BIODIVERSITY OF GUT MICROFLORA OF *Oreochromis niloticus* BASED ON CULTURE-INDEPENDENT rRNA GENE ANALYSES AT LAKE NASSER, EGYPT

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Lake Nasser is one of the largest artificial lakes in the world. The lake extends for about 300 km in Egypt, from 22°00` to 23°58` N and from 31°30` to 33°15` E (Gharib and Abdel-Halim, 2006). One of the main features of Lake Nasser is the presence of side extensions, locally named khors, mainly 85 known khors, 48 on the eastern side of the lake and 37 on the western side. The khors are, essentially, immersed desert streams (Van Zwieten *et al*., 2011). The khors cover about 79% of the total lake surface area (Latif, 1984).

Nile tilapia, *Oreochromis niloticus* is widely distributed in Africa and many parts of the world (Trewavas, 1983). It was introduced into Lake Victoria, the main Nile source, in the 1950s together with other tilapiines like *O. leucostictus* (Graham), *Tilapia zillii* (Gervais) and *T. rendalii* (Boulenger) (Trewavas, 1983).

The Tilapias are living mainly at khors of Lake Nasser, as favorite habitats due to high eutrophication (El-Shabrawy and Dumont, 2003). The dominance of *O. niloticus* over other tilapiines in the Lake Nasser is attributed to several factors including high fecundity and fast growth rates (Welcomme, 1967; Balirwa, 1998).

The gut is a reservoir of abundant and highly diverse microbial communities that have biological significances, like nutritional, health, etc., to the host fish (Xing *et al*., 2013). On the other hand, the gut microbiotas reflect the adaptation of the host fish to trophic niches. Tilapia fishes living at different Lake Nasser khors offer a particularly interesting system to explore the gut flora contribution to this adaptation.

Available gut microbiota research has primarily focused on single species, such as model systems, i.e. zebra fish (Roeselers *et al*., 2011), stickleback (Bolnick *et al*., 2014), and the Trinidadian guppy *Peocilia reticulata* (Sullam *et al*., 2015) or species relevant to aquaculture research, such as carp (Van Kessel *et al*., 2011), Atlantic salmon (Navarrete *et al*., 2009), turbot (Xing *et al*., 2013), Atlantic cod (Star *et al*., 2013) and the rainbow trout (Navarrete *et al*., 2012). Compara-

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tive gut microbiota studies across single fish species at different localities are rapidly increasing (Baldo *et al.*, 2015). Tilapia fishes from the three Eastern Africa Great lakes; Victoria, Malawi and Tanganyika (Salzburger, 2009) have largely diversified following adaptation to distinct trophic niches, some as specialists (e.g. feeding on plankton, fish larvae or scales), others as generalist feeders (Muschick *et al*., 2012). Such diet radiation represented a main drive in the process of ecological speciation of this group, as testified by the extensive diversity of intestine microflora, coupled with their feeding strategy (Muschick *et al*., 2014). On the other hand, a study has been done on the shifting in the food of Nile Tilapia, *O. niloticus*, in Lake Victoria, Kenya, (Njiru *et al*., 2004). Moreover, molecular analyses have showed shift in gut microbiota dynamics in Eastern African Cichlid fishes (Baldo *et al*., 2015).

Molecular studies on the composition of gut biota of *O. niloticus* have focused only on bacteria, leaving a gap in exploring archaeal and eukaryotic species. Moreover, monitoring of biota in guts of *O. niloticus* in Lake Nasser based on molecular tools has not yet done.

The main goal of this study was providing the first characterization of microbiome in the guts of *O. niloticus*, living at Lake Nasser khors, based on culture-independent rRNA gene analyses. PCR/Denatured Gradient Gel Electrophoresis, DGGE, followed by sequencing approach was applied to display the diversity of both eukaryotic and prokaryotic, bacteria and archaea, 18S and 16S rRNA genes, respectively, in guts of *O. niloticus* populations living at different khors of the Lake Nasser. We also introduced a vision of how the locality shapes the gut microbiota with respect to gut core microbiota.

MATERIALS AND METHODS

Sampling

The sites of sampling were four khors in Lake Nasser, Khor Kalabsha, 23°34' 6.5" N; 32°55' 1.4" E, Khor Wadi Abyad, 23°21' 49.5" N; 32°59' 32.6" E, Khor Korosko, 22°35' 9.2" N; 32°18' 8.9" E, and Khor Tushka, 22°28 '55.6" N; 31°43' 3.7" E (Fig. 1).

Ten individuals of *O. niloticus*, average weight of each specimen 1000 g, were collected from each studied khor. Once picking life fishes, the guts were removed, washed by sterile TE buffer and stored in absolute ethanol at 4°C for molecular analyses. The gut contents were evacuated using syringe filled with Tris-EDTA buffer, 10 mM Tris-HCL, 1 mM EDTA, pH 8, inside a clean sterile propylene tube.

Metagenomic DNA extraction and PCR amplification

Bulk microbial DNAs were extracted from gut contents using DNA isolation kit (MO BIO Laboratories, 12888-50, Carlsbad, CA), according to the manufacture's protocol with modifications of

Elsaied *et al*. (2002). Gut contents were fractionated using glass beads and strong vortex for 10 min. Chemical lysis was conducted using mixture of 5 M guanidine thiocyanate (Sigma) and 10% sodium dodecyl sulfate, SDS, and incubated at 75°C for 20 min with strong shaking. The homogenate was briefly centrifuged for 10 min at 10,000 rpm and the supernatant lysate was removed in a clean sterilized propylene tube. The DNA was purified from the crude lysate using a Sephadex column, provided in the kit. The purified metagenomic DNAs, extracted from all samples, were run on 0.9% agarose gel electrophoresis followed by staining with 20 µL/100 mL ethidium bromide (10 mg/mL) and UV visualization by Gel Doc™ XR^+ imager (Bio-Rad, UK).

