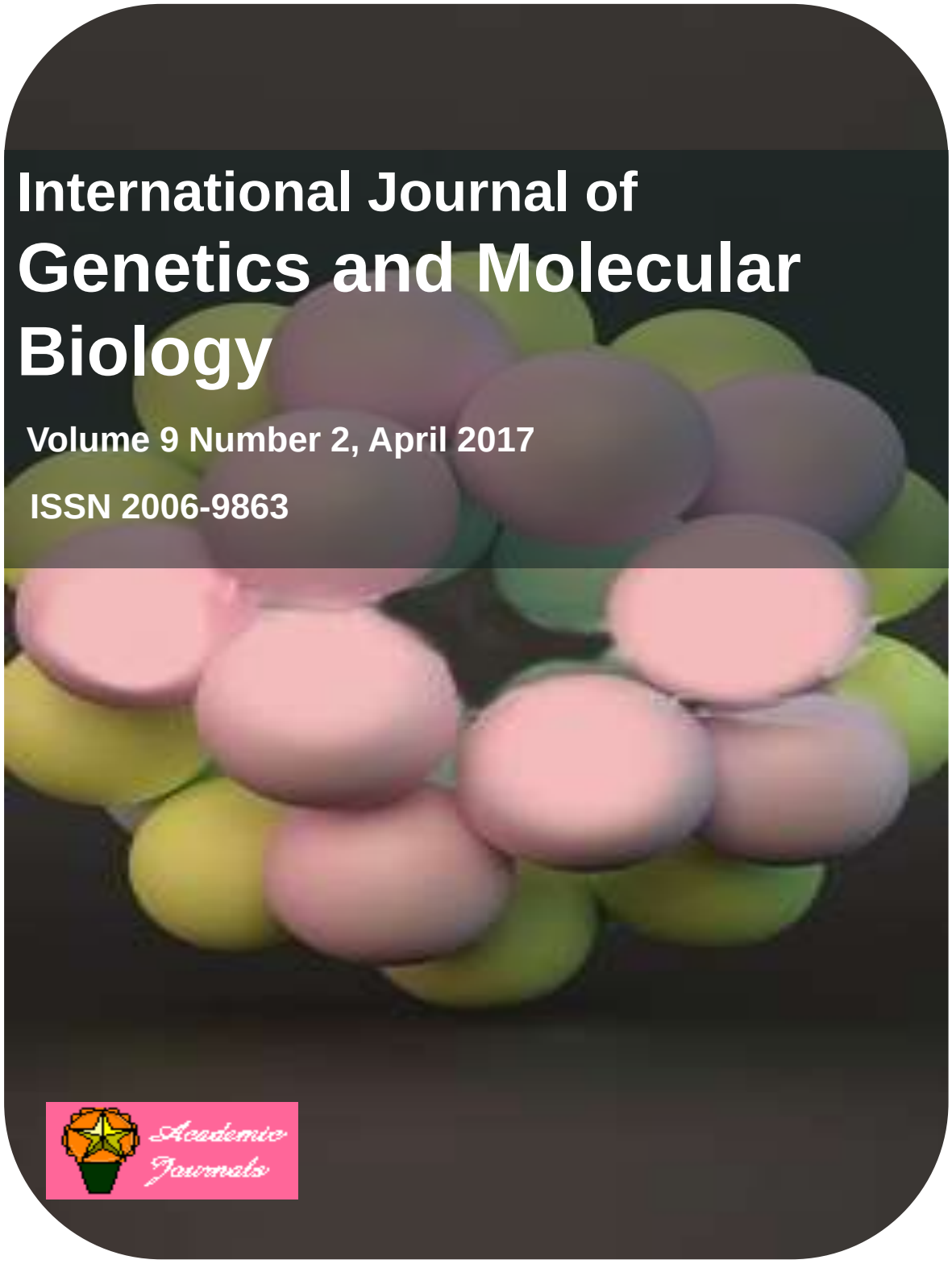


International Journal of Genetics and Molecular Biology

Volume 9 Number 2, April 2017

ISSN 2006-9863

Academic Journals

ABOUT IJGMB

The **International Journal of Genetics and Molecular Biology (IJGMB)** (ISSN 2006-9863) is published Monthly (one volume per year) by Academic Journals.

International Journal of Genetics and Molecular Biology (IJGMB) provides rapid publication (monthly) of articles in all areas of the subject such as DNA and RNA, Influence of risk factors on onset of hyperlipidemia in people with cerebrovascular insult, Study of proteinase activity of Lactobacillus plantarum etc.

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

Contact Us

Editorial Office: ijgmb@academicjournals.org

Help Desk: helpdesk@academicjournals.org

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

Submit manuscript online http://ms.academicjournals.me/.

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.
Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJFS to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.
Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the International Journal of Genetics and Molecular Biology is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

International Journal of Genetics and Molecular

Table of Contents: Volume 9 Number 2 April, 2017

ARTICLE

academicJournals

Vol. 9(2), pp. 8-15, April 2017
DOI: 10.5897/IJGMB2016.0130
Article Number: 214A16063937
ISSN 2006-9863

http://www.academicjournals.org/IJGMB

International Journal of Genetics and Molecular Biology

Full Length Research Paper

Biodiversity of uncultured bacteria in hypersaline lakes, Siwa oasis, Egypt, as determined by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) of 16S rRNA gene phylotypes

Hosam E. Elsaied[1]*, Mohamad Saad Abd El-Karim[2] and Mai A. Wassel[1]

[1]Genetics and Genetic engineering research group, National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt.
[2]Phycology research group, National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt.

