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Characterization, Enzymatic Activity, and Secondary Metabolites of Fungal Isolates from Lake Sonachi in Kenya

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***Abstract*:** *The soda lakes of Kenya provide an extreme environment where diverse groups of microorganisms thrive. They are characterized by great variation in temperature, halophillic and alkaliphilic- extreme conditions. Lake Sonachi has been the study site for this research. The study sort to isolate, characterize and identify fungi, screen for potential exo-enzymes and secondary metabolites production that may be of industrial application. Malt extract agar was used in the isolation of fungi and six (6) isolates were recovered. Inhibition zones were used to measure the enzymatic and antimicrobial activity of the isolates. GC-MC analysis was done on the filtrates extracted from the fungi to identify secondary metabolites. Molecular characterization of the 18s rDNA was done using fungal primers and sequencing PCR products.s .Phylogenetic tree was inferred using neighbor- joining method. The fungal isolates were alighned to diferrent genera, Acrimonies sp., Scopulariopsissp, Verticilium sp. Fusariumsp and Paecilomyces sp. The fungal isolates produce different types of enzymes (cellulases, proteases, pectinases and lipases) and metabolites (acids, ketones, quinones, alcohols, esters etc) .Antimicrobial assay showed that most of the fungal isolates produced inhibition zones ranging from 0.1 to 4mm, an indication of presence of compounds with antimicrobial activity against most of the test organisms, E.coli, B. subtilis, S.aureasetc, used in this study. Results indicate that Lake Sonachi, a soda lake has fungal species that are capable of producing enzymes and metabolites with antimicrobial activity.*

Keywords: *Characterization, Enzymatic activity, Antimicrobial activity and, Secondary metabolite*

I. Introduction And Literatute Review

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 [1].

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 [2, 3], while the salinity of these lakes ranges from around 5 % total salts (W/V) in Lake Bogoria, Nakuru, Elementaita and Sonachi but saturated in Lake Magadi and Natron with roughly equal proportions of Na_2CO_3 and Nacl as major salts [1].

Lake Sonachi is a small meromictic volcanic crater lake in Naivasha, Kenya. The factors that contribute to the maintenance of meromixis are basin morphometry, the diurnal periodicity of the winds and of thermal stratification, biological decomposition, and seasonal and yearly changes in rainfall. It is sheltered from wind by crater walls 30-115 m above its surface. Wind speeds have a diurnal pattern and typically were maximal when the lake was thermally stratified. Higher values of hydrogen sulfide, soluble reactive phosphate, and ammonia in the deeper waters, as well as a lower pH value, suggest that biological processes contributed to the meromixis. Freshening of the surface waters by rain contributed to the increased stability, and the conductivity and the volume of water below the chemocline had increased substantially.The lake is dominated by cyanobacterium, Synecoccusbacilaris[4]. Hypersaline environments are found in a wide variety of aquatic and terrestrial ecosystems. They are inhabited by halotolerant microorganisms but also halophilic microorganisms ranging from moderate halophiles with higher growth rates in media containing between 0.5 M and 2.5 M NaCl to extreme halophiles with higher growth rates in media containing over 2.5 M NaCl.Moderate and extreme halophiles have been isolated not only from hypersaline ecosystems (salt lakes, marine salterns and saline soils) but also from alkaline ecosystems (alkaline lakes). The most widely studied ecosystems are the Great Salt Lake (Utah, USA), the Dead Sea (Israel), the alkaline brines of WadiNatrun (Egypt) and Lake Magadi (Kenya) [5]. It is noteworthy that low taxonomic biodiversity is observed in all these saline environments [6], most probably due to the highly salt concentrations measured in these environments. 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 [7].

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 Rhizumucormiehei, Chaetomium thermophile, Melanocarpusalbomycesetc, have been isolated from compost, soils and other sources [8, 9]. Recently, different species of black yeast have been isolated from hyper- saline waters of solar saltans[10]. These new fungi were described as new groups of eukaryotic halophiles, and they are represented by Hortaeawerckii, Phaeothecatriangularies,Trimmasrostromasalinum,andhalotorelantAureobasidiumpulluns[11].
Cladosporiumglycolicumwas found growing on submerged wood in the Great salt lakes. [12], reported twenty six (26) fungal species representing thirteen (13) genera of Zygomycetes (Absidiaglauca), Ascomycotina (Chaetomiumaureum, C.flavigenum, Emericellanidulans, Eurotiumamstelodami and mitosporic fungi (Acremoniumpersicinum, Stschbotryschartarum, Ulocladiumchlamydosporum) from the Dead Sea and hence therefore high chances of isolating fungi from the Kenyan soda lakes. However the literature cited shows little or no information on the soda lake fungi in the Kenyan Rift Valley.

Alkaliphiles isolated in soda lakes have been analyzed and used for their various alkali-tolerant enzymes in many industrial processes [13]. Because these enzymes have the ability to function at high levels of pH, they are particularly useful in processes that require these extreme conditions. These alkaliphiles are thought to have significant economic potential because their specialized enzymes are already "used in detergent compositions and in leather tanning, and are foreseen to find applications in the food, waste treatment and textile industries; additionally, (they) are potentially useful for biotransformations, especially in the synthesis of pure enantiomers" [13]. Specific examples of such enzymes are proteases (used as detergent additives), starch-degrading enzymes, cellulases (laundry detergent additive), and pectinases (used to improve production of paper as well as waste treatment)[14]. Another important application is the industrial production of cyclodextrin by alkaline cyclomaltodextringlucanotransferase. This enzyme has reduced the production cost and paved the way for cyclodextrin use in large quantities in foodstuffs, chemicals, and pharmaceuticals.

