OPEN ACCESS

Journal of Yeast and Fungal Research

June 2018
ISSN 2141-2413
DOI: 10.5897/JYFR
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

ABOUT JYFR

The Journal of Yeast and Fungal Research (JYFR) (ISSN 2141-2413) is published Monthly (one volume per year) by Academic Journals.

Journal of Yeast and Fungal Research (JYFR), provides rapid publication (monthly) of articles in all areas of the subject such as Yeast physiology, fermentation and biotechnology, Bioremediation, Ethanol fermentation, economic importance of yeast etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JYFR are peer-reviewed.

Contact Us

Editorial Office: jyfr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/JYFR

Editorial Board

Journal of Yeast and Fungal Research

Table of Content: Volume 9 Number 1 June, 2018

ARTICLE

Vol. 9(1), pp. 1-13, June 2018
DOI: 10.5897/JYFR2018.0182
Article Number: 4647C1157500
ISSN: 2141-2413

http://www.academicjournals.org/JYFR

ACADEMIC JOURNALS
expand your knowledge

Journal of Yeast and Fungal Research

Full Length Research Paper

Isolation and characterization of fungi from a hot-spring on the shores of Lake Bogoria, Kenya

Odilia Atamba Salano[1*], Huxley Mae Makonde[2], Remmy Wekesa Kasili[1], Hamadi Iddi Boga[3]

[1]Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000, 00200, Nairobi, Kenya.
[2]Department of Pure and Applied Sciences, Technical University of Mombasa, P. O. Box 90420 - 80100, GPO, Mombasa, Kenya.
[3]Permanent Secretary, Ministry of Agriculture Research, Kilimo house, P. O. Box 30028-00100, Nairobi, Kenya.

Received 11 January, 2018; Accepted 26 March, 2018

Hot springs are aquatic environments with high temperatures. They harbor diverse groups of micro-organisms like fungi that have developed mechanisms to thrive at wide temperature ranges, according to their optimal growth requirements. Fungi are a large group of eukaryotic organisms with worldwide distribution, inhabiting a diverse range of extreme habitats from deserts to hyper saline environments. The fungal diversity of the hot spring along the shores of Lake Bogoria was investigated using a culture-dependent approach. Microbial mats and wet sediments were collected from three sampling points along the hot spring while water samples were gotten from the mouth of the hot spring on the shores of the lake. Physicochemical characteristics were recorded at the study sites. Samples from the hot spring were isolated on four different media at pH 10 and at a temperature of 30°C. A total of seventeen fungal isolates were identified using morphological, physiological and molecular characters. Furthermore, the isolates were screened for production of extracellular enzymes. All the fungal isolates variedly grew at pH ranging from 5 to 10, temperature range of 25 to 35°C and sodium chloride range of 5 to 30%. Based on experimental analysis, isolate B61 exhibited significant growth in the four parameters tested. Also all the seventeen isolates produced different extracellular enzymes such as amylases, lipases, proteases and esterases. Analysis of partial sequences using Blastn showed that about 11.7 and 29.1% of the isolates were affiliated with members belonging to the genera *Penicillium* and *Aspergillus*, respectively. A total of 12% of the isolates belonged to the genera *Alternaria* and *Fusarium* while another 41% of the isolates clustered closely with uncultured fungus. Fungal endophyte comprised 6%. Although the culture dependent method did not reveal the true diversity of the fungal community, the isolates recovered were a representation of thermotolerant, alkalitolerant and halotolerant microorganisms found in soda lake environments. The isolates have the potential to produce useful enzymes for biotechnological applications.

Key words: Hot springs, tropical mycology, diversity, extremophiles.

INTRODUCTION

Lake Bogoria is a soda lake in the Kenyan Rift valley andis a host to flamingoes and a variety of geochemically distinct hot springs (McCall, 2010). It is a deep, hyper alkaline and saline habitat that lies at an altitude of 1,000

m above sea level. The shoreline of the lake is fringed by geysers and hot springs with water temperatures ranging from 39 to 98.5°C. The hot springs and geysers found on the shores supplement water recharge for the lake. Lake Bogoria has been adversely affected by climate change that has resulted in long periods of drought that have led to fluctuation in the water levels. The shoreline of lake is fringed by geysers and almost 200 hot springs that discharge waters into the lake and this confirms the volcanic origin of this harsh landscape (Renaut and Owen, 2005).

Hot springs are a type of extreme environments that are unique with respect to physical, chemical and geographical characteristics (Spear et al., 2005). They are widely distributed all over the world and represent a wide range of microbial niches of highly diverse microorganisms (Song et al., 2013). Most of the hot springs within the Kenyan lakes harbor extremophiles. Most of the described extremophiles are characterized only by one distinctive extreme condition such as temperature or pH. However, others are multi-extremophiles, for example alkalithermophiles (Kevbrin et al., 1998).

Soda lakes represent the naturally occurring environments on earth, with pH values generally greater than 10. They are considered to exhibit high productivity rates (410 g cm^2 per day) more than their freshwater counterparts with salinity that is up to saturation concentrations (Grant, 2006). Recent studies have demonstrated that a wide range of fungal diversity inhabit the hyper alkaline and saline hot springs of soda lakes in Kenya (Kambura et al., 2016; Salano et al., 2017). Previously, combinations of both culture-dependent and culture-independent methods were used to document the microbial diversity of soda lakes (Baumgarte, 2003; Kambura et al., 2016) and some other lakes (Rees et al., 2004). Culture-independent methods have revolutionized our general view of microbial ecology and thus revealed diverse communities in the ecosystem (Bell et al., 2014; Kambura et al., 2016; Salano et al., 2017).

Although the use of next-generation sequencing (NGS) is playing a major role in exploring and detection of new species; in this study, four different growth media were used to assess the effectiveness of culture-dependent method at recovering fungal microorganisms from sediments, mats and water collected from a hot spring found along the shores of Lake Bogoria. Earlier studies conducted using culture-dependent method on the soda lakes of the East African Rift Valley have shown that there are dense and diverse populations of halophilic, alkaliphilic and alkalitolerant microorganisms (Zavarzin et al., 1999; Grant et al., 1999).

MATERIALS AND METHODS

Authority to conduct research

Permission to conduct research on Lake Bogoria was granted by the National Commission for Science, Technology and Innovation (NACOSTI). All other necessary documents to access and collect samples from the Soda Lake were obtained from the National Environment and Management Authority (NEMA) and the Kenya Wildlife Services (KWS).

