MICROBIAL ECOLOGY

Microb Ecol (1999) 38:273–284
DOI: 10.1007/s002489900159

Soil Type and Maize Cultivar Affect the Genetic Diversity of Maize Root–Associated *Burkholderia cepacia* Populations

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Received: 22 January 1999; Accepted: 7 April 1999

ABSTRACT

Burkholderia cepacia populations associated with the *Zea mays* root system were investigated to assess the influence of soil type, maize cultivar, and root localization on the degree of their genetic diversity. A total of 180 *B. cepacia* isolates were identified by restriction analysis of the amplified 16S rDNA (ARDRA technique). The genetic diversity among *B. cepacia* isolates was analyzed by the random amplified polymorphic DNA (RAPD) technique, using the 10-mer primer AP5. The analysis of molecular variance (AMOVA) method was applied to estimate the variance components for the RAPD patterns. The results indicated that, among the factors studied, the soil was clearly the dominant one in affecting the genetic diversity of maize root–associated *B. cepacia* populations. In fact, the percentage of variation among populations was significantly higher between *B. cepacia* populations recovered from maize planted in different soils than between *B. cepacia* populations isolated from different maize cultivars and from distinct root compartments such as rhizoplane and rhizosphere. The analysis of the genetic relationships among *B. cepacia* isolates resulted in dendrograms showing bacterial populations with frequent recombinations and a nonclonal genetic structure. The dendrograms were also in agreement with the AMOVA results. We were able to group strains obtained from distinct soils on the basis of their origin, confirming that soil type had the major effect on the degree of genetic diversity of the maize root–associated *B. cepacia* populations analyzed. On the other hand, strains isolated from distinct root compartments exhibited a random distribution which confirmed that the rhizosphere and rhizoplane populations analyzed did not significantly differ in their genetic structure.

Introduction

Biological diversity is defined as the variety of species in ecosystems as well as the genetic variability within each species and is a function of the number of species present as well as the distribution of individuals among them [15, 28]. Genetic transfer and mutation represent the basis of genetic diversity, which, in turn, leads to organisms better adapted to changing habitats where a series of environmental factors and stresses act as selective agents. Ecosystem functioning is

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largely governed by soil microbial dynamics, since soil microorganisms, being involved in elemental cycles of carbon, phosphorus, nitrogen, and others [17, 34], have a role in nutrient cycling, plant decomposition, soil formation, toxin removal [8, 48], and mycorrhizal association [41], as well as being able to influence plant growth [29] and susceptibility to pathogens [14, 49]. Diversity investigations help clarify the functional role of indigenous microorganisms and the biological changes associated with environmental perturbations in order to identify the relationships among microbial diversity, soil and plant quality, and ecosystem sustainability [1, 46].

Among soil microorganisms, rhizobacteria have received increasing attention as they play a key role in agricultural environments. In particular, the biocontrol plant growth-promoting bacteria (biocontrol-PGPB) and plant growth-promoting bacteria (PGPB) [3] are promising for their potential use in sustainable agriculture [13, 22, 29]. An understanding of the mutual influence between the rhizosphere environment and genetic diversity patterns of indigenous microbial populations could be useful to increase our knowledge of natural environments and to evaluate the impact produced by a microbial inoculum, which could affect a preexisting balance among resident populations [4, 47]. A series of abiotic and biotic factors such as soil and plant type are reported to affect the size and composition of the rhizosphere microbiota. The influence of soil type on rhizosphere microbiota has been shown on natural communities at both inter- and intraspecific levels. In a recent study, we demonstrated that the microbial density and community structure of maize rhizobacteria varied significantly among different sampling sites [12]. Latour et al. [31] have shown that the phenotypic diversity of populations of fluorescent pseudomonads was largely influenced by soil texture and composition, and Bashan et al. [2] found that soil type affected the survival of *Azospirillum brasilense.* In general, the rhizosphere microbiota is also plant dependent, as the root exudates play a key role in the selective stimulation of microorganisms, which, in turn, affect their composition [11, 40]. In a previous study, we found a correlation between plant growth and a progressive decrease of genetic diversity in a *Burkholderia cepacia* population associated with maize roots, confirming that changes occurring during plant development can affect rhizobacteria [16]. Lemanceau et al. [32] suggested a plant role in selecting specific populations of fluorescent pseudomonads, since isolates from different plant species showed different metabolic characteristics, and a selective influence of plant species on Pseudomonad phenotypic diversity has recently been assessed [21]. Moreover, not only plant species but also cultivar can affect the genetic polymorphism of rhizobacteria [37]. Temporal changes in composition of bacterial communities associated to cucumber roots have also been observed in rhizosphere, rhizoplane, and endorhiza [35], suggesting that differences can be detected between populations colonizing these different habitats.

