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Genetic diversity and salt tolerance of bacterial communities from two Tunisian soils

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ABSTRACT - Microbial ecology studies on arid soils are particularly important for the analysis of biological functions during desertification. Although much is known about the arid saline flora, few researches have directly compared the bacterial communities of saline arid soils with cultivated soils in Northern Africa. Bacterial communities present in two soils from Soliman (north of Tunisia), one salty and neglected, and the other cultivated, were investigated by using both cultivation dependent and independent approaches. The first approach was used to assess the presence of salt tolerant bacteria and the relationships among salt (NaCl) resistance phenotype, soil characteristics and phylogenetic assignment of strains. Total community analysis, performed by T-RFLP on total DNA, was carried out to investigate the relationships between total community fingerprinting with cultivated isolates diversity. The cultivated isolates from salty soil were more genetically diverse, harbouring strains that can grow at high salt concentration. Moreover, the salt resistance of isolates was found not to be related to any particular phylogenetic group, being widespread among isolates belonging to different bacterial subdivisions. Ribotype richness, evaluated as number of different T-RFLP bands (TRFs), was shown to be higher in the agricultural soil than in the salty soil and several agricultural soil-specific TRFs were detected.

Key words: bacterial communities, T-RFLP, salt tolerance, arid soil.

INTRODUCTION

Soil is a very complex system that comprises a variety of microhabitats with different physicochemical gradients and discontinuous environmental conditions where microorganisms adapt to microhabitats and live together in communities interacting with each other and with other components of the soil biota. Determining factors, such as temperature, pH, or geographic location, that correlate with differences between diverse microbial communities revealed how easily microbes tolerate different kinds of environmental changes (Girvan *et al*., 2003; Lozupone and Knight, 2007). Moreover, a recent meta-analysis found that the major environmental determinant of microbial community composition is salinity rather than extremes of temperature, pH, or other physical and chemical factors (Lozupone and Knight, 2007). In several studies, the investigation of microbial communities in soils undergoing salinization has stirred much of the attention because of the relevance for agriculture and as an example of "extreme" environment (Liu *et al*., 1997; Corpet, 1988; Zahran, 1999; Smit *et al*., 2001; Caton *et al*., 2004). Bacteria living in unusual extreme conditions might in fact offer an important research tool for investigating the relationships and interactions between environmental factors and microbial evolution at metabolic and gene levels (Gould and Corry, 1980).

Current analyses of soil communities are often performed through culture-independent DNA based methods. Among them, Terminal-Restriction Fragment Length Polymorphism (T-RFLP) is an extensively exploited tool used to analyze the genetic diversity of amplified 16S rRNA genes of a microbial community (Liu *et al*., 1997; Marsh, 1999; Dunbar *et al*., 2001; Berg *et al*., 2005; Mengoni *et al*., 2004, 2007). However, cultivation-independent approaches cannot provide insights about the phenotypes of the bacteria within the community, which are the most directly linked to community function and fitness with respect to environmental stressful conditions. In particular, salt resistance of bacteria, is an important parameter for assessing functionality of soil ecosystems in arid regions. The selection of salt tolerant strains and their subsequent inoculation was a strategy suggested for improvement of soil fertility especially in relation to plant-associated bacteria (Zahran, 1991; Lal and Khanna, 1994; Bouhmouch *et al*., 2001) and for crop cultivation of the saline lands in arid regions and of the salt-affected soils. Moreover, interest in microbes living in extreme environments, as arid soils has been fuelled by the discovery of novel taxa in these ecosystems and by the potential for discovery of valuable enzymes, polymeric

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materials, and bioactive compounds (Gould and Corry, 1980). For instances, in recent studies, salt-tolerant actinomycetes have been explored for their capacity to produce numerous bioactive molecules, particularly antibiotics (Vasavada *et al*., 2006), and enzymes as alkaline protease (Thumar and Singh, 2007). Finally, bacteria from saline environments may be considered models for biological salt tolerance (Lanyi, 1979). These organisms have evolved in saline environments and are able to overcome the deleterious effects of salts up to saturating concentrations.

