SECONDARY METABOLITES OF *ASPERGILLUS* SP ISOLATED FROM LAKE ELEMENTAITA

F. I. Ndwigah[1], H. Boga[1], W. Wanyoike[1] andR. Kachiuri[2]
[1]Department of Botany, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
[2]Institute of Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
E-mail: irerifin@gmail.com

Abstract
The soda lakes of Kenya provide an extreme environment where diverse groups of microorganisms thrive. Soda lakes are characterized by great variation in temperature, halophillic and alkaliphilic- extreme conditions. Fungi from such extreme environment may have modified their biosynthetic pathways to produce enzymes and compounds with antimicrobial activity. A fungus in the genus *Aspergillus* was isolated from the sediments of Lake Elementaita and screened for the ability to produce secondary metabolites. The objective of this study was to characterize and identify the fungus and screen for potential production of secondary metabolites. Malt extract agar was used for the isolation of fungus from the sediments, morphological studies as well as the effect of temperature, salinity and pH on growth. Molecular characterization of the 18s rDNA was done using fungal primers in this study. Fermentation of fungal isolate in 500ml conical flasks was done in fourteen days duration under a shaker. Extraction of the filtrate was done using the solvents, Ethyl acetate and hexane in the ratio 4:1. A rotary evaporator was use to evaporate the filtrate. The dry pellet was eluted in 1ml ethyl acetate, Antimicrobial activity was assayed using six millimeter paper disc loaded with the elute. GC-MS was used to analyze and identify the secondary metabolites produced by the fungus. The fungus grew well in alkaline pH and a temperature range of 26º C to 30 ºC. Growth was only observed on 0% to 10% NaCl.M olecular analysis showed that the isolate is closely aligned to the genus *Aspergillus* GC-MS identified a range of groups of metabolites from the isolates and some are kwon to have antimicrobial activity. The results shows that this fungus can only tolerate relatively low NaCl concentration of up to 10% .and grows best at alkaline conditions. The fungus has potential for producing metabolite which can be explored for future application in industries and pharmaceuticals.

Key words*:* *Aspergillus*, characterization, saline-alkaline, secondary metabolites

1.0 Introduction

The alkaline saline, soda lakes of Kenyan Rift valley include Lakes Bogoria, Elementaita, Magadi, Nakuru, Natron and Sonachi (formerly Naivasha Crater Lake). Their development is a consequence of geological and topological factors (Mwatha, 1991).

Soda Lakes are formed by unusual combination of environmental factors, which result in large amount of sodium carbonate and have very high concentration of Ca^{2+} and Mg^{2+}, which are insoluble as carbonates minerals under alkaline conditions. The pH of the lakes range from 8 to12 (Grant and Mwatha, 1989, Jones *et al*., 1994), while the salinity is around 5 % total salts (W/V) in Lake Bogoria, Nakuru, Elementaita and Sonachi but saturated in Lakes Magadi and Natron with roughly equal proportions of Na_2CO_3 and NaCl as major salts (Mwatha, 1991).

The soda lakes also exhibit active volcanism with numerous hot springs (some boiling) on the shores of some of the lakes. Microorganisms found in the soda lakes have to endure extreme environmental conditions in terms of pH, salinity and temperature at the hot springs. The soda lakes of East African provide an extremophilic environment; super-adoption to such extremely harsh environment could make such micro-organisms ideal candidates for exploitation of secondary metabolites of biotechnological applications. The catchments areas of some the soda lakes have been degraded due to human activities. Industrial and domestic effluents also find their way into the lakes. These pollutants may affect the biodiversity of these ecosystems.

The extreme environment may stimulate the microorganisms to produce a range of metabolites of which some may be potential antimicrobial agents. The fungi from the extreme environment have a great potential to produce natural antimicrobials and enzymes. Such fungi have been adapted to alkaline saline condition and relatively high temperatures and in this process they might have changed or modified their metabolic pathways to produce different or improved metabolites, which may have antimicrobial activity.

The literature cited shows little or no information on the soda lake fungi of the East African Rift Valley. However some thermophilic fungi such as *Rhizumucor miehei, Chaetomium thermophile*, Melanocarpus *albomyces* etc, have been isolated from compost, soils and other sources (Tansey, 1978, Reysenbach, 2002). This study is expected to accomplish the secondary metabolites of a fungal isolate from Lake Elementaita. Soda Lakes are formed by unusual combination of environmental factors, which result in large amount of sodium carbonate ($Na_2CO_3.10H_2O$ or $Na_2CO_3.Na_2HCO_3.2H_2O$) and very high concentration of calcium and magnesium ions, which are insoluble as carbonates minerals under alkaline conditions.