Received 1 August; 2016, Accepted 12 October, 2016

Bacterial populations within hypersaline lakes often exhibit uncultured unique species. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), followed by sequencing of 16S rRNA gene approach was applied in order to explore, for the first time, the bacterial communities within the hypersaline Lakes, Aghormy, Zeiton and Maraqi, Siwa Oasis, Egypt. The DGGE profile displayed 12 phylotypes of 16S rRNA gene, representing the total species richness within the three studied lakes. The phylotypes were varied among lakes, but were restricted to two phylogenetic groups, *Bacteroidetes*, which occurred in all lakes, and *Alphaproteobacetria*, which showed abundance in only Lakes Zeiton and Maraqi. Single spirochete-like phylotype characterized the Lake Aghormy. A phylotype, which was recorded in both of Aghormy and Zeiton, represented a first recorded of *Lewinella agarilytica* in hypersaline inland lakes. Sequence homology results suggested novel indigenous bacterial phylotypes.

Key words: Siwa lakes, halophilic bacteria, 16S rRNA gene, molecular diversity.

INTRODUCTION

Hypersaline environments are distributed worldwide, and saturated salt inland lakes are among the most physical and chemical demanding habitats on earth. Few groups of organisms survive in them; rare eukaryotes and many halophilic prokaryotic cells are capable of living in saltern crystallizers (DasSarma and DasSarma, 2012).

Halophiles are salt-loving organisms, capable of growing in salt concentrations more than 150 g/L (15% w/v, 2.5 M), which flourish in saline environments (Ollivier et al., 1994). Halophiles are generally more specialized

*Corresponding author. E-mail: hosameasa@yahoo.com.

organisms and are found in all three domains of life, that is, bacteria, archaea and eukaryotes.

Halophilic bacteria are abundant in many phylogenetic subgroups, most of which belong to Halomonadaceae, a family of Proteobacteria (Oren, 2002). Also, hypersaline environments are dominated by members of Bacteroidetes, often *Salinibacter ruber* (Anton et al., 2002). These bacterial species are aerobic, anaerobic, chemoheterotrophic, photoheterotrophic, and/or photoautotrophic. They are ubiquitously found in lakes and coastal areas worldwide, e.g., in the Dead Sea (Wei et al., 2015), Lake Urmia, Iran (Mehrshad et al., 2015), solar salterns in Tunisia (Boujelben et al., 2012), the Tuzkoy salt mine, Turkey (Mutlu and GUVen, 2015), Rambla Salada, Murcia, Spain (Luque et al., 2014), the Great Salt Lake, Utah, USA (Tazi et al., 2014), Dagong Brine Well, China (Xiang et al., 2008) and the Capuchin Catacombs, Italy (Pinar et al., 2014).

Halophilic bacteria have been studied by cultured- and uncultured-depending techniques, with sequence-based approaches for phylogenetic and taxonomic classification (DasSarma and DasSarma, 2012). For uncultured halophiles, sequencing and phylogenetic analyses of 16S ribosomal ribonucleic acid (rRNA) gene expanded the breadth of diversity of unknown species, among halophiles (Edbeib et al., 2016). Hence, application of these molecular approaches to study hypersaline environments have presented unique prokaryotic communities and found to be a valuable source of novel prokaryotic diversity.

The lakes in Siwa Oasis, western desert, Egypt, are athalasohaline environments, characterized by high salt concentrations and unique water parameters (Abd El-Karim and Goher, 2016). The low raining and high evaporation rates within those lakes favored the development of hypersaline environments. The Siwa lakes have been studied from the views of physicochemical characteristics and as a source of some environmental biotechnological applications (Abd El-Karim, 2016, Abd El-Karim and Goher, 2016, Mahmoud and Abd El-Karim, 2016). However, the uncultured bacterial communities within those lakes remained yet to be uncovered. In this study, application of bacterial 16S rRNA gene analysis, through PCR/DGGE and sequence approach, was done in order to explore the halophylic bacterial richness within those lakes.

MATERIALS AND METHODS

Sampling

Sampling was done from three Lakes, Aghormy, Zeiton and Maraqi, Siwa Oasis, Egypt (Figure 1). The lakes are characterized by water depths between 0.5 m, Aghormy and Zeiton, and 0.7 m, Lake Maraqi. The water pH values were 7.83, 7.36 and 7.61, for Aghormy, Zeiton and Maraqi, respectively. The most feature which characterizes those lakes is hypersalinity, where the recoded total dissolved solids, TDS, mainly sodium chloride, were 220.03, 239.29 and 280.45 gL^{-1} in the water of Aghormy, Zeiton and Maraqi, respectively. One liter of surface water microbial mat was collected from the margins of each of the three studied lakes. The collected microbial mats were treated with sterile TE buffer, 50 mM EDTA, 50 mM Tris-HCl, pH 7.6 to chelate as much as possible of cations in the collected saline water, and consequently, protect DNA from degradation inside the microbial cell. The treated microbial mates were stored at 4°C for molecular analyses.

