (SUV). SUC is defined by the mean conversion rate (in % of maximum conversion) of 95 sole C-sources (BIOLOG-GN) and 39 different substrates (API 20E, API 20NE). SUV values represent the number of substrates that were 100% converted in the respective tests. Levene and *t*-tests (independent samples, 2-tailed) were performed using SPSS 10.0 for the BIOLOG-GN dataset to compare SUC and SUV variance and mean values of *O. anthropi* and *Ochrobactrum* spp. isolates from bulk soil and from the rhizoplane. The respective API dataset was not evaluated statistically as the rate of positive reactions was too low in these tests and did not provide sufficient resolution.

Results and discussion

Genetic diversity

Forty-five *Ochrobactrum* isolates (*O. anthropi*: 32, *O. intermedium*: 7, *O. tritici*: 4, and *O. grignonense*: 2) were subjected to genetic profiling using BOX-PCR (Fig. 1). All isolates and strains clustered exactly according to their species affiliation, with several subclusters forming within species. Similarity values obtained with the Pearson correlation coefficient started at 46.6%, with the lowest similarity value for strains of one species being 57.6% (*O. intermedium* LMG 3301 vs. LMG 5426). Intraspecies diversity was examined with an emphasis on *O. anthropi* because sufficient isolates from different habitats were available for this species. *O. anthropi* isolates and strains of clinical, bulk soil, and wheat rhizoplane origin were intermingled in the clusters, giving no indication of a genetic diversification in clinical, rhizoplane or soil clusters. At the level of 80% profile similarity (90% value on the scale in Fig. 1), soil isolates of *O. anthropi* were distributed on four BOX-PCR subclusters (REP-PCR: 5), whereas rhizoplane isolates were distributed only on two BOX-PCR subclusters (REP-PCR: 4). Some rep-groups contained only few isolates; however, using other genetic markers, a lower diversity of *O. anthropi* at the rhizoplane has also been found previously (Lebuhn *et al*., 2000) as well as in another recent study (Lebuhn *et al*., 2006). There was no difference in the ELISA reaction to mAb2.11 between *O. anthropi* strains or soil and rhizoplane isolates (60–100%), ruling out sampling bias by immunotrapping. This indicates that the genetic diversity of *O. anthropi* was higher in soil than on the rhizoplane. Comparable results were obtained in the study by Laguerre *et al*. (1997) in which the diversity of rhizobial legume symbionts of different geographic origin and host plant cultivars was investigated. Similarly, the genetic diversity of *Paenibacillus polymyxa* was highest in bulk soil and lowest on the rhizoplane (Mavingui *et al*., 1992). Lower genetic diversity on the rhizoplane than in bulk soil may therefore generally be a consequence of rhizodeposition of easily