The soda lakes also exhibit active volcanism with numerous hot springs (some boiling) on the shores of some of the lakes and geologically, alkaline trachyte lavas predominate. The salinity of the soda lakes varies according to the geographical location and also according to seasonal weather conditions. These are extreme environments where only adapted microorganism can survive.

Extremophiles have been defined based on the nature of environment where they are found. Extremophiles that are adapted to high temperatures are called thermophiles, acidophiles are adapted to low pH and alkaliphiles are adapted to highly alkaline conditions (high pH). Some organisms occur in one or more environmental extremes simultaneously and are called polyextremophiles. An example is the archeabacterial species, *Sulfolobusacidocalderius*. It can survive at temperatures exceeding 80^oC and at acidities less than pH 3. Halophiles can survive in high salt concentration environments (Horikoshi, 1998), a characteristic of the Kenyan soda lakes.

The lake is located in a basin whose water budget is maintained by recharge from hot springs located on the southern lakeshore, two in flowing rivers, surface run off, direct rainfall and evapo-transpiration. The lake has no surface outlet or underground seepage for releasing its water to other aquifers. The lake is not protected and is surrounded by private and communal lands. It is also part of a wider human catchments basin where human population has been increasing rapidly in recent years. Various anthropogenic disturbances have been recorded in the Lake Elementaita area, like the degradation of the catchments through deforestation of the natural forest and woodlands have either been removed or modified into shrubs and bush land by cultivation, grazing and fires.

The catchment's area is under diverse land use practices, which include mining of sand, (salt and diatomite) and agriculture, ranching, forest conservation, urbanization, transportation and human settlements among others.Private ranches e.g., Soysambu Wildlife Sanctuary cover about 75% of the shoreline and is well protected though there is overgrazing during periods of drought especially in the southern eastern sector which accelerates erosion after heavy rains. Urban effluent disposal into streams is noticeable, particularly in the absence of any sewage treatment, Pollution from the factories like the Diatomite Company that undertakes diatomite mining and the treatment of poles by Cabro East African Ltd Company involves in seasoning of poles to about 25% moisture content and impregnation with Copper Chromium Arsenate (CCA) in a pressurized treatment cylinder (Mwaura, 1999).

1.1 Heavy Metal and Pesticide Pollution of Soda Lakes

Water, sediment, fish and algae collected from the lake and its feeder rivers during the dry and rainy seasons were analysed for heavy metals and organochlorine pesticide residues to identify possible sources of contaminants. Atomic absorption spectrophotometry and gas chromatography showed that more contaminants were added to the lake during the rainy season than the dry season. Heavy metals and pesticide residues were found in higher concentrations in the sediments than in the water.

Contamination by most of the pollutants has increased over the past 25 years. Dichloro diphenyl dichloro ethane (DDD) was more prevalent in the sediments and particulates in the water, but Dichloro diphenyl chloroethylene (DDE) was predominant in fish, suggesting two different mechanisms of Dichloro diphenyl trichloroethane (DDT) degradation in the biotic and abiotic environments. Whereas heavy metals tend to be distributed almost uniformly within the lake, pesticide residues are found predominantly in its northern and southern river inlets. Some contaminants occur in slightly higher concentration upstream, perhaps due to contamination from point sources, followed by dilution (Mavura and Wangila, 2003).

1.2 Fungi

Fungi are eukaryotic organisms that have a heterotrophic mode of nutrition. They are adapted to different types of environments such as fresh water, high temperatures and alkaline- saline environments (Alexopoulos and Mims, 1979).The literature available shows little or no information on the soda lake fungi of the East African Rift Valley. Studies from other extreme environments have shown that fungi can be isolated from thermophilic environment. However some thermophilic fungi such as *Rhizumucor miehei, Chaetomium thermophile*, *Melanocarpus albomyces* etc, have been isolated from compost, soils and other sources (Tansey,1978, Reysenbach, 2002). Recently, different species of black yeast have been isolated from hyper- saline waters of solar saltans (Gunde-Cimeman*et al.,* 2000). These new fungi were described as new groups of eukaryotic halophiles, and they are represented by *Hortaea werckii*, *Phaeotheca triangularies, Trimmasrostroma salinum*, and halotorelant *Aureobasidium pulluns* (De-Hoog *et al.,* 1999). *Cladosporium glycolicum* was found growing on submerged wood in the Great salt lakes. Buchalo *et al.,* (2000) reported twenty six (26) fungal species representing thirteen (13) genera of Zygomycetes (*Absidia glauca*), Ascomycotina (*Chaetomium aureum, C.flavigenum, Emericella nidulans, Eurotium amstelodami* and mitosporic fungi (*Acremonium persicinum, Stschbotrys chartarum, Ulocladium chlamydosporum*) from the Dead Sea and hence therefore high chances of isolating fungi from the Kenyan soda lakes.

