The organic components of the CH_2Cl_2 phase were fractionated by column chromatography (i.d. 15 mm, length 35 mm, Merck Silica gel 60, 70–230 mesh) and eluted with 2 column volumes of n-hexane ("total hydrocarbon") and 3 column volume of CH_2Cl_2 (alcohol/ketone fraction). The latter was derivatized with acetic acid anhydride in an equal volume of pyridine (14 h at room temperature) and fractions were analyzed by a Fisons Instruments MD 800 GC 8000 series GC/MS spectrometer equipped with a 50-m fused silica capillary column (DB5-HT, 0.32 mm i.d. 0.25-μm film thickness) using He as carrier gas. Temperature program: 5 min 80°C to 310°C at 4°C/min: 20 min at 310°C.

Nucleic Acid Extraction and 16S Ribosomal RNA Gene Libraries

Samples (water from mud pool, hot mud, and nonconsolidated crusts) devoted to the biological study of microbial diversity by molecular methods were kept in 80% ethanol at 4°C until DNA extraction. The DNA used in this study was extracted from nonconsolidated black crust at the edge of a mud pool in the central part of the crater (SOL7, 40°C) and from white, melted deposits adjacent to a chimney in the Northeast fumarole area of the crater (85°C, SOL18) by two methods, the SoilMaster DNA extraction kit (Epicentre) and a classical phenol-chloroform extraction. For the latter, prior to DNA extraction, samples were rehydrated with phosphate saline buffer (130 mM NaCl, 10 mM phosphate buffer, pH 7.7, PBS). PBS was also added to the sediment to a same final volume of 0.5 mL. Samples were then subjected to 6 freezing/thawing cycles in liquid nitrogen to facilitate cell lysis. Subsequently, 80 μg mL^{-1} proteinase K, 1% SDS, 1.4 M NaCl, 0.2 β-mercaptoethanol and 2% hexadecyltrimethylammonium bromide (CTAB) (final concentrations) were added sequentially.

Lysis suspensions were incubated overnight at 55°C. Lysates were extracted once with hot phenol (65°C), once with phenol-chloroform-isoamylalcohol, and once with chloroform-isoamyl-alcohol. Nucleic acids were concentrated by ethanol precipitation. 16S rRNA genes were amplified by PCR using the bacterial-specific primer 63F (CAGGCCTAACACATGCAAGTC) and the prokaryote-specific reverse primer 1397R (GGGCGGWGTGTACAAGGC). Polymerase chain reactions (PCR) were performed under the following conditions: 30 cycles (denaturation at 94°C for 15 s, annealing at 50°C for 30 s, extension at 72°C for 2 min) preceded by 2 min denaturation at 94°C, and followed by 7 min extension at 72°C. Dimethyl sulfoxide was added to a final concentration of 3–5% to the PCR reaction mix. rDNA clone libraries were constructed using the Topo TA Cloning system (Invitrogen) following the instructions provided by the manufacturers. After plating, positive transformants were screened by PCR amplification of inserts using flanking vector primers.

Sequence and Phylogenetic Analyses

A total of 63 (34 from SOL18 and 29 from SOL7) expected-size amplicons from these libraries was partially sequenced (lengths from 849 to 1050 nucleotides) with the primer 1387R (Genome Express). Closest relatives to our sequences were identified in databases by BLAST (Altschul et al. 1997) and retrieved from GenBank ⟨http://ncbi.nlm.nih.gov/⟩. Sequences were automatically aligned using the program BABA (H. Philippe, personal communication) to a 16S rRNA gene alignment containing ~17,000 sequences. The multiple alignment was then manually edited using the program ED from the MUST package (Philippe 1993). A preliminary phylogenetic analysis of all partial sequences was done by distance methods (neighbor-joining, NJ) using the program MUST, allowing the identification of identical or nearly identical sequences and the selection of representative clones for subsequent analysis. We then selected 15 representative sequences to be included in a phylogenetic tree, together with their closest relatives in GenBank and some cultivated species. A total of 710 positions were used in our analysis after removal of gaps and ambiguously aligned positions. The maximum likelihood (ML) tree was done using TREEFINDER (Jobb 2002) applying a general time reversible model of sequence evolution (GTR), taking among-site rate variation into account by using an eight-category discrete approximation of a Γgistribution (invariable sites are included in one of the categories). The α parameter of the Γ distribution estimated from the sequence set was 0.27. ML bootstrap proportions were inferred using 500 replicates. The sequences reported in this study were submitted to GenBank with accession numbers AY629323 to AY629339 (see also Figure 6).

RESULTS AND DISCUSSION

Petrological Characteristics of Solfatara Samples

The studied material from the Solfatara Crater is geologically young (~4,000 years) which, in principle, should facilitate the identification of unaltered biosignatures left by recent and present-day microbial communities. Hydrothermal springs in the center of the crater have their foundation on local faults that run radially through the crater, whereas the fumarolic activity is mainly dependent on the regional fault along the Northeast crater wall (Figure 2A, 2B). Products of Solfatara activity consisted mainly of breccia and stratified deposits with layers of pisolitic and coarse ashes, the basal surge structure being composed of beds of well-sorted pumice lapilli that were a few decimeters in size. The surges covered the East-Northeast wall of the crater and overflowed the Eastern and Western crater edges. Most volcanic products in the area were hydrothermally altered, giving raise to disordered trachytic deposits. Samples collected for this study included highly porphyritic scoria with trachytic appearance composed of sanidine, plagioclase, clinopyroxene, biotite, and opaque phenocrystals. Samples of emitted material around mud pools were mainly fine-grained clays, consolidated to some extent. All samples were very rich in sulfur, sulfates, and a variety of iron minerals. In the Northeastern zone of the crater, where fumarolic activity is intense, gypsum was also present. From the petrological and mineralogical characteristics, the observed