Study site

The study was conducted on one hot spring along the shores of Lake Bogoria. The sampling site was located in the Chemurkeu area at the western shore of Lake Bogoria (0° 13′ 33″ N, 36° 05′ 41″ E; 00° 13′ 46.1″N 36° 05′ 34.8″ E). At this site, hot springs (Cioni et al., 1992) drain waters directly into the lake. The waters of the lake originated from inflow from the Sandai and Emsos rivers and from about 200 alkaline hot springs that are present at four onshore sites. Lake Bogoria is shallow (about 10 × 10 m^2 depth), saline (up to 100 g/L total dissolved salts), alkaline (pH 10.5) and lies in a volcanic region in a basin south of Lake Baringo, and a little north of the equator. It lies at an altitude of approximately 990 × 1000 m above sea level and forms part of the Lake Bogoria National Reserve (Mugo, 2007). Although hyper saline, the lake is highly productive with abundant cyanobacteria that is food for the flamingos.

Measurement of physicochemical parameters

The geographical position of the site in terms of longitude, latitude and elevation was taken using Global Positioning System (GARMIN eTrex 20). During sampling, the temperature, electrical conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) were measured on site using Electrical Chemical Analyzer (Jenway - 3405), whereas the pH was measured with a portable pH-meter (Oakton pH 110, Eutech Instruments Pty. Ltd) and confirmed with indicator strips (Merck, range 5 to 10). Temperature was recorded at three distinctive points along the flow of the hot spring and the temperature assigned to all the sample types used in this study.

Sample collection

Samples analyzed in this study were collected from a hot spring located along the shores of Lake Bogoria (00° 13′ 46.1″N 36° 05′ 34.8″ E at an altitude of 1,000 m above sea level, at Chemurkeu area, at a place called “Mawe Moto’’ on 8th of July, 2014. Water samples were collected from the mouth (sampling point 1) of hot spring at 84.6°C and pH 9.0 in triplicates using 1 L sterile bottles. Wet sediments (500 g) and microbial mats (500 g) were collecte randomly from the floor of the rivulet in triplicates using sterile

*Corresponding author. E-mail: salanoodilia@gmail.com.

Table 1. Physicochemical parameters and sample types of sampling points at Lake Bogoria measured before sampling.

Sampling point	Sample ID	Sample type	Temperature (°C)	pH	Dissolved oxygen (Mg/L)	EC (mS/cm)	TDS (Mg/L)
1	LB1	Mats	84.6	8.2	0.1	1	0.64
2	LB2	Mats	77.7	8.3	0.23	1	0.65
3	LB3	Mats	54	8.7	0.53	1	0.64
1	LBa	Wet sediments	84.6	8.2	0.1	1	0.64
2	LBb	Wet sediments	77.7	8.3	0.23	1	0.65
3	LBc	Wet sediments	54	8.7	0.53	1	0.64
1	LBd	Water	84.6	9	0.1	1	0.64

containers at three distinct points (rivulet points 1, 2 and 3) that differed in temperature and alkalinity levels shown in Table 1 at 84.6, 77.7 and 54°C, respectively. The samples were labeled properly and transported on dry ice in cool boxes to the laboratory at the Jomo Kenyatta University of Agriculture and Technology. Once in the laboratory, the samples were divided into two sets. One set was preserved at -80°C as a backup, while the other was used for work on the isolation, characterization and screening of the fungal isolates.

Isolation and culturing of fungi

Fungi were isolated from the microbial mats, sediments and water samples. The water from the mouth of the hot spring was used to prepare culture media fungi isolated from the samples on Potato dextrose agar (PDA), Malt extract agar (MEA), Potato glucose agar (PGA) and Sabourand dextrose agar (SDA). Water samples from the mouth of the hot spring were filtered through 0.45 and 0.22 µm membrane filters (Whatmann), autoclaved and used to prepare the culture media. All media were prepared according to the manufacturer's instructions (Difco Laboratories). In order to restrict any bacterial growth, the media were supplemented with streptomycin (100 and 200 mg/L) and mixed well before dispensing on petri dishes. One gram of microbial mats and sediments was separately suspended in 9 ml of sterile distilled water and vortexed thoroughly. From this stock solution of 10 ml, serial dilutions were performed to 10^{-7}. Aliquots of 100 µl from dilutions of 10^{-4} were plated in triplicates on each of the four culture media. Inoculation was done on the surface of the four types of media, inverted and incubated at 30°C. The plates were checked for growth of fungi every 5 days for two weeks. The fungal colonies were sub-cultured using appropriate media at 30°C for 1 to 2 weeks until axenic cultures were obtained (Dipal and Pandey, 2012). Pure isolates were maintained at 4°C in a refrigerator for further analyses.

Morphological characterization of the isolates

Colony morphologies of the isolates were described using standard microbiological criteria, with special emphasis on colour, shape, size, elevation, form and pigmentations on both obverse and reverse (Table 3). Cell characteristics were described for cultures grown at optimum temperature, pH and salt concentration. Preliminary characterization by simple staining (using lacto phenol cotton blue dye) of each of the pure isolates was done and observed under a light microscope at ×100 (Keast et al., 1984) in order to differentiate the isolates based on their morphology (Cappuccino and Sherman, 2002). Colony morphologies of the isolates were described with special emphasis on colour, shape, size, elevation, form and pigmentations on both obverse and reverse (Table 3). Preliminary characterization by staining each of the pure isolates was done and observed under a light microscope at ×100 (Keast et al., 1984; Cappuccino and Sherman, 2002).

Physiological characterization

Determination of optimum growth temperature

To determine the ability of the isolates to grow over a range of temperatures, PGA media at pH 8.0 was prepared, sterilized and dispensed in sterile petri dishes. Each isolate was inoculated and incubated at different temperature levels (25, 30, 35, 40 and 45°C) for 7 days (Nazina et al., 2001). Two un-inoculated plates for each temperature were used as controls. Experiments were done in triplicates and the growth of isolates was observed and noted by measuring the diameter of the colony in millimeters.

Determination of optimum pH for growth

In order to determine the optimum pH requirements for the growth of the isolates, four batches of PGA media were prepared and pH was adjusted to 5.0, 7.0, 8.5 and 10.0 using 1 M HCl and 1 M NaOH solutions, respectively. The media were then autoclaved and dispensed in petri dishes. Each medium was inoculated with the isolates in triplicates and incubated at 30°C, which was the maximum growth temperature for the isolates for a period of 7 days. The level of growth (diameter) was measured and recorded in millimeters. For each pH two uninoculated plates were used as controls.

Growth on different media

The growth requirements for fungi may vary from strain to strain, although cultures of the same species and genera tend to grow best on similar media. An optimal nutrient medium should provide not only adequate growth but also the best possible growth in order to allow the fungi to express all phenotypes. This experiment was to determine the effect of media on the cultivation of the fungal isolates. Four types of media were used namely MEA, SDA, PDA and PGA. The isolates were then inoculated in triplicates and incubated at 30°C for a period of 7 days and the diameter of the

fungal colonies measured in millimeters. The fungal isolates were inoculated on the four different types of media (MEA, PGA, PDA and SDA) and incubated at 30°C for a period of 7 days. The diameter of the fungal colonies was measured in millimeters.