1.3 Halophilic Fungi

The importance of halophilic fungi, have long been neglected as members of hypersaline ecosystems. The biology of the most widespread and most halophilic or halotolerant fungi and yeasts has been reviewed. These include the black yeasts *Hortaea werneckii* which grows up to 5M NaCl, the true halophile Wallemia ichthyophaga that requires at least 1.5M NaCl and grows up to saturation, and *Aureobasidium pullulans* that grows up to 3M NaCl. All of these are commonly found in hypersaline lakes and in a great variety of other, often unexpected, environments: domestic dishwashers, polar ice, and possibly even on spider webs in desert caves. (Gunde-Cimerma*et al* 2009).

The halophilic and halotolerant fungi use polyols such as glycerol, erythritol, arabitol, and mannitol as osmotic solutes and retain low salt concentrations in their cytoplasm. Molecular studies on osmotic adaptation of *Hortaea werneckii* and *Wallemia ichthyophaga* have been presented. Identification and structural features of Na+-sensitive 3'-phosphoadenosine-5'-phosphatase HwHal2, one of the putative determinants of halotolerance in *H. werneckii* and a promising transgene to improve halotolerance in crops, was presented. (Vaupotič *at al* 2007). An in-depth understanding has been obtained of the HOG (high osmolarity glycerol) pathway, and this understanding may be applied in the future to the development of improved salt-resistant crops. Glycerol-3-phosphate dehydrogenase is involved in glycerol synthesis by both *Wallemia* and *Hortaea,* had heterologous expression of the gene encoding the enzyme can restore halotolerance in *Saccharomyces cerevisiae* deficient in glycerol production .Most of the fungi that can be found in extreme environments belong to the imperfect stage of the Ascomycota, which have been reported in mangroves, saline soils, marine sediments, sea water, salt marshes, and sand dunes.(Hyde and Pointing 2000). Because fungi were only recently isolated from hypersaline environments, their function in these extreme conditions is still unclear (Gunde-Cimerman *et al.,* 2004).

Reports on presence of filamentous fungi in the hypersaline waters of the Dead Sea (340 gL^{-1} total dissolved salts), survival of their spores and mycelia in this hostile environment have invoked great interest (Buchalo *et al*., 1998; Kis-Papo *et al.,* 2003). Further, a gene responsible for High Osmolarity Glycerol (HOG) response pathway from one such Dead Sea-fungus *Eurotium herbariorum* has been identified for stress tolerance to freezing and thawing (*Jin et al*., 2005). This has further led to production of a recombinant yeast Saccharomyces cerevisiae containing the gene HOG (Norbeck *et al*., 1996).

A diverse fungal fauna was recently discovered in environments with salinities ranging between 15–32 %, where it was so far assumed that bacteria only were able to grow. These fungi were first isolated in hypersaline waters of Secovlje salterns in Slovenia (Gunde-Cimerman *et al.,* 2000). The majority of species isolated belonged to melanized meristematic and yeast-like fungi, and a few different genera of filamentous fungi were also identified (Méjanelle *et al.,* 2001).The fungi from the extreme environment have a great potential to produce natural antimicrobials and enzymes. (Pilnik and Rombouts, 1985; Falch, 1991; Rao *et al.,* 1998).

1.4 Secondary Metabolites

Secondary metabolisms of fungi generate diverse and seemingly less essential or non-essential by-products called secondary products. The secondary products, having no role in the basic life process, are produced by pathways derived from primary metabolic routes. Secondary metabolic products constitute a wide array of natural products. They are derived from the primary products, such as amino acids or nucleotides, by modifications, such as: methylation, hydroxylation, and glycosylation (Bentley and Bennet, 1988)., fungi have only been surpassed by actinomycetales as a source for biologically active metabolites. The fungal biodiversity on land seems to be nearly exhausted. Thus, nowadays, researchers throughout the world have paid increasingly attention toward the potential of marine microorganism as an alternative source for isolation of novel metabolites. The estimated 3000 to 4000 known fungal secondary metabolites have been isolated, possibly not more than 5000 to 7000 taxonomic species have been studied in this respect. Genera such as:*Aspergillus, Penicillium, Fusarium,* and *Acremonium* are among fungi highly capable of producing a high diversity of secondary metabolites.

