Microbial Diversity in Two Egyptian Soils

Said M. Badr El-Din[1], Samir M. Radwan[1], Moawad K. Zahra[2], and Wafaa M. H. Zidan[1*]
[1]Agricultural Microbiology Department, National Research Centre, Dokki, Cairo, Egypt
[2]Agricultural Microbiology Department, Faculty of Agriculture, Cairo University, Egypt

ABSTRACT
Microbial DNA extracted from two soil samples collected from Beni-Suef and Kafr El-Sheikh were subjected to PCR amplification with primers specific for 16S rDNA gene and cloned in linear pCR 2.1 plasmid vector. Recombinants were transformed into *Escherichia coli* competent cells. Sixty clone inserts (30 from each soil sample) were sequenced and subjected to phylogenetic analyses. Forty sequences of the sixty clones were affiliated with previously recognized bacterial groups. Thirty six of these had closest relatives among cultured taxa and clustered primarily with three divisions containing microrganisms commonly associated with soil: *Proteobacteria,* Gram-positive organisms, and *Cytophaga-Flexibacter-Bacteroides* group. The results also showed the presence of one clone related to *Nirospira* retrieved from Beni-Suef soil, one clone from Archaea kingdom retrieved from Kafr El-Sheikh soil, and three clones affiliated to the newly described *Holophaga-Acidobacterium* phylum in both Beni-Suef and Kafr El-Sheikh soils. Seven sequences grouped with known divisions but had closest relatives among soil taxa known only from rDNA sequences analysis. Twelve clone sequences were distantly related to known sequences. Many of these sequences may represent new bacterial divisions.
Key words: Archaea, Beni-Suef, Holophaga-Acidobacterium, Kafr El-Sheikh, microbial diversity, phylogenetic analysis, *Proteobacteria*, soil DNA, 16S rDNA.

INTRODUCTION

The understanding of the soil microbial community is probably the most challenging because of the exceptionally high microbial diversity in soil (Borneman *et al.*, 1996), and the complex and variable matrix in which soil microbes are embedded. The unculturability of microorganisms and the lack of specificity and sensitivity of classical detection methods have hampered progress in microbial ecology, particularly with regard to complex ecosystems such as soils and sediments, in which the proportion of culturable cells seldom reach more than 5% of the total number, and that most of the diversity is located in the part of the community which cannot be cultivated by standard techniques (Torsvik *et al.,* 1990; Amann *et al.*, 1995).

Consequently, molecular biology techniques have been used by a lot of microbial ecologists to investigate the ecological functions of certain characterized genes that encode important metabolic pathways and reveal microbial community structure and diversity. Since the mid-1980s, the use of 16S ribosomal ribonucleic acid (rRNA) based techniques has facilitated the molecular identification of a wide variety of as yet uncultivated microorganisms and novel microbial groups in various environments. The stepwise strategy of this approach is to isolate total community DNA and use this DNA as a template for PCR amplification of 16S rRNA genes with universal or domain-specific primers. This is usually followed by construction of a clone library based on genes encoding rRNA (rDNAs) and rapid screening of the library based on sequence differences. The present work aims to examine the microbial diversity in two Egyptian soils by phylogenetic analysis of rDNA sequences.

MATERIALS AND METHODS

Two different types of soil samples were collected from Beni-Suef and Kafr El-Sheikh. Soil samples were immediately placed on dry ice, mixed, and then stored at -20°C prior to DNA extraction. Subsamples (approximately 250 g) of soil were set aside to be used in physicochemical and microbial analysess.

Soil analysis

Mechanical analysis of soils was determined by the standard method described in Piper (1950). Organic carbon, total nitrogen, pH, and electric conductivity (EC) of the soils were determined according to Jackson (1973). The total bacterial cell count for each sample was estimated by using an acridine orange epiflourescence direct counting method (Beloin *et al*., 1988; Haldeman *et al.,* 1995), and a Zeiss standard epiflourescence microscope equipped with a Zeiss 09 filter combination.

DNA extraction from soil

Microbial DNA was extracted from soil using FastPrep[TM] system (Bio101, CA, USA; Borneman *et al.*, 1996). A 978 μl of sodium phosphate buffer, 122 μl of MT buffer (Bio 101, catalog no. 6010-450), and 0.5 g of soil were added to a FastDNA tube containing a matrix designed to lyse most cell types. The mixture was vortexed for 2 min and then centrifuged at 16,000 xg for 5 min at room temperature. For DNA purification**,** a volume of 250 μl of the supernatant was added to a spinfilter with 500 μl of binding matrix. This tube was gently inverted five times, incubated for 5 min at room temperature, and then centrifuged for 30 sec at 16,000 xg. For this step and all other purification steps, the elute in the catch tube was discarded after

centrifugation. The pellet in the spinfilter was washed twice. Each wash was done by adding 500 µl of salt-ethanol wash solution and then centrifuging for 30 sec. at 16,000 xg. The spinfilter was then centrifuged for 1 min at 16,000 xg to dry the pellet. The DNA was eluted by transferring the spinfilter to a new catch tube, adding 50 µl of DNA elution solution, gently flicking the tube five times, and then centrifuging for 1 min at 16,000 xg. To minimize DNA shearing, vortex mixing was avoided.