PCR amplifications of eukaryotic 18S rRNA gene were done using the primers EukaryaF with GC clump, 5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G TCT GT GAT GCC CTT AGA TGT TCT GGG-3', and EukaryaR, 5'-GCG GTG TGT ACA AAG GGC AGG G-3' (Van Hannen *et al.*, 1998). Bacterial 16S rRNA gene was amplified using the primers 968F-GC, 5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G AAC GCG AAG AAC CTT AC-3' and 1401R, 5'-CGG TGT GTA CAA GAC CC-3' (Nubel *et al*., 1996). The archaeal 16S rRNA gene was amplified using the primers Arch344F-GC, 5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G ACG GGG YGC AGC AGG CGC GA-3' and 915R, 5'-GTG CTC CCC CGC CAA TTC CT-3' (Casamayor *et al.*, 2000). All primers were manufactured using nucleotide synthesizer and purified through HPLC (Life technology, England).

The PCR mixture contained 2.5 mM dNTP each, 10X Ex Taq™ buffer (Mg^{2+} free), 25 mM $MgCl_2$, 0.25 µM of each primer, 250 U Takara Ex-Taq™ Polymerase (Takara, Japan) and 500 ng DNA template. PCR was performed on a ProFlex™ PCR System (Life technology, USA), using an initial denaturation step of 3 min at 95°C. The touchdown PCR reaction continued with 30 cycles of 50 sec at 95°C, at the desirable annealing temperatures, 60°C with decreasing 0.1°C every 1 cycles and 1 min at 72°C, with a final extension of 12 min at 72°C. The PCR produced 18S rRNA and 16S rRNA, for bacteria and archaea, gene amplicons with approximate sizes, 210 bp, 433 bp and 590 bp, respectively.

Denatured gradient gel electrophoresis, DGGE

DGGE was done according to the method of (Muyzer *et al.*, 1993) with some modifications. The 18S rRNA gene amplicons were run on 9% polyacrylamide gel while the 16S rRNA gene amplicons were run on 6% polyacrylamide gel, the ratio of acrylamide to bisacrylamide was 37.5:1; 40% w/v, in 7 liters of 1X TAE running buffer (Tris-acetate 0.04 M, EDTA 0.002 M, pH 8.5).

Electrophoresis was run for 17 h at 120 V and 35 Amp under a constant tem-

perature of 60°C in a DCode™ Universal Mutation Detection System (Bio-Rad, UK). After electrophoresis, the gels were stained with (20 µL/100 mL) ethidium bromide (10 mg/mL) for 20 min, washed with distilled water for 20 min, and visualized under UV light by Gel Doc™ XR$^+$ imager (Bio-Rad, UK). The recovered bands were purified using the QIAquick® PCR Purification Kit (Catalog no. 28104, Qiagen, Germany) and undergoes analyses by sequencing. Sequencing was done on the Applied Biosystems 3500 Genetic Analyzer Sequencer (Hitachi, Japan).

Bioinformatics analyses

DGGE patterns were analyzed, including binary matrix, using AlphaView Software v.3.4.0 (http://www. Proteinsimple.com). The designation of the bandclasses was based on their position in the gel patterns. The DGGE fingerprinting was manually scored by the presence (1) and absence (0) of co-migrating bands, independent of intensity. To ensure gel-to-gel comparability, the bands, common and specific, were furthermore inspected manually for consistency. Similarities in gel patterns were determined using the cluster analysis with Euclidian distance measure. Analyses of DGGE profiles was done using both of Jaccard's similarity coefficient, S_{Jaccard} (Jaccard, 1908) and Dice coefficient, S_{Dice} (Heyndrickx *et al*., 1996) which were calculated according to the formula:

$$S_{\text{Jaccard}} = N_{AB} / (N_A + N_B - N_{AB})$$

$$S_{\text{Dice}} = 2\, N_{AB} / (N_A + N_B)$$

Where: N_{AB} is the number of bands common to both patterns; N_A and N_B represent the total number of bands in sample A and B, respectively.

Sequences were analyzed by FASTA screening to determine their similarity to the known sequences in the DNA database (http://www.ebi.ac.uk/Tools/sss/fasta/).

Phylogenetic trees were constructed through two bioinformatics processes. In the first processes, the nucleotide sequences of the recovered rRNA gene phylotypes and their homologous sequences from the DNA database, beside out-group sequences, were aligned using the online program "Clustal Omega" software (http://www.ebi.ac.uk/Tools/msa/clustalo/). In the second processes, the aligned sequences, including the sequence gaps, were submitted to MEGA software, V. 6.0.6, (http://www.megasoftware.net), for construction of consensus phylogenetic trees, using maximum likelihood, neighbor joining and maximum parsimony algorithms. Bootstrap method, provided as a phylogeny test, in the MEGA software was performed using a number of 500 bootstrap replications.

Nucleotide sequence accession numbers and nomenclature of phylotypes

We have adopted a nomenclature whereby each phylotype had the descriptor of the number of the recovered phylotype followed by sample type and sampling site, for example,

1.Bac.Kl.Ab.Ts, means a phylotype, which recorded in three locations, Kl, Kalabsha; Ab, Wadi Abyad; Ts, Tushka. The phylotype, which was recorded in all studied locations, was taken a synonym "Common" phylotype for example 2.Euk.Common.