DNA extractions and PCR amplifications

Bulk microbial DNAs were extracted from treated microbial mats, using DNA isolation kit (MO BIO Laboratories, 12888-50, Carlsbad, CA), according to the manufacture's protocol with modifications of Elsaied (2013). Three replicates of 50 ml of each collected microbial mat were centrifuged for 5 min, with speed 10000 rpm, at 4°C to concentrate the microbes. 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 homogenates were briefly centrifuged for 10 min at 10000 rpm and the supernatant lysates were removed in clean sterilized propylene tubes. The bulk DNA, of the three replicates for each lake water sample, was purified from the crude lysates, using Sephadex columns and finally combined with each other. The purified DNAs, extracted from all lake water 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 bacterial 16S rRNA gene were done, using the primers 341F-GC, 5'- CgC CCg CCg CgC CCC gCg CCC gTC CCg CCg CCC CCg CCC g CCT ACg ggA ggC AgC Ag -3' and 907R, 5'- CCg TCA ATT CMT TTg AgT TT -3' (Nubel et al., 1996). All primers were manufactured by 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 s at 95°C, with starting annealing temperature, 57°C, followed by decreasing 0.1°C every cycle, and 1 min at 72°C , with a final extension for 12 min at 72°C. Moreover, the bias in PCR was minimized by doing PCR replicates, for each lake water sample, using number of cycles ranged from 25 to 30 cycles (Suzuki and Giovannoni, 1996). These conditions gave the flexibility for the primers to anneal with various 16S rRNA gene templates with different GC contents and obtained PCR amplicons with sizes ranged from 547 to 561 bp. 16S rRNA gene amplicons were stained with ethidium bromide and visualized on 1.28 % agarose gel.

Denatured gradient gel electrophoresis, DGGE

DGGE was done according to the method of Muyzer et al. (1993), with modifications. 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 L 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 temperature of 60°C in a DCode™ Universal Mutation Detection System (Bio-Rad, UK). After electrophoresis, the gel was

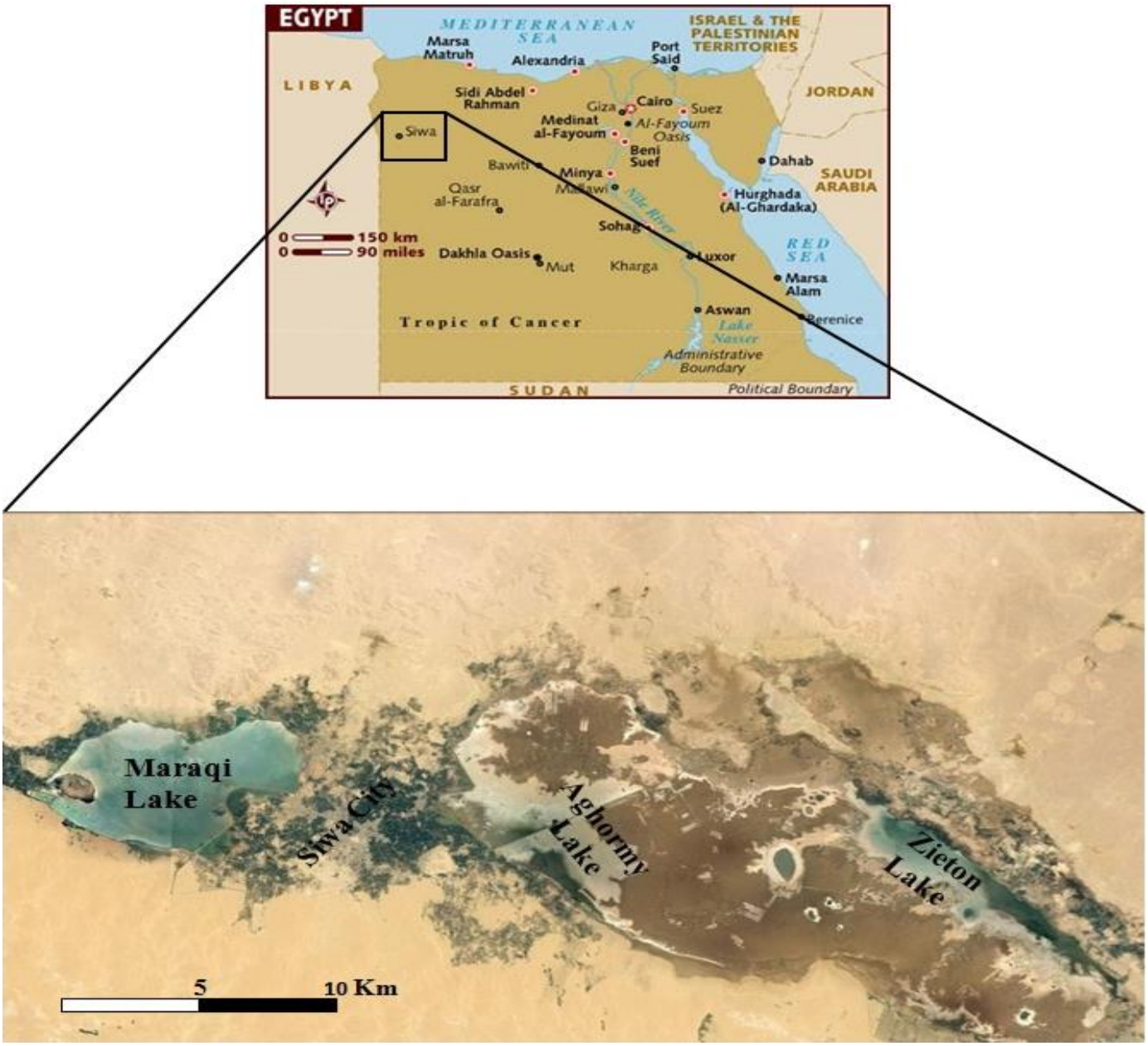


Figure 1. A map showing the sites of sampling at Siwa Lakes, Egypt.

stained with 20 µL/100 mL ethidium bromide concentration, 10 mg/mL, for 20 min, and visualized under UV light. The recovered clear bands were purified using the QIAquick® PCR purification kit (Catalog no. 28104, Qiagen, Germany) and analyzed by sequencing. Sequencing was done by the Applied Biosystems 3500 Genetic analyzer sequencer (Hitachi, Japan).