Determination of purity and yield of DNA

The concentration of the DNA in the sample can be measured by monitoring the absorbance of a dilute solution of the sample at 260 nm, and can be calculated based on the value of 1.0 A_{260} unit = 50 µg/ml of DNA, taking into account the dilution factor of the sample (Sambrook *et al.*, 1989). Purity of the DNA was determined by taking absorbance reading at 230, 260, and 280 nm. A_{260}/A_{280} and A_{260}/A_{230} ratios were calculated to evaluate levels of protein and humic acid impurities, respectively, in both soil samples.

PCR amplification of soil extracted DNA

Soil DNA was amplified by PCR using a GeneAmp PCR system 2400 (Perkin Elmer). The reaction mixture contained the following final concentration or total amounts: 3 µl of DNA (65 ng/µl), 10 mM Tris (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 nM deoxynucleoside triphosphate, and 200 nM of each universal SSU rRNA primer and 3 U of Taq polymerase. The primers used to amplify SSU ribosomal genes: 63F: 5'-CAGGCCTAACACATGCAAGTC-3', and 1387R: 5'-GGCGG(AT)GTGTAC -AAGGC-3', (Marchesi *et al.*, 1998). The following program was used: 94ºC for 5 min, 35 cycles consisting of 94ºC for 2 min, 55ºC for 1 min, and 72ºC for 2 min, and a final step consisting of 72ºC for 7 min. Successful amplification was confirmed by agarose gel electrophoresis and ethidium bromide staining.

Construction of 16S rDNA gene libraries

Clone libraries of small-subunit rDNA gene copies from each of the two soil samples, Beni-Suef and Kafr El-Sheikh, were generated. TA cloning kit (Invitrogen, Carlsbad, Calif., USA) was used for cloning PCR products. The 1.5-kb PCR product was ligated into the pCR 2.1 plasmid vector (Invitrogen) in a ligation reaction mixture (consisting of 1 µl of T4 DNA ligase, 2 µl of the vector, 2-3 µl of the amplicon, 1 µl ligation buffer and final volume 10 µl reached by distilled H_2O) and overnight incubation at 14ºC.

Recombinant plasmids were transformed into competent *E. coli* Top 10F cells (Invitrogen). Competent cells were thawed before the transformation step. 2-3 µl of the ligation mixture were added to *E. coli* competent cells, which were incubated on ice for 30 min. Cells were permeabilized by immersing the tube in a 42ºC water bath for exactly 30 sec. After this heat shock, cells were kept on ice for 2 min. A volume of 250 µl of sterile SOC medium (Invitrogen) was added. Transformed cells were shaked at 37ºC for 1 h. A volume of 50 µl of transformed cells was plated onto LB agar plate media containing 100 µg/ml ampicillin and X-gal, incubated at 37ºC overnight and then at 4ºC for 1 h. Clones containing plasmid with inserts appeared as white colonies and clones containing no inserts were blue (Sambrook *et al.*, 1989). Individual white colonies were subjected to PCR of insert sequences to ensure that an insert of the correct size was present in each clone (Frothingham *et al.*, 1991). Thirty clones of the correct size for each of the two samples were stored as glycerol stock at -70ºC. Each clone was designated B (Beni-Suef soil) and K (Kafr El-Sheikh soil) followed by clone number (01 to 30).

Determination of nucleotide sequences

Recombinant plasmids having 1.5 kb inserts were isolated from overnight culture by the alkaline lysis miniprep (Sambrook *et al.*, 1989). A single bacterial colony was transferred in 2 ml LB medium containing the appropriate antibiotic in a loosely capped 15-ml tube; the culture was incubated overnight at 37ºC with vigorous shaking. 1.5 ml of the culture was poured into a microfuge tube, centrifuged at 12,000 xg for 30 sec at 4ºC. Medium was removed by aspiration, leaving the bacterial pellet as dry as possible. The bacterial pellet was resuspended in 100 µl of ice-cold Solution (I) and vortexed vigorously. 200 µl of freshly prepared Solution (II) was added. The tube was closed tightly and the contents were mixed by inverting the tube rapidly five times without vortexing, then stored on ice. 150 µl of ice-cold Solution (III) were added. The tube was closed and vortexed gently for 10 sec, then stored on ice for 3-5 min, and centrifuged at 12,000 xg for 5 min at 4ºC. The supernatant was transferred to a fresh tube. The DNA yield was precipitated with 2 volumes of ethanol at room temperature, and mixed by vortexing. The mixture was allowed to stand for 2 min at room temperature, then centrifuged at 12,000 xg for 5 min at 4ºC. The supernatant was removed by gentle aspiration, and the tube standed in an inverted position on a paper towel to allow all of the fluid to drain away. Pellet of double-stranded DNA was rinsed with 1 ml of 70% ethanol at 4ºC. The supernatant was removed as before, and the pellet of nucleic acid was allowed to dry in the air for 10 min. Nucleic acids were redissolved in 50 µl of TE buffer (pH 8.0) containing DNAase-free pancreatic RNAase (20 µg/ml), and briefly vortexed. The DNA was finally stored at -20ºC.