As mentioned above, so far, studies on biodiversity have been performed especially on microbial communities, assessing the effects of environmental perturbations mainly at the genus and species level. Indeed, the investigation of factors affecting the biodiversity at intraspecific level is essential for evaluating the influence of exogenous microorganisms on closely related resident bacteria. In the present study, we have investigated the genetic diversity of *B. cepacia* populations associated with maize roots isolated from different soils, maize cultivars, and root compartments (rhizosphere or rhizoplane). The *B. cepacia* species was chosen as a model system of rhizobacteria to study the influence of different factors on genetic rearrangements and variability because of its ecological and genomic properties. In fact, this bacterial species has an unusual genomic organization, characterized by the presence of multiple chromosomes regulated by separate control systems and by an extensive array of insertion sequences, which plays a fundamental role in the ability to adapt to different environments by genetic transfer and mutation [33]. The *B. cepacia* species is also characterized by an extraordinary nutritional versatility that favors the ability to colonize highly different habitats, among which are soil and plant rhizosphere. *B. cepacia* is reported to be closely associated with *Zea mays* roots [26], to promote plant growth [6, 26, 45] and to antagonize and repress soilborne maize pathogens of the genus *Fusarium* [5, 25].

The aim of the present study was to assess how factors such as soil type, maize cultivar, and bacterial localization on the root system (rhizosphere or rhizoplane) affected the degree of genetic variability of *B. cepacia* populations.

Materials and Methods

Experimental Design

Samplings were carried out in different Italian fields with the soil characteristics reported in Table 1. Three maize cultivars, with a ripening period of about 130 days, were used in this study: Airone (Agra), Goldiane (Agra), and Eleonora (Pioneer); four plants of each cultivar were collected from each field after 60 days of plant growth. The origin of bacterial isolates is reported in Table 2.

Table 1. Soil characteristics of the different sites

Site	Clay (%)	Silt (%)	Sand (%)	Organic carbon (%)	Nitrogen (%)	Sodium (ppm)	Mag-nesium (ppm)	Phosphorus (ppm)	Potassium (ppm)	Sulfur (ppm)	Calcium (ppm)	C/N	pH
Casal Buttano	11	30	59	1.20	0.112	17	111	73	117	168	2100	10.70	6.31
Pieve d'Olmi	8	22	70	1.20	0.100	18	121	114	201	149	1108	12.00	6.08
Dragoni	27	15	58	1.15	ND	ND	ND	ND	ND	ND	ND	ND	5.43

ND, not determined.

Pairwise comparisions between *B. cepacia* populations were performed to investigate how the genetic diversity of these maize root-associated bacteria was influenced by factors such as soil type, maize cultivar and root compartment. These factors were investigated as follows:

1. *Soil type:* Cv. Airone was cultivated in two different fields located in Val Padana at Pieve d'Olmi and Casal Buttano, Cremona, Italy. Maize had been cultivated for 5 years in both fields.
2. *Cultivar:* Two cultivars (Airone and Goldiane) were planted in the same field located at Pieve d'Olmi.
3. *Root compartment:* Isolates were obtained from rhizosphere (the zone of soil affected by exudates and other materials supplied by the plants) and from rhizoplane (root surface) of cv. Eleonora planted at Dragoni, Caserta, Italy, where maize had been cultivated for 8 years.

Isolation and Identification of Burkholderia cepacia Strains

In order to evaluate the influence of soil type and maize cultivar on *B. cepacia* variability, bacterial populations from rhizosphere and rhizoplane were recovered together from maize roots following the procedure described by Di Cello et al. [16]. In order to study the influence of root compartment on *B. cepacia* variability, rhizosphere and rhizoplane bacterial populations were recovered separately according to the procedure described by Nacamulli et al. [36]. Samples were platted onto the selective medium PCAT [9] and incubated for 48 h at 28°C to estimate colony-forming units of microorganisms belonging to *B. cepacia* species per gram fresh weight (fwt) of root tissue. According to Di Cello et al. [16], colonies showing the characteristic *B. cepacia* morphology were randomly isolated from PCAT plates with 50 to 100 colonies, purified through serial transfers on the same medium, and cryopreserved at −80°C in 30% glycerol.