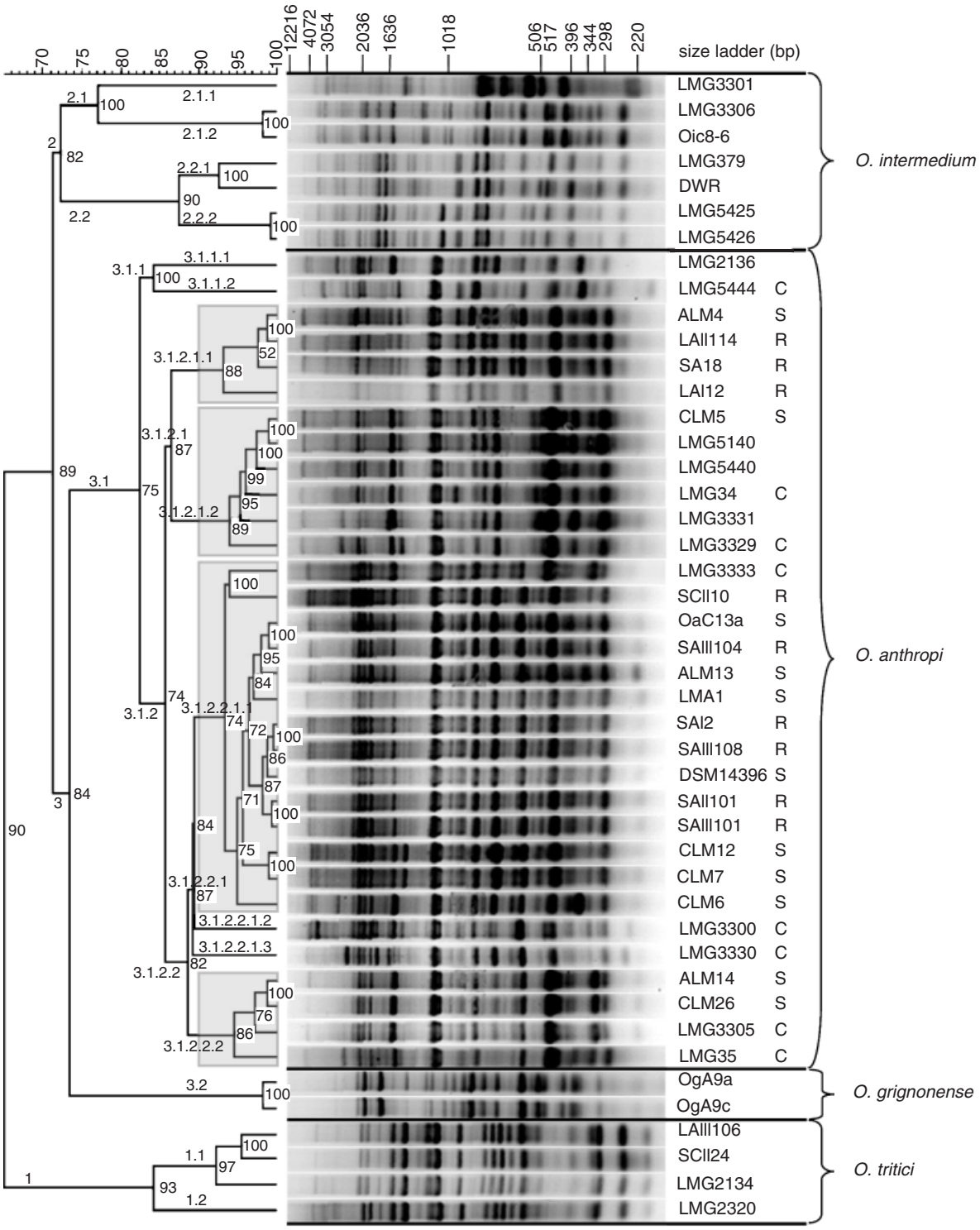

Fig. 1. Cluster analysis of BOX-PCR profiles of *Ochrobactrum* isolates using the Pearson correlation coefficient. Where possible, the origin of *Ochrobactrum anthropi* isolates is indicated: clinical (C), soil (S) and rhizoplane (R). Bootstrapping probabilities from 100 resamplings are shown. The scale shows percentage of profile similarity. Areas shaded in grey mark *O. anthropi* isolates falling within one BOX group on the basis of 80% profile similarity. Cluster/profile numbers are indicated.