DGGE analyses

DGGE patterns were analyzed by binary matrix, using AlphaView Software v.3.4.0 (http://www.proteinsimple.com/). The designation of the band-classes 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. Distances between gel patterns were determined using the cluster analysis with Euclidian measure. Similarity analysis of DGGE profiles was done using both of Jaccard coefficient, $S_{Jaccard}$ and Dice coefficient, S_{Dice}, which were calculated according to the formulas:

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

Table 1. Binary matrix of DGGE phylotypes and sequence accession numbers.

Phylotypes	Lakes			Accession numbers
	Aghormy	Zieton	Maraqi	
Siwa 1.Z.M	0	1	1	LC164659
Siwa 2.Z	0	1	0	LC164660
Siwa 3.Z	0	1	0	LC164661
Siwa 4.M	0	0	1	LC164662
Siwa 5.A	1	0	0	LC164663
Siwa 6.A	1	0	0	LC164664
Siwa 7.A	1	0	0	LC164665
Siwa 8.Z	0	1	0	LC164666
Siwa 9.A	1	0	0	LC164667
Siwa 10.Z	0	1	0	LC164668
Siwa 11.A	1	0	0	LC164669
Siwa 12.A.Z	1	1	0	LC164670

Where N_{AB} is the number of bands common to both patterns; N_A and N_B, representing 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 tree was constructed through two bioinformatics processes. In the first process, the nucleotide sequences of the recovered rRNA gene phylotypes and their homologous sequences from the DNA database were aligned using the online program "Clustal Omega" software (http://www.ebi.ac.uk/Tools/msa/clustalo/). In the second process, 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 tree, using maximum likelihood algorithm. The branching patterns of the constructed phylogenetic tree were confirmed by reconstruction of the phylogenies using the two other algorithms, neighbor joining and maximum parsimony. Bootstrap method, provided as a phylogeny test, in the MEGA software, was performed using a number of 500 bootstrap replications.

Nomenclature of phylotypes and sequence accession numbers

Each phylotype was marked by a number, showing its position on the DGGE profile, followed by the first letter of the source lake, where Aghormy, A, Zeiton, Z, and Maraqi, M, for example, phylotype 7.A, means phylotype number 7, belonging to Lake Aghormy. The phylotype synonym 1.Z.M indicated the phylotype number 1, which occurred in both of Zeiton and Maraqi Lakes, and so on.

The current 16S rRNA gene sequences have been registered in the DNA database under accession numbers listed in (Table 1).

RESULTS AND DISCUSSION

DGGE displayed specific bacterial 16S rRNA gene phylotype patterns for the studied lakes

All genomic DNAs from the lakes showed positive PCR amplifications of the bacterial 16S rRNA gene, variable regions, V3-V5. The DGGE displayed total of 12 bands, each band represented a phylotype. Each of the Lakes Aghormy and Zieton displayed 6 phylotypes (Figure 2 and Table 1). The Lake Maraqi had the lowest bacterial richness, representing only two phylotypes (Figure 2 and Table 1). Maraqi showed the highest accumulation of total dissolved solid contents, 280.45 gm/L, among the studied Siwa lakes, which may shaped the bacterial richness by occurrence of only limited species that could tolerate this very extreme environment (Abd El-Karim and Goher, 2016).

Some studies on hypersaline lakes showed numbers of DGGE bands greater than those of recorded in the current study. The DGGE banding patterns from hypersaline Lake Faro, Italy, showed a greatest difference of bands, 10 to 20 bands, was observed between depths of 0 and 30 m, respectively (Gugliandolo et al., 2011).

Richness of bacteria in Aran-Bidgol salt Lake, a thalasohaline lake in Iran showed 10 bands on DGGE profile (Makhdoumi-Kakhki et al., 2012).The results of 16S rRNA gene-based DGGE displayed 10 phylotypes from the hypersaline water of Lake Red, Romania (Borsodi et al., 2013). The PCR–DGGE method obtained 11 different bands, which were related to several distant taxonomic groups in central European hypersaline lakes (Keresztes et al., 2012). This difference in differential display of bacterial 16S rRNA gene DGGE patterns between current sampling lakes and other studied hypersaline lakes may be due to environmental conditions specific to each sampling location and methodology used in each study. Generally, the hypersaline environments have low bacterial diversity due to harsh conditions, which favor the dominance of specific bacterial community.

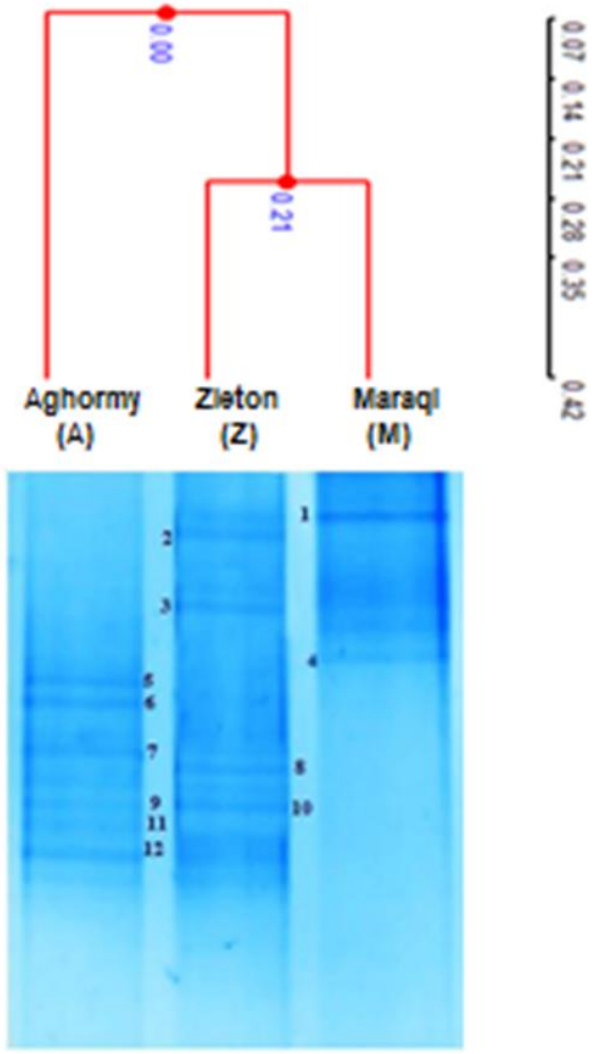


Figure 2. DGGE profiles of bacterial PCR-amplified 16S rRNA genes obtained from microbial mats collected from studied Siwa Lakes, Aghormy, A, Zieton, Z, and Maraqi, M. Sequenced bands, phylotypes, are indicated by numbers, from 1 to 12, as follow: 1. Z.M; 2. Z; 3. Z; 4. M; 5. A; 6. A; 7. A; 8. Z; 9. A; 10. Z; 11. A; and 12. A.Z. Euclidian distances among DGGE profiles are shown.

Dominance of bacteriodetes- and alphaproteobacteria-like phylotypes in the studied lakes

Phylogenetic analysis recorded 7 phylotypes, which were affiliated to the phylum Bacteriodetes (Figure 3). Bacteriodetes occur in wide range of hypersaline habitats (Oren, 2002; Gugliandolo et al., 2011; Borsodi et al., 2013). The phylotypes, 6.A, 7.A, 9.A and 4.M formed unique phylogenetic cluster with uncultured *Salinibacter* (Figure 3). Halobacterial genus, *Salinibacter*, has since been occurred ubiquitously in numerous hypersaline environments (Anton et al., 2008; Makhdoumi-Kakhki et al., 2012). *Salinibacter* survives in these harsh environments because of its adaptations in order to cope with the high salt concentrations (Mongodin et al., 2005). It was found that *S. ruber* made up from 5 to 25% of the total prokaryotic community of the Spanish saltern ponds (Anton et al., 2002). The phylotype, 12.A.Z, formed monophyletic clade with *Lewinella agarilytica* (Figure 3) (Buerger et al., 2012). The genus *Lewinella* was proposed by Sly et al. (1998) to encompass the marine species of the genus *Herpetosiphon* (Holt and Lewin, 1968) under the phylum Bacteriodetes. The current phylotype, 12.A.Z represented the first record of *Lewinella*-like phylotypes in hypersaline inland lakes.

The phylotypes, 1.Z.M, 2.Z, 3.Z and 8.Z were located in the branch of Alphaproteobacteria and linked with *Rhodovibrio* sp. from a saltern Mediterranean lake and uncultured Alphaproteobacterium from the hypersaline Sidi Ameur, Algerian Sahara (Moune et al., 2003; Boutaiba et al., 2011). Alphaproteobacteria is a diverse taxon and comprises several phototrophic genera, which occur in hypersaline habitats (Edbeib et al., 2016). The genus *Rhodovibrio* represents 35.3% of the total bacterial composition in Aran-Bidgol salt, the largest hypersaline lake in Iran (Makhdoumi-Kakhki et al., 2012). On the other hand, the current phylotypes had homologies, 85 to 90%, with corresponding species in the branches of Bacteriodetes and Alphaproteobacteria, representing new phylogenetic lineages, and consequently, expanding the global diversity of these bacterial groups in hypersaline environments.

Composition of 16S rRNA gene phylotypes implicates endemic bacterial communities within the studied lakes

The bacterial phylotype compositions were different among the studied lakes, showing very low similarity indices (Table 2). The similarity matrix value between Aghormy and Maraqi was zero, representing completely different bacterial communities. These results may implicate that each lake has the special physicochemical characteristics, which may shape the bacterial composition, to be specific for each lake (Abd El-Karim and Goher, 2016). This concept was strengthened by co-occurrence of Alphaproteobacteria-like phylotype, 1.Z.M, in the Lakes Zeiton and Maraqi, while lacked in Lake Aghormy (Figure 3). Moreover, the recorded bacterial phylotypes showed distant phylogenetic lineages within the branches of Bacteriodetes and Alphaproteobacteria (Figure 3). On the other hand, the phylotype 5.A, characterized only the Aghormy Lake, forming a monophyletic clade with uncultured spirochaete isolated from hypersaline site, Guerrero Negro, Mexico (Figure 3) (Sahl et al., 2008). The spirochaetes have phylogenetic

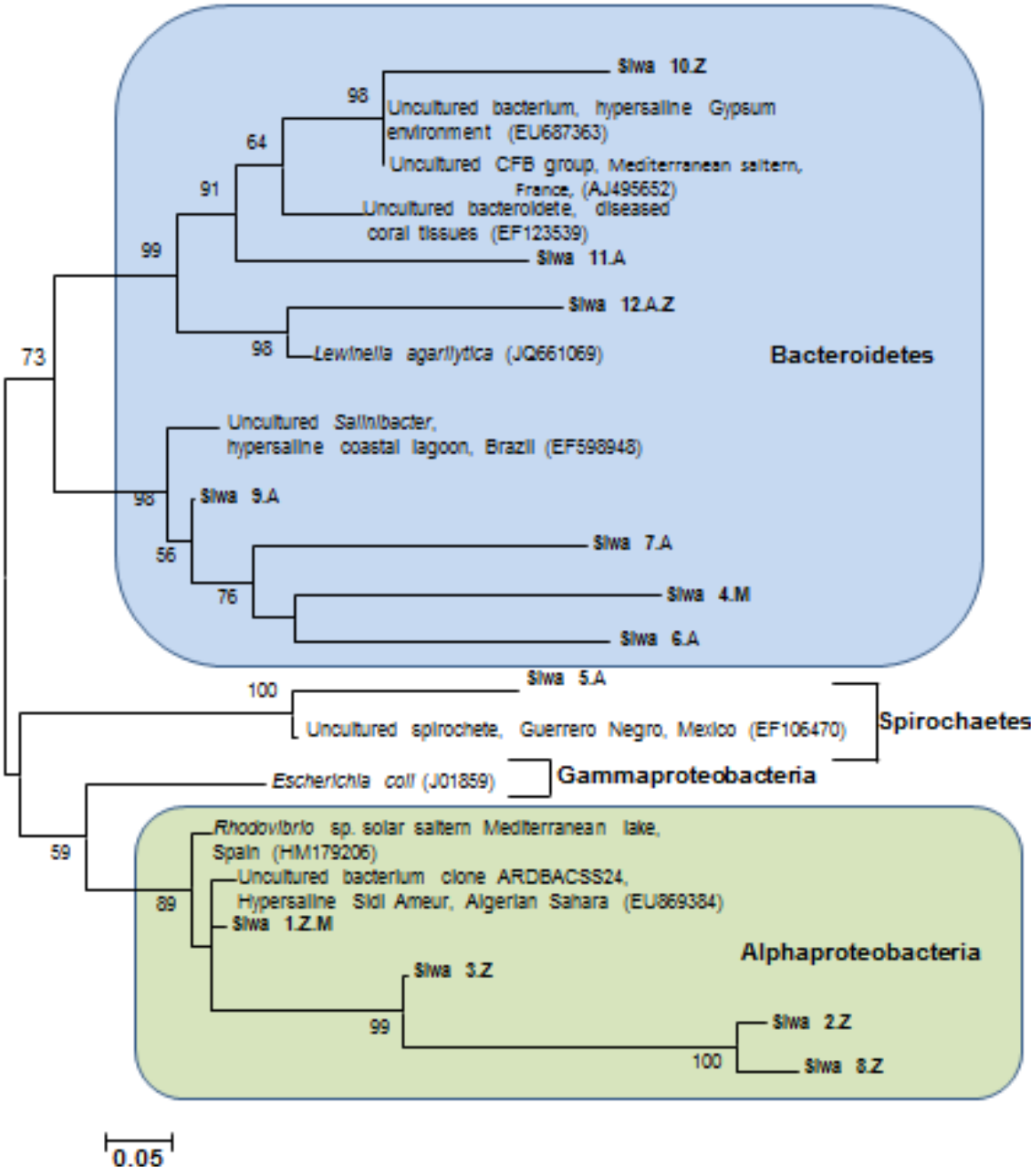


Figure 3. A consensus phylogenetic tree based on 16S rRNA gene sequences of current recorded bacterial phylotypes, from the studied Siwa lakes; beside their corresponding sequences from database. Bootstrap values, more than 50%, of compared algorithms, are indicated at the branch roots. The bar represents 0.05 changes per nucleotide. Accession numbers of database extracted sequences are in brackets.

Table 2. Similarity matrix, using Jaccard coefficient (Dice coefficient).

Lakes	Aghormy	Zieton	Maraqi
Aghormy	100.0	-	-
Zieton	9.0 (16.6)	100.0	-
Maraqi	0(0)	14.28 (25)	100.0

relationships at the level of gross phenotypic characteristics. They possess some genomic features that help those bacteria in living at hypersaline habitats (DasSarma and DasSarma, 2012).

Conclusion

The approach of uncultured-dependent PCR/DGGE followed by sequencing of DGGE bands, targeting the 16S rRNA gene, was applied to study bacterial richness in three Lakes, Aghormy, Zeiton and Maraqi, Siwa oasis, Egypt. The bacterial compositions in all three lakes had low diversity, a common characteristic of hypersaline environments. Bacteriodetes-like phylotypes dominated in all lakes, while Alphaproteobacteria-like phylotypes were occurred in Zeiton and Maraqi. Each lake showed novel endemic bacterial phylotype composition, with low nucleotide homologies with other previous studied halophilic taxons. However, this study opened a window to explore the bacterial phylotypes in hypersaline lakes of western desert, Egypt. Future phylogenetic studies must be done in order to cover the total diversity of all microbial communities, from other domains, archaea and eukaryotes, living within those lakes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors thank all technical and administrative stuff at National Institute of Oceanography and Fisheries, Egypt, for help in sampling and experimental work. This work was funded by both of basic fund of National Institute of Oceanography and Fisheries, NIOF, and Grant-in-Aid for scientific research, project number 5592, Science and Technology Development Fund, STDF, Egypt.

REFERENCES

Abd El-Karim M (2016). Chemical composition and antimicrobial activities of cyanobacterial mats from hyper saline Lakes, northern western desert, Egypt. J. Appl. Sci. 16(1):1.

Abd El-Karim M, Goher M (2016). Heavy metal concentrations in cyanobacterial mats and underlying sediments in some northern western desert lakes of Egypt. J. Fish. Aquat. Sci. 11:163-173.

Anton J, Oren A, Benlloch S, Rodriguez-Valera F, Amann R, Rossello-Mora R (2002). *Salinibacter ruber* gen. nov., sp. nov., a novel, extremely halophilic member of the bacteria from saltern crystallizer ponds. Int. J. Syst. Evol. Microbiol. 52:485-491.

Anton J, Peña1 A, Santos F, Martínez-García M, Schmitt-Kopplin P, Rosselló-Mora, R (2008). Distribution, abundance and diversity of the extremely halophilic bacterium *Salinibacter ruber*. Saline Syst. 4:15.

Borsodi A, Felfoldi T, Mathe I, Bognar V, Knab M, Krett G, Jurecska L, Toth E, Marialigeti K (2013). Phylogenetic diversity of bacterial and archaeal communities inhabiting the saline Lake Red located in Sovata, Romania. Extremophiles 17:87-98.

Boujelben I, Yarza P, Almansa C, Villamor J, Maalej S, Anton J, Santos F (2012). Virioplankton community structure in Tunisian solar salterns. Appl. Environ. Microbiol. 78:7429-7437.

Boutaiba S, Hacene H, Bidle K, Maupin-Furlow J (2011). Microbial diversity of the hypersaline Sidi Ameur and Himalatt salt lakes of the Algerian Sahara. J. Arid Environ. 75:909-916.

Buerger S, Spoering A, Gavrish E, Leslin C, Ling L, Epstein S (2012). Microbial scout hypothesis and microbial discovery. Appl. Environ. Microbiol. 78:3229-3233.

DasSarma S, DasSarma P (2012). Halophiles. Encycl. Life Sci. pp. 1-9.

Edbeib M, Abdul Wahab R, Huyop F (2016). Halophiles: biology, adaptation, and their role in decontamination of hypersaline environments. World J. Microbiol. Biotechnol. 32:135.

Elsaied H (2013). Monitoring of uncultured *Dunaliella* sp. in an Egyptian solar saltern field based on RuBisCO-encoding gene *cbbL*. Afr. J. Biotechnol. 12:5361-5369.

Gugliandolo C, Lentini V, Maugeri T (2011). Distribution and diversity of bacteria in a saline meromictic lake as determined by PCR-DGGE of 16S rRNA gene fragments. Curr. Microbiol. 62:159-166.

Holt J, Lewin R (1968). *Herpetosiphon aurantiacus* gen. et sp. n., a new filamentous gliding organism. J. Bacteriol. 95:2407-2408.

Keresztes Z, Felfldi T, Somogyi B, Szekely G, Dragos N, Marialigeti K, Bartha C, Voros L (2012). First record of picophytoplankton diversity in Central European hypersaline lakes. Extremophiles 16:759-769.

Luque R, Bejar V, Quesada E, Llamas I (2014). Diversity of halophilic bacteria isolated from Rambla Salada, Murcia (Spain). Can. J. Microbiol. 60:839-846.

Mahmoud A, Abd El-Karim M (2016). Fatty acid profiles, pigments and biochemical contents of cyanobacterial mat in some lakes of North Western Desert, Egypt. Am. J. Biochem. Mol. Biol. 6:15-24.

Makhdoumi-Kakhki A, Amoozegar M, Kazemi B, Pasic L, Ventosa A (2012). Prokaryotic diversity in Aran-Bidgol Salt Lake, the largest hypersaline Playa in Iran. Microbes Environ. 27:87-93.

Mehrshad M, Amoozegar MA, Makhdoumi A, Rasooli M, Asadi B, Schumann P, Ventosa A (2015). *Halovarius luteus* gen. nov., sp. nov., a novel extremely halophilic archaeon from Urmia salt lake. Iran. Int. J. Syst. Evol. Microbiol. 65:2420-2425.

Mongodin EF, Nelson KE, Daugherty S, Deboy RT, Wister J, Khouri H, Weidman J, Walsh DA, Papke RT, Sanchez Perez G, Sharma AK, Nesbø CL, MacLeod D, Bapteste E, Doolittle WF, Charlebois RL, Legault B, Rodriguez-Valera F (2005). The genome of *Salinibacter ruber*: convergence and gene exchange among hyperhalophilic bacteria and archaea. Proc. Natl. Acad. Sci. USA. 102:18147–18152.

Moune S, Caumette P, Matheron R, Willison J (2003). Molecular sequence analysis of prokaryotic diversity in the anoxic sediments underlying cyanobacterial mats of two hypersaline ponds in Mediterranean salterns. FEMS Microbiol. Ecol. 44:117-130.

Mutlu MB, GUVen K (2015). Bacterial diversity in Camalt Saltern, Turkey. Polish J. Microbiol. 64:37-45.

Muyzer G, De Waal EC, Uitterlinden AG (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes encoding for 16S rRNA. Appl. Environ. Microbiol. 59:695-700.

Nubel U, Engelen B, Felske A, Snaidr J, Wieshuber A, Amann RI, Ludwig W Backhaus H (1996). Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. Bacteriol. 178:5636–5643.

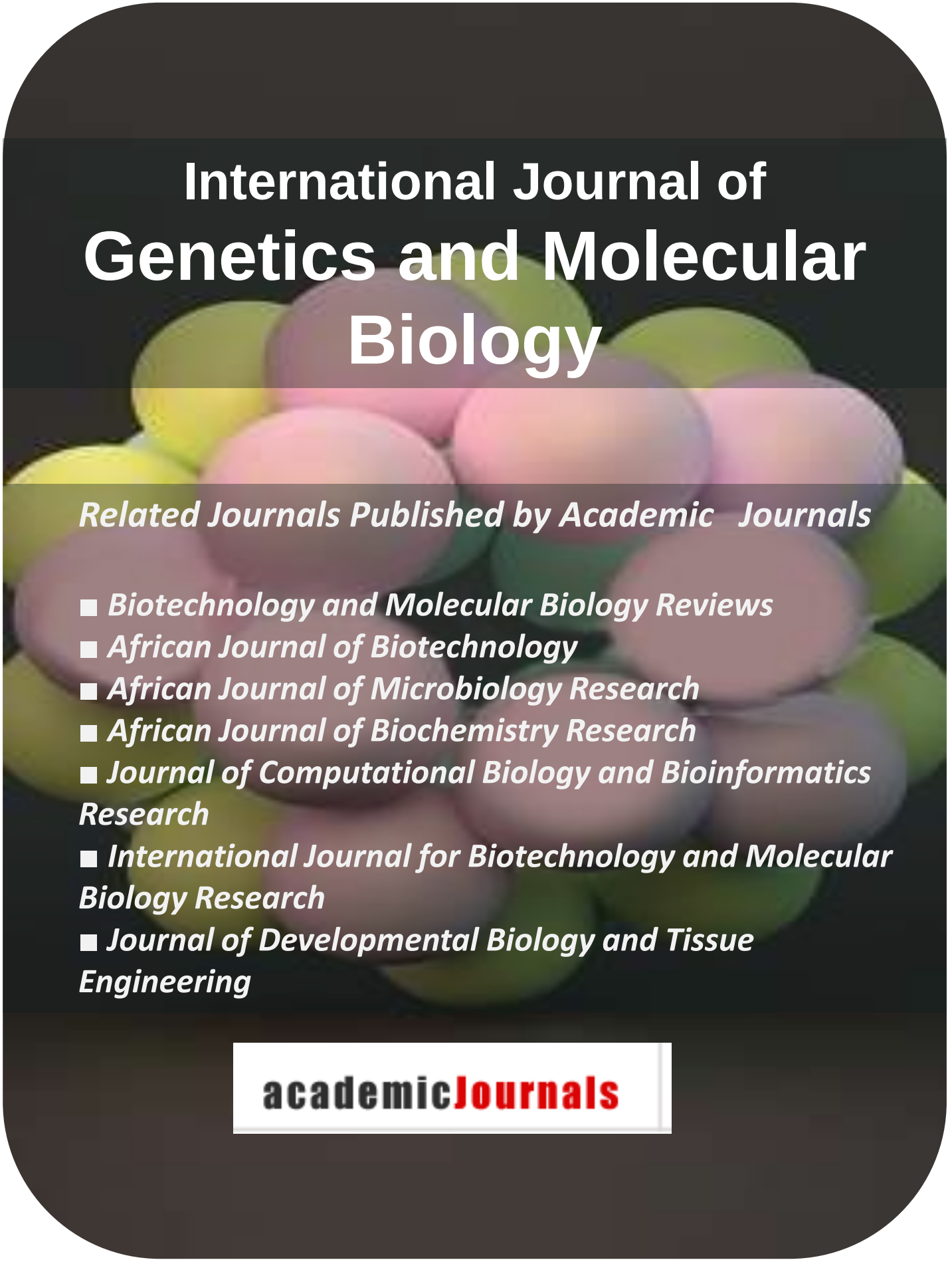
Ollivier B, Caumette P, Garcia JL, Mah RA (1994). Anaerobic bacteria from hypersaline environments. Microbiol. Rev. 58:27-38.

Oren A (2002). Molecular ecology of extremely halophilic archaea and bacteria. FEMS Microbiol. Ecol. 39:1-7.

Pinar G, Krakova L, Pangallo D, Piombino-Mascali D, Maixner F, Zink A, Sterflinger K (2014). Halophilic bacteria are colonizing the exhibition areas of the Capuchin Catacombs in Palermo, Italy. Extremophiles18:677-691.

Sahl J, Pace N, Spear J (2008). Comparative molecular analysis of endoevaporitic microbial communities. Appl. Environ. Microbiol.

74:6444-6446.
Sly L, Taghavi M, Fegan M (1998). Phylogenetic heterogeneity within the genus *Herpetosiphon*: transfer of the marine species *Herpetosiphon cohaerans*, *Herpetosiphon nigricans* and *Herpetosiphon persicus* to the genus *Lewinella* gen. nov. in the Flexibacter– Bacteroides–Cytophaga phylum. Int. J. Syst. Bacteriol. 48:731-737.
Suzuki M, Giovannoni S (1996). Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl. Environ. Microbiol. 62:625-630.
Tazi L, Breakwell DP, Harker AR, Crandall KA (2014). Life in extreme environments: microbial diversity in Great Salt Lake. Utah. Extremophiles 18:525-535.
Wei X, Jiang Y, Chen X, Jiang Y, Lai H (2015). *Amycolatopsis flava* sp. nov., a halophilic actinomycete isolated from Dead Sea. Antonie Van Leeuwenhoek.
Xiang W, Guo J, Feng W, Huang M, Chen H, Zhao J, Zhang J, Yang Z, Sun Q (2008). Community of extremely halophilic bacteria in historic Dagong brine well in southwestern China. World J. Microbiol. Biotechnol. 24:2297-2305.



International Journal of Genetics and Molecular Biology

Related Journals Published by Academic Journals

- *Biotechnology and Molecular Biology Reviews*
- *African Journal of Biotechnology*
- *African Journal of Microbiology Research*
- *African Journal of Biochemistry Research*
- *Journal of Computational Biology and Bioinformatics Research*
- *International Journal for Biotechnology and Molecular Biology Research*
- *Journal of Developmental Biology and Tissue Engineering*

academicJournals