PCR was then used to produce the sequencing templates. The 15 µl reaction volumes contained the following final concentration or total amount: 1 µl of DNA (diluted 1: 100 in H_2O), 50 mM Tris (pH 8.3),

250 μg of BSA per ml, 2.5 mM $MgCl_2$, 200 nM deoxynucleoside triphosphates, 200 nM each primer, and 0.75 U of *Taq* polymerase. All reagents were mixed and then heated to 92°C for 1 min. Thirty-five cycles of PCR were then run at 92°C for 5 sec, 50°C for 30 sec, and 72°C for 60 sec, followed by 72°C for 3 min. These PCR products were purified by using Wizard Preps (Promega).

The sequencing reactions were done by using an ABI PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer). Excess dye terminator was removed from the reaction mixture with Centri-Sep spin column (Princeton Separation). The reactions were run on an ABI 310 sequencer (Applied Biosystems Incorporation, California, USA).

Sequences analysis

The rDNA clone sequences were aligned with representative rRNA sequences from GenBank by using PILEUP (Genetic Computer Group [GCG]). Distance matrices and phylogenetic trees were constructed by using the Jukes-Cantor algorithm (Jukes and Cantor, 1969) and the neighbor-joining method (Saitou and Nei, 1987), respectively, using MEGA package (Kumar *et al.*, 1993). Taxonomic assignments were done by comparing the soil clone sequences with the nonredundant nucleotide database at GeneBank by using BLAST (NCBI). Only clones whose identities were corroborated were given a taxonomic assignment; all others were classified as unknown soil organisms.

RESULTS

The physical and chemical properties of the two soils used in DNA extraction were quite different (Table 1). Soils of Beni-Suef and Kafr El-Sheikh were classed as silty clay loam and silty clay, respectively.

Table (1): Physico-chemical characteristics of soils used for DNA extraction.

Soil location		Beni-Suef	Kafr El-Sheikh
Mechanical analysis	**Clay %**	32.5	38.7
	Silt %	50.8	42.1
	Sand %	16.2	18.2
Texture		Silty clay Loam	Silty clay
CaCO₃ %		1.3	1.2
Total-N %		0.108	0.085
Organic carbon %		1.18	0.92
EC		1.6	5.8
pH		7.6	7.95
Bacterial Counts x 106g-1		140	90

Phylogenetic assignments and molecular diversity

In the present work sixty clone inserts (30 from each soil sample) were sequenced and subjected to phylogenetic analyses. A phylogenetic tree from the analysis of the 60 clone sequences, together with those of their closest relatives in the database and representatives of major bacterial divisions are given in Figures (1 and 2). Most sequences are of bacterial origin; one clone was from Archaea kingdom, while

none were from the kingdom Eucarya. The analysis also showed that none of the recovered sequences are identical to each other and none are identical to sequences in the databases.

Forty sequences of sixty were affiliated with previously recognized bacterial group (figures 1 and 2). Thirty six of these had closest relatives among cultured taxa and clustered primarily with three divisions containing organisms commonly associated with soil: *Proteobacteria,* Gram-positive organisms, and *Cytophaga-Flexibacter-Bacteroides* group. These taxonomic groups have been commonly found in other studies of soil microbial diversity that were based on the identification of cultured organisms (Alexander, 1977; Borneman *et al.*, 1996). Seven sequences grouped with known divisions, but had closest relatives among soil taxa known only from rDNA sequences analysis. Clones B25, B28 and B30 retrieved from Beni-Suef soil had the highest sequences identity to AB186468.1, AY171310.1, and AY493971.1, respectively. These clone sequences were found to group within the family δ *Proteobacteria*, *Cytophaga-Flexibacteria-Bacteroides,* and Low-G+C Gram-positive bacteria, respectively. Clones K14, K24, K25, and K27 found in Kafr El-Sheikh soil exhibiting highest sequence similarity with AF423236.1, AY850302.1, AY959126.1, and DQ190362.1, respectively. These sequences of clones related to high-G+C Gram-positive bacteria, *Holophaga-Acidobacterium,* γ *Proteobacteria*, and low-G+C Gram-positive bacteria, respectively.

Between 16.7 and 20% of clones from Beni-Suef and Kafr El-Sheikh soils, respectively, were related to low-G+C Gram-positive bacteria (Table 2). These results are in agreement with the previous studies (Stackebrandt and Liesack, 1993; Marilley and Aragno, 1999). Gram-positive bacteria accounted for 28% (Borneman *et al.*, 1996), 15% (Rheims *et al.*, 1996), 7% (Kuske *et al.*, 1997) or 17% (Marilley and Aragno, 1999) of soil clones retrieved from different geographical locations. Half of the clones related to low-G+C Gram-positive bacteria in both soils were affiliated to *Bacillus*. However, clones related to *Clostridia* were only found in Kafr El-Sheikh soil.

