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Isolation and characterization of actinobacteria from Lake Magadi, Kenya

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Actinobacteria are ubiquitous and have been detected in terrestrial and aquatic ecosystems. Although, various studies have focused on the ecology of this phylum, data on the ecology of actinobacteria endemic to soda lakes are scanty. This study aimed to determine actinobacterial diversity in Lake Magadi. Four different sampling points were selected randomly within the lake to cover a broad range of sample diversity. Wet sediments and water samples were collected from each sampling point. Good success in isolation of actinobacteria was obtained using a variety of strategies designed to select against fast growing bacteria. Serially diluted samples were plated on growth media containing complex substrates such as malt and chitin. The media was prepared using sterile lake water and pH kept at 8 which is high for most bacteria. DNA was extracted from the isolates and the 16S rRNA genes amplified using primers described to be domain specific for actinobacteria. The isolates were characterized both physiologically and biochemically and were screened for the presence of polyketide synthase-1 genes. The amplified 16S rRNA gene sequences were used for sequence analysis to identify the isolates and establish their phylogeny. Four actinobacteria isolates affiliated with the genera *Rhodococcus*, *Dietzia*, *Microbacterium* and *Nocardia* were obtained. BLAST analysis showed that all the isolates had between 97 and 99% similarity to their close relatives. Three of the isolates were positive for polyketide synthase genes. These results increase the number of actinobacteria isolates from soda lakes for potential use in biotechnology research.

Key words: Actinobacteria, diversity, 16s rRNA, Lake Magadi.

INTRODUCTION

Actinobacteria are morphologically and phylogenetically diverse Gram positive bacteria. They are generally aerobic with a high genomic guanine-plus-cytosine (G+C) content (>55 mol %) and traditionally associated with soil (Rheims et al., 1996). However, studies have shown that they are widespread in nature and are able to occupy a wide range of habitats (Allgaier and Grossart, 2006; Holmfeldt et al., 2009). Some are pathogenic to both plants and animals (Valverde et al., 2012), while others form symbiotic associations with plants and insects (Kaltenpoth, 2009). The search for novel actinobacteria capable of producing novel compounds or catalyzing unique reactions (Sarethy et al., 2011) has pushed microbiologists to look in unique previously unexplored environments. The polyketides, comprising a large and structurally diverse family of bioactive natural products, have been isolated from actinomycetes (Savic and Vasiljevic, 2006). Polyketide synthase (PKS) genes

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are known to encode at least three domains corresponding to a ketosynthase, acyltransferase and acyl carrier protein (Ayuso and Genilloud, 2004) involved in the synthesis of biologically active compounds including antibiotics produced by actinobacteria. The soda lakes in Kenya, with their steep salinity, pH and temperature gradients, present unique habitats which may be colonized by novel microorganisms containing novel PKS genes not previously described.

There have been a few reports of actinobacteria in soda lakes where most studies have reported Archaea, *Bacillus* species and Cyanobacteria as the dominant groups (Zavarzin et al., 1999; Jones et al., 1998; Baumgarte, 2003; Rees et al., 2004; Mwirichia et al., 2010). Valverde et al. (2012) reported the detection of actinobacteria from hot springs in Lake Magadi. The extreme physical and chemical properties of Lake Magadi raise the possibility of recovering novel actinobacteria with unique adaptations to their habitat which may be correlated with synthesis of novel types of bioactive compounds (Zhao et al., 2008). In this study, selective media was used to isolate and characterize actinobacteria from Lake Magadi in Kenya, screen the isolates for the presence of polyketide synthase genes and for production of antimicrobial compounds.

MATERIALS AND METHODS

Study site

Lake Magadi (1°52′S 36°16′E; 1.867°S 36.267°E) is the southernmost lake in the Kenyan Rift Valley lying in a catchment of faulted volcanic rocks. Lake Magadi is a saline, alkaline lake, approximately 100 km^2 in size. The Magadi Lake brines are normally saturated with a dissolved solids content of approximately 32 %w/w. Of this, the sodium chloride content varies from 30 to 34 %, the rest being almost exclusively sodium carbonate compounds with some sodium fluoride in small quantities. The lake is recharged mainly by saline hot springs (temperatures up to 86°C) that discharge into alkaline lagoons around the lake margins, where there is little surface runoff in this arid region (Behr, 2002).