Determination of optimum salt concentration for growth

Four batches of PGA media medium were prepared in 1 L of distilled water and each supplemented with 5, 10, 20 and 30% sodium chloride concentration. This was to determine the ability of the isolates to grow at different sodium chloride concentration. Three sets of experiments for each concentration were conducted. These were incubated at 30°C then checked for growth after 7 days by measuring the diameter of the colony in millimeters.

Enzymatic characterization

Determination of amylase activity

The ability to degrade starch was tested used as a test for production of amylolytic enzymes as described by Hankin and Anagnostakis (1975). The isolates were inoculated on nutrient agar (NA) with 0.2% of soluble starch (g L^{-1}), pH 8.0 in triplicates. After 5 days of incubation, the cultures were flooded with iodine solution (1% w/v). A yellow zone around a colony in an otherwise blue medium indicated amylolytic activity while negative isolates indicated a blue black colour all over the plate (Castro et al., 1993).

Determination of esterase activity

The medium used was as described by Sierra (1957). It contained (g L^{-1}): 10.0 g peptone, 5.0 g NaCl, 0.1 g $CaCl_2.2H_2O$, 18.0 g agar at pH 8.0. To the sterilized culture media, sterilized Tween 80 was added in a final concentration of 1% (v/v). This medium was inoculated with the isolates in triplicates and incubated at 30°C. The presence of a precipitation of calcium crystals around the colonies showed the presence of esterase enzyme activity.

Determination of lipase activity

A previously described method to determine the esterase activity (Sierra, 1957) was used; however, Tween 80 was substituted by Tween 20. This medium was inoculated with the isolates in triplicates. Positive isolates for lipase production were indicated by a precipitation of calcium crystals around the colonies. Esterase activity was measured following the method of Sierra (1957). However, Tween 80 was substituted by Tween 20. The experiment was set up in triplicates and positive isolates for lipase production were indicated by a precipitation of calcium crystals around the colonies.

Determination of protease activity

For the determination of protease activity, the isolates were cultured on a media containing g/L 8.0 g Nutrient Broth, 1.0 g glucose, 18.0 g agar and the pH was adjusted to 8.0 (Vieira, 1999). After autoclaving the media, 15.0 ml of autoclaved skimmed milk was added. The microorganisms were inoculated in triplicates and incubated at 30°C for and after the growth period of 7 days, after which 2.0 ml of 0.1 M HCl was added to the plates. Positive isolates exhibited a clearing zone, indicative of proteolysis (Cappuccino and Sherman, 2002).

Determination of cellulase activity

The medium media (7.0 g KH_2PO_4, 2.0 g K_2HPO_4, 0.1 g $MgSO_4.7H_20$, 1.0 g $(NH_4)_2SO_4$, 0.6 g yeast extract, 10 g microcrystalline cellulose and 15 g agar per liter) by of Stamford et al. (1998) was used. The plates were inoculated in triplicates and incubated at 30°C for 7 days. After incubation, the plates were stained with 0.1 % (w/v) Congo red dye and the diameter of each cleared zone was recorded. The present appearance of a clear halo around the fungal growth indicated cellulose activity.

Determination of xylanase activity

The medium (5.0 g Birch wood xylan, 5.0 g Peptone, 5.0 g Yeast extract, 1.0 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$ and 20.0 g agar per litre) as described by Nakamura et al. (1993) was used. The inoculated plates were incubated at 30°C for 7 days. The plates were then flooded with 0.1% (w/v) Congo red dye and left for 30 min before they were washed with 1 M NaCl solution. Positive xylanolytic isolates were detected based on the clear zones of hydrolysis on the xylan.

Molecular characterization of the isolates

DNA extraction

Total genomic DNA of the isolates was extracted from fungal mycelia mycelial cells in duplicate using two lysis buffers, solution A (50 mM Tris pH 8.5, 50 mM EDTA, pH 8.0 and 25% sucrose solution) and solution B (10 mM Tris pH 8.5, 5 mM EDTA, pH 8.0 and 1% SDS). The mycelia cells were scrapped aseptically using a sterile surgical blade by taking care not to pick the media. These were crushed separately in 200 µl solution A using sterile mortar and pestle, and resuspended in 100 µl of solution A. This was followed by addition of 30 µl of 20 mg/L Lysozyme and 15 µl of RNase A. The mixture was gently mixed and incubated at 37°C for 2 h to lyse the cell wall. Subsequently, solution B (600 µl) was added and gently mixed by inverting the tubes several times. 10 µl of Proteinase K (20 mg/L) was added and the mixture was incubated at 60°C for 1 h. Extraction followed the phenol/chloroform method (Sambrook et al., 1989). The quality of DNA was checked using a 1% agarose stained with ethidium bromide. The genomic DNA was used as templates for subsequent PCR amplification.

PCR amplification and sequencing of ITS gene region

The purified DNA from each isolate was used as a template for amplification of the ITS gene region using a fungal primer pair: ITS1 (5´TCCGTAGGTGAACCTTGCGG3´) and ITS4 (5´TCCTCCGCTTATTGATATGC3´) (White et al., 1990). Amplification was performed using DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD) PCR machine. Amplification was carried out in a 40 µl mixture containing 5 µl of PCR buffer (×10), 3 µl dNTP's (2.5 mM), 1 µl (5 pmol) of ITS1 forward primer, 1 µl (5 pmol) of ITS4 reverse primer, 0.3 µl taq polymerase, 1.5 µl of template DNA and 28.2 µl of water. The control contained all the aforementioned except the DNA template. Reaction mixtures were subjected to the following temperature cycling profiles repeated for 35 cycles: Initial activation of the enzyme at 95°C for 5 min, denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s,

chain extension at 72°C for 1 min and a final extension at 72°C for 10 min.

The quality of PCR products was assessed on 2% agarose gel and visualized under ultraviolet by staining with ethidium bromide (Sambrook et al., 1989) to determine the success of amplification and the relative intensity of bands. Sequencing of the PCR products was performed using a commercial service provider (Macrogen, Korea). The PCR products were purified using the multiscreen filter plate (Millipore Corp and sequenced by a Big Dye (R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to manufacturer's instructions.

Phylogenetic analysis

The ITS partial gene sequences obtained were manually edited. The sequence data were BLAST (www.ncbi.nlm.nih.gov/BLAST/) analyzed against the GenBank 18S rDNA database. Alignments were checked and corrected manually based on conserved regions. The 18S rDNA gene sequences were compared to sequences in the public database using Basic Local Alignment Search Tool (BLAST) in the National Center for biotechnology Information (NCBI) website (http://www.ncbi.nih.gov) in order to determine similarity to sequences in the Gene bank database (Shayne et al., 2003). The 18S rDNA gene sequences with high similarities to those determined in the study were retrieved based on BLASTn results and added to the alignment.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model (Jukes and Cantor, 1969). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2015).