Sequences related to the high-G+C Gram-positive bacteria, represented by *Streptomyces*, ranked among the main phylogenetic group in both soils. It represented 16.7 and 13.3% of total clones derived from Beni-Suef and Kafr El-Sheikh soils, respectively. Clones of *Micrococcus* sp. and *Acidimicrobium ferrooxidans* were retrieved from Beni-Suef soil. However, clones of *Arthrobacter* sp. and *Corynebacterium* sp. were detected in Kafr El-Sheikh soil.

A clone related to alpha *Proteobacteria* affiliated to *Rhizobium leguminosarum bv trifolii* was detected in Kafr El-Sheikh soil (Fig. 2). However, a clone related to beta *Proteobacteria* affiliated to *Nitrosomonas europea* was obtained from Beni-Suef soil (Fig. 1). Gamm
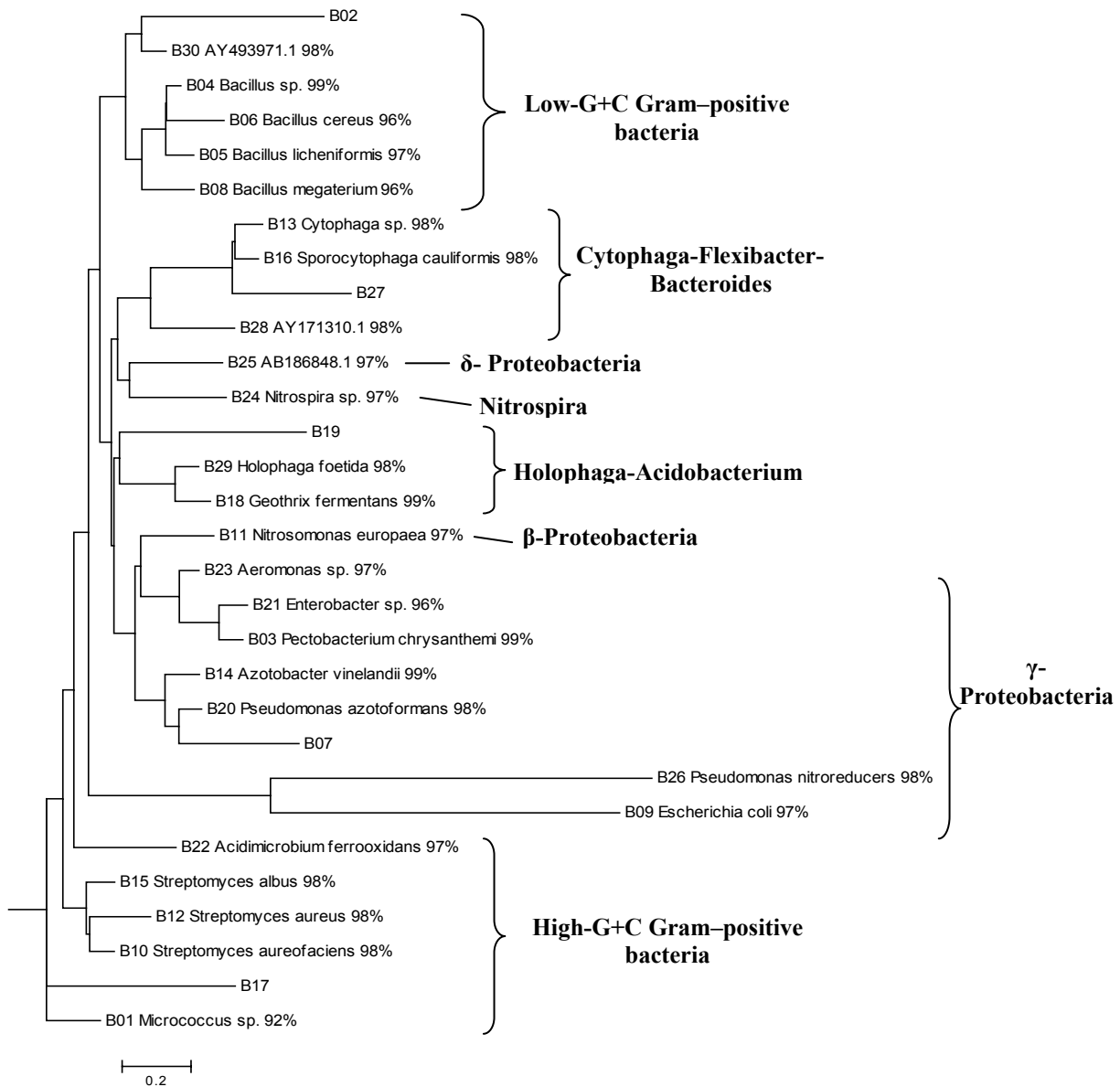
Figure (1): phylogenetic analysis of rDNA clone sequences from Beni-Suef soil. Soil clones are represented by B with its clone number followed by the most closely related sequence obtained from database and by corresponding homology value, as determined by FASTA. Database sequence accession number is given for clones to yet-uncultured bacteria. Names of the major taxa are shown in bold face type.