In the present work we investigated the bacterial communities of two soils, one salty and abandoned, the other one cultivated, which are in close proximity in Soliman (north of Tunisia) by using both cultivation dependent and independent approaches. The aim was: i) to isolate and characterize salt resistant strains and the cultivable communities in the two soils, and ii) to determine the presence and extent of taxonomic differences between the total bacterial communities of agricultural and saline soil.

MATERIALS AND METHODS

Soil sampling and characterization. Two soils, one saline and one agricultural (1 km far from each other) from Soliman (longitude, 10°29′30″E; latitude, 36°41′47″N), North Tunisia were studied. On each site (at about 10 cm depth from "naked" soil) soil samples of 500 g were taken in March 2006 with a clean steel spatula sterilized with ethanol. Three replicates were sampled across the site for both soils, at a distance of about 35 m between each sampling point. The agricultural soil was a fallow field with sandy soil type previously used for intense production of onion (*Allium cepa*), olive (*Olea europea*) and barley (*Hordeum vulgare*). The saline soil was clay, without agricultural activity, with a few vegetation (*Medicago* spp.) and some halophytes *Arthrocnemum indicum* (Willd.) Moq., *Suaeda fruticosa* Forsk., and *Sesuvium portulacastrum* L. Soil samples were transported to the laboratory in a sealed polyethylene bag at ambient temperature and stored at 4 °C in sterile containers, until analysis the next day.

Moisture content, pH, organic matter, texture and electrical conductivity of the soil samples were determined. Soil texture was determined by wet-sieving and sedimentation using the Kohn-Pipette method after organic C destruction with H_2O_2 and chemical dispersion using $Na_4P_2O_7$ (Hartge and Horn, 1992). The oven dry method was used for moisture content, weighing samples before and after drying. The percent of weight loss was used as the percent moisture content of the sample. The pH of each sample was determined by using a pH meter method: ten grams of soil were shaken in 25 ml of de-ionized water for 10 min and then allowed to settle for 30 min. A pH meter was then used to determine the pH of the supernatant water without disturbing the settled soil. The amount of carbon or organic matter was determined using the weight loss on ignition test (Dean 1974). For the electrical conductivity, direct fields measurements were taken with the Veris EC sensor (Veris, Inc., Salina, Kan).

Isolation of bacteria. Triplicate subsamples (1 g) were taken from each soil sample, kept in sterile plastic boxes at 4 °C for a few hours before using. Then, homogenized in 10 ml of physiological solution and 100 µl of 10-fold serial dilutions of the suspension were spread in triplicate on low nutrient R2A medium for heterotrophs (Difco) (Øvreås and Torsvik, 1998). In order to inhibit the growth of fungi, 300 µg ml^{-1} cycloheximide was added. Plates were incubated at 27 °C for 2 days; after titres of aerobic heterotrophic bacteria were evaluated as colony-forming units (CFUs). Each CFU determination was performed in triplicate and an average value of bacterial titre was determined. Colonies having different morphology (colour, shape, etc.) were picked up and isolated from each sample. Each colony was purified on R2A and stored at −20 °C in R2A broth medium containing 50% glycerol.

Salt tolerance of bacterial isolates. Isolates were incubated in liquid Luria Broth (LB) medium with increasing concentration of NaCl (no salt, 100, 300, 600 mM and 1 M). In each tube, 200 µl from 24 h preculture (OD_{620} = 1.0) was added to 5 ml of fresh growth medium. Growth was measured by turbidity at 620 nm after 24 h incubation at 27 °C in a rotary shaker at 150 rpm. Growths were performed in triplicate for each isolate. Bacterial isolates were classified by their growth at different NaCl concentration in liquid medium. To confirm result, salt tolerance was also estimated on solid LB medium with the same NaCl concentrations and scored for growth after 24 and 48 h incubation at 27 °C.