To identify *B. cepacia* strains, amplification of 16S rDNA was performed on 2 µl of cell lysate obtained from a single colony from each isolate, according to the procedure described by Di Cello et al. [16]. Restriction analysis of the amplified 16S rDNA with the enzyme *Alu*I made it possible to assign to *B. cepacia* species the isolates showing the same ARDRA pattern as that obtained from reference strain *B. cepacia* LMG11351 [16].

Random Amplified Polymorphic DNA (RAPD) Fingerprinting

Amplification reactions of genomic DNAs were performed on the same lysates used to amplify the 16S rDNA, as previously described [16]. The 10-mer primer AP5 (5′-TCCCGCTGCG-3′), with a GC content of 80%, was used. The amplification patterns were analyzed manually.

Statistics

Data from root colonization were log converted and analyzed using one-way ANOVA (StatView 512+, BrainPower Inc.; CA, USA).

Analyses of population genetic structure were performed using the Arlequin ver. 1.1 software provided by L. Excoffier [42]. The measure of the genetic distance for each pair of strains was calculated by using the vectors of presence and absence of RAPD markers (1 for the presence or 0 for the absence of each band in the gels) for each strain. The Euclidean metric measurement (E) of Excoffier et al. [18], as defined by Huff et al. [27], was used: $E = \varepsilon^2_{xy} = n(1 - 2n_{xy}/2n)$, where $2n_{xy}$ is the number of markers shared by two individuals, and n is the total number of polymorphic sites. The analysis of molecular variance (AMOVA) procedure, based on 10,000 permutations, was applied to estimate the variance components associated with the different possible levels of genetic structure (within individuals or within populations). Pairwise fixation indices F_{ST} [53] were obtained by variance components among populations; transformed F_{ST} [39, 44] were used as short-term genetic distances between some pairs of populations.

The Euclidean distances calculated between all possible combinations of strains taken in pairs were further analyzed using one-way ANOVA to compare the level of internal variability among populations taken in pairs.

The genetic relationships among all the *B. cepacia* isolates were also investigated using the FITCH program for applying the Fitch–Margoliash method of the PHYLIP 3.5c software package [20], with

Table 2. Origin of *B. cepacia* strains isolated from maize roots in different samplings

Sample	Site	Maize cultivar
MVP/B1	Casal Buttano	Airone
MVP/C1	Pieve d'Olmi	Airone
MVP/C2	Pieve d'Olmi	Goldiane
MD1-rz[a]	Dragoni	Eleonora
MD1-rp[b]	Dragoni	Eleonora

[a] Rhizosphere.
[b] Rhizoplane.

Table 3. Data from root colonization, isolation, identification and RAPD fingerprinting of *B. cepacia* isolates recovered from maize roots in each sampling

	Isolates					
	MVP/B1	MVP/C1	MVP/C2	MD1-rz	MD1-rp	Totals
Log cfu/g root fwt[a]	5.03 ± 0.73	6.20 ± 0.22	6.50 ± 0.24	6.33 ± 0.50	6.40 ± 0.35	
Number of *B. cepacia* isolates	20	59	49	26	26	180
Number of RAPD haplotypes	13	48	41	26	23	145
% haplotypes[b]	65.0	81.3	83.7	100	88.4	80.0
G.D.[c]	5.52 ± 2.77	11.14 ± 4.54	9.47 ± 4.41	10.76 ± 3.35	10.68 ± 3.70	

[a] Log colony forming units (cfu) of *B. cepacia* per gram root fresh weight (fwt) measured on PCAT medium. Values are the means of four replicates ± standard deviation.
[b] Percentage of distinct haplotypes among the total RAPD patterns obtained.
[c] Mean values of genetic distances (G.D.) ± standard deviation.

the Euclidean distance matrix as input file. The TREEVIEW program was used to display the trees obtained [38].