Sampling points

Samples were collected in four different locations selected to cover a broad range of sample diversity. The first station (S2° 00.060 min; E36° 13.925 ± 3 min) was a shallow area of the lake with hot springs. Microbial mats were sampled near the hot springs. Mud was collected from where the lake had receded. The second station (S01° 57.879 min; E36° 16.594 ± 3 min) was a shallow area of the lake and sediments were sampled. The third station (S01° 43.802 min; E36° 16.843 ± 3 min) sediments were collected deeper into the lake. The fourth station (S01° 53.931 min; E36° 17.614 ± 3 min) was at the salt lagoons where salt pans are concentrated. Salt liquor (water) and salt crystals were sampled. Temperature, pH and conductivity readings of the sampling sites were taken in triplicates and average measurements recorded. Samples were collected at various points at every station and put in sterile plastic containers and placed in a cool box to be transported to the laboratory, where they were kept at 4°C.

Media and cultivation

Isolation of cultures was performed by the serial dilution plate technique (Ellaiah, 1996) using International Streptomyces Project (ISP) medium 5 (starch casein agar) and malt extract-yeast extract agar (ISP medium 2). The pH was adjusted to 8.2. The media containing 50% filtered lake water were supplemented with nystatin, 75 mg/ml to inhibit fungal contamination. All plates were incubated at 28°C for 2 to 7 days depending on the rate of growth. Isolates were then stored at 4°C for future use (Panchagnula and Terli, 2011).

Screening for actinobacteria using group specific probes

Genomic DNA was extracted from pure isolates following a modification of the method described by Wilson (1987). Young liquid cultures were harvested by centrifugation. The cells were then disrupted using enzyme digestion and detergent lysis and then extracted using organic solvents. Group specific primers, F-Act 243 (5´-GGATGAGCCCGCGGCCTA-3´) and R-Act A3 (5´-CCAGCCCCACCTTCGAC-3´) described by Monciardini et al. (2002) were used to screen for actinobacteria. Reactions were performed in a final volume of 50 µl, containing 3 µl of genomic DNA, 5 µl of 10x Genescript *Taq* Buffer, 5 µl of 2.5 mM dNTPs, 0.4 µl of 20 mg/ml BSA, 0.4 µl of 5U Genescript *Taq* polymerase and 2.5 µl of 20 pmole each primer. Amplifications were carried out according to the following profile: 10 min at 95°C and 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, 45 s, followed by 10 min at 72°C. Amplification products were analyzed by electrophoresis in 2% (w/v) agarose gels stained with ethidium bromide. Isolates that produced an amplification product at this stage were recognized as being actinobacteria and were thus characterized further.

Amplification of polyketide synthase genes type-1(PKS-I)

Genomic DNA preparations were used as template DNA for *Taq* polymerase. Degenerate primers K1F (5´-TSAAGTCSAACATCGGBCA-3´) and M6R (5´-CGCAGGTTSCSGTACCAGTA-3´) were used for amplification (Ayuso-Sacido and Genilloud, 2004). The K1F and M6R primers anneal to the ketosynthase and acyl transferase (methyl-malonyl-CoA for type-I PKS) modules, respectively. The size range of the amplified fragments depends on the variable inter-domain region extension.

Reactions were performed in a final volume of 50 µl containing 3 µl of template DNA, 5 µl of 10x Genescript *Taq* Buffer, 5 µl of 2.5 mM dNTPs, 0.4 µl of 20 µg/ml BSA, 0.4 µl of 5U Genescript Taq polymerase, 2.5 µl of 20 pmole each primer. Amplifications were then performed according to the following profile: 5 min at 95°C and 35 cycles of 1 min at 95°C, 1 min at 55°C, 2 min at 72°C, followed by 5 min at 72°C. Reactions were confirmed through gel documentation.

Following confirmation of PCR products, successful reactions were cleaned using the QUAquick® PCR purification Kit from Qiagen as was specified by the protocol sent with the kit and the samples were sent to ILRI, Nairobi for sequencing.

Sequencing and phylogenetic analysis

Partial sequences of 16S rDNA were BLAST analyzed against the GenBank 16S rRNA gene sequence database in the National Centre for Biotechnology Information (NCBI) website (htt://www.ncbi.nih.gov). Phylogenetic analyses were conducted in MEGA version 4 (Tamura et al., 2007). The sequences were

Table 1. Similarity values (%) for 16S rRNA gene sequences of isolates from Lake Magadi and their BLAST close relatives.