PCR-amplification, ARDRA sequencing and analysis of bacterial 16S rDNA. DNA was extracted from each isolate after growing in liquid LB medium at 27 °C for 24 h by using the cetyltrimethylammonium bromide (CTAB) method (Ellis *et al.*, 1999). Extracted DNA was quantified after agarose gel electrophoresis and staining with ethidium bromide (1 µg/ml). Amplification of 16S rDNA was performed as previously described (Mhamdi *et al*., 2002) using primer (27f, 5′ GAGAGTTTGATCCTGGCTCAG, and 1495r, 5′ CTACGGCTACCTTGTTACGA). For the ARDRA (Amplified Ribosomal DNA Restriction Analysis) (Vaneechoutte *et al*., 1992), 1 µg of the amplified 16S rDNA was digested with 2 units of the restriction enzyme *Alu*I (Invitrogen, San Giuliano Milanese, Italy) in a total volume of 15 µl at 37 °C for 3 h. The reaction mixture was then incubated at 65 °C for 10 min to inactivate the restriction enzyme. Reaction products were separated by agarose gel (2.5% w/v) electrophoresis in TAE buffer run at 10 V cm^{-1} for 1.5 h and stained with 1 µg ml^{-1} ethidium bromide. For the sequencing reaction of isolates from selected ARDRA groups the amplified 16S rDNA was purified from salts and primers with the MinElute PCR purification kit (Qiagen Inc., Chatsworth, CA, USA) according to the manufacturer's instructions. Direct sequencing of amplified 16S rDNA, of three isolates per ARDRA group, was performed with the 27f primer on an ABI310 automated sequencer (Applied Biosystems, Foster City, CA, USA) using the Big Dye Terminator kit, version 2 (Applied Biosystems). Obtained sequences were matched against the GenBank-EMBL-DDBJ database with the BLAST program (Altschul *et al*., 1997) to provide taxonomic interpretation (that is to identify the bacterial groups) of the selected ARDRA groups.

Shannon index of diversity was calculated by using the on-line calculator present at http://www.changbioscience.com/genetics/shannon.html.

T-RFLP analysis. Total soil DNA from the same samples used for the isolation of bacteria was extracted as previously described (Yeates *et al*., 1998). DNA was quantified by agarose gel eletrophoresis and by spectrophotometric reading. PCR reactions were performed in 50 µL volume containing 20 ng of template DNA and 2 U of *Taq* DNA polymerase (Invitrogen) using primer 27f labeled with 6-carboxyfluorescein (FAM) and primer 1495r as previously reported (Mengoni *et al*., 2002). Amplified 16S rDNA was digested separately with 20 U of *Msp*I, *Rsa*I and *Taq*I (Promega, New England Biolabs, Beverly, MA, USA) restriction enzymes for 3 h at 37 °C (or 65 °C for *Taq*I). A 200 ng aliquot of

the digested product was resolved by capillary electrophoresis on an ABI310 Genetic Analyzer (Applied Biosystems) using ROX 500 (Applied Biosystems) as a size standard. Fragment sizes from 35 to 500 bp were considered for profile analysis.

TRFs, with appropriate size, derived from *Msp*I restriction digestion were cloned by using an adaptor-based method (Mengoni *et al*., 2002) and MiCA3 web tool (http://mica.ibest.uidaho.edu/) to allow their taxonomic interpretation. Three clones, for each selected TRF size, were selected for sequencing (performed as previously described).

Statistical analysis of T-RFLP profiles. Analysis of T-RFLP profiles was performed with the software GelComparII 3.0 (Applied Maths, Kortrijk, Belgium) directly from chromatogram files. Only fragments with fluorescence intensity >50 arbitrary units of fluorescence were considered. Alignment of the profiles was performed and a binary matrix, in which the presence or absence of peaks was scored as string of ones or zeros. Matrices from the three different restriction digestions were linearly combined to obtain a unique binary vector for each sample. The matrix of binary vectors was then used to compute the community similarity values based on the Jaccard coefficient of similarity, which only takes into account band sharing between vectors. The matrix of mean Jaccard similarity was then used for Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering and Principal Component Analysis (PCA) using the modules present in NTSYSpc 2.02 (Rohlf, 1990).