RESULTS

Physical characteristics at sampling site

The metadata collected before sampling included the geographical position of each sampling point in terms of latitude, longitude and elevation, temperature, pH, electrical conductivity, total dissolved solids and dissolved oxygen. The samples collected from the Soda Lake and their parameters are summarized in Table 1.

Fungal isolates

A total of 17 fungal isolates, 9 from wet sediments, 7 from microbial mats and 1 from water 17 fungal isolates, 9 from wet sediments, 7 from microbial mats and 1 from water were recovered from the three sampling points along the hot spring at Lake Bogoria as summarized in Table 2.

Colony and cell morphology of the isolates

Morphological characterization was based on classical macroscopic techniques of color, form, shape, margin and elevation of the pure colonies (Table 3). Most colonies were able to grow within 4 to 7 days of incubation at 30°C. The colony characteristics recorded for the various isolates are as shown in Table 3. The isolates showed reproductive spores, mycelia and hyphae (Figure 1). The hyphae were either septate or aseptate and in some, the spores or conidia were in a chain at the end of aerial hyphae or in a sac like structure and in some the spores were formed externally on a base or just scattered in the media.

Physiological characterization

All the isolates grew at 25, 30, and 35°C. Growth at 40°C was observed only for the isolates B23, B25 and B61. Generally, the isolates did not show any growth at 45 and 50°C. The optimum growth of the isolates was observed between 30 and 35°C (Table 4). Different isolates showed different growth rates on the four types of media used. Isolates B3, B5, B41, B61, and B63 recorded average growth values of 80, 82.67, 80.33, 80.67 and 81.33 mm respectively in on PGA, isolates B5, B41, and B63 had average values of 70, 66.67 and 64 mm in Sabourand dextrose agar, respectively while isolates B5, B61 and B61 B63 recorded average values of 80.7 76.67 and 71.67 mm in potato dextrose agar media. In Malt extract agar isolates B5, B31 and B61 had average values of 80.33, 79.33 and 80.33 mm, respectively. Generally, isolates growing in potato glucose agar media showed significant growth in the four types of media used in this study (Table 4). Although the isolates were from an alkaline environment, all showed grow at all the pH values tested, pH values including acidic pH 5 and neutral pH 7. There was significant growth for all the isolates at pH 10 with five isolates: B3, B5, B17, B21 and B61 (B3, B5, B17, B21 and B61) recording a mean value of over 78 mm (Table 5). All the 17 isolates were able to grow in varying concentrations of sodium chloride. Growth was improved with the decrease in salt concentration from 30% up to 5% sodium chloride. The highest growth was recorded at 5% sodium chloride followed by 10 then 20% and gradually decreased towards 30%. The isolates marked a and b in Tables 4 and 5 have the same standard errors of ±0.9 and ±1.2, respectively even though the mean values and growth parameters are different. The same standard error values implied that the growth of the isolates were not significantly different in their various growth parameters; media, pH, salinity and temperatures. Statistical analysis showed that the growth of isolate B4 and B17 (marked c) were significantly different in salt concentration of 5, 10

Table 2. Summary of isolates recovered from Lake Bogoria and their sampling point, type, and temperature.

Sampling point	Sampling type	Temperature (°C)	Isolate
1	Wet sediments	84.6	B1, B3, B4, B5
2	Wet sediments	77.7	B17
3	Wet sediments	54	B21, B23, B24, B25
1	Water	84.6	B31
1	Mats	84.6	B41
2	Mats	77.7	B52, B54
3	Mats	54	B61, B62, B63, B64

Table 3. Morphological (colony) characteristics of the 17 isolates from Lake Bogoria.

Isolate	Colour		Margin	Elevation	Form
	Top	Bottom			
B1	white	yellow	Entire	Raised	circular
B3	Green	Cream	Entire	Flat	circular
B4	Yellow and white	red	Undulate	Raised	Irregular
B5	Green with white margin	cream	Entire	Flat	circular
B17	Cream	Cream	Entire	Raised	circular
B21	Cream with purple rings	Brown	Entire	Flat	circular
B23	Grey	Cream	Entire	Flat	circular
B24	Grey	Black	Undulate	convex	Irregular
B25	Green	Purple	Entire	convex	circular
B31	Pink	Pink	Entire	Raised	circular
B41	Green with black spots	Cream	Undulate	Raised	Irregular
B52	Green and cream	brown	Entire	Flat	circular
B54	Green and cream	Green and cream	curled	Umbonate	Irregular
B61	green	Cream	Undulate	Raised	Irregular
B62	green with yellow margin	Cream	Entire	Flat	circular
B63	Green and white	Cream	curled	Raised	Irregular
B64	white	Cream	Entire	Umbonate	circular

and 20% and MEA, PDA and PGA media, respectively as shown by high standard error values of above ±8.0 (Tables 4 and 5).

Screening the isolates for production of extracellular enzymes

All the isolates apart from B3, B4, B17, B21, B23, B31 and B41 were positive for the amylase test. Only isolates B21, B23, B31, B41 and B61 gave negative results for esterase test. Five isolates out of the 17 isolates tested were negative for lipolytic activity while 6 isolates gave positive results for protease test. Nine isolates B4, B17, B23, B24, B25, B52, B54, B62 and B64 were positive for xylanases, while the rest of the isolates were negative. All the 17 isolates tested negative for the production of cellulase enzyme shown in Table 6 with + and - signs for positive and no production of the enzyme tested, respectively.

Molecular characterization of fungal isolates

Phylogenetic analysis of sequences

The BLASTn search results showed that all the isolates belonged to the fungal domain. 90% of the isolates were affiliated to the phylum *Ascomycota* and this suggested that diverse groups of fungi in this phylum have the springs. Isolates B4, B21, B31, B41 and B63, closely clustered with members of the genus *Aspergillus* including

Figure 1. Colony and cell characteristics of some of the 17 isolates from the hot spring at Lake Bogoria.

Aspergillus versicolor and *Aspergillus fumigatus* both at 99% similarity and *Aspergillus flavus* at 97% similarity. Isolate B17 was affiliated to *Penicillium pinophilum* (with sequence identity of 99%), while isolate B61 clustered closely with members of the genus *Alternaria*. The phylum *Basidiomycota* was represented by a single filamentous fungi (Isolate B1) of the genus *Trametes* and family Polyporaceae.

DISCUSSION

In this study, culture-dependent approach was used to investigate the fungal community in a hot springs on the

Table 4. Impact of culture media and temperature on the growth of fungi isolated from Lake Bogoria. The values represent the mean in millimeters ± standard errors for the various isolates.