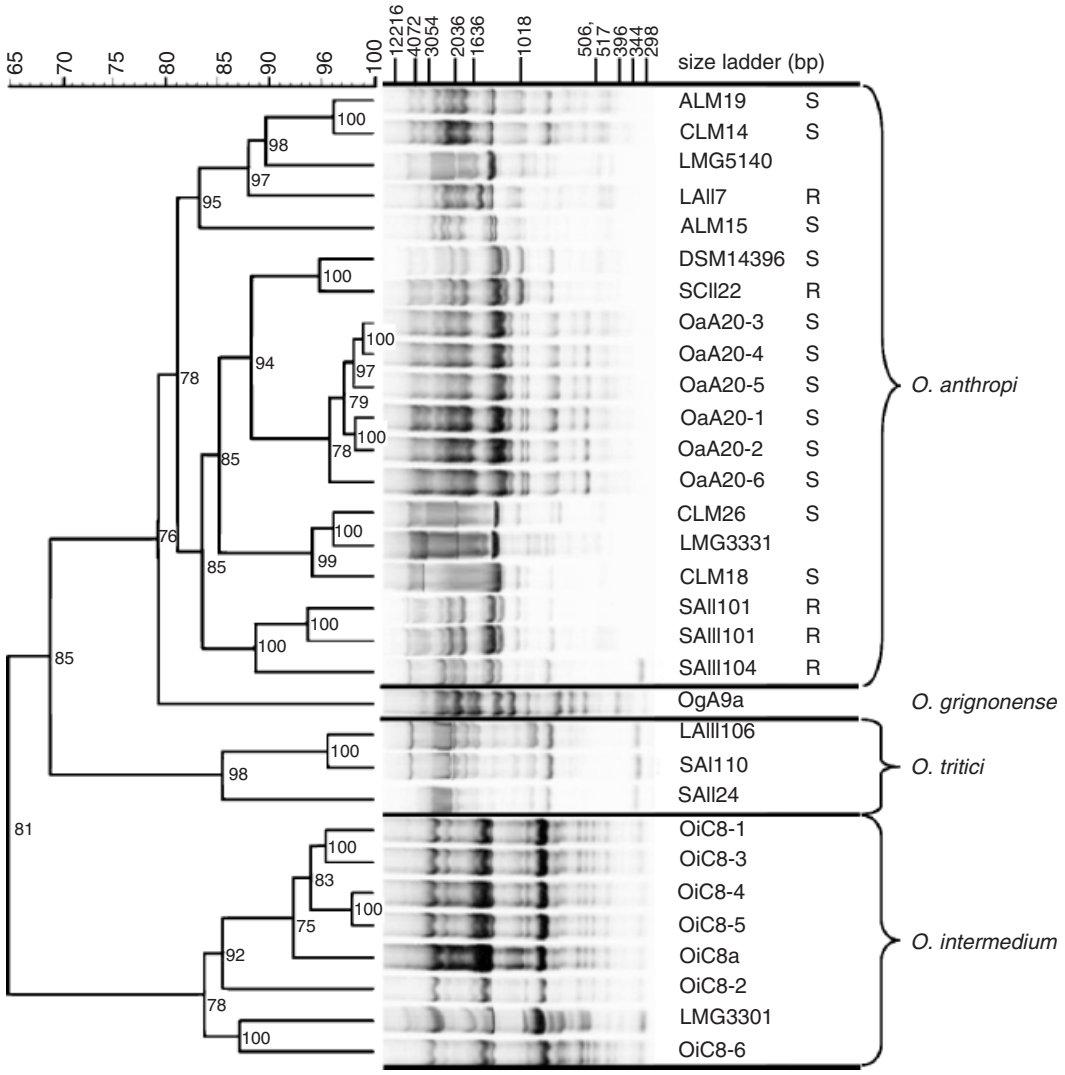

Fig. 2. Cluster analysis of REP-PCR profiles of *Ochrobactrum* isolates using the Pearson correlation coefficient. Where possible, the origin of *Ochrobactrum anthropi* isolates is indicated: soil (S) and rhizoplane (R). Bootstrapping probabilities from 100 resamplings are shown. The scale shows percentage of profile similarity.

degradable substrates that support clonal proliferation of specifically adapted subpopulations (Lynch, 1990).

Ochrobactrum strains LMG 2134 and LMG 2320, both listed in the LMG database as *O. anthropi*, clustered with *O. tritici*, and are considered to be strains of this species (Fig. 1). (Lebuhn *et al.*, 2006) corroborated this using ITS and 16S rDNA sequence analysis.