To assess the amount and the significance of the genetic differences between saline and agricultural soil community profiles, the Analysis of Molecular Variance (AMOVA, Excoffier *et al*., 1992) was applied to the T-RFLP matrix described above. This test, originally developed to infer the genetic structure of populations (Mengoni and Bazzicalupo, 2002), was here applied as an alternative to the classical ANOVA (Miller, 1997) in deriving a measure of genetic differentiation between bacterial communities. The software ARLEQUIN 3.1 (Excoffier *et al*., 2006) was used for AMOVA computation and statistical significance was assessed after 100000 permutations.

RESULTS

Soil characteristics and bacterial titres

Physical and chemical characteristics of the soil samples are reported in Table 1. As expected, a major difference between soil types was found in the electrical conductivity (EC) with the saline soil having far higher values than the agricultural one. Bacterial titres were on average 1.5 x 10^7 CFU/g for the agriculture soil and higher, 8 x 10^7 CFU/g for the saline soil. Plates were visually inspected and, on plates of the same soil site, colonies with different morphology were picked up and re-isolated on R2A agar plates. A total of 208 colonies were selected. In general, colonies from agricultural soil appeared more similar to each other, and this is why we selected more colonies from saline than from agricultural soil (140/68).

Salt tolerance of bacterial isolates

Growth of triplicate culture of bacterial isolates at various salt concentrations was evaluated (Table 2). As there was no difference in the results obtained on solid and liquid medium, data in Table 2 refer only to liquid cultures. Among 208 isolates, five main phenotypic classes were scored. Phenotypic class P5 was the most abundant and comprised isolates growing up to 1 M NaCl. However, no correlation was found between OTUs (see below and Table 3) and salt-tolerant phenotype, each OTU including different phenotypes, that is each phenotypic class for salt tolerance included isolates from different taxonomic groups. However, OTU 6, 35 and 36 isolated from the saline soil samples, were included in the most salt resistant phenotype (P5). The distribution of salt tolerant phenotypes between the two soil types showed that a higher proportion of isolates from saline soil was found to be resistant to high NaCl concentrations compared with those from agricultural soil (one-way ANOVA, $P < 0.0001$). The distribution of isolates resistant to 600 mM and 1 M NaCl (76 isolates), revealed that they were represented by 17 major OTUs.

16S rDNA profiling

208 bacterial isolates were taxonomically typed by using ARDRA and 16S rDNA sequencing; these isolates were also phenotypically characterized for NaCl tolerance.

Fifty different ARDRA restriction patterns (Operational Taxonomic Units, OTUs or haplotypes) were found after digestion of amplified 16SrDNA with *Alu*I. Thirty of the 50 OTUs were represented by less than four isolates. The remaining twenty OTUs accounted for 160 (more than 74%) of the total number of isolates, and are referred to as "major OTU", being represented by a minimum of 4 isolates.

In Fig. 1 the number of OTUs of each soil samples is reported. Twenty-nine OTUs were found among 68 isolates from agricultural soil samples and 39 OTUs were scored for 140 isolates from saline soil samples. Within the 20 major OTUs, six were exclusively recovered from saline soil (OTU6, OTU7, OTU8, OTU15, OTU31, and OTU35) and just one was recovered from agriculture soil (OTU26). Among these 20 major OTU, 3 isolates per OTU were chosen for a partial 16SrDNA sequencing of the first 400-

TABLE 1 - Soil physical and chemical characteristics and bacterial titres

Samples	Agricultural soil	Saline soil
pH	8.46 ± 0.13	8.93 ± 0.13
Moisture content (%)	32.33 ± 0.65	54 ± 4
Electrical conductivity (EC) (mmho/cm)*	0.7 ± 0.11	4.66 ± 1.72
Organic matter (%)	0.75 ± 0.4	1.1 ± 0.16
Carbon (%)	0,53 ± 0.23	0.66 ± 0.06
Texture	Sandy	Clay
Bacterial titres (CFU/g of soil)	1.5 ± 0.97 x 10^7	8.13 ± 1.36 x 10^7

* 1 dS/m = 1 mmho/cm (Bauder *et al*., 2004).
Values are means ± Standard deviation of triplicate measures.