Polyketides are natural products which provide a staggering range of clinically effective drugs. Acetyl-CoA is the most precursor of fungal secondary metabolites, leading to polyketides, terpenes, steroids, and metabolites derived from fatty acids. Other secondary metabolites are derived from intermediates of the shikimic acid pathway, the tricarboxylic acid cycle, and from amino acids. Current research in drug discovery from medicinal higher fungi involves a multifaceted approach combining mycological, biochemical, pharmacological, metabolic, biosynthetic and molecular techniques. In recent years, many new secondary metabolites from higher fungi have been isolated and are more likely to provide lead compounds for new drug discovery, which may include chemo preventive agents possessing the bioactivity of immune modulatory, anticancer, etc. However, numerous challenges of secondary metabolites from higher fungi are encountered including bioseparation, identification, biosynthetic metabolism, and screening model issues, etc (zhong and Xiao. 2009).However the literature cited shows little or no information on the soda lake fungi in the Kenyan Rift Valley.

2.0 Materials and Method

Sediment samples were collected from Lake Elementaita in the Keyan Rift Valley atelevation 1774m above sea level. Samples were collected at different sites and then pulled together as a single sample which was transferred to the laboratory for fungal isolation. Malt Extract Agar (MEA) and Potato Dextrose Agar media were used to isolate fungi from the sediment sample and the establishment of pure cultures from which morphological studies were conducted. Colony diameter were measured in mm per fungal isolate and recorded. Effect of PH and Sodium Chloride (NaCl) concentration on growth was done by measuring the radial growth of isolate on malt extract medium in a peridish.

2.1 DNA Extraction

DNA was extracted using the bead beater machine method and two lyses buffers as solution A (50mM Tris pH 8.5, 50mM EDTA pH 8.0 and 25% sucrose solution) and solution B (10mM Tris pH 8.5, 5mM EDTA pH 8.0 and 1% SDS). Total genomic DNA of the isolates was extracted from these cells in duplicate using two lysis buffers as solution A (50mM Tris pH 8.5, 50mM EDTA pH 8.0 and 25% sucrose solution) and solution B (10mM Tris pH 8.5, 5mM EDTA pH 8.0 and 1% SDS). The cells were scrapped asceptically using a sterile surgical blade taking care not to pick the media. These were crushed separately in 200µl solution A using sterile mortar and pestle, and resuspended in 100µl of solution A. This was followed by addition of 30µl of 20mg/l Lysozyme and 15µl of RNase, gently mixed and incubated at 37^{o}C for two hours to lyse the cell wall. 600µl of Solution B was then added and gently mixed by inverting the tubes severally, followed by the addition of 10µl of Proteinase K (20mg/l) and the mixture incubated at 60^{o}C for 1 hour. Extraction followed the phenol/chloroform method (Sambrook *et al.,* 1989). The presence of DNA was checked on 1% agarose and visualized under ultraviolet by staining with ethidium bromide. The remaining volume was stored at -20^{o} C. The genomic DNA was used as templates for subsequent PCR amplification.

Total DNA from each isolate was used as a template for amplification of the 18S rRNA genes. Nearly full-length 18S rDNA gene sequences were PCR-amplified using fungal primer pair Fung5f forward 5'-GTAAAAGTCCTGGTTCCCC-3' and FF390r reverse, 5'-CGATAACGA ACGAGA CCT-3'(Vainio and Hantula, 2000) and Lueders *et al.* (2004). Amplification was performed using Peqlab primus 96 PCR machine. Amplification was carried out in a 40µl mixture

containing 5µl of PCR buffer (×10), 3µl dNTP's (2.5mM), 1µl (5 pmol) of Fung5f forward primer, 1µl (5pmol) of FF390r reverse primer, 0.3µl taq polymerase, 1.5µl of template DNA and 28.2µl of water. The control contained all the above except the DNA template. Reaction mixtures were subjected to the following temperature cycling profiles repeated for 36 cycles: Initial activation of the enzyme at 96^{o} C for five minutes, denaturation at 95^{o} C for 45 seconds, primer annealing at 48^{o}C for 45 seconds, chain extension at 72^{o} C for 1.30 minutes and a final extension at 72^{o} C for 5 minutes. Amplification products (5µl) were separated on a 1% agarose gel in 1× TBE buffer and visualized under ultraviolet by staining with ethidium bromide (Sambrook *et al*., 1989). PCR products for each isolate was purified using the QIAquick PCR purification Kit protocol (Qiagen, Germany) and then sent for sequencing at ILRI.

2.2 Fermentation of Fungi in Liquid Medium

Each of the fungal isolate was grown in liquid medium composed of 15g malt extract, 5g Bacteriological peptone, 5g Glucose, 2% NaCl in 1l of distilled sterile water at pH 8.5.2500ml of the stile medium was dispensed into sterile 500ml conical flasks. Each flask was inoculated with a four millimeter agar disc cut from two days fungal isolate culture and incubated at ± 28^{o} C in a shaker (1000RP/Minute) for fourteen days. The crude filtrate was recovered for each fungal isolate and subjected to ethyl acetate/hexane extraction (ratio 2:1) three times. The precipitate was eluted with 1ml ethyl acetate. Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis was done for secondary metabolites identification in isolates extract filtrate.