Results

Isolation and Root Colonization of B. cepacia Populations

Restriction analysis of the amplified 16S rDNA with the enzyme *Alu*I enabled the recognition of a total of 180 isolates belonging to the *B. cepacia* species (Table 3); 20 isolates were obtained from cv. Airone in the field of Casal Buttano (MVP-B1); 59 isolatse were recovered from cv. Airone (MVP-C1) and 49 from cv. Goldiane (MVP-C2) in the field at Pieve d'Olmi; 26 isolates were recovered from the rhizosphere (MD1-rz) and 26 from the rhizoplane (MD1-rp) of cv. Eleonora in the field of Dragoni. The initials used to describe the isolates were followed by progressive numbers of isolation to describe each *B. cepacia* strain.

B. cepacia populations density ranged from 6.20 ± 0.22 to 6.50 ± 0.24 log cfu (g fwt)$^{-1}$ in almost all the root samples examined (Table 3), and no statistical differences were found in the level of maize root colonization by *B. cepacia* populations ($P > 0.05$). Only the *B. cepacia* population recovered at Casal Buttano (MVP/B1) showed a level of root colonization [5.03 ± 0.73 log cfu (g fwt)$^{-1}$] significantly lower ($P < 0.001$) than the others.

RAPD Fingerprinting

All the 180 isolates assigned to the *B. cepacia* species were RAPD fingerprinted. The DNA of the same lysed-cell suspensions previously used to amplify the 16S rDNA was amplified by the RAPD technique using the 10-mer primer AP5. The reproducibility of the results was verified in independent experiments (data not shown). Amplifcation of genomic DNAs of *B. cepacia* strains gave rise to 59 bands, with dimensions ranging from 150 to 2,300 bp. The presence or absence of these RAPD markers represented the RAPD patterns. A total of 145 different haplotypes were found, showing a high variability among the *B. cepacia* strains investigated. As reported in Table 3, the highest percentage of haplotypes was found within the populations isolated at Dragoni and, in particular, each of the strains MD1-rz showed haplotypes different from each other. The lowest percentage of different haplotypes was recovered among the MVP/B1 strains. On checking for similar haplotypes shared between two or more populations, only a few were recovered in two distinct populations: three haplotypes shared between MVP/B1 and MVP/C2, one between MVP/C1 and MVP/C2, and two between MD1-rz and MD1-rp.

Genetic Variability among B. cepacia Isolates

The RAPD patterns were compared to each other and the Euclidean distance matrix (E) was constructed for the five *B. cepacia* populations examined (MVP/C1, MVP/B1, MVP/C2, MD1-rz, and MD1-rp) to analyze the RAPD variation among and within them using the AMOVA method. Results obtained showed that most of the total molecular variance was attributable to divergences among strains, although highly significant ($P < 0.001$) genetic differences (9.15%) were observed among the five samplings.

The F_{ST} values, varying from 0 (absence of differentiation) to 1 (complete differentiation), were calculated using Arlequin software. The $F_{ST}P$ values were <0.001 (significance level = 0.05) between all pairs of population samples, with the one exception of the value found between rhizosphere and rhizoplane populations, MD1-rz and MD1-rp ($P = 0.883$; see Table 4).

Table 4. AMOVA on *B. cepacia* strains

Source of variation	Samples	Total (%)[a]	P[b]	F-statistic[c]
Soil type	MVP/C1 vs MVP/B1	11.98	<0.001	0.119
Maize cultivar	MVP/C1 vs MVP/C2	5.72	<0.001	0.057
Root compartment	MD1-rz vs MD1-rp	−1.28	0.853	−0.012

[a] Percentage of variation among populations.
[b] P (rand. value > obs. value): probability of having a more extreme variance component than the observed value and F-statistic.
[c] Fixation index: F_{ST} (F-statistic).

The mean genetic distances were calculated for each of the five populations (Table 3). Almost all the values obtained ranged from 9.47 ± 4.41 (MVP/C2) to 11.14 ± 4.54 (MVP/C1), except the value obtained for MVP/B1 isolates (5.35 ± 3.86), which was significantly lower than the others ($P < 0.001$). This result confirms the lowest variability of the MVP/B1 population among all those analyzed, as clearly demonstrated by their having the lowest percentage of haplotypes.

Genetic Diversity in Population Isolated from Different Soils, Cultivars, and Root Compartments

Two-by-two group analyses were performed on *B. cepacia* populations to assess the amount of genetic diversity attributable to divergences among soil, cultivar, or rhizosphere–rhizoplane origin (Table 4). Results showed that microbial diversity was affected to a different degree by the various parameters investigated.