TABLE 2 - Salt resistance phenotypes of the 208 bacterial isolates

Phenotype code	Number of isolates	NaCl*			
		100 mM	300 mM	600 mM	1 M
P1	17	-	-	-	-
P2	28	+	-	-	-
P3	37	+	+	-	-
P4	50	+	+	+	-
P5	76	+	+	+	+

* Salt tolerance in liquid cultures is designated are as follows: + = good growth, as in the absence of salt; - = no growth. Growth was measured by means of OD at 620 nm after 24 h incubation at 27 °C in a rotary shaker at 150 rpm. Growths were performed in triplicate for each isolate. Bacterial isolates were classified by their growth at different NaCl concentration. The OD at 620 nm ≥ 1.0 was considered as good growth.

TABLE 3 - Shannon index estimates of bacterial diversity and number of ARDRA OTUs for isolates from agricultural and saline soil

Sample	Number of OTUs	Shannon index	Number of major OTUs*	Shannon index Major OTUs
Total agricultural	29	2.95	13	2.27
Total saline	39	3.09	20	2.52

* Major OTUs are defined as those represented by a minimum of 4 isolates.

500 bases from 27f primer annealing site in order to phylogenetically assigning them to bacterial taxonomic groups. Results obtained (Table 4) allowed to recognize 7 bacterial genera, the most represented being the genus *Bacillus* (10 out of 20 OTUs).

T-RFLP community profiles

T-RFLP analysis of 16SrRNA gene fragments amplified from total DNA was used to evaluate and compare total bacterial community diversity in the above described soils. The three restriction endonucleases applied to 16S rDNAs amplicons yielded a total of 86 different peaks or TRFs (Terminal Restriction Fragments). *Msp*I produced the highest number of peaks (45), while *Taq*I gave the lowest (17). The largest number of TRFs was observed in the agricultural soil samples (70 TRFs). Among the 86 TRFs, nine were ubiquitous in all soil types, six TRFs (produced after restriction digestion with *Msp*I) were exclusively present in the agriculture soils, whereas no TRFs were exclusive to the saline soils. The six TRFs, identified as specific of agricultural soil type were taxonomically interpreted after cloning and sequencing and were assigned to *Bacillus* (EU122184), *Rubrobacter* (EU122187), *Betaproteobacteria* (EU122185 and EU122186), *Acidobacteria* (EU122188) and *Actinobacteria* (EU122189) (Table 5). A matching of TRFs with RDP release 9.60 sequences with MiCA3 web tool gave similar results for most the TRFs.

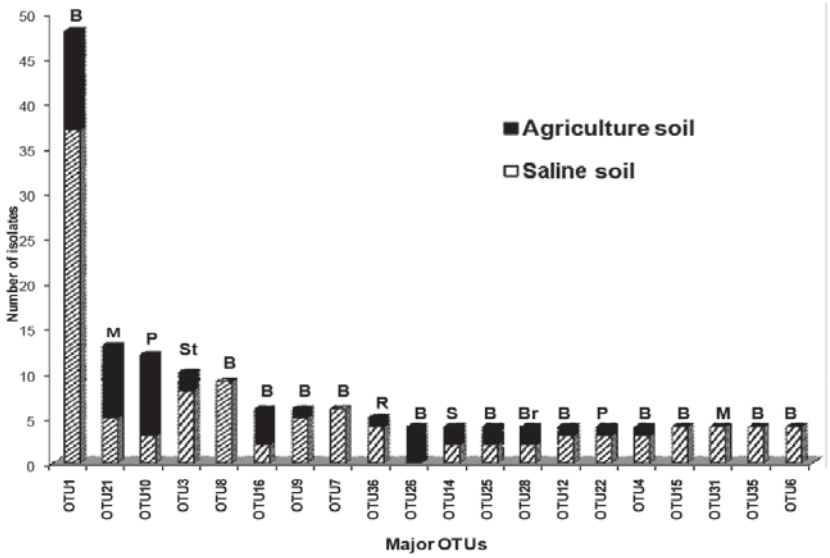

FIG. 1 - Distribution of different bacteria isolates among the major OTUs in the two soil types, and putative genus assignment of the major ARDRA types. B, *Bacillus*; St, *Stenotrophomas*; P, *Pseudomonas*; M, *Microbacterium*; S, *Sphinogomonas*; Br, *Bordetella*; R, *Rhizobium.*