The fungi from the extreme environment have a great potential to produce natural antimicrobials and enzymes. The importance of the microorganisms in enzyme production is due to high production capacity, low cost and susceptibility to genetic manipulation. Actually, the enzymes of microbial origin have high biotechnological interest such as in the processing of foods, manufacturing of detergents, textiles, pharmaceutical products, medical therapy and in molecular biology [15].

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, areproduced by pathways derived from primary metabolic routes.

Secondary metabolic products constitute a wide array of natural products. They are derived from the primaryproducts, such as amino acids or nucleotides, by modifications, such as: methylation, hydroxylation,and glycosylation., fungi have only been surpassed by Actinomycetales as a source for biologically active metabolites. The fungal biodiversity on land seemsto be nearly exhausted. Thus, nowadays, researchers throughout the world have paid increasinglyattention toward the potential of marine microorganism as an alternative source for isolation of novelmetabolites.Although the estimated 3000 to 4000 known fungalsecondary metabolites have been isolated,possibly not more than 5000 to 7000 taxonomic species have been studied in this respect. Generasuch as:Aspergillus,Penicillium, Fusarium, andAcremonium 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. These include antibiotics and anticancer drugs .Acetyl-CoA is the most precursor of fungal secondary metabolites, polyketides, terpenes, steroids, and metabolitesderived from fatty acids. Other secondarymetabolites are derived from intermediates of the shikimic acid pathway, the tricarboxylic acid cycle and from amino acids.

II. Materials And Methods

Sediment samples were collected from Lake Sonachi in the Kenyan Rift Valley, position, 37 19 5 036 E and 9991 334 7N, elevation 1885m 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) medium was 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 NaCl on concentration on growth was done by measuring the radial growth of isolate on malt extract medium in a petri-dish.

DNA Extraction

DNA was extracted using the bead beater machine methodand twolyses 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 aseptically using a sterile surgical blade taking care not to pick the media. These were separately put in a tube containing 100µl solution A and beads the subjected to bead beater machine protocol. 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 [16]. 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 rDNA 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'[17]. 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 [16]. PCR products for each isolate was purified using the QIAquick PCR purification Kit protocol (Qiagen, Germany) and then sent for sequencing at ILRI.

Biochemical Tests

Determination of Esterasic and lipolytic activity

The media used was described by [18], containing (gl^{-1}): peptone 10.0, NaCl 5.0, $CaCl_2 2H_2O$ 0.1, agar18, PH 9.To the sterilized culture media, previously sterilized Tween 80 was added in a final concentration of 1% (v/v. This was incubated at 28ºC for 5 days and presence of a halo was an indicative of esterasic activity. The determination of lipolytic activity was done by replacing Tween 80 with Tween 20 in the medium.

Determination proteolytic

To determine the hydrolysis of gelatin, (frazie'rs gelatin agar) the medium contained malt extract agar and bacteriological gelatin (4.0g 1). This was incubated at 28^{0}C for 5days. The plates flooded with Frazier's revealers (distilled water100ml, Hull 20.0g and mercury dichloride 15.0g),modified from [19]. The presence of a clear halo around the fungal colony indicated a positive result.

Determination of pectinolytic activity

Malt extract medium was added 5g pectin and incubated at 30 ^{0} Cfor 5 days and the plate was added 5.0 ml of HCl (2ml l^{-1}). The presence of a clear halo around the fungal colony was indicative of the degradation of pectin. [20].

Determination of Amnylolytic activity.-

The ability to degrade starch was used as the criterion for determination of ability to produce amylolytic enzymes. The medium used contained malt extract plus 0.2% soluble starch, pH9. After 3-5 days of incubation the plates were flooded with an iodine solution and a yellow zone around a colony in an otherwise blue medium indicated amylolytic activity [21].

Determination of the cellulolytic activity

The media used contained 7.0g KH_2PO_4, 2.0g K_2HPO_4, 0.1g $MgSO_4.7H_20$, 1.0g $(NH_4)_2SO_4$, 0.6g yeast extract, 10g carboxy methyl cellulose and 15g agar per liter [22]. The plates were inoculated in duplicates and incubated at 28 °C for 7 days. For best viewing area for clarification the plates were stored at 50 °C for one night after the incubation period. The presence of a clear halo around the fungal growth indicated positive results

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 and was used for antimicrobial bioassay by inhibition zone method.

The test organisms used in bioactivity were; Escherichia coli(ATCC25922), Bacillus subtilis (ATCC11778), Staphylococcus aureas(ATCC25923), Pseudomonas aeroginosa (ATCC27853) Salmonellatyphimurium (ATCCC700931) andCandidaalbicans (ATCC9008).

Gaschromatography-MassSpectrophotometry (GC-MS) analysis was done for secondary metabolites identification in the extract filtrate.