1. *Soil:* The comparison between *B. cepacia* populations isolated from roots of the cv. Airone planted in different soils (MVP/C1 vs. MVP/B1) resulted in a significant ($P < 0.001$) value of variance components among samplings (11.98%). The means of the genetic distances of MVP/C1 and MVP/B1 populations differed significantly ($P < 0.001$), as shown in Table 3. Relationships among the *B. cepacia* strains, calculated using the distance matrix E and the Fitch–Margoliash method, were represented as an unrooted tree (Fig. 1). The dendrogram looks more like a bush than like a tree, as is described for bacterial populations adapted to live in soil or lotic environments where genetic recombination seems to be frequent [16, 52]. Moreover, two main clusters can be recognized where MVP/C1 and MVP/B1 isolates were mainly grouped.
2. *Cultivar:* Maize cultivar significantly ($P < 0.001$) affected the genetic diversity between the *B. cepacia* populations MVP/C1 and MVP/C2 isolated from cv. Airone and Goldiane, respectively, both planted at Pieve d'Olmi, as demonstrated by a variance component among populations of 5.72%. The means of genetic distances were significantly different ($P < 0.001$). The distribution of the strains was represented in a dendrogram in which different clusters can be recognized where strains isolated from each cultivar were predominant (Fig. 2).
3. *Root compartment:* AMOVA analysis of *B. cepacia* populations separately isolated from rhizosphere and rhizoplane (MD1-rz vs. MD1-rp) resulted in a variance component among populations of −1.28% ($P = 0.853$), suggesting that the entire molecular variance was attributable to divergences among strains. No significant difference of the mean GD values were detected between these two samplings ($P = 0.773$). Furthermore, MD-rz and MD-rp strains were randomly distributed in the dendrogram obtained from the distance matrix E of these populations (Fig. 3).

The effect of soil and cultivar was further analyzed by using the parameter F_{ST} according to Reynolds [39], to evaluate the short-term genetic distances between bacterial populations recovered from different soils and cultivars. The results obtained are in general agreement with those reported above, showing that the distance between MVP/C1 and MVP/C2 populations isolated from different cultivars was lower than the distance between MVP/C1 and MVP/B1 populations isolated from different soils (data not shown).

Discussion

In recent years, the study of bacterial population structure in soil and root systems has attracted great interest because of the positive response of many plant species to biocontrol-PGPB and/or PGPB strains. Many studies have been performed on the biodiversity of natural communities in rhizosphere, although only a few attempts have been made to assess the diversity within a species [23, 24, 37, 43]. Indeed,