TABLE 4 - Taxonomic interpretation of the major OTUs

OTU number	GenBank accession number	Best matched sequence (accession number, % of similarity)*	Number of isolates **
OTU1	EU122164	*Bacillus* sp. (AF539671, 89%)	48
OTU3	EU122165	*Stenotrophomonas* sp. (AJ011332, 92%)	10
OTU4	EU122166	*Paenibacillus* sp. (AY336562, 91%)	4
OTU6	EU122167	*Bacillus* sp. (AF157696, 96%)	4
OTU7	EU122168	*Bacillus* sp. MK03 (AB062678, 83%)	6
OTU8	EU122169	*Bacillus* sp. MP2 (DQ985283, 92%)	9
OTU9	EU122170	*Bacillus* sp. (AF295302, 99%)	6
OTU10	EU122171	*Pseudomonas* (DQ862549, 94%)	12
OTU12	EU122172	*Bacillus* sp. (DQ275178, 94%)	4
OTU14	EU122173	*Sphingomonas* sp. (AB033944, 99%)	4
OTU15	EU122174	*Bacillus* sp. (EF152359, 95%)	4
OTU16	EU122175	*Pseudomonas* sp. (EF528294, 94%)	6
OTU21	EU122176	*Microbacterium* sp. (AB271048, 94%)	13
OTU22	EU122177	*Pseudomonas* sp. (EF424136, 96%)	4
OTU25	EU122178	*Bacillus* sp. (EF656456, 98%)	4
OTU26	EU122179	*Bacillus* sp. (DQ993678, 92%)	4
OTU28	EU122180	*Bordetella* sp. (EF442019, 96%)	4
OTU31	EU122181	*Microbacterium* sp.(AY974047, 94%)	4
OTU35	EU122182	*Bacillus* sp. (EF638801, 97%)	4
OTU36	EU122183	*Rhizobium* sp. (EF070127, 96%)	6

* The bacterial group shown is that comprising the best matched sequences after BLAST search. In brackets the accession numbers and percentage of similarity of the best matched sequences are reported.

** Number of isolates with ARDRA pattern identical to that of the sequenced strain. From each major OTU, three isolates were sequenced.

To assess the distribution of ribotypic diversity between the two soil types, Principal Component Analysis (PCA), UPGMA clustering (Fig. 2) and AMOVA were used. Both analyses recognized a separation between the community profiles of the two soil types and correctly clustered T-RFLP profiles of soil samples according to their origin. In particular UPGMA showed a large separation of saline vs. agricultural soil (32% of similarity between the two clusters). The most similar samples were SS1 and SS3 (63% of similarity). Triplicate samples from saline soil type appeared slightly more heterogeneous than agricultural ones (42.5 and 45% of similarity, respectively). The first principal component of PCA, in agreement with UPGMA clustering, separated saline soil samples (in particular SS1 and SS3) from the agricultural soil samples. In the second principal component, the heterogeneity within soil types was shown. To quantitatively assess the degree of heterogeneity within soil samples and between soil types, an AMOVA was performed in order to detect an uneven distribution of ribotypic T-RFLP variance with respect to the different soil type. Obtained results showed that soil heterogeneity within soil type was the most important factor affecting variance (within soil

TABLE 5 - Taxonomic interpretation of TRFs specific of agricultural soil type*

Size (nt)	Best matched sequence (accession number, % of similarity)**	MiCA 3 matching***
167	*Bacillus* sp. (AY211124, 99%)	*Bacillus, Chloroflexi, Helicobacter, Finegoldia, Brevibacter, Staphylococcus, Corynebacterium*
494	Uncultured betaproteobacterium (EF219646, 95%)	Uncultured betaproteobacteria, *Acinetobacter, Enterobacter, Pantoea, Escherichia coli*
488	Uncultured betaproteobacterium (AM691112, 97%)	*Acidovorax, Comamonas*, Uncultured betaproteobacteria
286	Uncultured *Rubrobacter* (AY571811, 99%)	Uncultured bacteria
196	Uncultured *Acidobacteria* (EF664827, 98%)	*Halomonas, Streptomyces*
297	Uncultured actinobacterium (EF651774, 100%)	Uncultured bacteria

* The 6 TRFs, due to *Msp*I digestion of amplified 16SrDNA, were taxonomically interpreted after cloning and MiCA3 web tool.