Isolate ID	Nearest neighbor of partial 16s rDNA sequence	GenBank accession number	Sequence Similarity values (%)
MS-2	*Dietzia maris*	X79290	98
MS-3	*Microbacterium lacus*	AB286030	98
MS-5	*Rhodococcus erythropolis*	JF327477	98
MS-11	*Norcadia cerradoensis*	AF060790	97

aligned using the CLUSTAL W program against the nearest neighbours (Higgins and Sharp, 1988). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Maximum Composite Likelihood method (Robertson et al., 2009).

Sequence analysis of polyketide synthase genes

Full sequences of polyketide synthase genes were edited and translated using the program ChromasPro© version 1.6 (Technelysium pty ltd). The translated FASTA sequences were analyzed by BlastP algorithm in the NCBI databank to check their identity to known PKS sequences in the database.

Physiological and biochemical characterization

The Gram's stain reaction was carried out using the Dussault (1955) modification with safranin as counterstain. Physiological characterization was carried following the methods described by Kutzner (1981). Temperature, pH range and NaCl range of growth were determined by measuring optical density at wavelength of 600 nm. Isolates were inoculated in ISP medium 6 broth and incubated at 28°C for three days in a shaking incubator at 100 rpm.

Biochemical characterization was carried out using standard methods described by Shirling and Gottlieb (1966). Gelatin liquefaction was determined after forty eight hours incubation at 28°C on a medium composed of gelatin, glucose and peptone. After having been incubated, the cultures were placed in a refrigerator to detect liquefaction. Resistance to 1 mg/ml lysozyme was done by inoculating the isolates in glycerol broth with lysozyme. Tyrosine hydrolysis was done in tyrosine agar plates. Urease production was determined by observing colour change in urea broth. Carbohydrate utilization was examined by the method of Pridham and Gottlieb (ISP medium 9). Catalase production was determined by adding hydrogen peroxide to incubated trypticase soy agar slants. Nitrate reduction and hydrogen sulphide production were done according to the protocol described by Harold (2002).

Isolates were tested for production of lipase, xylanase, cellulase, amylase and protease following the method of Lee et al., (2005). Basal media supplemented with starch, xylan, cellulose, skimmed milk and olive oil were used.

RESULTS AND DISCUSSION

Phylogenetic analysis based on 16S rRNA gene sequences showed that isolates MS-2, MS-3, MS-5 and MS-11 are members of the phylum Actinobacteria with 16S rRNA gene sequence similarity values between 97 and 98% with their close relatives (Table 1). They were affiliated with the genera *Dietzia*, *Microbacterium*, *Rhodococcus* and *Nocardia*. To our knowledge, these isolates represent the first actinobacteria isolates from Lake Magadi. The results of 16srDNA sequences phylogeny demonstrated distinct positions of the isolates in the phylogenetic tree (Figure 1). Previous studies have documented actinobacteria isolates from other Kenyan soda lakes. Members of the genera *Arthrobacter* and *Terrabacter* (Duckworth et al., 1996) have been described from Lake Oloiden. Others include *Bogoriella caseilytica* (Groth et al., 1997) *Dietzia natronolimnaea* (Duckworth et al., 1998) and *Cellulomonas bogoriensis* (Jones et al., 2005) from Lake Bogoria.

The Sequence results of the polyketide synthase genes for three of the isolates belonged to the peptidase family (Table 2). Isolate MS-3 did not produce an amplification product. Lack of amplification may be an indication of the absence of the PKS-1 system or occurrence of methylmalonyl-CoA transferase domain with less conserved sequences and therefore lower homology with the primers (Ayuso-Sucido and Genilloud, 2004). The fragments obtained exhibited low similarity to sequences in the GenBank closest neighbours and were not identical to each other. Adamalysin and matrixin domains exhibited 48% identities to their closest matches, while dihydropteroate synthase showed 52% similarity. None of the sequences showed higher than 80% identity to GenBank sequences. This may imply their chemical and functional novelty. It may also indicate that the isolates with these domains have not yet been analyzed for their PKS genes pathways (Zhao, 2008).