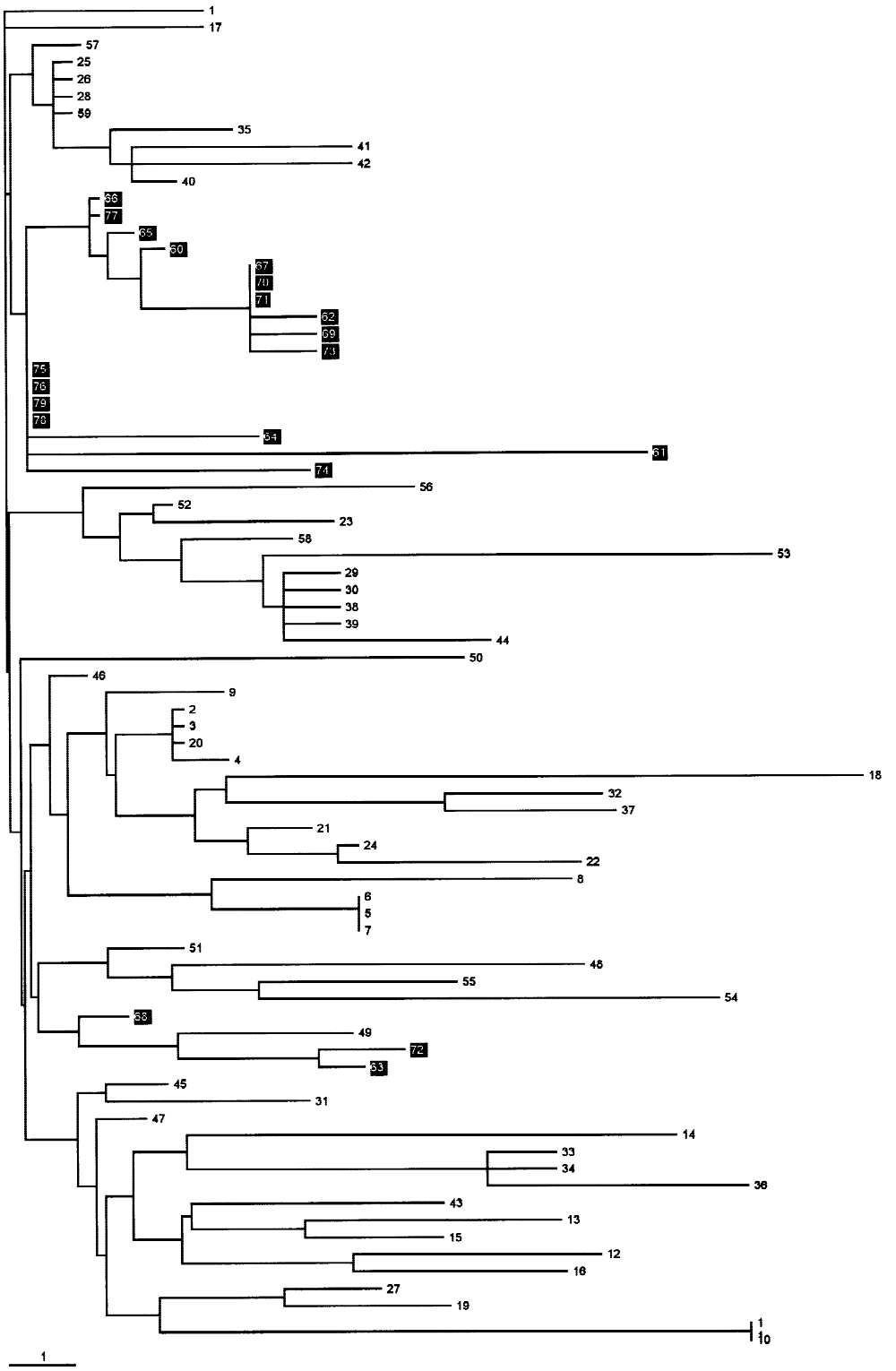

Fig. 1. Dendrogram showing the genetic relationships among *B. cepacia* strains isolated from roots of maize cv. Airone planted in two different fields located at Pieve d'Olmi, MVP/C1 strains (black), and at Casal Buttano, MVP/B1 strains (white on black background). Genetic distances were calculated by using the Euclidean distance matrix *E.*