** The bacterial group shown is that comprising the best matched sequences after BLAST search. In brackets the accession numbers and percentage of similarity of the best matched sequences are reported.

*** For the MiCA3 matching, the names of the matched organisms with Ribosomal Database release 9.60 (comprising 511847 sequences) is reported.

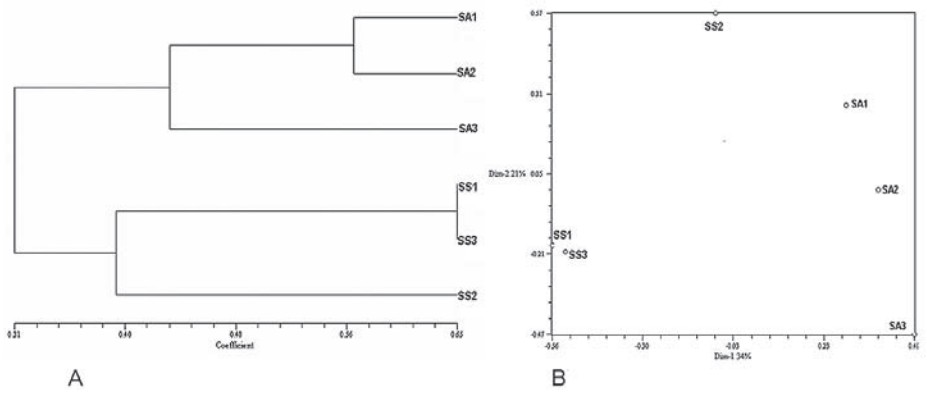

FIG. 2 - A: UPGMA dendrogram computed from Jaccard similarity matrix among T-RFLP profiles of amplified 16S rDNA genes from the different soils. Scale bar indicates Jaccard similarity values. B: Principal component analysis of the T-RFLP profiles of agricultural (SA) and saline (SS) soil types. Dim-1: first principal component, Dim-2: second principal component. The percentage of variance due to the displayed component is reported.

type 68.57%) (Table 6). However, in agreement with previous analyses, a clear separation of T-RFLP profiles with respect to soil type was found, estimating the among soil variance component to more than 30% ($P < 0.0001$) of the total genetic variance.

DISCUSSION

Salinity is one of the main factors shaping bacterial community composition. Here we isolated heterotrophic bacteria from two soil types in Northern Tunisia, one undergoing salinity and desertification, the other one cultivated, aiming to determine their genetic diversity and salt resistant phenotypes. Moreover we investigated total community composition aiming at characterizing the differences between the bacterial communities present in the two soils.

Obtained results on cultivated bacteria showed that, in our conditions, saline soil had the highest titres and the highest diversity (as Shannon index) for heterotrophic cultivable bacteria.

On the 208 bacterial isolates, 50 ARDRA restriction patterns were found after digestion of amplified 16SrDNA. 16SrDNA sequencing of main ARDRA groups showed that bacterial isolates, for both saline and agricultural soil, consisted mainly of organisms taxonomically close to the genera *Bacillus*, by far the most frequent, *Pseudomonas, Microbacterium, Rhizobium, Bordetella*, *Sphingomonas* and *Stenothrophomonas*. These genera were found to dominate the cultivated bacterial communities in several other arable and forest soils (Smit *et al.*, 2001; Axelrood *et al.*, 2002; Zhou *et al*., 2004) and were reported also in saline habitats (Rosenberg, 1983; Rodriguez, 1991). Among the 50 ARDRA groups, 6 of them were found in saline soil only and were assigned to the genera *Bacillus* and *Microbacterium*. Previous reports indicated that Gram-positive bacteria are well represented in saline habitats, and members of the genera *Bacillus* and *Micrococcus* are dominant among other Gram-positive bacteria in saline soils (Zahran *et al*., 1992).