Intra-species microdiversity was also observed when REP-PCR was used for analysis of a partially different subset of 31 strains and isolates (Fig. 2). Similarity values started at 39.2%, and the lowest similarity value for members of the same species was 70.3%. Species-conform clustering was also obtained in the REP analysis, but subclusters below the species levels were partly different from the BOX subclusters. There was also no clear indication of a separation in soil and rhizoplane clusters in the REP analysis. As, in agreement with other studies, BOX-PCR performed and resolved better than REP-PCR (compare Figs 1 and 2), we rely on the results from the BOX profile comparisons.

Correlation of BOX- and REP-genotype fingerprinting with DNA–DNA hybridization

Figure 3 presents scatter-plots of similarity values obtained by pairwise comparison of BOX- and REP-PCR profiles with the corresponding data from DNA–DNA reassociation analyses (Lebuhn *et al.*, 2000), which have priority for the species-level classification of bacterial isolates (Stackebrandt *et al.*, 2002). The highest DNA–DNA reassociation value for isolates belonging to different *Ochrobactrum* species was

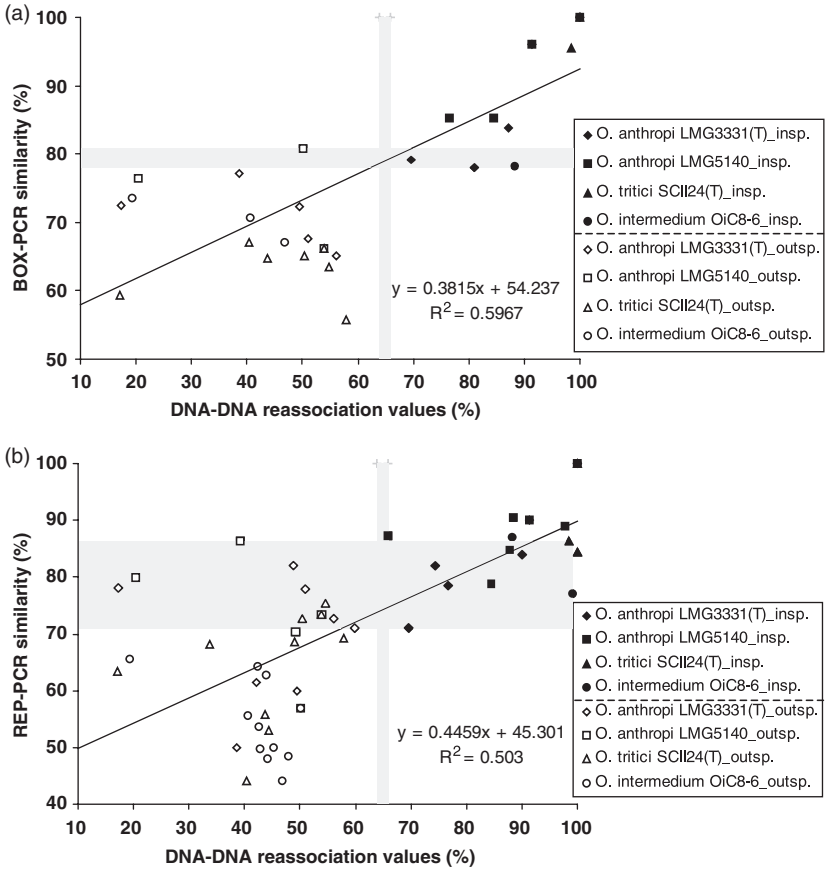

Fig. 3. Scatter-plots of (a) DNA–DNA reassociation vs. BOX-PCR similarity values and (b) DNA–DNA reassociation vs. REP-PCR similarity values of *Ochrobactrum* spp. isolates. rep-profile similarity values are from the cluster analyses in Figs 1 and 2, and DNA–DNA reassociation data from (Lebuhn *et al.*, 2000). Closed symbols (_insp.) denote data pairs of the indicated probe (in DNA–DNA reassociation) and *Ochrobactrum* isolates of the same species, open symbols (_outsp.) denote data pairs of the indicated probe and *Ochrobactrum* isolates of different species.