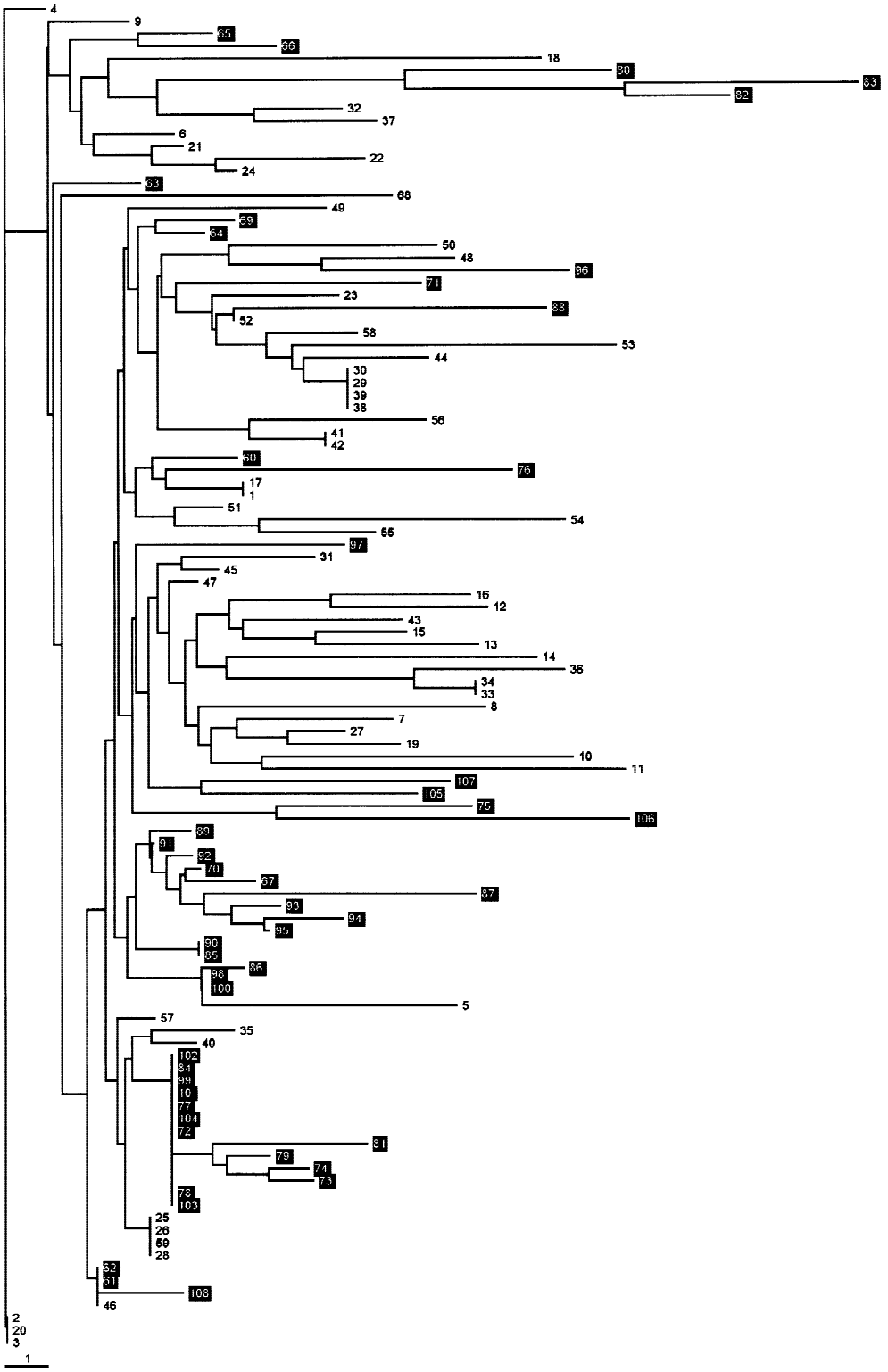

Fig. 2. Dendrogram showing the genetic relationships among *B. cepacia* strains isolated from roots of maize cv. Airone, MVP/C1 strains (black), and Goldiane, MVP/C2 strains (white on black background), planted at Pieve d'Olmi. Genetic distances were calculated by using the Euclidean distance matrix *E.*

Fig. 3. Dendrogram showing the genetic relationships among *B. cepacia* isolated from rhizosphere, MD-rz strains (black) and rhizoplane, MD-rp strains (white on black background) of maize cv. Eleonora Pioneer planted at Dragoni. Genetic distances were calculated by using the Euclidean distance matrix *E.*

an improvement in the study of biodiversity within single species seems to be fundamental to the understanding of the population dynamics of bacterial species in fluctuating environments such as the rhizosphere. This knowledge is essential for the development of effective strategies for delivery and maintenance of exogenous microorganisms in association with root systems.

The present study was aimed to show if factors known to affect bacterial mixed communities have some influence also on the individuals belonging to a single species, and if the mechanisms that cause or favor genetic variability at interspecific and intraspecific level may be considered similar. An analysis of the genetic diversity of *B. cepacia* populations associated to maize roots was performed to investigate whether their degree of polymorphism was affected by environmental factors such as soil type, maize cultivar, and localization on roots. Isolated strains were subjected to RAPD fingerprinting, and the data obtained were analyzed using statistical and phylogenetic methods. A high intraspecific genetic diversity was found within the *B. cepacia* populations investigated, in agreement with previous studies suggesting that soil microbial populations do not follow the clonal model of population structure characteristic of bacterial species [50]. Indeed, the potential for bacterial adaptation to highly heterogeneous and fluctuating environments such as soil depends on their genetic diversity, which is favored by genetic transfer and recombination [19, 30, 51]. In addition, the high level of genetic polymorphism found in the *B. cepacia* populations investigated in this study is in agreement with the results of our previous work where populations of this bacterial species were collected at different stages of maize development [16]. In both cases, high numbers of haplotypes were found among strains isolated in all the samplings, and the statistical analysis of RAPD data showed that most of the molecular variance was attributable to divergences among strains, although a significant part was dependent on other factors.