Table (2): Phylogenetic assignment of 60 clone sequences of Beni-Suef and Kafr El-Sheikh soil rDNA.

Phylogenetic group	Beni-Suef soil		Kafr El-Sheikh soil		Total	
	No.	%	No.	%	No.	%
Archaea	0	0	1	3.3	1	1.7
Bacteria	30	100	29	96.7	59	98.3
Proteobacteria	9	30	8	26.7	17	28.3
α subdivision	0	0	1	3.3	1	1.7
β subdivision	1	3.3	0	0	1	1.7
γ subdivision	7	23.3	5	16.7	12	20.0
δ subdivision	1	3.3	2	6.7	3	5.0
Nitrospira	1	3.3	0	0	1	1.7
High-G + C Gram-positive	5	16.7	4	13.3	9	15.0
Low-G + C Gram-positive	5	16.7	6	20.0	11	18.3
Cytophaga-Flexibacteria-	3	10.0	2	6.7	5	8.3
Holophaga-Acidobacterium	2	6.7	2	3.3	4	6.6
Unclassified	5	16.7	7	23.3	12	20.0

Proteobacteria dominated Beni-Suef and Kafr El-Sheikh soils; it represented 23.3 and 16.7% of total clones, respectively. *Pseudomonas* represented the major members of this phylogenetic group (Table 2). More than 28.5 and 60% of Gamma *Proteobacteria* related to *Pseudomonas* in Beni-Suef and Kafr El-Sheikh soils, respectively. Clones related to *Pectobacterium chrysanthemi, Escherichia coli, Azotobacter vinelandii, Enterobacter* sp*., Aeromonas* sp. were detected in Beni-Suef soil and *Acinetobacter* sp. in Kafr El-Sheikh soil. Two clones related to δ-*Proteobacteria* were detected in Kafr El-Sheikh soil affiliated to *Polyangium cellulosum*, and *Pelobacter acetylenicus* (Figure 2).
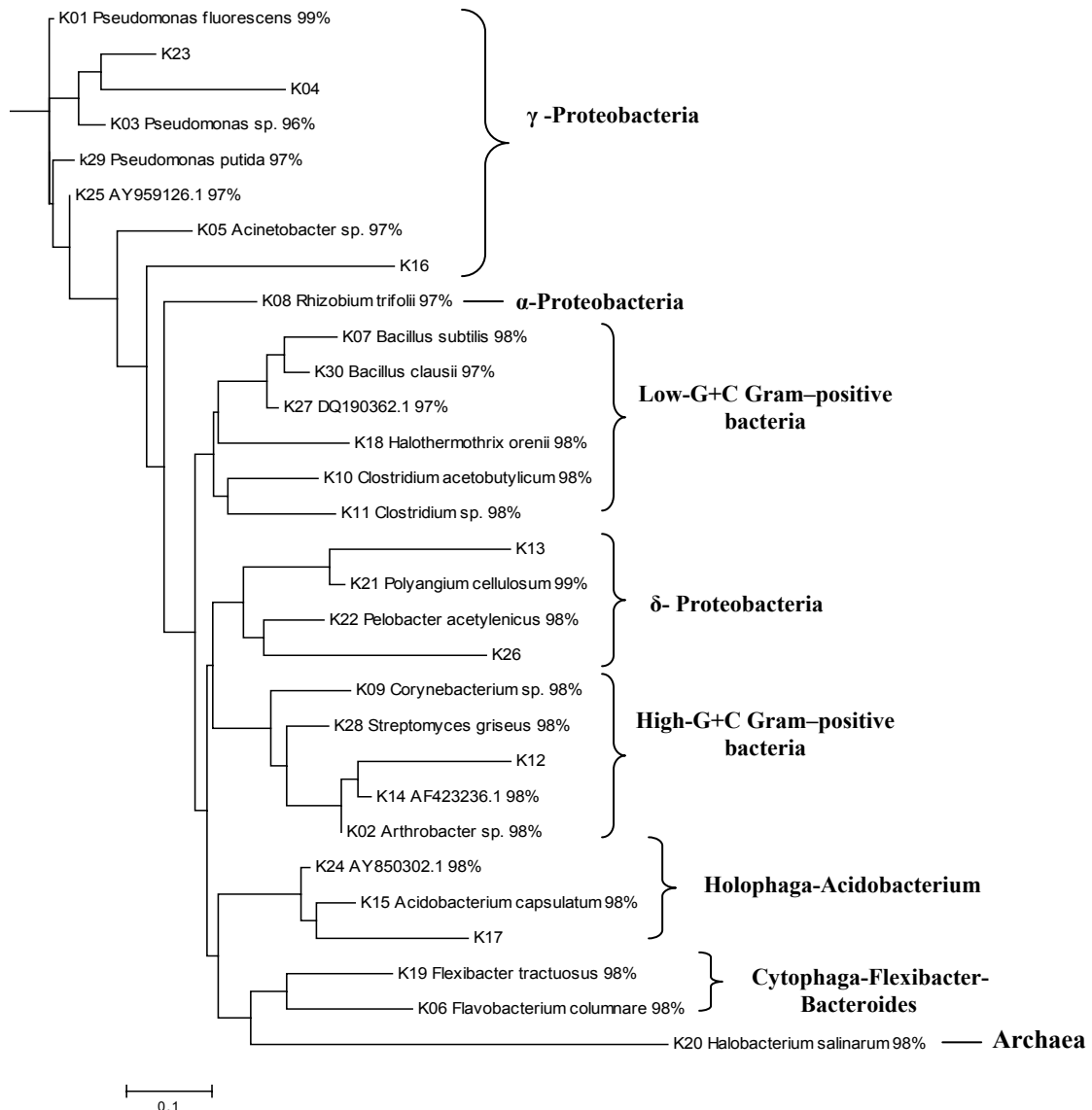
Figure (2): phylogenetic analysis of rDNA clone sequences from Kafr El-Sheikh soil. Soil clones are represented by K with its clone number followed by the most closely related sequence obtained from database and by corresponding homology value, as determined by FASTA. Database sequence accession number is given for clones to yet-uncultured bacteria. Names of the major taxa are shown in bold face type.