Fungal isolate	Media				Temperature			
	MEA	PDA	SDA	PGA	25°C	30°C	35°C	40°C
B1	59±1.5	60.7±1.2[b]	59.33±1.8	79.67±0.9[a]	33±1.2[b]	67.67±0.9[a]	34.67±2.2	0
B3	78.33±1.7	66±8.9	58.67±3.7	80±1.5	72±0.6	68±1.5	67±0.6	0
B4	19.67±1.2[b]	24.3±0.3	30.33±0.9[a]	44.33±2.3	66±1.7	71±1.2	68±1.5	0
B5	80.33±0.3	80.7±1.2[b]	70±2.1	82.67±0.9[a]	52.67±2.0	66±1.5	56.33±1.5	0
B17	47.67±8[c]	30.33±8[c]	19±1.2[b]	50±8.1[c]	59.33±4.3	80±1.2	70.66±1.9	0
B21	39.33±1.5	38.7±3.8	27.67±3.5	55.33±3.2	37±0.6	27.33±2.2	25±1.2[b]	0
B23	64.33±0.9[a]	57±4.7	62.33±2.7	79.33±1.2[b]	71.33±1.2[b]	76.67±0.9	74.33±2.9	23±1.2[b]
B24	44.33±2.3	20.67±1.9	44.33±2.3	60.67±0.9[a]	29±1.2[b]	20.33±0.9[a]	15.33±0.9[a]	0
B25	37±0.6	27.33±2.2	25±1.2[b]	51±3.2	22±1.0	75.33±0.9[a]	63.67±1.5	15.33±0.9[a]
B31	79.33±0.7	66±1.5	56.33±1.5	72±0.6	61.33±2.9	75.67±2.4	72.67±1.2[b]	0
B41	70±5.8	71.33±4.4	66.67±1.2[b]	80.33±1.2[b]	39.67±0.9[a]	18.67±1.5	15±1.5	0
B52	39.67±0.9[a]	18.67±1.5	15±1.5	32.67±2.3	76.67±0.9[a]	26.67±1.2	23±1.2[b]	0
B54	40.67±1.7	26.33±1.8	29.33±2.9	44.33±2.3	17±1.2[b]	24.67±1.5	15.67±0.9[a]	0
B61	80.33±1.5	76.67±0.7	62±2.1	80.67±1.5	51.67±2.3	77±0.6	79.67±0.9[a]	13.33±1.2[b]
B62	47.33±1.5	72±0.6	51±3.2	79±0.6	44.33±2.3	20.67±1.9	44.33±2.3	0
B63	78.33±1.3	71.67±5.9	64±1.5	81.33±0.9[a]	19±1.2[b]	17±1.5	32.67±2.3	0
B64	19±1.2[b]	17±1.5	32.67±2.3	47.67±8	18.67±1.5	33.33±1.2[b]	37±1.2[b]	0

Table 5. Influence of pH and salinity on growth of fungi isolated from lake Bogoria. The values represent the mean in millimeters ± standard errors for the various isolates.

Fungal isolate	Salinity				pH			
	5%	10%	20%	30%	pH 5.0	pH 7	pH 8.5	pH 10
B1	25.33±0.9[a]	19±1.2[b]	12.67±0.9[a]	10±0.6	65±1.5	62±1.2	68±1.2	67.67±0.9[a]
B3	39.67±0.9[a]	32.67±2.3	18.67±1.5	15±1.5	64±2.3	80.33±0.3	81.33±1.2	81.67±1.2[b]
B4	47.67±8.0[c]	50±8.1[c]	30.3±8.0[c]	19±1.2	20±1.5	21±1.2[b]	72.67±0.9[a]	73.67±1.8
B5	60.67±0.9[a]	44.33±2.3	44.33±2.3	20.67±1.9	33±1.7	30.67±0.7	31±1.2	81±1.5
B17	75.33±0.9[a]	63.67±1.4	22±1	15.33±0.9[a]	33±2.7	82±0.6	32.33±0.9[a]	81±1
B21	51±3.2	37±0.6	27.33±2.2	25±1.2[b]	18±0.6	24±1	18.33±0.7	78±1.5
B23	74±1.7	66.67±1.2[b]	24±1.7	20.33±1.9	44±2.7	47.67±0.9[a]	62.33±0.9	62.33±1.2[b]
B24	24±1.7	22±1	17.67±1.2[b]	17.67±0.9[a]	25.67±1.5	28±0.6	67.33±0.9	28.33±1.2[b]
B25	28±1	24.33±0.3	21.33±1.5	16.33±1.2[b]	22.67±1.2[b]	22.67±1.5	25.33±1.2	23±1.2[b]
B31	39.67±0.9[a]	32.67±2.3	18.67±1.5	15±1.5	28.33±1.2[b]	66.67±1.2[b]	32.67±1.8	51.33±0.9[a]
B41	44.33±2.3	40.67±1.8	29.33±3.0	26.33±1.8	65.33±1.2[b]	76.33±0.3	80±0.6	68.33±1.2[b]
B52	74.67±1.2[b]	54.33±1.5	26.33±1.5	15.33±0.9[a]	25±2.5	20±1.2	17.33±0.9[a]	22±1
B54	53.67±1.5	43.33±2.0	27.33±0.7	16.67±1.5	28±1.2[b]	18±0.6	18.67±0.9[a]	61.67±1.2[b]
B61	78.33±1.2[b]	81.33±0.9[a]	71.67±5.9	64±1.6	75.33±1.2[b]	80.67±1.2[b]	81±1.2	80.67±0.7
B62	19±1.2[b]	17±1.5	32.67±2.3	47.67±8.0	67.67±0.9[a]	80.33±0.7	73±1.5	81±1.7
B63	48.67±1.9	38±0.6	27.67±1.9	24±1.5	32.33±1.2[b]	73±1.2[b]	61±1.5	30.33±0.9[a]
B64	81.33±0.9[a]	51±0.6	27.67±1.2[b]	18.67±1.2[b]	27.33±0.3	16±1.2[b]	17.67±0.9	18.67±0.3

shores of Lake Bogoria. Although culture-dependent methods generally recover only a small portion of the diversity from environments, they are still a critical component in research (Malaviya and Rathore, 2007).

From this study, morphological observations indicated that different species of fungi were isolated from the hot spring. The isolates exhibited different colours and the spores observed were anamorphic with different shapes

Table 6. Biochemical characterization of the 17 isolates from lake Lake Bogoria**.**

Isolate	Amylases	Esterases	Lipases	Xylanases	Proteases	Cellulases
B1	+	+	-	-	-	-
B3	-	+	+	-	+	-
B4	-	+	+	+	+	-
B5	+	+	+	-	-	-
B17	-	+	+	+	-	-
B21	-	-	-	-	+	-
B23	-	-	+	+	-	-
B24	+	+	+	+	+	-
B25	+	+	-	+	-	-
B31	-	-	+	-	-	-
B41	-	-	+	-	-	-
B52	+	+	-	+	-	-
B54	+	+	+	+	-	-
B61	+	-	+	-	-	-
B62	+	+	+	+	-	-
B63	+	+	+	-	+	-
B64	+	+	-	+	+	-

and structures belonging to the sub-division of *Ascomycotina* and *Basidiomycotina*. Cellular morphology showed long, branching filamentous (Figure 1) hyphae (Madigan et al., 2005). According to classical mycology, most species of endophytic fungi have been described based on their morphological features such as ascospore morphology, colour, odour, and other organoleptic characteristics (Barseghyan and Wasser, 2010). Toledo et al. (2013) used morphological and molecular characteristics of fungal isolates to identify a fungus of *Hirsutella* species from plant hoppers.