Among the factors analyzed in this study, soil type influenced the genetic diversity of root-associated *B. cepacia* populations more than maize cultivar and root compartment. In fact, the variance component among populations was higher in the comparison performed between populations isolated from different soils than those isolated from different cultivars. Therefore, soil type seems to play an important role in controlling the biodiversity of *B. cepacia* populations, i.e., in selecting strains with different degrees of polymorphism. This result is in agreement with a series of data reported in the literature showing the effects of soil type on microbial composition: at the level of soil bacterial communities [7, 12], at the genus level, as seen for *Pseudomonas* spp. in a study on plant-associated microorganisms [31] and in a study on microorganisms isolated from agricultural and industrial soils [10], and, finally, at the intraspecific level, as shown in a population of *Sinohizobium melitoti* (formerly *Rhizobium melitoti*) [24]. It was also observed that, among the two *B. cepacia* populations isolated from cv. Airone, the mean value of genetic distances resulted significantly lower for the strains isolated at Casal Buttano than for those isolated at Pieve d'Olmi. It is noticeable that the density of root colonizing *B. cepacia* in the field of Casal Buttano was significantly lower than in the other bacterial samplings. Both the low level of root colonization and the low intraspecific variability seen in the *B. cepacia* population isolated at Casal Buttano might be due to a reduced ability of this bacterial species to colonize maize roots in a given soil as compared to others, although further studies are necessary to determine the mechanisms by which soil type may affect rhizosphere community.

Maize cultivar also influenced the intraspecific genetic diversity of *B. cepacia* populations associated to the root system, in agreement with a series of published data indicating a plant role in determining the composition of rhizosphere bacteria. Biodiversity of fluorescent pseudomonad populations has been shown to be affected by plant species, although at a lower level if compared to soil type [31]. Plant growth has also been reported to influence the genetic diversity of bacterial populations associated to maize roots. We have previously shown that the biodiversity of rhizosphere *B. cepacia* populations decreases over time [16], just as Seldin et al. [43] have shown that the diversity of root-associated *Paenibacillus azotofixans* populations is significantly affected by plant development. In general, little is known about the influence exerted by plant cultivar on the degree of genetic polymorphism of rhizosphere populations. To our knowledge, the effect of plant cultivar has been reported on *Sinorhizobium melitoti* populations isolated from nodules of different *Medicago sativa* cultivars [37]. Our study demonstrated the effect of plant cultivar on the polymorphism degree of a free-living rhizobacterial population. In particular, it was observed a lower influence on *B. cepacia* polimorphism degree by maize cultivar in comparison to soil type.

The last factor examined in this study was root localization. Analysis of soil bacterial communities revealed some differences among ecosystems represented by rhizosphere, endorhiza, and soil [35]. We studied *B. cepacia* populations separately isolated from maize rhizosphere and rhizoplane,

and results of statistical analysis conducted on RAPD patterns showed that the entire molecular variance was attributable to divergences among strains. This means that no separation could be detected between the populations of *B. cepacia* species that colonize rhizosphere and rhizoplane, probably because these root compartments, as defined in this study, do not represent really different habitats for *B. cepacia* populations. Also in the above-mentioned study on biodiversity of *Paenibacillus azotofixans* [43], populations isolated from maize rhizozosphere and rhizoplane were compared and no significant difference was found among them.

In conclusion, the methods used in this study were useful to evaluate the diversity among the strains tested and are potentially helpful for understanding the genetic structure of microbial species in natural ecosystems. We have shown that both soil type and plant cultivar have some effect on the genetic diversity of root-associated *B. cepacia* populations. These results suggest that soil type and metabolites produced by root systems, depending on the plant species as well as the cultivar, may act as selective agents for rhizosphere bacteria. These factors might select not only some species among all those present in the entire rhizosphere [31], but also, within one species, some strains characterized by distinct haplotypes. Further genetic and phenotipic analyses are necessary to improve the knowledge of soil bacterial diversity, as well as to understand the ecological role of the haplotype distribution of rhizobacteria, i.e., to correlate phenotypic characters to different haplotypes.

Acknowledgments

We thank G. Seri and L. Ferrandi for technical assistance and Dr. Bonora, Dr. Tatini, Dr. A. Desideri Gaveglio, Dr. M.L. Desideri Gaveglio, and Dr. C. Nacamulli for assistance in providing plant samples. This study was partially supported by Consiglio Nazionale delle Ricerche (C.N.R.), ECOMI 0639 n. 96.00627.CT0.

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