Members of the *Cytophaga-Flexibacter-Bacteroides* phylum occurred in Beni-Suef and Kafr El-Sheikh soils represented 10 and 6.7%, respectively (Table 2). Clones related to the *Cytophaga-Flexibacter-Bacteroides* phylum were commonly found in an agricultural soil in Wisconsin (Borneman *et al*., 1996) and in arid soil in Southwestern United States (Kuske *et al.,* 1997).

Results in table (2) show one clone related to *Nitrospira* was retrieved from Beni-Suef soil.

The results also showed the presence of three clones (5%) of total clones, affiliated to the newly described *Holophaga-Acidobacterium* phylum (Ludwig *et al.,* 1997) in both soils of Beni-Suef and Kafr El-Sheikh (Figure 1 and 2). Their sequences have homology to the rDNA sequences of *Holophaga foetida*, *Geothrix fermentans*, and *Acidobacterium capsulatum*.

Comparison of clones obtained from Beni-Suef soil and Kafr El-Sheikh soil suggests differences between the two sites (Table 2). A greater number of unclassified organisms were found in Kafr El-Sheikh soil than in Beni-Suef soil. Two clone sequences of Kafr El-Sheikh soil related to *Clostridium*, while none of Beni-Suef soil clone sequences were similar to sequences of that genus. Conversely, clone sequences found in Beni-Suef soil related to *Bacillus* sequences were more than those found in Kafr El-Sheikh soil. High G+C Gram positive–like sequences were found to be more in Beni- Suef soil than in Kafr El-Sheikh soil.

Novel rDNA sequences

Results in Table (2) show that twelve clone sequences (20%) are distantly related to known sequences.

Similarities between these sequences and those of most closely related cultivated organisms ranged from 70 to 85%. Thus, many of these sequences may represent new bacterial divisions, based on an interkingdom identity range from 70 to 85% (Woese, 1987).

Previous reports have suggested that *Holophaga foetida* belongs to an unclassified group of *Proteobacteria,* and that the *Acidobacterium capsulatum* may represent a new bacterial division (Hiraishi *et al.,* 1995; Liesack *et al*., 1994). This group was retrieved from many geographical locations (Stackebrandt and Liesack, 1993; Kuske *et al*., 1997; Felske *et al.*, 1998; Marilley and Aragno, 1999).

The analysis of the sequences of this phylum indicated the existence of yet-uncultivated organisms phylogenetically related to *Acidobacterium capsulatum*, a chemo-organotrophic bacterium isolated from an acidic environment by Hiraishi *et al.* (1995). Members of this new line of descent are thought to rank among the most important metabolizers in soil (Felske *et al.*, 1998).

DISCUSSION

Soil microbial communities are among the most complex, diverse, and important assemblages of organisms in the biosphere, yet little is known about the species that comprise them, due to the limitations of culture-based studies. One approach for defining the components of a microbial community in an environmental sample is the phylogenetic analysis of the small subunit rRNA gene sequences present in the crude pool of DNA isolated from that environment. This method allows identification of microbial species without prior cultivation, and studies using this approach in many different environments have identified new microorganisms that may be abundant or physiologically significant (Kuske *et al.* 1997; Borneman *et al.*, 1996; Marilley and Aragno, 1999).