3.0 Results

The morphology of the fungal isolate was grey, septate hyphae and phialidic conidia.

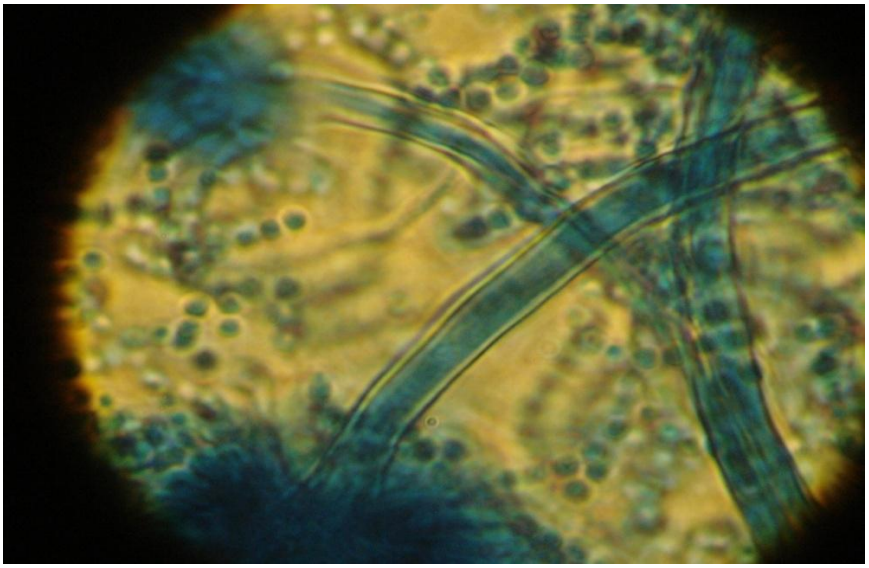

Figure 1: Shows fruiting body, conidiophores and conidial chains

3.1 Growth of Isolates

Effect of growth on isolate at 28 ºC±1 for eight day incubation.

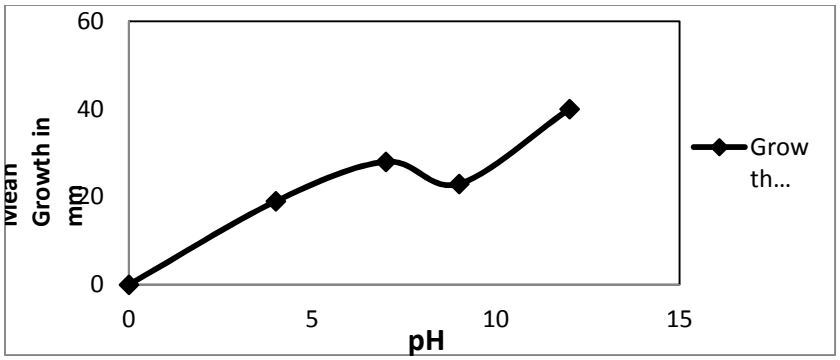

Figure 2: Growth effect on pH of the isolates

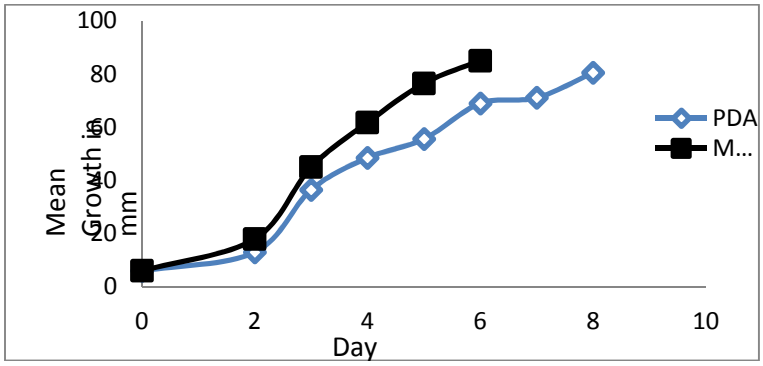

Figure 3: Growth effect on malt extract agar (M*EA) and potato dextrose agar (PDA) media*