63.8%, and the lowest value for isolates of one *Ochrobactrum* species was 65.9% (as 65.9% was lower than the 70% 'gold standard,' species affiliation was confirmed by ΔTm analysis and phenotyping) (Lebuhn *et al.*, 2000). Borderline regions where no clear assignment was possible based on the similarity values are shown as gray-shaded areas in Fig. 3. BOX- as well as REP-PCR similarity values correlated positively with the DNA–DNA reassociation data ($R^2 = 0.6$ for BOX-PCR and $R^2 = 0.5$ for REP-PCR when using linear regression). As indicated by the higher R^2 value, the grouping of the isolates by BOX-PCR fingerprinting was in better accordance with DNA–DNA hybridization, and the borderline region smaller, than that of the REP-PCR scatter-plot, suggesting better discriminatory performance and higher reliability of the BOX fingerprints. Table 2 shows BOX and REP profile similarity cut-off values that agreed with DNA–DNA hybridization data for *Ochrobactrum* isolates belonging to both different and the same species. In Fig. 3, the BOX profile similarity level of 80.7% (borderline for species delineation) crosses the regression curve at 70% DNA–DNA hybridization, and the REP profile similarity level of 86.4% crosses at 92% DNA–DNA hybridization. This confirms the suitability of these rep-profile analyses as substitutes for DNA–DNA hybridization in the genus *Ochrobactrum*. As studies for other genera came to the same conclusion (Rademaker *et al.*, 2000; Mergaert *et al.*, 2003; Lanoot *et al.*, 2004), rep-PCR profile comparisons may generally be suitable not only to group isolates, but also generally to delineate genospecies in bacteriology (Schloter *et al.*, 2000) if the respective calibrations with DNA–DNA hybridization are performed.

Phenotypic diversity and metabolism

On the phenotype level, a representative subset of the *Ochrobactrum* spp. isolates was compared regarding their

Table 2. Cut-off values for *Ochrobactrum* species borderlines as determined by the three different methods

Method	Different species	Borderline*	Within species
DNA–DNA hybridization[†]	≤63.8%[‡]	63.8–65.9%	≥65.9%[§,*]
BOX-PCR	≤78.1%	78.1–80.7%	≥80.7%
REP-PCR	≤71.0%	71.0–86.4%	≥86.4%

*Additional tests are required to confirm the species status.
[†]Data from Lebuhn *et al.* (2000).
[‡]Highest and
[§]lowest value obtained in Lebuhn *et al.* (2000).

Table 3. Occurrence of *Ochrobactrum* spp. in soil and on the wheat rhizoplane

	Soil*	Wheat rhizoplane†
Total CFU	$1–2 \times 10^7$	$10^9–10^{10}$
Ochrobactrum spp.	$2–4 \times 10^5$	$1–2 \times 10^7$
O. anthropi	3×10^5	4×10^6
	4 BOX groups	2 BOX groups
O. intermedium	2×10^4	–
O. grignonense	2×10^4	–
O. tritici	–	10^7

*CFU per gram dry weight of soil.
†CFU per gram dry weight of root.

occurrence and abundance in the different habitats. *O. anthropi* was the only species isolated from both environments, soil and rhizoplane. *O. intermedium* and *O. grignonense* were only cultivated from bulk soil, and *O. tritici* only from the wheat rhizoplane. In soil, *Ochrobactrum* was found to constitute approximately 2% of the bacteria cultivable on 1/10 strength tryptone-soy agar, whereas on the wheat rhizoplane, this fraction was only about 0.3% (Table 3).

A subset of these isolates was analyzed for their metabolic capacity and versatility by BIOLOG GN plates (indirect test), and a subset of these by API 20E, NE (direct) substrate utilization tests (Table 4). At the genus level, SUC and SUV were higher for the *Ochrobactrum* collective from bulk soil than from the rhizoplane, regardless of the system used (significantly in the BIOLOG tests). For *O. anthropi*, for which an intra-species comparison was possible, the highest SUC and SUV values were also obtained for the bulk soil isolates (significantly in the BIOLOG tests, Table 4). From these common trends we concluded that any bias introduced by the indirect colorization method (BIOLOG) was negligible. The highest SUC and SUV values were always obtained for isolates from bulk soil, for *O. anthropi* using the BIOLOG-system, and for *O. grignonense* using the API-system. This was also true for the mean values for the examined *Ochrobactrum* species (Table 4).