III. Results

Lake Sonachi is a soda lake in the Kenyan rift valley where sampling of fungal isolates was done during the wet season. Water temperature on shallow water was recorded at 23 ºC and 20.8^{o}C in deep water. The lake is at 1885M above sea level and a pH of 10.4 was recorded.

Morphological studies

There were six fungal isolates recovered from the pulled sediment sample .Figure 1-6 shows morphological characteristics of fungal isolates.

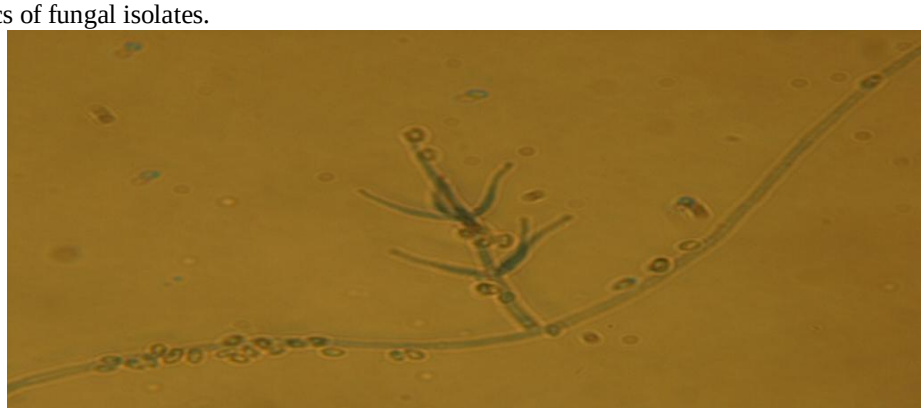

Figure 1: Fungus isolate SON 1. Showing conidia and conidiophores

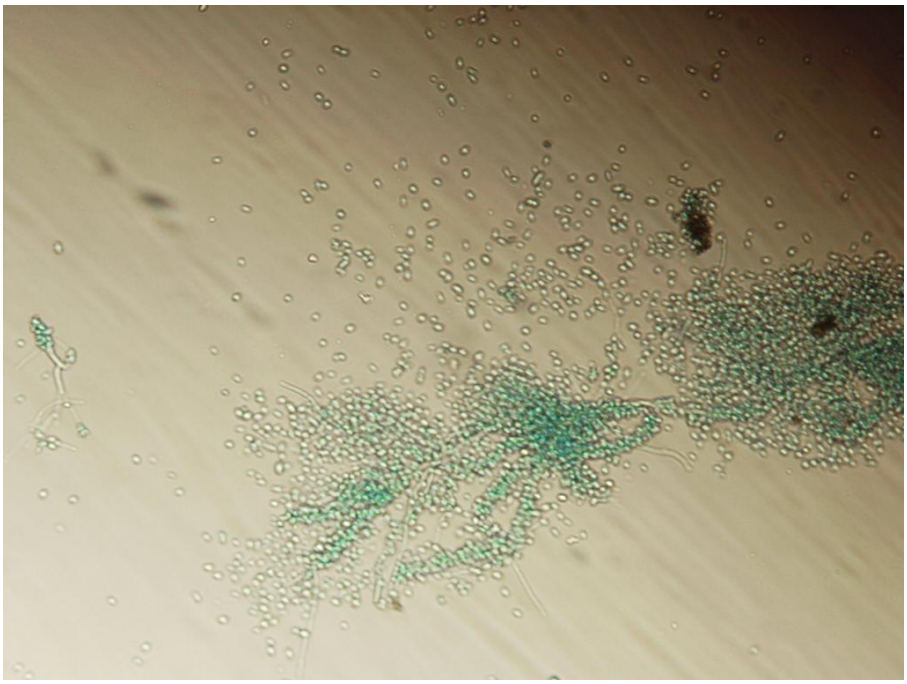

Figure 2: Fungus isolate SON 2. Shows small numerous conidia and conidiophores

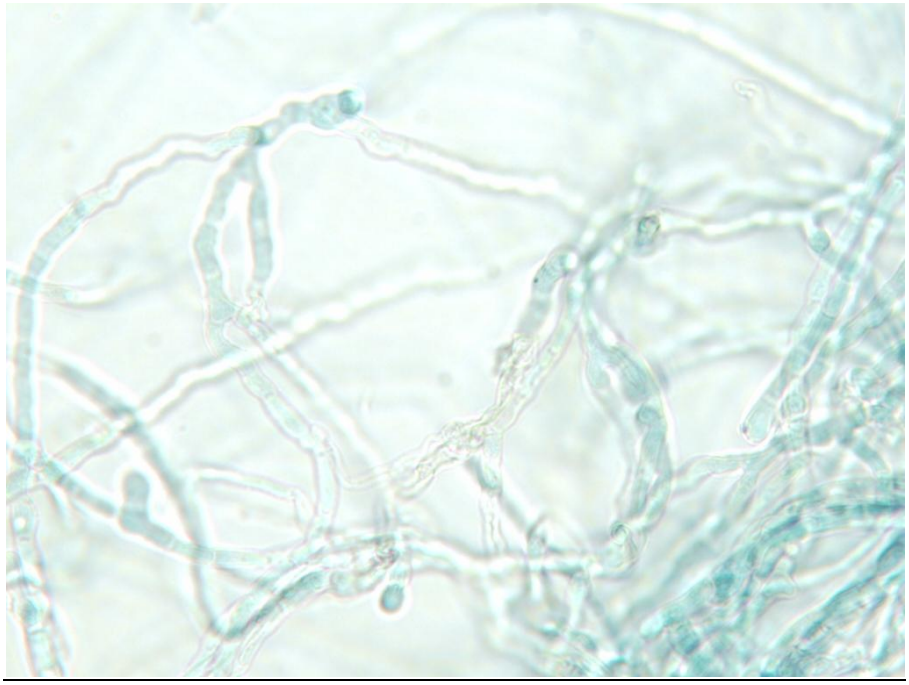

Figure 3: Fungal isolate SON 3 Shows septatehypae, and chlamydospore formation

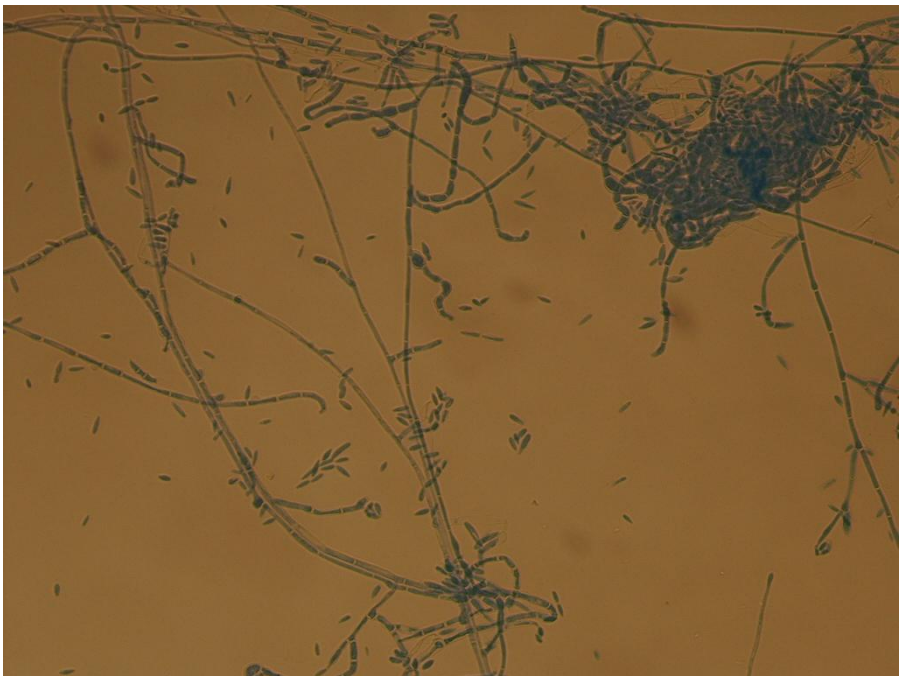

Figure 4: Fungal isolate SON4.Showsseptate,microconidia and macroconiia (3-4 celled)