All the isolates were found to be Gram-positive, non-motile rods. On the basis of physiological characteristics, the isolates differed slightly with the reference strains (Table 3). This could be due to difference in the physical parameters of their original sites of isolation. The pH range of growth was found to be between 5 and 11 with an optimum at 9 and 11 (Table 3). The observation that the isolates are alkaliphilic is expected since the site of isolation is alkaline. All the isolates could grow between 25 and 50°C with an optimum temperature of 30°C. Isolate MS-11 had a higher optimum temperature of 40°C (Table 3). The isolates were able to grow at sodium chloride concentration of up to 20%, with optimum growth at 5%. This wide range of salt tolerance could be an adaptation to salt fluctuation within the lake when there is

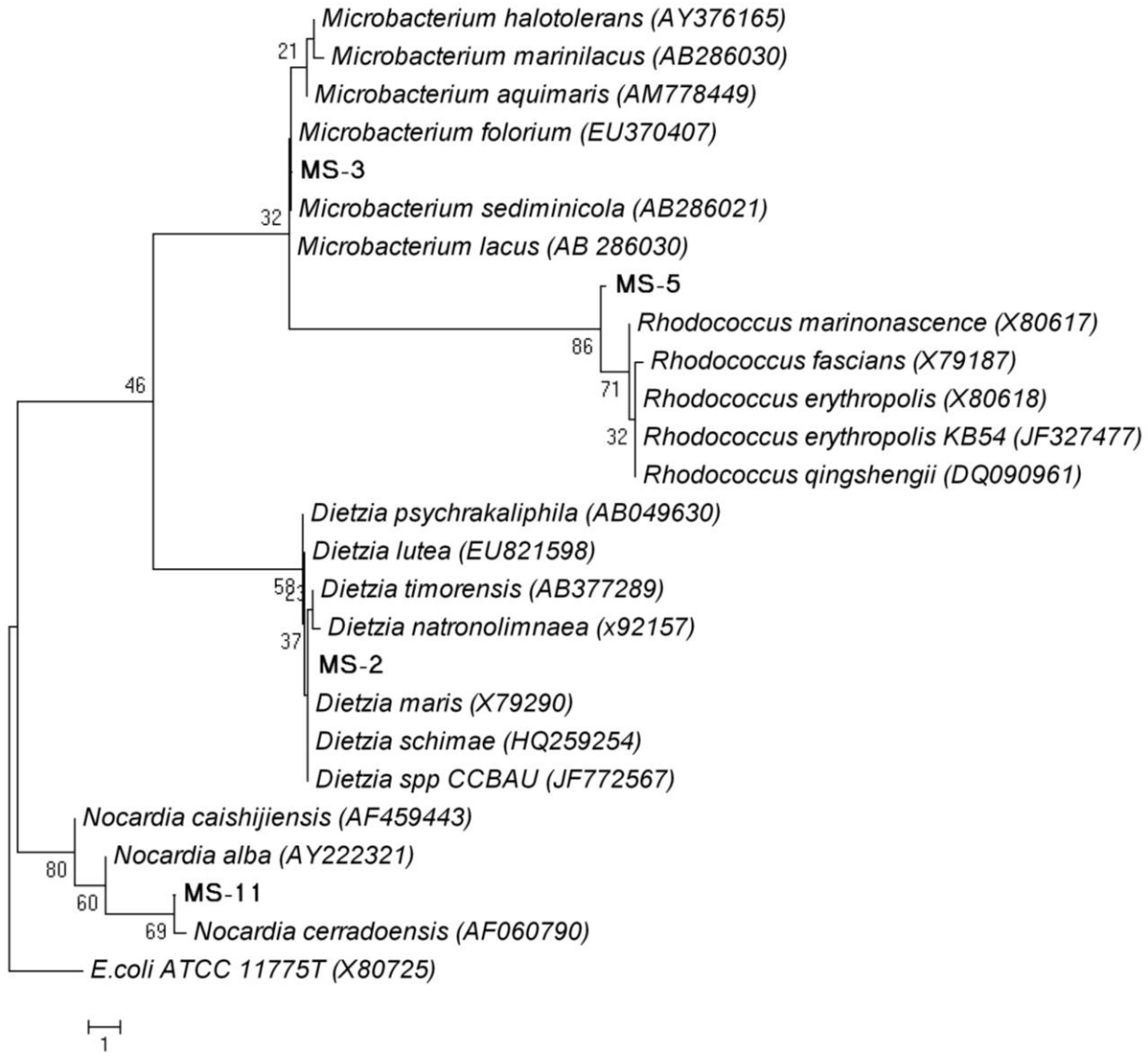

Figure 1. Neighbor joining tree showing the phylogenetic positions of isolates from Lake Magadi rooted using 16S rDNA of *Escherichia coli* as outgroup. Only bootstrap values above 20 are shown. The scale bar represents the expected number of changes per homologous nucleotide position.