Our results suggest that plant root colonizers may have lost unnecessary metabolic capabilities and became specialized to more easily degradable substrates provided by rhizodeposition (*r*-selection (Andrews & Harris, 1986), see also below), whereas strains favouring bulk soil (*K*-selection Andrews & Harris, 1986) may not be able to compete at the rhizoplane (see below) but seem to be equipped with a more diverse battery of degradation genes. Such genetic differences, however, were not detected by the rep-profile comparisons (see *Genetic diversity*).

Effects in the gradient rhizoplane–soil

The lower genetic diversity as well as the lower metabolic versatility and capacity of the rhizoplane isolates in comparison with isolates from soil are clear indicators of a 'rhizosphere effect' caused by the various gradients between rhizoplane and bulk soil (Lynch, 1990). Data on bacteria per gram dry weight (Table 4) suggested a higher population density on the rhizoplane, as described in Lynch (1990), although it should be taken in account that the comparison of soil and rhizoplane colonization in terms of population densities is problematic, as soil is rich in inorganic particles and roots shrink a lot by drying. However, similar differences between soil and rhizosphere microbial populations have also been observed in other studies (Mavingui *et al.*, 1992; Latour *et al.*, 1996; Frey *et al.*, 1997; Laguerre *et al.*, 1997). The main explanation is probably the higher amount of easily degradable nutrients at and near the rhizoplane due to rhizodeposition. We assume that the favourable conditions (high *r* (Andrews & Harris, 1986)) select for specifically adapted populations with reduced and specialized

Table 4. Metabolic capacity and versatility of *Ochrobactrum* isolates immunotrapped from soil and the wheat rhizoplane

	BIOLOG-GN			API 20E, API 20NE	
	Soil	$p(F)$; $p(T)$	Wheat rhizoplane	Soil	Wheat rhizoplane
Substrate utilization capacity (SUC, mean conversion rate in % ± SD)					
O. anthropi	77.6 ± 3.0	0.065; 0.016	74.9 ± 1.5	23.8	20.9 ± 0.4
O. intermedium	71.9 ± 2.4		–	25.4	–
O. grignonense	73.4 ± 0.8		–	29.3 ± 1.4	–
O. tritici	–		68.8 ± 1.2	–	29.0 ± 2.1
Ochrobactrum spp.	75.2 ± 3.8	0.048; <0.001	70.2 ± 2.9	26.2 ± 2.8	25.0 ± 4.0
Substrate utilization versatility (SUV, number of different converted substrates ± SD)					
O. anthropi	71.6 ± 2.6	0.457; 0.011	69.0 ± 1.9	16	12.5 ± 0.5
O. intermedium	66.0 ± 2.5		–	17	–
O. grignonense	64.0 ± 1.0		–	19 ± 1	–
O. tritici	–		63.7 ± 0.8	–	17 ± 1
Ochrobactrum spp.	69.0 ± 3.9	0.001; <0.001	65.0 ± 2.5	17.3 ± 1.5	14.8 ± 2.3

$p(F)$, significance of equality of variances; $p(T)$, significance of equality of means.

metabolic versatility. This can lead to, or be due to, loss of genes that may serve to utilize exotic compounds and survive in bulk soil (high *K* (Andrews & Harris, 1986)) but are not essential at or near the rhizoplane. Specific *r*-selection at the rhizoplane also appears to limit genetic and taxonomic diversity, as we observed in the comparison with bulk soil (Table 4, cf. *Genetic diversity*). This less diverse, *r*-selected and adapted rhizocompetent population is obviously able to cope with the conditions and should be competitive, e.g. with predators, by means of a (temporary) population boost at favourable conditions. This strategy could help the population to survive at the rhizoplane.

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