The current 18S rRNA gene and 16S rRNA gene sequences have been registered in the DNA database under accession numbers listed in (Table 1) for eukaryotic 18S rRNA gene phylotypes, (Table 2) for bacterial 16S rRNA gene phylotypes, while (Table 3) for archaeal 16S rRNA gene phylotypes.

RESULTS AND DISCUSSION

PCR profile of eukaryotic and prokaryotic rRNA genes in tilapia gut contents

All samples showed positive PCR amplifications of the eukaryotic 18S rRNA gene, bacterial and archaeal 16S rRNA gene. The amplified amplicons of eukaryotic 18S rRNA gene phylotypes from each sample had sizes ranged from 180 bp, as in phylotype 1.Euk.Kl.Kr, to 244 bp, as in phylotype 2.Euk.Common. Bacterial 16S rRNA gene amplicons had sizes ranged from 341 bp, as in phylotype 5.Bac.Kl.Kr, to 412 bp, as in phylotypes 6.Bac. Common and 10.Bac.Ab.Ts, while archaeal 16S rRNA gene amplicons had sizes ranged from 456 bp, as in phylotype 2.Arc.Common, to 496 bp, as in phylotype 5.Arc.Kl.Kr. These amplification results may indicate that the possibility of bias in PCR amplification was minimized. This is because the PCR was tested on the bulk DNA extracted from each sample by: a) Decreasing the annealing temperature 0.1°C every PCR cycle and b) Doing PCR using number of cycles ranged from 25 to 30 cycles. These conditions gave the flexibility for the primers to anneal with various rRNA gene templates with different GC contents (Suzuki and Giovannoni, 1996).

DGGE patterns displayed common and specific eukaryotic and prokaryotic rRNA gene phylotypes in tilapia guts from different studied khors

The DGGE method is particularly useful as an initial step in ecological studies to distinguish tilapia fish gut communities from different ecosystems and to determine if numerically dominant phylotypes are present. In order to validate this concept, it is necessary to create data representatives of the various origins.

The DGGE displayed total of 5 band groups, each band group represented a phylotype, of eukaryotic 18S rRNA gene in gut contents of tilapia from all studied khors (Fig. 2, Table 1). The gut content from Kalabsha showed the highest 18S rRNA gene phylotype diversity, representing 4 phylotypes (Fig. 2, Table 1). In contrast with Kalabsha, the gut contents from Wadi Abyad harbored only 1 phylotype of 18S rRNA gene, recording the lowest diversity of eukaryotesamong the studied gut contents. The phylotype 2.Euk. Common was occurred in gut contents from all khors. On the other hand, the phylotype

3.Euk.Kr characterized only gut contents of tilapia from Korosko.

The DGGE profile of bacterial 16S rRNA gene showed 12 phylotypes from gut contents of all studied khors (Fig. 3, Table 2). The DGGE patterns of 16S rRNA gene in the guts of tilapia from Vietnam have displayed 8-11 visible phylotypes (Maiwore *et al*., 2009), diversity in the range of that of in current study. On the other hand, DGGE patterns of the 16S rRNA gene in tilapia guts from three different lakes in Cameroon have revealed the presence of 4-9 bands of bacterial phylotypes (Tatsadjieu *et al*., 2010). The gut contents from Tushka harbored the highest diversity of bacterial phylotypes, representing 10 phylotypes (Fig. 3, Table 2). Korosko had the lowest bacterial phylotype diversity. Two phylotypes, 6.Bac.Common and 8.Bac.Common, were recorded in gut contents from all locations. Bacterial 16S rRNA gene DGGE common band has been recorded in the gut contents of tilapia from three studied lakes in Cameroon (Tatsadjieu *et al*., 2010). Also, three common DGGE bands, for bacterial 16S rRNA gene, have been recorded in guts of Tilapia from Cameroon and Vietnam (Maiwore *et al*., 2009). The gut contents from Khor Kalabsha were characterized by harboring the phylotype 4.Bac.Kl (Fig. 3, Table 2), which can be used as a specific marker of tilapia guts from that khor.

There were five DGGE band groups were recorded for archaeal 16S rRNA gene from gut contents of all studied khors (Fig. 4, Table 3). The archaeal 16S rRNA gene phylotypes 2.Arc.Common and 4.Arc.Common were displayed on the DGGE patterns of gut contents from all studied khors (Fig. 4, Table 3). Generally, the diversity of archaea in the gut contents is the lowest among other domains, eukaryotes and bacteria. However, molecular studies on archaea in the gut content of tilapia, and generally fishes, are still behind.

Zooplankton-like 18S rRNA gene phylotypes constituted the dominant eukaryote fraction in guts of tilapia at the studiedkhors

The recorded eukaryotic 18S rRNA gene phylotypes in the studied tilapia gut contents were distributed in the branches of 3 phylogenetic taxa (Fig. 5). Crustacea, genera *Darwinula* and *Vestalenula*, were represented by the phylotypes 2.Euk.Common, in guts from all khors, and 1.Euk.Kl.Kr, Khors Kalabsha and Korosko. In addition, *Ostracoda* species have been recorded as natural food of tilapia in Lakes Manzallah, Edku and Burullus (Ramdani *et al*., 2001), implicating a favorite food of tilapia from different Egyptian water sources. The phylotype 2.Euk. Common formed a distinct monophyletic lineage, implicating new species of *Ostracoda*, which preferred by tilapia in Lake Nasser (Fig. 5).