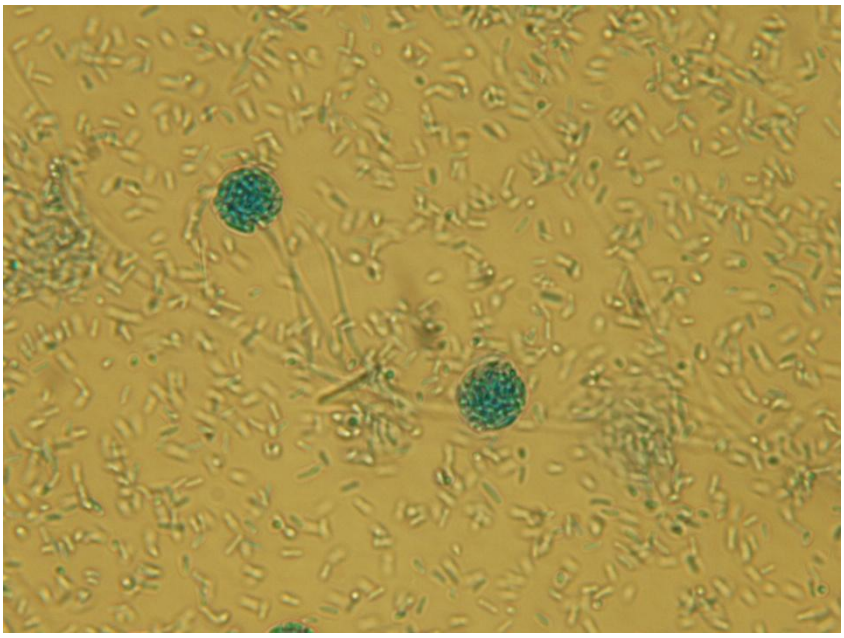

Figure 5: Fungal isolate SON 5. Shows fruiting body ,conidiophores and conid

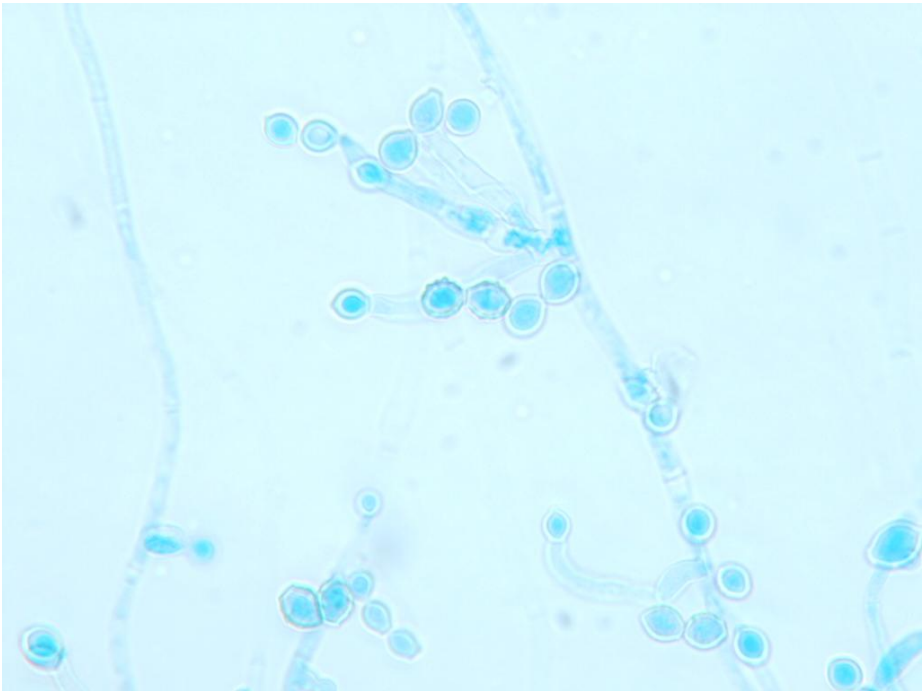

Figure 6: Fungal isolate SON 6. Shows septatehy phea, branching conidiophores and ridged conidia

Fungal enzymes

The six fungal isolates were able to produce different types of enzymes using different substrates.

Table 1.Enzymatic activity of fungal isolates

Isolate	Amylase(starch)	Amylase(CCC)	Esterase(T80)	Lipase(T20)	Pectinase(pectin)	Protease(gellatin)
SON 1	+++	+++	++	+	+	+++
SON 2	-	+++	+++	++	-	+++
SON 3	-	-	-	+	-	+++
SON 4	-	-	-	++	+	+++
SON 5	-	++	-	-	-	-
SON 6	-	-	-	++	+	+++

Key: - (no activity), + (0 – 3 mm), ++ (3.1 – 6 mm), +++ (> 6 mm)

Antimicrobial activity of fungal isolates

The six fungal isolate extracts showed antimicrobial activity on most of test organisms

Test organisms

Table 2. Antimicrobial activity

Isolates	E. coli	B. subtilis	S. aureus	P. aurogenosa	C. albicans	S. pneumonia	S. typhae
SON1	++	+++	+	++	+	+	++
SON2	++	+++	+	+	+	+	+++
SON3	++	++	+	+	-	+	+
SON4	+	++	+	++	+	+	+
SON5	+	+	-	+	+	+	-
SON6	+	++	+	+	+	++	-

Key: - (no activity), + (0 – 3 mm), ++ (3.1 – 6 mm), +++ (>6 mm)