One Archaeal clone was found in Kafr El-Sheikh soil, while no Archaea sequences were detected in Beni-Suef soil, which could be attributed to high salinity of Kafr El-Sheikh soil. This low ratio of archaeal to the bacterial sequences suggests that soil may be an inhospitable environment for Archaea, in contrast to the marine environment, where Archaea can constitute up to 34% of the prokaryotic population (DeLong *et al.,* 1994).

Comparing results obtained by DNA-based techniques with those by culturing techniques, however, was difficult since no attempts to identify bacteria in these soil samples by culture techniques have been made. Nevertheless, differences between traditional culturing studies and rDNA sequence analyses are expected, since most bacteria (90 to 99%) in environmental samples are not culturable (Alexander, 1977; Amann, *et al*., 1995; Borneman *et al.*, 1996). In addition, over one-fifth of the sequences were distantly related to the known major taxa of Bacteria. As most bacteria in soil are not culturable, it is not surprising that some hitherto-unknown taxa would be found among the dominant groups of bacteria in soil.

Despite the fact that these soils show enormous diversity among the Bacteria, this diversity is not random. A majority of the sequences fall into just three major taxa, *Proteobacteria,* Gram-positive organisms, and *Cytophaga-Flexibacter-Bacteroides*. In addition, since the unidentified sequences generated in this study (Figures 1 and 2) have low-level sequence homology (70-85%) to identify sequences in the GeneBank, it is apparent that the diversity of microorganisms in soil is extensive and that the phylogenies of many dominant soil bacteria remain uncharacterized.

This work, as well as others, provides evidence indicating that soil serve as habitat for novel microorganisms. The vast majority of soil microorganisms cannot be cultivated by current techniques and therefore have remained unclassified (Torsvik *et al.*, 1990; Tsuji *et al.*, 1995). The molecular phylogeny surveys of soil have all shown immense and previously undescribed diversity (Ueda *et al*., 1995; Borneman *et al*., 1996). The phylogenetic analysis of PCR-amplified rDNA gene from Egyptian soils provides an evidence for unusual organisms which may represent new bacterial divisions.

REFERENCES

ALEXANDER, M. 1977. Introduction to soil microbiology, second edition. John Wiley and Sons, Incorporation, New York.

AMANN, R.I., W. LUDWIG, AND K.H. SCHLEIFER. 1995. Phylogenetic identification and *insitu* detection of individual microbial cells without cultivation. Microbiological Reviews **59:** 143-169.

BELOIN, R.M., J.L. SINCLAIR, AND W.C. GHIORSE. 1988. Distribution and activity of microorganisms in subsurface sediments of a pristine study site in Oklahoma. Microbial Ecology **16:** 85-97.

BORNEMAN, J., P.W. SKROCH, K.M. O'SULLIVAN, J.A. PALUS, N.G. RUMJANEK, J.L. JANSEN, J. NIENHUIS, AND E.W. TRIPLETT. 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. Applied Environmental Microbiology **62:** 1935-1943.

DELONG, E.F., K.Y. WU, B.B. PRÉZETIN, AND R.V.M. JOVINE. 1994. High abundance of Archaea in Antarctic marine picoplankton. Nature **371:** 695-697.

FELSKE, A., A. WOLTERINK, R.V. LIS, AND A.D.L. AKKERMANS. 1998. Phylogeny of the main bacterial 16S rRNA sequences in Drentse a grassland soils (The Netherlands). Applied Environmental Microbiology **64:** 871-879.

FROTHINGHAM, R., R.L. ALLEN, AND K.H. WILSON. 1991. Rapid 16S ribosomal DNA sequencing from single colony without DNA extraction or purification. Biotechniques **11:** 40-44.

HALDEMAN, D.L., P.S. AMY, D. RINGELBERG, D.C. WHITE,

R.E. Garen, and W.C. Ghiorse. 1995. Microbial growth and resuscitation alter community structure after perturbation. FEMS Microbiology Letters **17:** 27-38.

Hiraishi, A., N. Kishimoto, Y. Kosako, N. Wakao, and T. Tano. 1995. Phylogenetic position of the menaquinone-containing acidophilic chemoorganotroph *Acidobacterium capsulatum*. FEMS Microbiology Letters **132:** 91-94.

Jackson, M.L. 1973. Soil chemical analysis. Prentice Hall, New Delhi.

Jukes, T.H., and C.R. Cantor. 1969. Evolution of protein molecules, p.21-132. In: Munro H.N. (ed.), Mammalian protein metabolism. Academic Press, New York.

Kumar, S., K. Tomura, and M. Nei. 1993. MEGA: Molecular Evolutionary Genetics Analysis, version 1.0. The Pennsylvannia State University, University Park.

Kuske, C.R., S.M. Barns, and J.D. Busch. 1997. Diverse uncultivated bacterial groups from soils of the arid southwestern United States that is present in many geographic regions. Applied Environmental Microbiology **63:** 3614-3621.