Physiochemical characterization of the isolates showed that the highest growth was observed at pH 10. However, the isolates were able to grow at pH 5, which suggests that they are also acido-tolerant (Moreira and Siqueira, 2002)**.** This growth at pH range of 5 to 10 is consistent with the earlier study by Horikoshi, (1998) which showed a low to high pH range of 5.7 to 9.0, favours growth of alkaliphiles and that a pH range of 9.0 to 10 may serve as their selective optimum pH. Another study done by Jaouani et al. (2014) found that all isolates obtained from ashes collected from Sebkha El Melah, a Saharan salt flat located in southern Tunisia, were considered as alkali-halotolerant since they were able to grow in media containing 10% of salt with an initial pH 10. Therefore, regarding the stress of pH, the capacity of the majority of isolates to growth at pH 10 implies that some habitats along the hot spring may have a higher pH and secondly, that fungi can tolerate a wide pH range. It also suggests adaptation of the fungi to the alkali environments as characteristic of alkalophilic fungi (Horikoshi, 1999).

Culture media is an essential growth factor for controlling the growth and sporulation of fungi. Different fungal species can grow on different media, which may be related to the preference of the microorganisms for some nutrients in the different media (Barreto et al., 2011). Therefore, to obtain many pure cultures, it is necessary to provide an appropriate substrate for the growth of microorganisms. The composition of a particular medium plays a great role in the growth and sporulation of fungi. Zhao and Shamoun (2006) suggested that culture media significantly affected the growth, sporulation and conidial discharge in fungi. In this study, the data revealed that maximum mycelial growth was observed on PGA followed by MEA with a mean maximum growth of 64.8 and 54.4 mm, respectively. On the contrary, Jain (2001) studied the effect of four culture media on filamentous fungal growth and found that SDA medium showed maximum growth and sporulation of all fungi. Also several workers investigating the influence of culture media on growth, colony character and sporulation of fungi stated that PDA is the best media for mycelial growth (Saha et al., 2008).

From this study the optimal temperature for conidial germination of the fungal isolates was approximately 25°C, with an upper limit at 35°C, though some grew at temperatures above 35°C. Studies have also shown that filamentous fungi may experience elevated temperatures throughout their growth period (Kalsbeek et al., 2001).

Most of the fungi isolated in this study were able to produce extracellular enzymes such as amylases, lipases, proteases, esterases and xylanases (Figure 2).

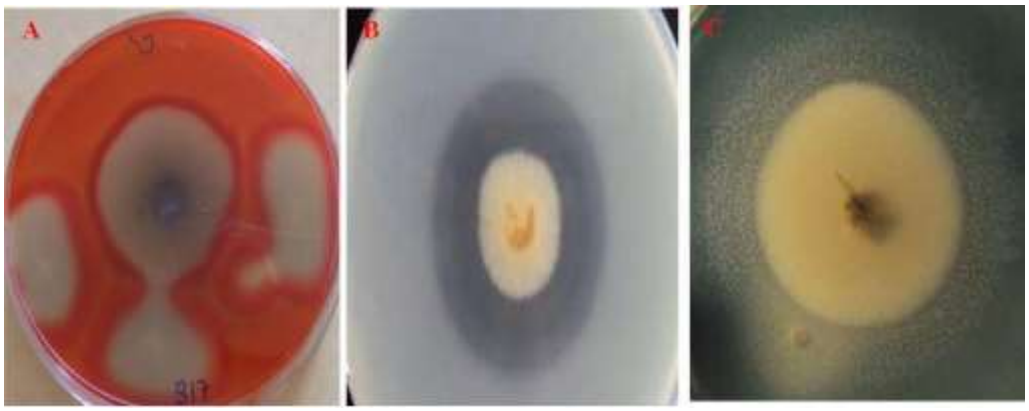

Figure 2. (A) Shows positive results for xylan utilization in isolate B17, (B) isolate B4 showing production of protease enzyme, and (C) isolate B1 showing precipitates of calcium salts around the colony showing indicating esterase production of esterase enzyme. The isolates were grown on potato glucose agar media.

The stability of these enzymes at alkaline pH is attributed to their habitat (alkaline lake) and growth profile in a wide range of pH. This is a characteristic feature that confirms their role in the decomposition of organic matter in these habitats (Kieser et al., 2000). Alkaline proteases, chitinases, amylases, lipases and caseinases have also been reported in a wide range of microorganisms isolated from Soda Lake environments, such as Rift valley Soda lakes (Joshi et al., 2008).

In this study, all isolates were sensitive to salt and generally their growth rates were clearly inhibited with increasing concentrations NaCl (salt concentrations), although the mycelium remained viable. Growth of the fungi was considerably more inhibited in the presence of increased concentrations of NaCl. Only a small number of isolates were able to grow at higher NaCl concentrations. It could be possible that Na^+ is poorly taken up by fungi, and could have caused alkaline stress to the fungi inhibiting their growth at high salt concentrations (Yuan et al., 2017). This is consistent with earlier data by Maciá-Vicente et al. (2012) that fungi isolated from halophytes are more likely halo tolerant but not halophilic.

Phylogenetic analysis of 18S rDNA gene sequences placed most of the fungal isolates in the phylum Ascomycota. The isolates were affiliated with most abundant genera within the phylum Ascomycota including of the genera *Aspergillus* (29.1%), *Penicillium* (11.7%), *Alternaria* (6%) and *Fusarium* (6%). While the phylum *Basidiomycota* was represented by a single member of the genus *Trametes* belonging to family Polyporaceae (Figure 3). Jaouani et al. (2014) isolated fungi belonging to the genera *Cladosporium, Alternaria, Aspergillus, Penicillium, Ulocladium, Engyodontium* and *Cladosporium cladosporioides* that were able to grow in media containing 10% of salt with an initial pH 10 from Sebkha El Melah, a Saharan Salt Flat in Southern Tunisia. Also, Damare et al. (2006) showed that genera *Penicillium*, *Aspergillus* and *Cladosporium* are the most abundant fungal species in aquatic environments. According to studies by Christensen et al. (2000) and Asan (2004), the species of *Aspergillus* and *Penicillium* are among the most abundant and widely distributed microfungi in nature.