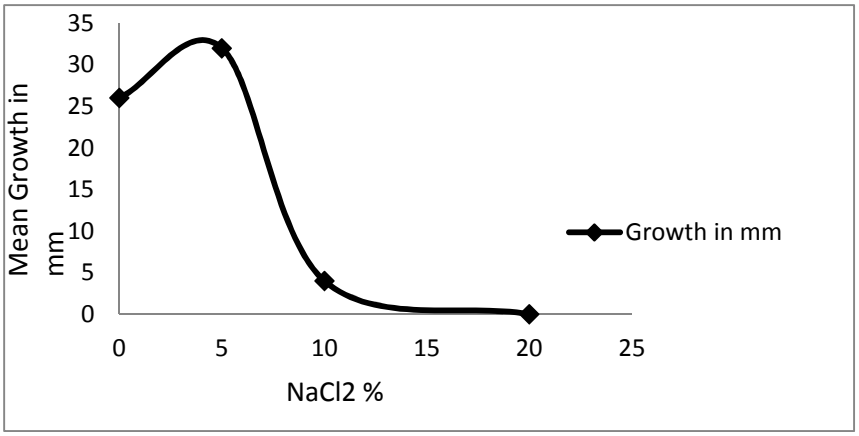

Figure 4: Growth effect of NaCl on isolates

3.2 Molecular Characterization

The 18s DNA gene sequence for the isolate.

TTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGG
AATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCTAGGACCGCCGTAATGATTA
ATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTG
AAGACTAACTACTGCGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGT
TAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGG
GATCGGGCGGTGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGG
GTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCAC
AAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACA
AAATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTGGATGGTGGTGCATGGC

Figure 5: Shows the nucleotide sequence representing the conserved genes of the fungus.

3.3 BlastResults Showing the Closest Neighbors

Table 1: Showing the closest neighbors and percentage similarity

Fungi- 15	Accession	Class	% Similarity
Aspergillus sp	KC120773	Ascomycota; Pezizomycotina; Eurotiomycetes;	99
Aspergillus terreus strain	JQ812052	Ascomycota; Pezizomycotina; Eurotiomycetes	99
Aspergillus fumigatus strain	HQ871898	Ascomycota; Pezizomycotina; Eurotiomycetes	99
Aspergillus flavus isolate	JF824683	Ascomycota; Pezizomycotina; Eurotiomycetes	99

3.4 Secondary Metabolites from the Isolate

Literature comparison of mass spectra was used in compound identification. A total of seventeen different compounds were identified in fungal isolate extract (Table 2, Fig 6). Concentration of compounds for this isolate ranged between 0.1 % to 3.9 %. Isopentyl alcohol (3.9%) was the most abundant compound followed by Cresol<meta-> (3.3%) and Propanoic acid, 2-methyl-(2.5%). The least two in abundance were 2,3-Butanediol (0.2%) and N-(1-Cyclopenten-1-yl)-morpholine (0.1%).

Table 2: Metabolite Profile for the isolate

Peak no.	Rt (min)	Metabolite	% area
1	3.905	2-Butanone, 3-hydroxy-	0.940
2	4.699	Isopentyl alcohol	3.909
3	5.192	1,8-Nonadien-3-ol	0.197
4	5.617	Propanoic acid	0.540
5	7.611	2,3-Butanediol	0.178
6	8.820	Propanoic acid, 2-methyl-	2.508
7	9.335	Isovaleric acid	1.439
8	11.390	Trimethyl benzene<1,2,4->	1.015
9	12.426	Cyclopentene<3,5-dimethylene-1,4,4-trimethyl->	0.731
10	12.538	Cymene<ortho->	0.510

11	13.613	Pentanoic acid, 2-hydroxy-4-methyl-,	0.728
12	14.979	2-Coumaranone	0.647
13	18.272	4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-	1.875
14	19.772	Benzaldehyde, 2-hydroxy-5-methoxy-	0.614
15	19.862	N-(1-Cyclopenten-1-yl)-morpholine	0.147
16	22.751	Flopropione	2.463
17	28.082	Cresol<meta->	3.348

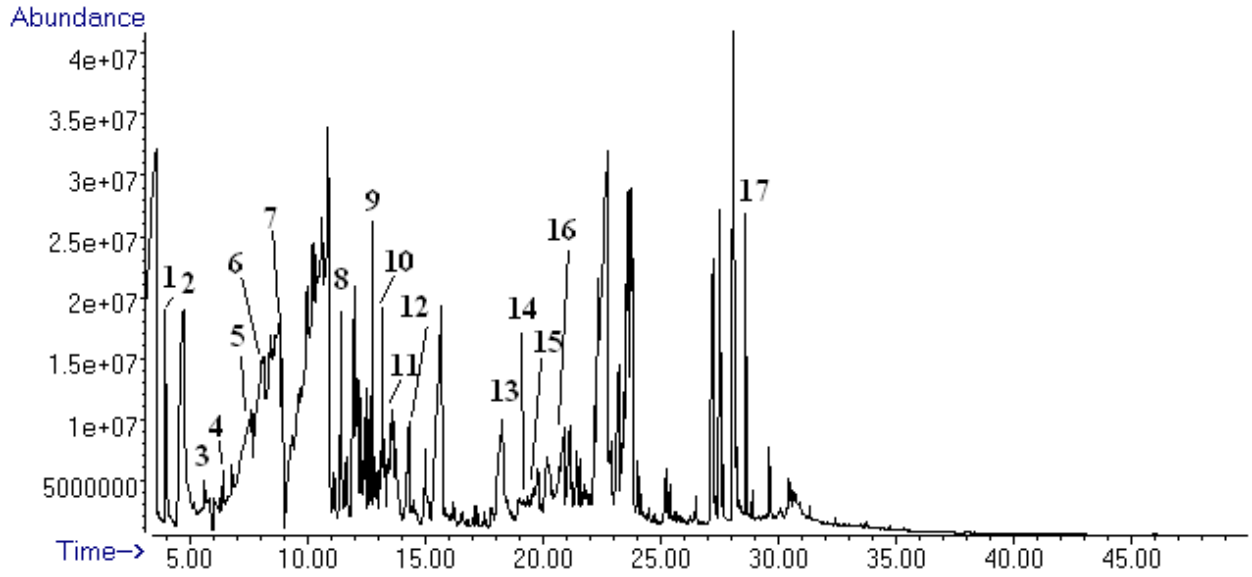

Figure 6: Mass spectrum for the isolate showing the seventeen metabolites

4.0 Discussion

The objective of this study was to characterize, identify and screen for secondary metabolites produced by the fungal isolate. Morphology of the isolate reviewed anamorphic structure of the genus *Aspergillus.*

The isolate grew best on MEA and least on PDA media at pH9, and 5% NaCl. on MEA at pH9 (Figure 3). Growth of the isolateincrease with increasing pH but shot up at alkaline PH 10and 12 indicating that the isolate is well adapted for alkaline environment (Figure 2). This growth at pH range of 5 to10 is in consistent with an earlier study by (Horikoshi, 1998) which showed that a low to high pH range of 5.7 to 9.0, favours growth of alkaliphiles and that a pH range 9.0 to 10 may serve as their selective optimum pH.

The effect of NaCl concentration showed that the isolate grew well at the range of 0-10% NaCl. An increase in salt concentration resulted in a drastic decrease in growth, which was arrested at 20% NaCl. (Figure 4) thus the fungal isolate thrives in low saline condition. Recently it has been shown that fungi are not only able to sustain, but also to propagate at different environmental extremes such as hypersaline waters (Gunde-Cimerman *et al.,* 2000).

Molecular characterization showed that the isolateis plased in phylum Ascomycota, sub phylum Pezizomycotina, and class EurotiomycetesIt was closelyaligned to the genus *Aspergillus sp*. (KC120773), *Aspergillus terreus* (JQ812052),*A.fumgatus* (HQ871898), and *A.flavus* (JF824683) all with 99% similarity.Result indicates that the fungal isolate an *Aspergillus* species but more molecular information is required to specify the correct genus. (Table 1).

GC-MS analysis identified seventeen different metabolites from the isolate extract filtrate. The compounds are in the chemical groups of acid, alcohols, ketones, aldehydes and heterocyclic compounds among others, such

chemical groups of compounds are known to have antimicrobial activity. These results could suggest that the isolate has high potential of producing metabolites that may be applicable in biotechnological application.

5.0 Conclusions and Recommendations

The fugal isolate from the sodaLake Elementaita belongs to the genus *Aspergillus* in the phylum Ascomycota. The fungus grew well at alkaline P^H 9 to12 range and at salt (NaCl) concentration of 0 to 5% on MEA medium. The isolate has high potential of producing a range of metabolites which can be explored for future application in industries. There is need for extensive studies to characterize the isolate further and to characterize the secondary metabolites using fraction guided GC-MS. The individual metabolites should be assayed for antimicrobial activity.

References

Alexopoalos, C.J. and Mims, C.W. (1979). Introductory mycology 3rd edition. John and sons, New York.

Zhong J. J. and Xiao J.H.(2009). Secondary metabolites from higher fungi: discovery, bioactivity, and bioproduction.*Advanced Biochemical Engineering/ Biotechnology*, **113**, pp 79-150.

Hibbett, D.S., *et al.* (2007). "A higher level phylogenetic classification of the Fungi". *Mycological Research,* **111** (5), pp 509–47.