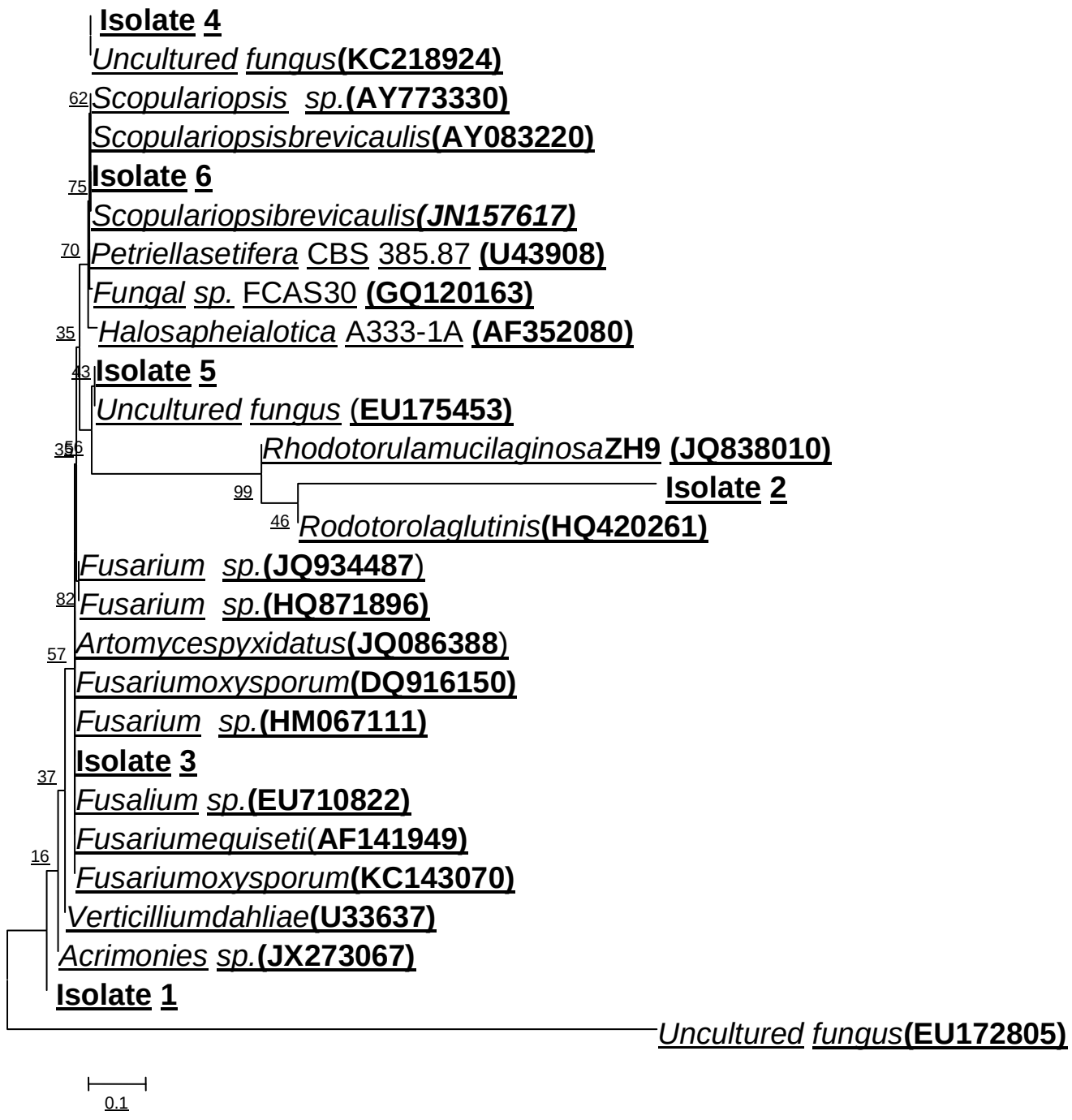

Figure 7. Evolutionary relationships of 27 taxa

The evolutionary history was inferred using the Minimum Evolution method [23]. The optimal tree with the sum of branch length = 1.14131214 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown next to the branches [24]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [25] and are in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm [26] at a search level of 1. The Neighbor-joining algorithm [27] was used to generate the initial tree. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 429 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [28].

Secondary metabolitesof fungal isolates

Literature comparison of mass spectra was used in compound identification. A total of six different compounds were identified in fungal isolate SON 1 extract (Table 3). Butanediol<2,3-> (61%) was the most abundant compound followed by Benzeneacetic acid (2.6%) with the least being Phenol, 3,5-dimethoxy- (1.3%). Alcoholic class of compounds was the majority with their number totaling to three (Phenol, 3, 5-dimethoxy- (1.3%), Propanol<3-methylthio-> (1.5%), Butanediol<2,3-> (61%). The extract also comprised of other groups of compounds like esters, l-Leucine, N-cyclopropylcarbonyl-, hexadecyl ester (1.6%), and carboxylic acid, Benzeneacetic acid (2.6%).

Table 3.Metabolites Profile for isolate SON 1

Peak no.	Rt (min)	Metabolite	% area
1	3.804	Propanol<3-methylthio->	1.4699
2	6.670	Butanediol<2,3->	61.4751
3	15.405	Benzeneacetic acid	2.6317
4	22.147	l-Valine, n-propargyloxycarbonyl-, heptadecyl ester	1.8138
5	22.416	Phenol, 3,5-dimethoxy-	1.2722
6	23.132	l-Leucine, N-cyclopropylcarbonyl-, hexadecyl ester	1.6747

Literature comparison of mass spectra was used in compound identification. A total of nine different compounds were identified in fungal isolate SON 2 extract (Table 4). Butanediol<2,3-> (87.1%) was the most abundant compound followed by 5-Nitroso-2,4,6-triaminopyrimidine (9.7%) and 2-Furanmethanol (3.4%) with the least being (2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans- (0.2%). About 50% of compounds were present in very minute quantities in terms of proportion each having less than 1% of total.

Table 4:Metabolites Profile for isolate SON 2

Peak no.	Rt (min)	Metabolite	% area
1	3.803	Propanol<3-methylthio->	0.7896
2	5.842	Butanediol<2,3->	87.138
3	7.230	1H-Pyrazole, 3,5-dimethyl-	0.3797
4	7.902	2-Furanmethanol	3.4023
5	13.143	Isophorone	1.8639
6	23.535	5-Nitroso-2,4,6-triaminopyrimidine	9.7046
7	23.893	2,4-Dimethoxyamphetamine	0.6012
8	27.883	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	0.2315
9	28.373	Cresol acetate<para-	0.6585

Table 5: Metabolites Profile for isolate SON 3

Literature comparison of mass spectra was used in compound identification. A total of seventeen different compounds were identified in fungal isolate SON 3 extract (Table 5). Concentration of compounds for this isolate ranged between 0.2 % and 10.5 %. Butanediol<2,3-> (10.5%) was the most abundant compound followed by 2-Furanmethanol (3.0%) and N-Acetyltyramine (1.9%) while Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)- (0.2%) was the least in abundance.

PEAK	RT (MIN)	METABOLITE	% AREA
1	6.5807	Butanediol<2,3->	10.5166
2	8.0381	2-Furanmethanol	3.022
3	10.6794	Furfural<5-methyl->	0.4052
4	13.1202	Maltol	0.31114
5	14.3077	2-Thiophenecarboxylic acid hydrazide	0.8944
6	15.5396	3-Pyridinecarboxylic acid, 1,2,5,6-tetrahydro-1-nitroso-	0.4494
7	16.1667	Thymohydroquinone	0.7898
8	17.1074	Skatole	0.5108
9	18.273	N-Isopropylcyclohexylamine	0.3859
10	18.6752	Thujaplicin<beta->	0.7557

11	19.2127	2-Acetylpyrido[3,4-d]imidazole	0.2199
12	20.131	Italicene	1.4407
13	21.318	Diaminopyridine	0.8711
14	22.7291	N-Acetyltyramine	1.9755
15	24.3417	Geranyl-citronellol	0.24226
16	26.335	1,3,5-Triazin-2-amine, 4-(2-furyl)-6-(1-piperidyl)-	0.4711
17	30.7473	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	0.1822

Table 6: Metabolites Profile for isolate SON 4

Literature comparison of mass spectra was used in compound identification. A total of thirteen different compounds were identified in fungal isolate4 extract (Table 6). Concentration of compounds for this isolate ranged between 0.2 % and 10.8 %. Benzeneacetic acid (10.8%) was the most abundant compound followed by Thujaplicin<beta-> (1.5%) while Furfural<5-methyl-> (0.2%) was the least in proportion.

PEAK	RT (min)	METABOLITE	% AREA
1	6.648	Butanediol<2,3->	1.603
2	8.439	Isopentyl acetate	0.6I54
3	10.186	Mesitylene	0.2513
4	10.343	Furfural<5-methyl->	0.2031
5	10.679	2-Cyclopenten-1-one, 3-methoxy-4-methyl-	1.0386
6	10.837	Mesitylene	0.6078
7	12.404	Cymene<para->	0.6845
8	13.613	Maltol	2.6396
9	15.069	2-Coumaranone	0.7926
10	16.160	Benzeneacetic acid	10.809
11	18.787	Thujaplicin<beta->	1.4916
12	19.369	N-Acetyltyramine	0.633
13	22.034	4,5,6,7-Tetrahydro-benzo[c]thiophene-1-carboxylic acid allylamide	0.6828

Literature comparison of mass spectra was used in compound identification. A total of seven different compounds were identified in fungal isolate 5 extract (Table 7). Concentration of compounds for this isolate ranged between 0.4 % and 70.2 %. Phenol, 3-methyl- (70.2%) was the most abundant compound followed by 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (3.7%) and 2-p-Tolyl-2,3-dihydro-1H-benzo[1,3,2]diazaborole (3.2% while p-Formophenetidide (0.4%) was the least in proportion.

Table 7: Metabolites Profile for isolate 5

PEAK	RT (min)	METABOLITE	% AREA
1	22.0124	p-Formophenetidide	0.4089
2	22.1692	l-Valine, n-propargyloxycarbonyl-, heptadecyl ester	1.31
3	26.4694	Phenol, 3-pentadecyl-	0.4581
4	28.082	Phenol, 3-methyl-	70.2852
5	28.8883	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	0.5313
6	29.6275	Phenol, 2-methyl-	1.8502
7	30.5905	2-p-Tolyl-2,3-dihydro-1H-benzo[1,3,2]diazaborole	3.2295

Table 8:Metabolites profile for isolate SON 6

Literature comparison of mass spectra was used in compound identification. A total of twenty different compounds were identified in fungal isolate LSON 6 extract (Table 8). Concentration of compounds for this isolate ranged between 0.2 % and 3.7%. Maltol (3.7%) was the most abundant compound and Quinolin-2-ol, 4-amino- (0.2%) was the least.

PEAK	RT (MIN)	METABOLITE	% AREA
1	4.341	1-Butanol, 3-methyl-	0.4015
2	4.8114	Propanol<3-methylthio->	0.408
3	6.0656	Butanediol<2,3->	3.112
4	7.947	2-Furanmethanol	1.948
5	8.0589	Guanidine	0.4313
6	9.246	Butanoic acid, 4-hydroxy-	0.245
7	10.311	Furfural<5-methyl->	0.273
8	11.5977	1,2-Cyclopentanedione, 3-methyl-	0.903
9	13.1655	Maltol	3.7707
10	13.3671	5-Acetyl-4-methylthiazole	0.32
11	13.5015	Benzene, 1-methyl-4-(1-methylpropyl)-	0.4618
12	13.6134	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.7409
13	14.1062	2-(Dimethylaminomethyl)-3-hydroxypyridine	0.4496
14	14.5317	N-Aminopyrrolidine	0.4643
15	17.1074	Skatole	0.6904
16	20.4894	Quinolin-2-ol, 4-amino-	0.204
17	21.0049	Guaicol	0.3855

18	21.3181	Diaminopyridine	1.2097
19	23.1994	2,5-Dimethylhydroquinone	0.2146
20	25.4391	Ethyl Oleate	0.7013

IV. Discussion

The characterization of fungi was based on Morphological, biochemical characteristics and molecular analysis of 18s rDNAgene [29, 30]. The molecular analysis is an important tool for fungal taxonomy [29, 30, 31, 32, 33], it is based on important genes which are conserved during evolution.

Morphological studies indicated that different species of fungi were isolated from this lake. All the spores observed were anamorphic fungal spores with different shapes and structure, and septate hyphae, anindication of higher fungi,Ascomycotina and basidiomycotina**.**

Fungal isolates extract filtrate were tested for antimicrobial activity using seven test organisms, E. colli, B. subtilis, S. aureas, P. aurogenosa, C. abicans, S .pneumonia, S.typhi.

All the isolates tested positive for at leastfive test organisms. Isolate1, 2 and 4 were positive for all the test organisms in this study,whileIsolate 3didnot show antimicrobial effect on Candida abicans. Isolate 5 and 6 were active against S.typhi. (Fig.8)

Enzymatic activities shoed that all isolates were capable of producing at least two types of enzymes with exception of isolate5 which was only positive for amylase test. Isolate 1 produced all the enzymes while 2 was positive for amylase, esterase,lipase and protease. 4 and 11 were positive for lipase, pectinase and protease respectively. All the isolates were positive for protease and lipase except 5. (Fig 7).

The phylogenetic tree (Fig.9) shows the evolutionary relationships of the six isolates from Lake Sonachi. Blast results showed isolate 4 was aligned to Scopulariopsis. Sp (AY77330) and S.brevicauis (AYO83220) both with 99% similarity, while isolate 6 showed S. brevicaulis (JNI57617), and Scopulariopsis sp. (AY77330) both with 99% similarity.

This genusScopulariopsis belongs to phylum Ascomycota, and class sordariomycetes, it is cosmopolitan and has been isolated fron alkaline and saline environments (Kladwang, et al 2003).

The isolate 5 was aligned to Paecilomyces sp. (JN546116), Sarocladium kiliense (HQ232198) and Acrimonium strictun strain (HM216183) all with 100% similarity and are sordariomycetes.

Isolate 1 and 3 blast result showed Fusariumoxysporum. Fusariumsp, all with a similarity of 100%. Isolate 1, blast results showed Acrimonies sppJX273067), Plectosphaella sp. (HQ871886) And Verticiliumdaliae (U33637) all with 99% similarity.

Isolate 2 blast results showed uncultured eukaryotic fungus clone with 87% similarity and Rodotorula glutinis(HQ420261), (Basidiomycota, Pucciniomycotina)with 77% similarity. The percentage similarity is very low and this may be a new novel fungal species.

The blast search results showed that all the isolates belong to the fungal domain and were clustered mainly within phylum Ascomycota ,and only isolate 2 was clustered within the phylum Basidiomycoca. Ascomycota, and Basidiomycetes comprise the subkingdom Dikarya (often referred to as the "higher fungi") within the Kingdom Fungi. The Ascomycota is the largest phylum of Fungi, with over 64,000 species and inhabit different ecosystems [34].

The six isolates were able to produce a wide range of groups of secondary metabolites such as ,alcohols, acids, aldehydes, esters, hydrocarbons,bases,terpenes,phenols and heterocyclic hydrocarbons, ketones, isopropyl etc. Some of these compounds have been documented to have antimicrobial activity.

Thujaplicins**,** α-thujaplicin, β-thujaplicin and γ-thujaplicin.are known for potent anti-fungal and anti-bacterial properties[35] and they are also known to be potent antioxidants [36].

Isophorone (3, 5, 5-trimethyl-2-cyclohexen-1-one), a monoterpene, and the structurally related 1,8-cineole and camphor, have demonstrated a protective effect against cancer, biological activity against a variety of microorganisms, and anti-oxidant properties [37].

Antifungal effect of thymol, thymoquinone and thymohydroquinone against yeasts, dermatophytes and non-dermatophyte molds isolated from skin and nails fungal infections[38].

However these are just few examples of fungal secondary metabolites cited to have antimicrobial activity otherwise many more are known and documented. GC-MS results has review that the fungal isolates in this study are able to produce a wide range of metabolites and some are well documented as antimicrobial, thus a great potential for compound for future exploitation in pharmaceutical, food and agricultural industries.

V. Conclusion

Lake Sonachi, a soda lake in the Kenyan rift valley has high diversity of anamorphic fungi mainly in the Phylum ascomycota and Basidiomycota which have a high potential of producing different types of enzymes and secondary metabolites that can be exploited for future biotechnological applications in industries. Different methods of isolation and identification should be usedin order to meet the full fungal biodiversity of the lake.

There are possibilities of isolating novel species in this lake, an indication of isolate 2. Extensive research on individual enzymes and secondary metabolites need more attention.

VI. Recommendation

There is need for extensive research onfungal diversity and environmental impact assessment in Lake Sonachi. More studies should be directed towards fungal communities to review the evolutionary trends of fungi in this lake.Further studies should be directed towards fraction guided GC- MS analysis for individual compounds antimicrobial activity. Extensive research on the specific enzymes and antimicrobial compounds produced by these microorganisms is of great importance. This will help to elucidate the structures and biochemical characteristics of any novel enzymes and bioactive metabolites detected.

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