Tilapia gut contents harbored unique bacterial 16S rRNA gene-like phylotypes

The bacterial 16S rRNA gene phylotypes were distributed in the branch-

es of 3 phylogenetic taxa (Fig. 6). Four phylotypes formeda unique cluster in the branch of uncultured cyanobacteria. The abundance of cyanobacteria observed here and in other fish guts, was possibly supported by their role as fish's major food source (Ye *et al*., 2014). A single phylotype, 5.Bac.Kl.Kr, which appeared in the gut contents from Khors Kalabsha and Korosko**,** was closely related to alpha proteobacterium, *Rhodobacter* sp., which has been recorded in the guts of tilapia larvae as one of preference gut bacterial species (Giatsis *et al*., 2015). Most of bacterial phylotypes, which recorded in the guts, especially from Khor Wadi Abyad, were linked with uncultured bacteria from environmental samples (Fig. 6), with no clear phylogenetic positions. Most of uncultured environmental bacteria have unclear phylogenetic characterization (Handelsman, 2004), especially those collected from an environment that never studied before, such as Lake Nasser.

Euryarcheota- like phylotypes constituted the main archaeal component in gut of tilapia

The composition of archaeal 16S rRNA gene pool of tilapia gut contents was characterized by the occurrence of 5 phylotypes, which were distributed in the branch of uncultured euryarchaeota (Fig. 7). Euryarchaeota constitute the main archaeal fraction in the guts of fishes (Van der Maarel *et al*., 1999). The phylotypes 2.Arc.Common, 3.Arc.Kl.A.Kr and 5.Arc.Kl.Kr, which represented all studied sites, formed a single cluster with uncultured methanogen, which collected from biogas plants (acc. no. DQ262635) (Fig. 7). The same observation was recorded in other current phylotypes in the branch of uncultured euryarcheota. Methanogens play an important role in fermentation of undigested food in the digestive tract of several fishes (Austin, 2002). Generally, studies on diversity of archaea in fish guts are very limited, comparing with higher animals. Accordingly, additional phylogenetic surveys must be done to explore the diversity of uncultured archaea in fish guts.

Guts contents and health of tilapia fish

The phylotypes, 4.Euk.Kl.Tsand 5.Euk.Kl.Ts, formed monophyletic clade with *Neoechinorhynchus*, *Acanthocephala*. Neoechinorhynchidae is a family of parasitic worms which infect several marine and freshwater fishes (Amin *et al*., 2011). *Neoechinorhynchus* has been isolated from Nile tilapia of Kivu Lake, Congo (acc. no. DQ181946) (Fig. 5). These records may implicate that this parasitic worm is commonly occurred in guts of tilapia, which lives a long Nile river. The phylotype 3.Euk.Kr, which characterized the gut content from Khor Korosko was cladded with *Catenula lemnae***,** Platyhelminthes (Larsson and Jondelius, 2008). The Catenulida are turbellarian flatworms, which has been recorded in silver croaker fish *Plagioscion squamosissimus* (Melo *et al*., 2013). Hence, worms could be common parasites of tilapia at Lake Nasser.

The gut contents from distant khors shared phylotype compositions, representing core gut microbiota

Both of DGGE patterns and phylogenetic analyses showed similarities of gut content rRNA gene compositions. The eukaryote 18S rRNA gene composition of Kalabsha and Tushka showed the highest similarity, 75% with Jaccard, 86% with Dice, among compared area (Table 4). On the other hand, identical archaeal 16S rRNA gene composition was recorded between Kalabsha and Korosko (Table 4). Khor Kalabsha is located at the northwest of the lake, with distance average 149 Km a way from Khor Tushka and Korosko, southeast of the lake (Fig. 1). Also, higher similarity was recorded in bacterial 16S rRNA gene phylotypes between distant Khors, Wadi Abyad and Tushka (Table 4). These observations may implicate that the biogeographic distribution of gut eukaryote and archaea is not affected by distance. These results are in agreement with those reported earlier in microbial diversity of *O. niloticus* of three distant lakes of Cameroon (Tatsadjieu *et al*., 2010). Generally, a fish species is characterized by core gut microbiota with respect to distant localities (Roeselers *et al*., 2011). This fact could be due to the importance of gut microbiota in species essential specific metabolisms, which are not affected by environment stress. However, this concept needs more studies on biodiversity of gut flora in tilapia from many localities in the Lake.

SUMMARY

The diversity of *Oreochromis niloticus* gut microbiome domains, eukaryotes, bacteria and archaea, was studied to understand the contribution of microbiota to the health of the fish. Fishes were collected from four different Khors, Kalabsha, Wadi Abyad, Tushka and Korosko, of Lake Nasser, Egypt. The approach of this study depends on culture-independent PCR/DGGE and sequence of small subunit of rRNA genes, 18S rRNA gene and 16S rRNA gene. The DGGE patterns displayed 5, 12 and 5 band groups, phylotypes, for eukaryotic 18S rRNA gene, bacterial and archaeal 16S rRNA genes, respectively, in gut contents from the studied khors. DGGE showed bands, which were common and specific for each site and could be used as a bar code to certify the origin of the fish. Statistical analyses, using binary matrix, showed numbers of DGGE bands, 1, 2 and 2, for eukaryotes, bacteria and archaea, respectively, were commonly occurred in all studied khors. The DGGE phylotype, 3.Euk.Kr characterized eukaryotes in Khor Korosko. Phylogenetic analyses showed that two of eukaryotic phylotypes, 1.Euk.Kl.Kr and 2.Euk.Common, were belonged to crustacean Ostracoda. Bacterial phylotypes in all studied khors were located in the branch of cyanobacteria, alpha proteobacteria, but most of them constituted unique phylogenetic lineages within the branch of uncultured environmental bacteria. All archaeal phylotypes were located in the branch of methanogenic uncultured euryarchaeota. Some

helminthes, of the genera *Neoechinorhynchus* and *Catenula*, -like rRNA gene phylotypes were recorded in guts from Kalabsha, Tushka and Korosko, suggesting common gut parasitic worms. The DGGE patterns and sequence analyses showed high similarities of eukaryote, bacteria and archaea rRNA gene phylotype compositions in fish guts from distant khors, implicating core gut microbiome. This is the first survey of all microbiome domains in tilapia guts at Lake Nasser based on molecular approaches.