Liesack, W., F. Bak, J.U. Kreft, and E. Stackebrandt. 1994. *Holophaga foetida* gen. nov., sp. nov., a new homacetogenic bacterium degrading methoxylated aromatic compounds. Archives of Microbiology **162:** 85-90.

Ludwig, W., S.H. Bauer, M. Bauer, I. Held, G. Kirchhof, R. Schulze, I. Huber, S. Spring, A. Hartmann, and K.H. Schleifer. 1997. Detection and *insitu* identification of representatives of a widely distributed new bacterial phylum. FEMS Microbiology Letters **153:** 181-190.

Marchesi, J.R., T. Sato, A.J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiom, and W.G. Wade. 1998 Design and evaluation of useful bacterium specific PCR primer that amplify genes coding for bacterial 16S rRNA. Applied Environmental Microbiology **64:** 795-799.

Marilley, L., and M. Aragno. 1999. Phylogenetic diversity of bacterial communities differing in degree of proximity of *Lolium perenne* and *Trifolium repens* roots. Applied Soil Ecology **13:** 127-136.

Piper, C.S. 1950. Soil and plant analysis. University of Adelaide, Australia.

Rheims, H., C. Sproer, F.A. Rainey, and E. Stackebrandt. 1996. Molecular biological evidence for the occurrence of uncultured members of the actinomycete line of descent in different environments and geographical locations. Microbiology **142:** 2863-2870.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for constructing phylogenetic trees. Molecular Biology and Evolution **4:** 406-425.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, second editioin. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Stackebrandt, E., and W. Liesack. 1993. Nucleic acids and classification, p. 151-194. In: GoodFellow M. and G.O'Donnell (ed.), Handbook of New Bacterial Systematics. Academic Press, London.

Torsvik, V., J. Goksoyer, and F.L. Daae. 1990. High diversity in DNA of soil bacteria. Applied Environmental Microbiology **56:** 782-787.

Tsuji, T., Y. Kawasaki, S. Takeshima, T. Sekiya, and S. Tanaka. 1995. A new fluorescence staining assay for visualizing living microorganisms in soil. Applied Environmental Microbiology **61:** 3415-3421.

Ueda, T., Y. Suga, and T. Matsuguchi. 1995. Molecular phylogenetic anaylsis of a soil microbial community in a soybean field. Eurpean Journal of Soil Sciences **46:** 415-421.

Woese, C.R. 1987. Bacterial evolution. Microbiological Reviews **51:** 221-271.

التنوع الميكروبى في عينتين من الأراضى المصرية

سعيد بدر الدين[1]، سمير رضوان[1]، معوض زهرة[2]، وفاء زيدان[1]
[1]قسم الميكروبيولوجيا الزراعية، المركز القومى للبحوث، الدقى، القاهره، مصر
[1]قسم الميكروبيولوجيا الزراعية، كليه الزراعه، جامعة القاهره، مصر

الملخص العربــى

تم استخدام الحامض النووى (DNA) المتحصل عليه من عينتى بنى سويف وكفر الشيخ فى تفاعل البلمرة المتسلسل (PCR) باستخدام بادئات متخصصة لجين 16S rDNA، وتم نسخ ناتج التفاعل في ناقل pCR 2.1 plasmid vector، ثم إيلاج هذا الناقل الحامل للجين 16S rDNA في خلايا بكتيريا *E. coli*. وتم الحصول على 60 كلون للجين 16S rDNA (30 من كل عينة تربة). وتم عمل تحليل تتابع القواعد الأزوتية ومقارنة تلك التتابعات بمثيلاتها في قاعدة البيانات الوراثية وعمل التحليل المبنى على درجة القرابة الوراثية بين الكائنات. تم نسب 40 كلون من الكلونات الستون الي مجاميع بكتيرية معروفة مسبقا، حيث وجد أن 36 منهم قد تشابهت مع أنواع معزولة مسبقا، وتنتمى الى ثلاثة أقسام شائعة بالتربة هي *Proteobacteria*، البكتيريا الموجبة لجرام، و *Cytophaga-Flexibacter-Bacteroides*، كما وجد سبع كلونات تتشابه مع الكلونات الموجودة في أقسام معروفة فى قواعد البيانات الوراثية. كما وجد كلون واحد فقط يشابه تتابع من مجموعة *Nitrospira* في عينة بنى سويف. أظهرت النتائج أيضا وجود ثلاث كلونات تم وضعهم فى مجاميع جديدة *Holophaga-Acidobacterium* فى كل من بنى سويف وكفر الشيخ، بالإضافة إلى وجود تتابع واحد فقط من مملكة Archaea. ويتضح من هذه الدراسة أن التنوع الميكروبى في الأراضى المصرية واسع المدي وقد تشابهت معظم الكلونات مع أنواع معزولة مسبقا وتنتمى إلي أقسام شائعة بالتربة. كما تم التعرف علي 12 نوعا جديدا من الميكروبات غير معروفة فى قواعد البيانات قد تكون ذات أهمية حيوية واقتصادية.