Concerning the salt resistant phenotype of isolates, the higher proportion and extent of salt resistance was retrieved within saline soil. Interestingly, salt tolerance phenotypes were not related to any particular ARDRA group. This fact may suggest the presence of a relatively high number of molecular/genetic ways salinity tolerances can evolve and spread into the community. Even though salt-tolerant strains occurred in both saline and non saline sites, the isolates showing the highest salt tolerance were found in saline soil. It is worth of note that, among the isolates identified as belonging to the genus *Rhizobium,* all were shown to be resistant to 600 mM and 1 M NaCl. A number of studies have shown that osmoadaptation in *Rhizobium* appears to be atypical compared to that found in many enteric bacteria (for a review see Miller and Wood, 1996) and rhizobia have been previously reported to colonize the saline soil and establish effective symbiosis with plants (Zahran, 1999).

The total community analysis, performed by T-RFLP, showed a general clustering of bacterial communities profiles accordingly with soil types. However, differences between profiles, especially for those of the agricultural soil, were found. Concerning the ribotypic diversity, the agricultural soil showed the highest values with 70 TRFs against 45 found in saline soil T-RFLP

TABLE 6 - Analysis of molecular variance (AMOVA) for 6 soil samples combined in two different soil types (saline and agricultural) using 86 T-RFLP bands*

Source of variation	d.f.	Sum of squares	Variance	Total (%)	*P*
Among soil types	1	31.667	6.11111	31.43	< 0.0001
Within soil types	4	53.333	13.33333	68.57	< 0.0001
Total	5	85.000	19.44444		

* The 6 samples were distributed between 2 soil types. AMOVA was performed attributing the grouping of samples according to their site of sampling that is AS1, AS2, AS3 for agricultural soil type and SS1, SS2, SS3 for saline soil type. The total variance derived from T-RFLP profiles was attributed to the three hierarchical partitions: first lane, among soil types; second lane, within soil types. Data show the degrees of freedom (d.f.), the sum of squared deviation, the variance component estimate, the percentage of total variance contributed by each component and the probability of obtaining a more extreme component estimate by chance alone (*P*-value). The *P*-values were estimated computing 100000 permutations.

profiles. Agricultural activities, such as tillage, intercropping, rotation, drainage, and fertilizers, can have significant implications for the microorganisms present in the soil (Øvreås and Torsvik, 1998). In the saline soil type, though some TRFs are specific for a particular sample, no TRFs were found common for all saline soil samples and exclusive of the saline soil type. On the contrary, six TRFs exclusive of the agricultural soil were detected and identified as belonging to bacterial domains like *Acidobacteria, Betaproteobacteria* and *Actinobacteria*. Such phylogenetic groups were found to dominate the bacterial community structures in several agricultural and forest soils (Galinski and Tindall, 1982). Although members of *Acidobacteria* were especially abundant in arable soils (Buckley and Schmidt, 2003), Smit *et al*. (2001) suggested that the ratio between the number of *Proteobacteria* and *Acidobacteria* might be indicative of the trophic level of the soil. Consequently, future analyses on those soils could specifically address this point by clone library and FISH analyses. Nevertheless, the functional role of the members of *Acidobacterium* division in soil is still unknown (Galinski and Tindall, 1982; Felske *et al*., 2000). Within agricultural soil samples, the largest number of TRFs was found for SA3 and could be related with the lower organic matter and low quantities of humic acids present there (Paul and Clark, 1989), in fact, it has been reported that humic acids may introduce a bias toward low diversity estimates (LaMontagne *et al*., 2002). However, to fully address the relationships between soil parameters and bacterial community profiles, more samples distributed along a gradient are needed to perform statistical analyses of association and determine the quantitative effect of soil chemical parameters over community composition in these soils.

In conclusion, this paper reports an initial analysis showing possible association between the presence of some bacterial flora and soil salinity. In particular, genetic diversity on T-RFLP pattern seems to be related to soil parameters. Moreover, in this work, a number of bacterial isolates resistant to high NaCl concentrations (1 M) has been isolated which can have a great impact on the performances of vegetation of saline arid soils. In particular plant-associated bacteria as those belonging to the genus *Rhizobium* have been found which could be useful for land restoration managements.

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