Table 2. BLAST results of polyketide synthase gene sequences using protein algorithm.

Isolate ID	Protein database homology	Similarity values (%)
MS-2	Adamalysin (Peptidase M10A)	48
MS-3	-	-
MS-5	Matrixin (M12B)	48
MS-11	Dihydropteroate synthase	52

dilution due to rain and high concentration as a result of increased rates of evaporation (Ulukanli and Digrak, 2002).

Utilization of a range of carbon sources and enzyme activities varied across the isolates (Table 4). These biochemical characteristics may be attributed to the ecological role they play in nutrient cycling in their environment. For example, bacterial nitrate reduction may play

Table 3. Physiological characteristics of isolates and their reference strains.

Isolate/Reference	Gram reaction	Cell form	Optimum temperature for growth (°C)	Optimum salt concentration for growth (%)	Optimum pH for growth
MS-11 (this study)	+	Rods	40	0	11
Nocardia cerradoensis (AF060790) (Albuquerque De Barros et al., 2003)	+	Rods	29	5	ND
MS-5 (this study)	+	Rods	30	5	9
Rhodococcus erythropolis (JF327477) (Yoon et al., 1997)	+	Rods	30	5	ND
MS-2 (this study)	+	Rods	30	5	9
Dietzia maris (X79290) (Rainey et al.,1995)	+	Rods	30	0	9
MS-3 (this study)	+	Rods	30	5	7
Microbacterium lacus (AB286030) (Kageyama et al., 2007)	+	Rods	25	2	ND

ND ≡ Not determined.

Table 4. Biochemical characteristics of isolates and their reference strains.

Utilization of:	MS-2	*Dietzia maris* (X79290)	MS-3	*Microbacterium lacus* (AB286030	MS-5	*Rhodococcus erythropolis* (JF327477)	MS-11	*Nocardia cerradoensis* (AF060790)
Xylose	+	-	-	-	±	-	±	-
Arabinose	+	-	+	-	+	-	+	-
Inositol	±	-	-	-	-	-	-	+
Mannitol	+	-	-	+	±	+	-	-
Fructose	+	+	+	+	+	+	+	-
Sucrose	+	-	+	+	-	+	+	-
Hydrolysis of								
Starch	+	-	-	-	+	+	-	+
Cellulose	+	ND	-	ND	-	ND	-	-
xylan	+	ND	-	ND	-	ND	-	ND
Casein	+	-	+	ND	+	+	-	-
Lipids	+	+	-	ND	-		-	ND
Urea	-	-	-	-	-	-	-	+
gelatine	-	-	-	+	-	-	-	-

Table 4. Contd.

Tyrosine reaction	+	ND	-	ND	-	-	-	ND
H_2S production	-	-	-	ND	-	-	-	ND
Nitrate reduction	+	-	+	-	+	+	-	+
Catalase	+	+	+	+	+	+	+	+
Sources	Soda lake sediments	soil	Soda lake sediments	Marine sediments	Soda lake sediments	Marine sediments	Soda lake sediments	soil

Hydrolysis tests: + = hydrolysis, - = no hydrolysis. Utilization test: + = growth, - = no growth, ± = minimal growth, ND ≡ not determined.

an important role in the nitrogen cycle within the lake ecosystem. Organotrophic actinobacteria are known to utilize nitrate, creating a link between nitrogen and carbon cycles (Foti et al., 2007).

In spite of its limitations, the culture dependent approach is still an effective method to study microbial ecology in natural environments. From the results of this study, it can be concluded that actinobacteria within Lake Magadi are active and play important roles in nutrients cycling. Novel isolation techniques will capture new groups and therefore broaden our understanding on their diversity in soda lake ecosystem.

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