Conclusion

The current study demonstrated the isolation and characterization of fungi from a hot spring at the shores of Lake Bogoria using culture dependent method. The results showed that culturing recovered a small proportion of the microorganisms from the hot spring. Although cultivation and isolation of microorganisms are essential for studying the physiological and metabolic characteristics of individual microbes, majority of microorganisms from natural environments like hot springs cannot be grown in the laboratory. It is therefore important to combine culture dependent and independent techniques so as to increase the recovery of fungi from these extreme environments. The findings also revealed that culture media differentially influenced the growth, colony character and sporulation of the isolated fungi. Out of the four test media employed in the present study, PGA was found to be most suitable growth media followed by MEA and PDA, respectively.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

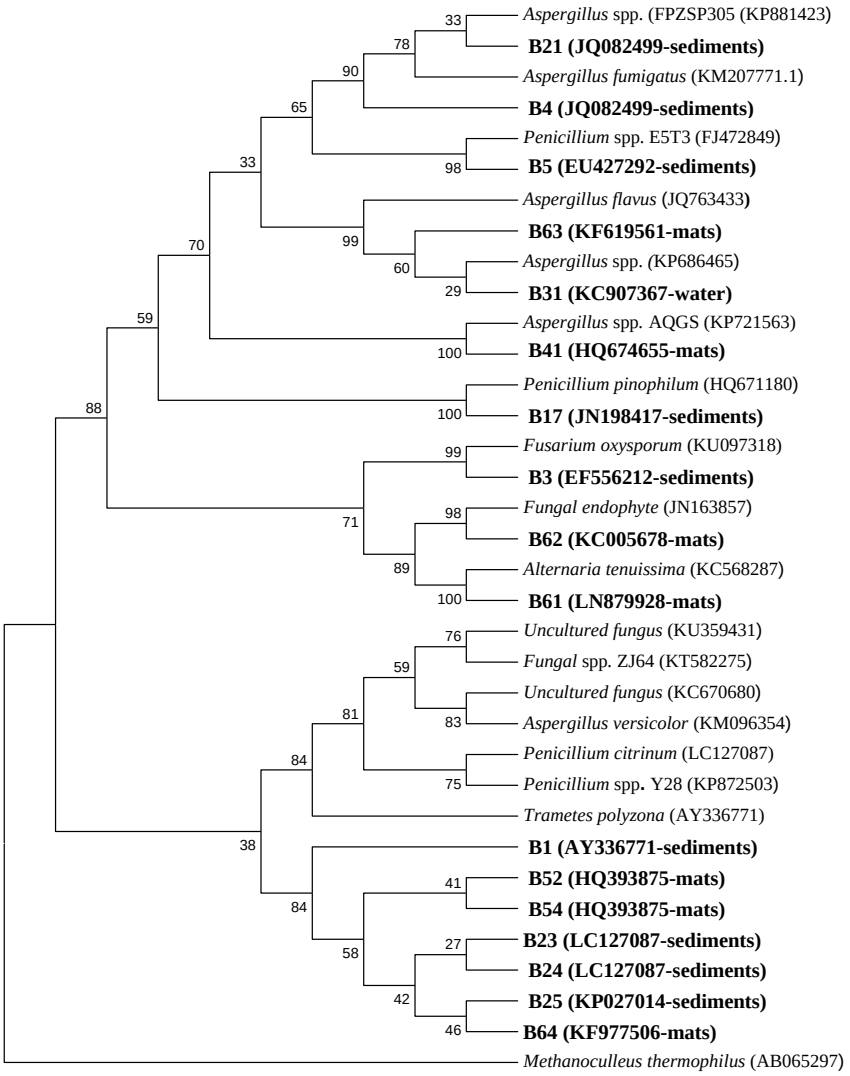

Figure 3. Molecular phylogenetic analysis by Maximum Likelihood method. Species names are followed by the GenBank accession numbers while the isolates are followed by the accession numbers and the source of isolation.

ACKNOWLEDGEMENTS

The author thanks the National Commission for Science, Technology and Innovation (NACOSTI) for the partial Research Grant towards this project and Kenya Wildlife Services (KWS) and from the National Environment and Management Authority (NEMA) for providing permits to access and collect samples from Lake Bogoria.

REFERENCES

Asan A (2004). Aspergillus *Aspergillus, Penicillium* and related species reported from Turkey. Mycotaxon 89:155-157. http://www mycotaxon.com/resources/checklists/Checklist001.pdf

Barreto MC, Frisvad JC, Larsen TO, Mogensen J, San-Romão MV (2011). Exo-metabolome of some fungal isolates growing on cork-based medium. European Food Research and Technology 232:575-582.

Barseghyan GS, Wasser SP (2010). "Species diversity of hypogeous Ascomycetes in Israel. Mycobiology 38(3):159-165.

Baumgarte S (2003). Microbial diversity of soda lake habitats. PhD thesis, Carolo-Wilhelmina University, Braunschweig.

Bell TH, El-Din Hassan S, Lauron-Moreau A, Al-Otaibi F, Hijri M, Yergeau E, et al. (2014). Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. The ISME Journal 8:331-343.

Castro GR, Ferrero MA, Méndez BS, Sineriz F (1993). Screening and selection of bacteria with high amylolytic activity. Acta Biotechnology. Acta Biotechnology 13:197-201.

Cappuccino JG, Sherman N (2002). Microbiology: A laboratory manual. 6th Edition. Pearson Education inc. San Francisco, California 2:15-224.

Christensen M, Frisvad JC, Tuthill DE (2000). Penicillium species diversity in soil and taxonomic and ecological notes. In: Samson RA, Pitt JI (Eds) Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification, pp. 309-320. Singapore: Harwood Academic Publishers.

Cioni R, Fanelli G, Guidi M, Kinyariro JKL (1992). Marini Lake Bogoria hot springs (Kenya): geochemical features and geothermal implications Journal of Volcanology and Geothermal Research 50(3):231-246.

Damare S, Raghukumar C, Raghukumar S. (2006). Fungi in deep-sea sediments of the Central Indian Basin. Deep-Sea Research Part 1 Oceanographic Research Papers 53(1):14-27.

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Grant S, Grant D, Brian EJ, Kato C, Li L (1999). Novel archaeal phylotypes from an East African alkaline saltern. Extremophiles 3:139-145.

Grant WD (2006). Alkaline environments and biodiversity, in extremophilies. In: Gerday Charles, Glansdorff Nicolas (eds). Encyclopedia of Life Support Systems (EOLSS). Developed under the Auspices of the UNESCO, Eolss Publishers: Oxford, UK. http://www.eolss.net.

Hankins L, Anagnostakis SL (1975). The use of solid media for the detection of enzyme production by fungi. Mycologia 67:597-607.

Horikoshi K. (1998). Alkaliphiles. In*:* K. Horikoshi and W. Grant (ed.). Extremophiles Microbial Life in Extreme Environments pp. 1-24. Wiley-Liss, Inc. New York.

Horikoshi K (1999). Alkaliphiles: some applications of their products for biotechnology. Microbiology and Molecular Biology Review 63:735-750.