Buchalo, A.S., Nevo, E., Wasser, S.P. and Volz, P.A., (2000). Newly discovered halophilic fungi in the Dead Sea (Israel). Journey to the Diverse microbial Worlds (ed. Seckbach, J.), Kluwr, Dordrecht, **2**, pp 241-252.

De Hoog, G.S., P. Zalar, C. Urzi, F. de Leo, N.A. Yurlova, and K. Sterflinger.(1999). Relationships of dothideaceous black yeast and meristematic fungi based on 5.8S and ITS2rDNA sequence comparison. *Studies in Mycology*,**43**,pp 31-37.

Grant, W.D. and Mwatha, W.E., (1989). Bacteria from alkaline saline environment. In recent advances in microbial ecology. Japan scientific press, 64-67.

Gunde-cimerman, N., P. Zalar, G.S. de Hoog, and A. Plemenitas. (2000). Hypersaline watersinsalterns: Natural ecological niches for halophilic black yeasts. *FEMS microbial. Ecol.***32**, pp 235240.

Horikoshi, K., and W.D. Grant, (1998). Extemophiles: Microbial Life in Extreme Environments. New York: Wiley Liss,

Horikoshi, K. and Akiba, T., (1982). Alkaliphilic microorganisms. Springer, Berlin. Heidelberg. Hydrobiol. 81: 71-86. **In:** Enzymes system for lignocellulose degradation (Coughlan, M.P., Ed.)
Elsevier science publishers Ltd: England.

Mavura,W. J. and Wangila, P.T. (2003). The pollution status of lake Nakuru,Kenya: heavy metals and pesticides. *Africanjounal ofAquatic Science*, **28**, PP 13-18.

Mwatha, W.E., (1991). Microbial Ecology of Kenyan soda lakes Ph.D. thesis university of Leicester.Mwura, F., (1999).A Spatial chemical survey of hydrogeothermal springs in Lake Elementaita.*International journal of salt lakes research*, **55,** PP 125-138.

Pirt, S.J. and Righelato, R.C., (1967). Effects of growth rate on the synthesis of penicillin in Penicillium chrysogenum in batch and chemostat cultures. *Applied Microbiology,* **15**, pp 1284-1290.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1987). Molecular cloning - a laboratory manual.Cold Spring Harbor, USA, Cold Spring Harbor Laboratory Press.

Buchalo, AS., Nevo, E., Wasser, S.P., Oren, A. and Molitoris, H.P. (1998). Fungal life in the extremely hypersaline water of the Dead Sea: first records. *Proceedings of the Royal Society of London*-B **265,** pp 1461-1465.

Gunde-Cimerman, N., Zalar, P., Petrovic,ˇ U., Turk, M., Kogej, T., de Hoog, G.S. and Plemenitas, A. (2004). Fungi in the Salterns. In: Ventosa A (ed), Halophilic Microorganisms. Springer-Verlag, Heidelberg, pp. 103–113.

Gunde-Cimerman, N., Zalar, P., de Hoog, S. and Plemenita, S. A. (2000). Hypersaline waters in salterns—natural ecological niches for halophilic black yeasts. *FEMS Microbiology of Ecology*, **32**, pp 235–240.

Hyde, K.D. and Pointing, S.B. (2000). Marine Mycology. A Practical Approach. Fungal Diversity Press, Hong Kong.

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

Jin, Y., Weining, S. and Nevo, E. (2005). A MAPK gene from Dead Sea-fungus confers stress tolerance to lithium salt and freezing-thawing: prospects for saline agriculture. *Proceedings of the National Academy of Sciences,* **102,** pp 18992-18997

Kis-Papo, T., Oren, A., Wasser, S.P. and Nevo, E. (2003). Survival of filamentous fungi in hypersaline Dead Sea water. *FEMS Microbiology Ecology*, **45, pp** 183–190.

Mejanelle, L., Lopez, J.F., Gunde-Cimerman, N., Grimalt, J.O., (2001). Ergosterol biosynthesis in novel melanized fungi from hypersaline environments. Journal of Lipid Research, **42,** pp 352 -358.

De Hoog, G.S., P. Zalar, C. Urzi, F. de Leo, N.A. Yurlova, and K. Sterflinger(1999). Relationships of dothideaceous black yeast and meristematic fungi based on 5.8S and TS2rDNA sequence comparison. *Studies in Mycology,* **43, pp** 31-37.

Vaupoti$\check{c}$ T, Gunde-Cimerman N, Plemenita.$\check{S}$A (2008). Novel 3'-phosphoadenosine-5'-phosphatases from extremely halotolerant Hortaea werneckii reveal insight into molecular determinants of salt tolerance of black yeasts. *Fungal Genetetics and Biology*, **44**, pp 1109 - 22.