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Table (1): Accession numbers and binary Matrix of DGGE bands of eukaryotic 18S rRNA gene phylotypes recorded in the gut contents of Nile tilapia.

Eukaryal phylotypes	Acc. No.	Khors			
		Kalabsha, Kl	Wadi Abyad, Ab	Tushka, Ts	Korosko, Kr
1.Euk.Kl.Kr	LC148634	1	0	0	1
2.Euk.Common	LC148635	1	1	1	1
3.Euk.Kr	LC148636	0	0	0	1
4.Euk.Kl.Ts	LC148637	1	0	1	0
5.Euk.Kl.Ts	LC148638	1	0	1	0

Table (2): Accession numbers and binary Matrix of DGGE bands of bacterial 16S rRNA gene phylotypes recorded in the gut contents of Nile tilapia.

Bacterial phylotypes	Acc. No.	Khors			
		Kalabsha, Kl	Wadi Abyad, Ab	Tushka, Ts	Korosko, Kr
1.Bac.Kl.Ab.Ts	LC148639	1	1	1	0
2.Bac.Kl.Ab.Ts	LC148640	1	1	1	0
3.Bac.Ab.Ts	LC148641	0	1	1	0
4.Bac.Kl	LC148642	1	0	0	0
5.Bac.Kl.Kr	LC148643	1	0	0	1
6.Bac.Common	LC148644	1	1	1	1
7.Bac.Kr.Ts	LC148645	0	0	1	1
8.Bac.Common	LC148646	1	1	1	1
9.Bac.Ab.Kr.Ts	LC148647	0	1	1	1
10.Bac.Ab.Ts	LC148648	0	1	1	0
11.Bac.Ab.Ts	LC148649	0	1	1	0
12.Bac.Kl.Ab.Ts	LC148650	1	1	1	0

Table (3): Accession numbers and binary Matrix of DGGE bands of archaeal 16S rRNA gene phylotypes recorded in the gut contents of Nile tilapia.

Archaeal phylotypes	Acc. No.	Khors			
		Kalabsha, Kl	Wadi Abyad, Ab	Tushka, Ts	Korosko, Kr
1.Arc.Kl.Kr.Ts	LC148651	1	0	1	1
2.Arc.Common	LC148652	1	1	1	1
3.Arc.Kl.Ab.Kr	LC148653	1	1	0	1
4.Arc.Common	LC148654	1	1	1	1
5.Arc.Kl.Kr	LC148655	1	0	0	1

Table (4): Similarity percentages, Jaccard coefficient and (Dice coefficient) between PCR-DGGE profiles of eukaryotic 18S rRNA gene, bacterial and archaeal 16S rRNA gene in gut content according to the sampling regions.

	Kl			Ab			Kr		
	Euk.	Bac.	Arc.	Euk.	Bac.	Arc.	Euk.	Bac.	Arc.
Ab	25.0 (40.0)	45.5 (62.50)	60.0 (75.0)						
Kr	40.0 (57.0)	33.3 (50.0)	100.0	33.3 (50.0)	27.3 (42.9)	60.0 (75.0)			
Ts	75.0 (86.0)	41.7 (58.8)	60.0 (75.0)	33.3 (50.0)	90.0 (94.7)	50.0 (66.7)	20.0 (33.3)	36.4 (53.3)	60.0 (75.0)

K1: Kalabsha, Ab: Wadi Abyad, Kr: Korosko, Ts: Korosko

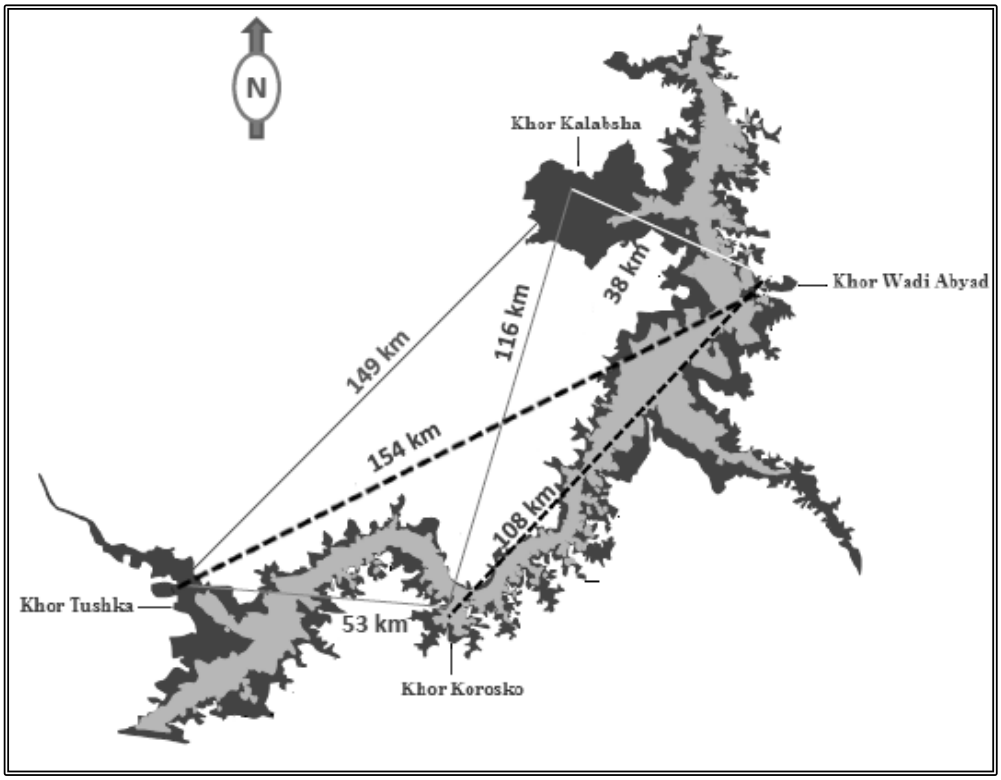

Fig. (1): A map showing the khor sites of sampling at Lake Nasser.

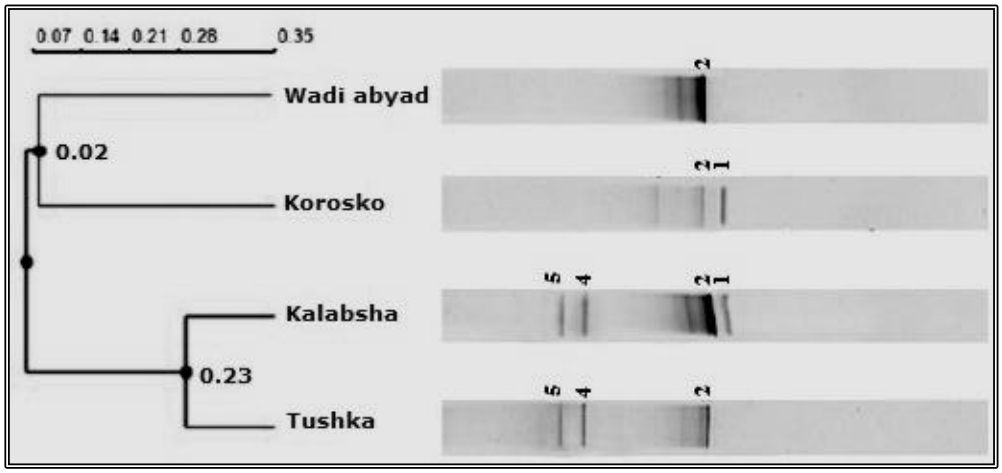

Fig. (2): PCR-DGGE of eukaryotic 18S rRNA gene banding profile of DNA samples of gut contents from the studied Khors. 1- Euk.Kl.Kr; 2- Euk.Common; 3- Euk.Kr; 4- Euk.Kl.Ts; 5- Euk.Kl.Ts.

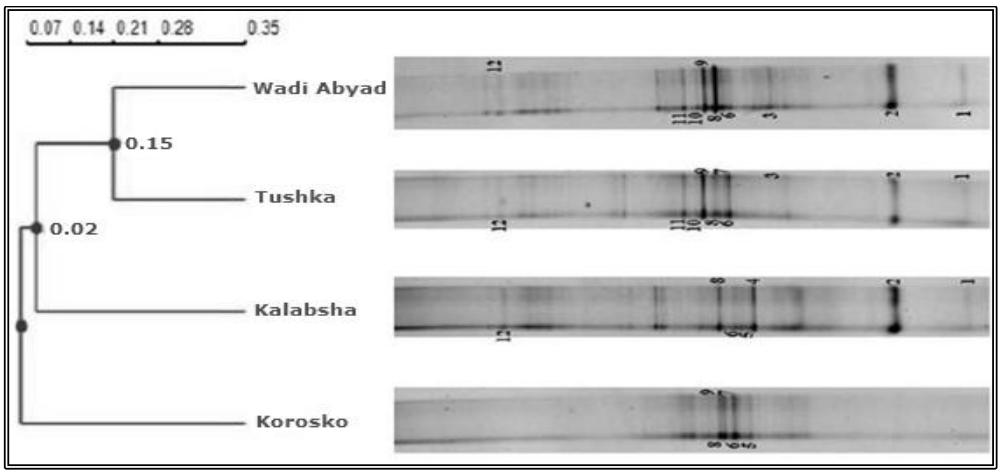

Fig. (3): PCR-DGGE of bacterial 16S rRNA gene banding profile of DNA samples of gut contents from the studied Khors. 1- Bac.Kl.Ab.Ts; 2- Bac.Kl.Ab.Ts; 3- Bac.Ab.Ts; 4- Bac.Kl; 5- Bac.Kl.Kr; 6- Bac.Common; 7- Bac.Kr.Ts; 8- Bac.Common; 9- Bac.Ab.Kr.Ts; 10- Bac.Ab.Ts; 11- Bac.Ab.Ts; 12- Bac.Kl.Ab.Ts.

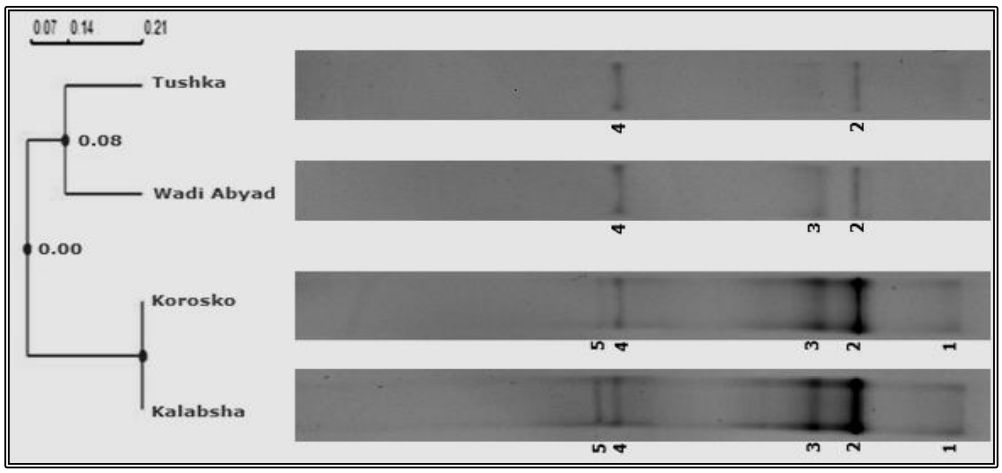

Fig. (4): PCR-DGGE of archaeal 16S rRNA gene banding profile of DNA samples of gut contents from the studied Khors. 1- Arc.Kl.Kr.Ts; 2- Arc.Common; 3- Arc.Kl.Ab.Kr; 4- Arc.Common; 5- Arc.Kl.Kr.

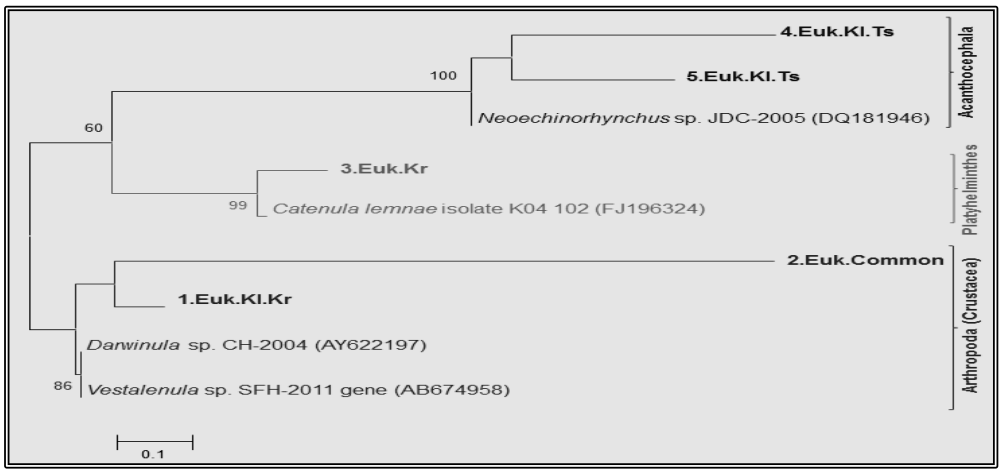

Fig. (5): A consensus phylogenetic tree based on 18S rRNA gene sequences of current eukaryote phylotypes in tilapia guts; beside their corresponding sequences from database. Bootstrap values, more than 50%, of compared algorithms, are indicated at the branch roots. The bar represents 0.1 changes per nucleotide. Accession numbers of database extracted sequences are in brackets

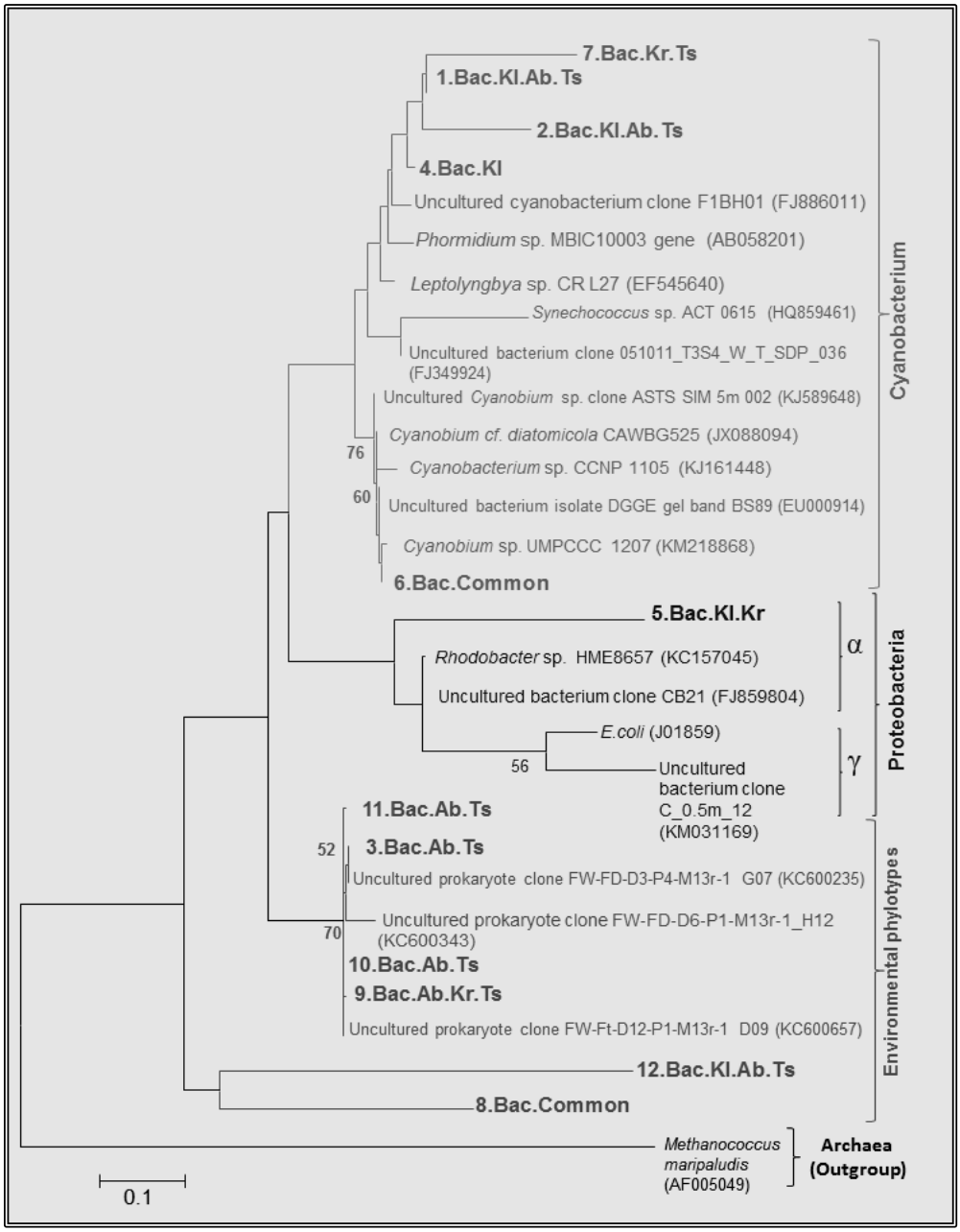

Fig. (6): A consensus phylogenetic tree based on 16S rRNA gene sequences of current bacterial phylotypes in tilapia guts; beside their corresponding sequences from database. Bootstrap values, more than 50%, of compared algorithms, are indicated at the branch roots. The bar represents 0.1 changes per nucleotide. Accession numbers of database extracted sequences are in brackets.

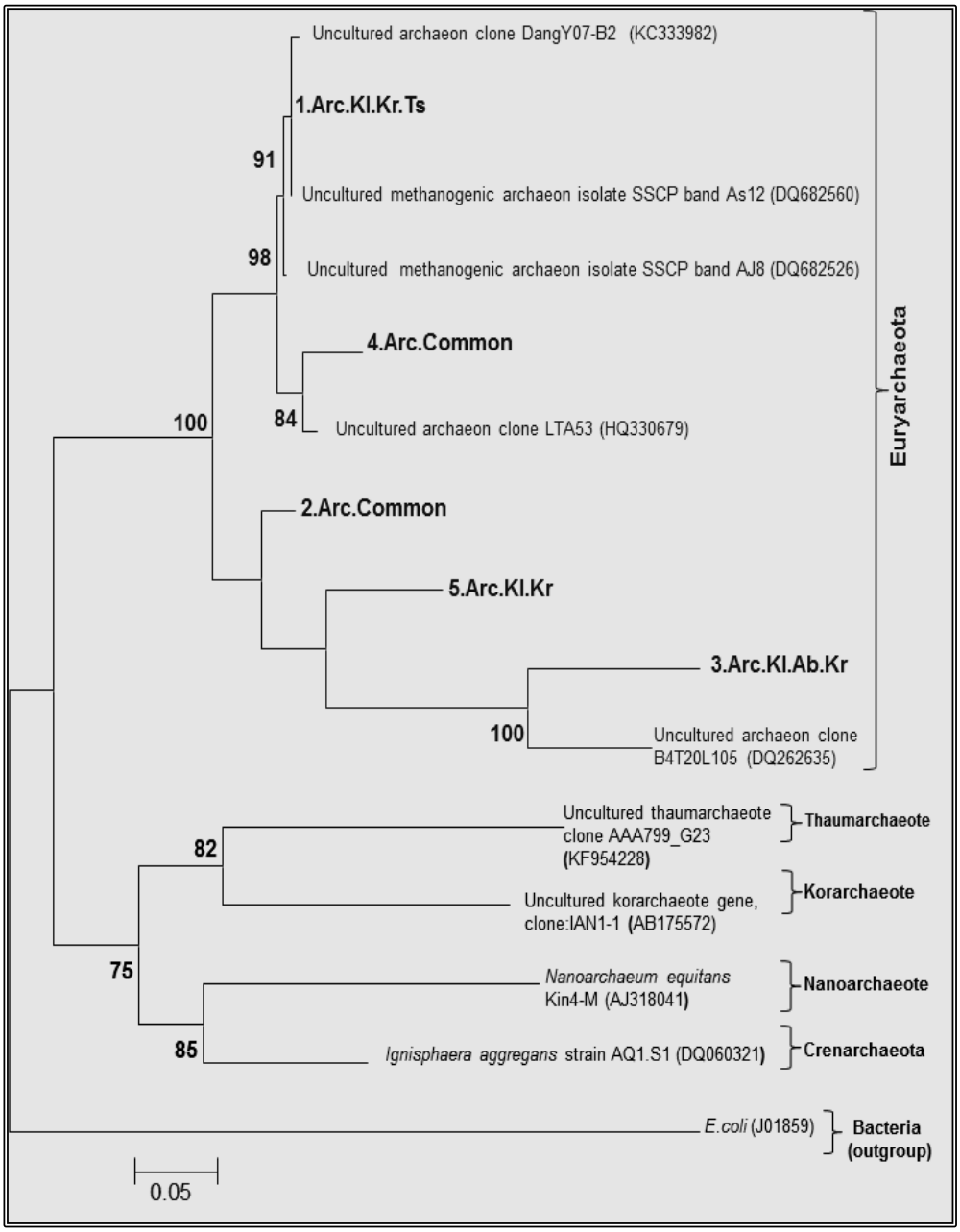

Fig. (7): A consensus phylogenetic tree based on 16S rRNA gene sequences of current archaeal phylotypes in tilapia guts; beside their corresponding sequences from database. Bootstrap values, more than 50%, of compared algorithms, are indicated at the branch roots. The bar represents 0.05 changes per nucleotide. Accession numbers of database extracted sequences are in brackets.