Jain N (2001). Antidermatophytic activity of some plant metabolites. PhD. Thesis, Botany Department, University of Rajasthan, Jaipur, India.

Jaouani A, Mohamed N, Valeria P, Amani A, Imed S, Sonia BA, Seifeddine BT, Giovanna CV, Ameur C, Maher G (2014). Diversity and Enzymatic Profiling of Halotolerant Micromycetes from Sebkha El Melah, a Saharan Salt Flat in Southern Tunisia. BioMed Research International 2014:11.

Joshi AA, Kanekar PP, Kelkar AS, Shouche YS, Vani AA, Borgave SB, Sarnaik SS. (2008) Cultivable bacterial diversity of alkaline Lonar Lake, India. Microbial Ecology 55:163-172.

Jukes TH and Cantor CR (1969). Evolution of protein molecules. In Munro HN, editor, Mammalian Protein Metabolism pp. 21-132, Academic Press, New York.

Kambura AK, Mwirichia RK, Kasili RW, Karanja EN, Makonde HM, Boga HI (2016) Diversity of fungi in sediments and water sampled from the hot springs of Lake Magadi and Little Magadi in Kenya African Journal of Microbiology Research 10(10):330-338.

Keast D, Rowe P, Sanfellieu L, Shhannan J, Bowra B, Skates S, Stapley EO, Woodruf HB (1984). Use of computers to group actinomycetes for studies on the ecology of soil microorganisms. Journal of Applied and Environmental Microbiology 48:791-796.

Kevbrin V, Romanek C, Wiegel J (1998). Alkalithermophiles: a double challenge from extreme environment. Biochemical Society Transaction 1:1-16.

Kieser T, Bibb MJ, Buttner MJ Chater KF (2000). Practical Streptomyces genetics. Hopwood D. A. (Ed.). John Innes Centre, Norwich, England.

Kumar S, Stecher G, Tamura K (2015). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7):1870-1874.

Kalsbeek V, Mullens BA, Jespersen JB. (2001). Field studies of *Entomophthora* (Zygomycetes: Entomophthorales) - induced behavioral fever in *Musca domestica* (Diptera: Muscidae) in Denmark. Biological Control 21:264-273.

Madigan M, Martinko J (2005). Brock Biology of Microorganisms (11th ed.). Prentice Hall. ISBN 0131443291.

Maciá-Vicente JG, Ferraro V, Burruano S, Lopez-Llorca LV (2012). Fungal assemblages associated with roots of halophytic and non-halophytic plant species vary differentially along a salinity gradient. Microbial Ecology 64:668-679.

McCall J (2010). Lake Bogoria, Kenya: hot and warm springs, geysers and Holocenestromatolites. Earth-Science Reviews 103 (1-2):71-79.

Malaviya P, Rathore V (2007). Bioremediation of pulp and paper mill effluent by a novel fungal consortium isolated from polluted soil. Bioresource Technology 98:3647-3651.

Moreira FMS, Siqueira JO (2002). Microbiologia e bioquímica do solo. Lavras: UFLA, P 626.

Mugo K (2007). Lake Bogoria National Reserve, World Ramsar Site (No. 1057). Integrated Management Plan 2007–2012. WWF-EARPO, County Council of Baringo, County Council of Koibatek. pp. 1-48.

Nakamura H, Kobayashi J'I, Nakamura Y, Ohizumi Y, Kondo T, Hirata Y (1993). Theonellamine B, a novel peptidal Na,K-ATPase inhibitor from an Okinawan marine sponge of the genus *Theonella*. Tetrahedron Letters 27:4319-4322

Nazina TN, Taurova TP, Poltaraus AB, Novikova EV, Grigoryan AA, Ivanova AE, Lysenko AM, Petrunyaka VV, Osipov GA, Belyaev SS, Ivanov MV (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus, Bacillus thermocatenulatus, Bacillus thermoleovorans,Bacilluskaustophilus, Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus, G.thermocatenulatus, G. thermoleovorans, G. kaustophilus, G. thermoglucosidasius and G. thermodenitrificans*. International Journal of Systematic and Evolutionary Microbiology 51:433-446.

Qin Y, Pan X, Kubicek C, Druzhinina I, Chenthamara K, Labbé JL, Yuan Z (2017). Diverse Plant-Associated Pleosporalean Fungi from Saline Areas: Ecological Tolerance and Nitrogen-Status Dependent Effects on Plant Growth. Frontiers in Microbiology 8:158.

Rees HC, Grant WD, Jones BE, Heaphy S (2004). Diversity of Kenyan soda Lake Alkaliphiles assessed by molecular methods. Extremophiles 8:63-71

Renaut RW, Owen RB (2005). The geysers of Lake Bogoria, Kenya Rift Valley, Africa. GOSA Transactions 9:4-18.

Saha A, Mandal P, Dasgupta S, Saha D (2008). Influence of culture media and environmental factors on mycelial growth and sporulation of Lasiodiplodia theobromae (Pat.) Griffon and Maubl. Journal of Environmental Biology 29(3):407-410.

Salano OA, Makonde HM, Kasili RW, Wangai LN, Nawiri MP, Boga HI (2017). Diversity and distribution of fungal communities within the hot springs of soda lakes in the Kenyan rift valley. African Journal of Microbiology Research 11(19):764-775.

Sambrook KJ, Fritsch, EF, Maniatis T (1989). Molecular Cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Shayne JJ, Hugenholtz P, Sangwan P, Osborne C and Janssen HP (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. Applied and Environmental Microbiology 69:7211-7214.

Sierra GA. (1957). A simple method for the detection of lypolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substracts. Antonine van Leeuwenhoeck 28:15-22.

Stamford TL, Araújo JM, Stamford NP (1998) Atividade enzimática de microrganismos isolados de jacatupé (*Pachyrhizus erosos* L. Urban). Ciência e Tecnologia dos Alimentos 18(4):382-385.

Song ZQ, Wang FP, Zhi XY, Chen JQ, Zhou EM, Liang F, Xiao X, Tang SK, Jiang HC, Zhang CL, Dong H, Li WJ (2013). Bacterial and archaeal diversities in Yunnan and Tibetan hot springs, China. Environmental Microbiology 15:1160-1175.

Spear JR, Walker JJ, McCollom TM (2005). Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. Proceedings of the National Academy of Sciences of the United States of America 102:2555-2560.

Vieira JDG (1999). *Purificação e caracterização de uma* a-*amilase de Streptomyces sp.* PhD. Thesis, Universidade de São Paulo, Brazil.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In

Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: a guide to methods and applications. New York: Academic Press.
Zavarzin GA, Zhilina TN, Kevbrin VV (1999). The alkaliphilic microbial community and its functional diversity. Microbiology 68:503-521.
Zhao S, Shamoun SF (2006). The effects of culture media, solid substrates, and relative humidity on growth, sporulation and conidial discharge of Valdensinia heterodoxa. Mycological Research 110(11):1340-1346.

Related Journals: